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FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

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The sustainability of endangered species under intensive management: the case of the scimitar-horned oryx *Oryx dammah*

by

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ABSTRACT

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THE SUSTAINABILITY OF ENDANGERED SPECIES UNDER INTENSIVE MANAGEMENT: THE CASE OF THE SCIMITAR-HORNED ORYX *Oryx dammah*

By Tania Gilbert

The world is facing an unprecedented loss of biodiversity caused by anthropogenic environmental change. Captive breeding and reintroduction can help mitigate the effects of biodiversity loss for some endangered species, but to accomplish this, captive populations need to be self-sustainable. Intensive population management aims to achieve sustainability by maximising the retention of genetic diversity, maintaining demographic stability, and reducing adaptation to captivity. Recent evaluations of captive populations have indicated that many are not meeting their genetic and demographic goals, and are not sustainable. Consequently, their contribution to biodiversity conservation is being undermined.

This thesis aims to evaluate the sustainability of captive populations using the scimitar-horned oryx as a case study. The European scimitar-horned oryx population experiences many of the challenges encountered by other captive populations, specifically, poor data quality in the international studbook resulting in less effective population management; rapid loss of genetic variation; and economic fragmentation.

This thesis presents a series of original studies that evaluate the sustainability of captive populations, examines the impact of poor quality data on population management, and tests the effects of population fragmentation. The results contribute knowledge to the management of small captive populations in general, and to the scimitar-horned oryx in particular. I propose solutions to some of the challenges faced by endangered species in captivity, and advocate a reorientation of the existing small population management paradigm. Finally, I challenge the international zoological community to fulfil its potential for biodiversity conservation, and sustainably manage the populations in its care.

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DECLARATION OF AUTHORSHIP

I, Tania Gilbert, declare that the thesis entitled 'The sustainability of endangered species under intensive management: the case of the scimitar-horned oryx *Oryx dammah*', and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
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- none of this work has been published before submission

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Date:…31/12/2011…….

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DNA extraction from blood, tissue, skin and faecal samples detailed in Chapter Five was carried out by Dr Arati Iyengar at the University of Southampton. Further information on this can be found in Appendix E and F. The microsatellite analysis detailed in Chapter Five was carried out by Rob Ogden at the Wildgenes laboratory at the Royal Zoological Society of Scotland.

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List of Acronyms

AE	United Arab Emirates (UAE)
AFLP	Amplified Fragment Length Polymorphisms
ARKS	Animal Record Keeping System
ASMP	Australasian Species Management Plan
ARAZPA	Australasian Regional Association of Zoological Parks and Aquaria (now ZAA)
AT	Austria
AWPR	Al Ain Wildlife Park and Resort
AZA	The Association of Zoos and Aquariums (USA)
BE	Belgium
BG	Bulgaria
BSE	Bovine spongiform encephalopathy
BT	Bluetongue
CBSG	Conservation Breeding Specialist Group IUCN/SSC
CD	Compact disc
CH	Switzerland
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CMS	Convention on the Conservation of Migratory Species of Wild Animals
CTT	Cotton-top tamarin
CZ	Czech Republic
DE	Germany
DK	Denmark
DNA	Deoxyribonucleic Acid
DOS	Disc Operating System
EAD	Environment Agency Abu Dhabi
EAZA	European Association of Zoos and Aquaria
EE	Estonia
EEP	European Endangered species Programme
ES	Spain
ESB	European Studbook

EU	European Union
FFEM	Fonds Français pour l'Environnement Mondial
FGE	Founder Genome Equivalents
FGS	Founder Genome Surviving
FI	Finland
FIC	Founder Importance Coefficient
FMD	Foot and Mouth Disease
FPA	Fenced Protected Area
FR	Faunal Reserve
FR	France
GB	United Kingdom
GD	Gene Diversity
GE	Georgia
GR	Greece
GU	Genome Uniqueness
GV	Gene Value
H_e	Expected Heterozygosity
HR	Croatia
HU	Hungary
IE	Ireland
IL	Israel
ISB	International Studbook
ISIS	International Species Information System
IT	Italy
IUCN	The World Conservation Union
JAZA	Japanese Association of Zoos and Aquaria
KZ	Kazakhstan
LR	Lynch-Ritland Relatedness Value
LT	Lithuania
LTF	Lost-to-follow-up
LV	Latvia
MAI	Maximum Avoidance of Inbreeding
MD	Republic of Moldova

MGA	Melengestrol Acetate
MHC	Major Histocompatibility Complex
MK	Mean Kinship
MtDNA	Mitochondrial DNA
N_e	Effective population size
NL	Netherlands
NO	Norway
NP	National Park
NR	Nature Reserve
OIE	World Organisation for Animal Health
PA	Protected area
PE	Probability of extinction
PL	Poland
PM2000	Population Management 2000 software
PMP	Population Management Plan
PMx	Population Management x software
PT	Portugal
PVA	Population Viability Analysis
QA	Qatar
RAPD	Random Amplified Polymorphic DNA
REGASP	Regional Animal Species Collection Plan
RFLP	Restriction Fragment Length Polymorphisms
RU	Russian Federation
SCF	Sahara Conservation Fund
SD	Standard deviation
SE	Sweden
SFR	Special Faunal Reserve
SHO	Scimitar-horned oryx
SI	Slovenia
SK	Slovakia
SNP	Single Nucleotide Polymorphisms
SPARKS	Single Population Analysis and Record Keeping System
SSC	IUCN Species Survival Commission
SSP	Species Survival Plan

TAG	Taxon Advisory Group
TE	Time to extinction
UA	Ukraine
ZAA	Zoo and Aquarium Association (formerly the Australasian Regional Association of Zoological Parks and Aquaria)
ZIMS	Zoological Information System

1.0 Chapter one: introduction

1.1 The biodiversity crisis

The world is currently facing a period of unprecedented loss of biodiversity, in which hundreds of species have already been driven to extinction by anthropogenic change, and thousands more are predicted to go extinct in the next few decades (Lande *et al.* 2003; Magin *et al.* 1994; Magurran & Dornelas 2010; Pereira *et al.* 2010; Rands *et al.* 2010; Stork 2010). The International Union for the Conservation of Nature (IUCN) documents that 47,677 species are now at risk of extinction (Conway 2011), but only an estimated 26% of known species have been assessed using the IUCN Red List of Threatened Species criteria (IUCN 2010), and most of the estimated 4-15 million species on the planet have not yet been described (Barnosky & Kraatz 2007; Jackson & Johnson 2001; Stork 2010). Current species extinction rates are 1,000-10,000 times higher than the background rates observed in the fossil record (Mace & Purvis 2008; Magurran & Dornelas 2010; Purvis et al. 2000a), but this underestimates the overall loss of biodiversity as local population extinctions, habitat loss, and erosion of genetic diversity are not included (Brooks *et al.* 2006; Frankham 1995a; Purvis *et al.* 2000a; Rands *et al.* 2010).

Unlike past mega-extinction events, which were the result of massive natural catastrophes such as asteroid collisions and global shifts in climate (Franklin 1980), the current extinction crisis is being caused by a rapidly increasing human population and its rising consumption of natural resources (Conway 2011; Primack 2002; Rands *et al.* 2010).

Human activity has resulted in habitat destruction, fragmentation and degradation, pollution, over-exploitation of resources, and the introduction of exotic species and diseases to naïve populations (Pullin 2002; Rands *et al.* 2010; Stork 2010; Venter *et al.* 2008). The threats posed by habitat destruction and over-exploitation are compounded by the potentially damaging effects of rapid anthropogenic global climate change (Godley 2009; Isaac 2009; Rubidge *et al.* 2011; Venter *et al.* 2008). All of these factors, individually and combined, have had a devastating impact on global biodiversity (Lee & Jetz 2010; Price & Gittleman 2007; Reed 2004).

Biodiversity has an intrinsic value, but it also provides human societies with food, fuel, fibre and medicine (Frankham 1995a; Mace *et al.* 2010). Biodiversity contributes to agriculture through crop pollination and pest control; it provides carbon storage and sequestration, and helps buffer against disturbance and environmental change (Rands *et al.*

2010). It is becoming increasingly clear that the loss of biodiversity has a major impact on ecosystem functions and services (Baillie *et al.* 2008; Mace *et al.* 2010). Added to this are non-material benefits such as recreation and a positive impact on human health and wellbeing (Rands *et al.* 2010; Sutherland 1998). Biodiversity is not only essential for human survival, but the cost of maintaining it is only a fraction of its economic value (Frankham 1995a; Rands *et al.* 2010).

1.2 Conserving biodiversity

There are a number of different approaches to conserving biodiversity, including the development of prominent international, regional and national legislation (Pullin 2002), such as the Convention on Biological Diversity (CBD) (UNEP 2011). Conservation action has helped protect biodiversity at both a local and global level. Orthodox conservation practices, such as habitat restoration, removal of anthropogenic pressures and invasive species, and establishment of protected area networks (Dawson *et al.* 2011; Hoffmann *et al.* 2010; Rands *et al.* 2010; Stork 2010), have resulted in notable successes (Hoffmann *et al*. 2010). Whilst protected areas are crucial for conserving ecosystems and biodiversity (Lee & Jetz 2010; Rands *et al.* 2010), they are not a guarantee of protection for all the species living within their borders (Bruner *et al.* 2001; Gordon 1991; Venter *et al.* 2008). Added to this is the fact that only approximately 13% of the Earth's land is formerly protected (WDPA 2011). Consequently, a more species driven approach to conservation is sometimes required (Shaffer 1981).

Endangered species often have declining and fragmented populations (Höglund 2009; Wilson *et al.* 2004), and targeted action includes mitigation of threats such as over-hunting, habitat protection, intensive captive management and reintroduction (Caro *et al.* 1998; Pullin 2002; Rands *et al.* 2010). It is widely acknowledged that species conservation is most effective when it takes place in the species' natural habitat (Caro *et al.* 1998; Redford *et al.* 2011). This also has the advantage that other species, and their interactions with each other and the environment, are also preserved, i.e. the focal species acts as an umbrella for wider conservation (Balmford *et al.* 1995; Caro 2003; Pullin 2002; Redford *et al.* 2011). Many species are now conservation dependent (IUCN 2010), and reliant on long-term action. For example, the whopping crane *Grus Americana* has been the recipient of targeted conservation action for 70 years, and the white rhinoceros *Ceratotherium simum*, for 115 years (Hoffmann *et al.* 2010). This species-specific approach has yielded some notable successes. The golden lion tamarin *Leontopithecus rosalia* was downgraded

from ‘Critically Endangered’ to ‘Endangered’ on the IUCN Red List of Threatened Species after 30 years of concerted conservation action (Ballou *et al*. 1995; Hoffmann *et al*. 2010; IUCN 2010; Rands *et al.* 2010).

1.3 Intensive management of endangered species and populations

Although conserving species in their natural habitat is preferable, the traditional notion of the ‘wild’ is disappearing, especially for large-bodies species with group social structures and extensive home ranges (Foose *et al.* 1995; Purvis *et al.* 2000b), such as antelope. These species are particularly vulnerable to habitat loss and degradation, edge effects, and over-exploitation by humans. Antelope often have low fecundity rates and population densities, and are more susceptible to extinction because they are less able to compensate for increased mortality (Purvis *et al.* 2000b). Approximately 62% of antelope have declining populations (Mallon & Chardonnet 2009; Mésochina *et al*. 2009), and half of all antelope species are threatened with extinction (Mésochina *et al.* 2003; Price & Gittleman 2007). Nine species of antelope are classified as ‘Vulnerable’, nine as ‘Endangered’, five as ‘Critically Endangered’, and one, the scimitar-horned oryx *Oryx dammah*, as ‘Extinct in the Wild’ by the IUCN (Mésochina *et al.* 2009). The status of the scimitar-horned oryx means that research into its status and conservation is particularly important, and this thesis contributes to this.

Increasing habitat destruction and fragmentation have resulted in many wildlife populations being reduced in size and restricted to isolated protected areas (PAs) (Lacy 1992; 1993a; Mace *et al*. 1992). Few PAs are large enough to support self-sustaining populations (Conway 2011; Lacy 1992), consequently, many species need some form of curatorial care such as veterinary support, supplemental feeding and population management (Conway 2011). In some instances monitoring and management is so intensive that every individual, and its pedigree, is known and managed, as can be seen with the Eastern mountain gorilla *Gorilla gorilla berengei* in Central Africa (Ballou *et al.* 1995). In such cases, conservation management of populations in their natural habitat is similar to those in captivity (Mace 1989). In essence, many PAs are becoming megazoos with fenced boundaries (Foose *et al.* 1995).

Some species are so severely threatened in the wild that saving a species and saving its habitat are no longer linked in space and time (Conway 2011). Successful species conservation often depends on complementary *in-situ* and *ex-situ* strategies including habitat protection and restoration, captive breeding and reintroduction (IUCN 2002; Ralls

& Ballou 1992; Redford *et al.* 2011). Article 9 of the CBD, the IUCN, and the US Endangered Species Act all recognise that *in-situ* and *ex-situ* conservation actions need to be combined in order to address species extinction (Conde *et al.* 2011a; UNEP 2011). This has lead some authors to reject the traditional binary classification of 'captive' and 'wild' in favour of one based on a gradient of human intervention. The proposed classification ranges from 'fully conserved' for species that are not reliant on direct human action for their survival, to 'captive managed' in which the species no longer occurs in the wild (Soulé 1980).

When threatened species have been removed from their natural habitat to establish intensively managed captive populations, the intention is often to return them to the wild at some later date when the cause of the species decline has been removed (Foose *et al.* 1995; Frankham *et al.* 2010; Hedrick & Miller 1992; Montgomery *et al.* 2010; Pullin 2002; Robert 2009; Williams & Hoffman 2009). Many captive populations are regarded as 'assurance populations' or an 'insurance policy' to protect against extinction if the species is extirpated in the wild (Bingaman Lackey 2010; Conde *et al.* 2011a; Frankham 2008; Mace 1989), and can make a valuable contribution to the conservation of species, sub-species, varieties and populations (Ballou & Lacy 1995; Caughley 1994; IUCN 2002; Philippart 1995).

There are strong arguments for the captive breeding and reintroduction of species that are extinct in the wild or threatened with imminent extinction (Philippart 1995; Snyder *et al.* 1996). In captivity, populations can be protected from over-exploitation, environmental variance can be moderated, and intensive genetic and demographic management can take place. Captive populations are safeguarded from many sources of natural mortality, so rapid population growth can provide stock for reintroduction to natural habitats (Foose *et al.* 1995; Lacy 1994). In some cases captive breeding may be the only solution for a species' survival (Briton *et al.* 1994; Foose *et al.* 1995; Hedrick & Miller 1992; IUCN 2002).

There is also an argument for the captive breeding and display of species for the purpose of public education, professional training, research, and fundraising for *in-situ* conservation projects. Alongside this, many zoological institutions are involved in the conservation of habitats and ecosystems associated with the species in their care (WAZA 2005c; 2005d). This type of captive breeding differs in its objectives to that of breeding for reintroduction, but it is no less valid in contributing to conservation (Foose *et al.* 1995; Gippoliti & Carpaneto 1997; Hutchins *et al.* 1997; Snyder *et al.* 1996).

Whilst the benefits of captive breeding are widely acknowledged, there are also concerns associated with this approach to biodiversity conservation. Captive breeding is undoubtedly expensive when compared to conserving species in their natural habitat (Balmford *et al.* 1995; 1996; Lacy 1994; Snyder *et al.* 1996), and there is some concern that captive breeding directs funds away from *in-situ* conservation initiatives (Bowkett 2009). Added to this is the failure of some species to breed well under captive conditions, genetic and behavioural adaptation to captivity, loss of genetic diversity, and alterations in the morphological, physiological, ecological, genetic and behavioural characteristics that define the species i.e. species integrity (Balmford *et al.* 1996; Frankham 1995a; Lacy 1994; Mcphee 2004; Philippart 1995; Snyder *et al.* 1996). The ecological interactions between animals and their environments are also disconnected (Junhold & Oberwemmer 2011). These factors, individually and combined, may result in poor captive breeding and reintroduction success (Bowkett 2009). Captive breeding can also become an end in itself (Ballou 1992; Snyder *et al.* 1996), and consequently should always be combined with other species conservation measures (Conde *et al.* 2011b; Ralls & Ballou 1992).

Whilst these concerns are all valid, captive breeding has made the difference between extinction and survival for a number of species. There are 38 animal species that are 'Extinct in the Wild' and only persist because of captive-breeding programmes (IUCN 2010). Examples of these include the scimitar-horned oryx, California condor *Gymnogryps californianus*, and Guam rail *Rallus owstoni*, Wyoming toad *bufo baxteri*, and American bison *Bison bison* (Conway 2011; Gilbert & Woodfine 2004a; Snyder *et al.* 1996).

The ongoing biodiversity crisis means that many more species will need intensive captive management to avoid extinction (Conway 2011; Frankham 2005b). Soulé *et al.* (1986) estimated that 2,000 large vertebrate species would require captive breeding assistance. The IUCN (2002) recommended that threatened taxa of scientific or cultural importance, and all taxa listed as 'Critically Endangered' and 'Extinct in the Wild' should be subject to intensive *ex-situ* management. This amounts to a minimum of 1695 species of Animalia (IUCN 2002; 2010). Captive breeding is likely to be a critically important tool for the conservation of many more species in the future (Ballou & Ralls 1982; Snyder *et al.* 1996; WAZA 2005e).

1.4 Captive breeding and reintroduction

Many of the arguments in support of captive breeding are centred on captive breeding for reintroduction as a species conservation tool (Gordon 1991; Mésochina *et al.*

2003; Robert 2009). The practice of releasing captive-bred animals into the wild to support, or re-establish, endangered populations is increasing (Conway 2011; Earnhardt 1999). Most reintroductions involve charismatic megafauna, for example the white-tailed sea eagle *Haliaeetus albicilla*, griffon vulture *Gyps fulvus*, and orang-utan *Pongo pygmaeus* (Sarrazin & Barbault 1996). Vertebrate reintroductions are over-represented with respect to their prevalence in nature, and this bias is not related to vulnerability to threat (Conway 2011; Seddon *et al*. 2005). The bias can be partially explained because reintroductions often use a flagship, or umbrella species, that confer protection to other fauna and flora through their reintroduction to their natural habitat (Sarrazin & Barbault 1996; Seddon *et al.* 2005). An ISI Web of Knowledge literature search in July 2009 using the search term 'reintroduction', returned papers on 218 species of plant and animal that have been reintroduced. One-hundred and fifty-six species were vertebrates, with mammals (50%) and birds (35%) dominating the species list. Not all the papers reported the source of animals (vertebrates) for reintroduction, but those that did stated that 67% came from captive-bred sources (Appendix A). This is likely to be an underestimate of species reintroductions, as many reintroduction practitioners have not reported findings in journals or conference proceedings, and are therefore not included in the literature search.

Despite the prevalence of reintroduction projects, many attempts have failed to establish self-sustaining populations (Beck *et al.* 1994; Bowkett 2009; Conway 2011; Mathews *et al.* 2005; Reading *et al*. 1997; WAZA 2005b), especially when captive-bred animals have been used (Campbell 1980; Mathews *et al.* 2005). Intensively managed populations may genetically adapt to captive conditions, resulting in the expression of deleterious traits, or maladaptation, when they are released to the wild (Campbell 1980; Frankham 2005b; Lacy 1994; Montgomery *et al.* 2010). Added to this is ecological naivety and behavioural adaptation to captivity that may result in inappropriate behavioural responses once animals are released (Hakansson & Jensen 2005; Lyles & May 1987). For example, reintroduced captive-bred bank voles *Clethrionomys glareolus* were unable to effectively forage for food, and were less dominant than wild-bred voles (Mathews *et al.* 2005), and golden-lion tamarins released to Brazil could not move quickly through the forest canopy and were confused by novel foods (Lacy 1994). Furthermore, some reintroduced animals, for example ruffed lemurs *Varecia variegate*, elk *Cervus canadensis*, houbara bustards *Chlamydotis undulate*, and black-footed ferrets *Mustela nigripes*, were unable to effectively avoid predators (Lacy 1994; Ralls & Ballou 1992; Rosatte *et al.* 2007; Zafar-ul Islam *et al.* 2010; WAZA 2005b). Although inappropriate behavioural responses

can lead to decreased survivorship for released animals (Conde *et al.* 2011a; Mathews *et al.* 2005), it varies between species. The annual survival rate of reintroduced captive-reared bighorn sheep *Ovis Canadensis* did not differ from wild-reared bighorn sheep (Ostermann *et al*. 2001). Captive-bred individuals may also have significantly reduced reproductive capacity compared to wild-bred counterparts (Wang & Ryman 2001). This has been particularly noticeable in supportive-breeding programmes for fish (Araki *et al*. 2007; Ardren & Kapuscinski 2003; Jensen *et al.* 2008).

Small closed populations such as those found in captivity lose genetic diversity through genetic drift and inbreeding (Ryman & Laikre 1991; Wilcken & Lees 1998). A lack of genetic diversity in a reintroduced population means that it may not be able to respond to selection pressures in the wild and adapt to the local environment (Armstrong & Seddon 2007; Arnold 1995; Lage & Kornfield 2006; Sarrazin & Barbault 1996).

Reintroductions are usually costly, there are considerable logistical difficulties and technical challenges, concerns over animal welfare, and a shortage of suitable habitat, making it an unfeasible option for many rare and endangered species (Kleiman 1989; Law & Linklater 2007; Mathews *et al.* 2005; Ralls & Ballou 1992).

Despite this, captive breeding and reintroduction can be an effective conservation strategy, and the number of successful reintroductions has increased in the last decade (Bowkett 2009). Examples of successful reintroduction projects include the American bison, bald eagle *Haliaeetus leucocephalus,* bean goose *Anser fabalis,* Peregrine falcon *Falco peregrinus,* and the European wisent *Bison bonasus* (Kleiman 1989; Ralls & Ballou 1992; Wolf *et al.* 1996). The European wisent was extinct in the wild in 1921, but a series of reintroduction projects has seen wild numbers reach approximately 3000 (Kleiman 1989). Similarly, the captive breeding and reintroduction of the peregrine falcon has resulted in the re-establishment of this species across much of North America (Ralls & Ballou 1992).Captive breeding and reintroduction has played a major role in the recovery of 17 of the 68 species whose IUCN Red List threat status has been reduced, including the Arabian oryx *Oryx leucoryx,* Przewalski horse *Equus przewalskii,* black-footed ferret, and Mauritius kestrel *Falco punctatus* (Abu Jafar & Hays-Shahin 1988; Bouman *et al.*1994; Conde *et al.* 2011a; Frankham *et al.* 2010; Snyder *et al.* 1996). Whilst successes are widely publicised, the definition of success varies and is limited in time (Seddon 1999). The reintroduction of the Arabian oryx to Oman was widely hailed as a great success story. However, two decades after the first releases, epidemic poaching has meant that a free-ranging population is no longer viable (Seddon 1999; WAZA 2005b).

1.5 Challenges for captive populations

There are a number of factors that influence the success of a reintroduction project, but a viable, well-managed, sustainable captive population with substantial genetic diversity is a pre-requisite when utilising captive-bred animals (Kleiman 1989; Lees & Wilcken 2009; Ralls & Ballou 1992; Wisely *et al.* 2003).

Captive populations are often small, fragmented and closed (Ballou & Lacy 1995; Ballou & Foose 1996; Williams & Hoffman 2009) and more vulnerable to extinction than large contiguous populations subject to migration (Ballou & Foose 1996; Lacy 1993a, 2000a). Generally, large populations lose genetic diversity more slowly than small populations, and on average retain more genetic variation (Frankham 1995a; Thompson 2004). Population size (*N*) is a major determinant of extinction risk, and larger populations have a better chance of survival (Purvis *et al.* 2000b; Reed *et al.* 2003c; Traill *et al.* 2010). The minimum *N* needed to ensure persistence will vary depending on the biological characteristics of the population (Pollak *et al.* 2005; Ralls & Ballou 1992; Reed *et al.* 2003c), but essentially, the larger the *N,* the better the chance of survival (Foose *et al.* 1995; Lees & Wilcken 2009). However, merely ensuring a large census size may not be adequate to retain essential genetic variation (Briscoe *et al.* 1992). In general, captive populations should be as large as practicable without depriving other species of valuable, but limited resources (Ballou *et al.* 2010; Mace 1989; Reed & Hobbs 2004; Traill *et al.* 2010).

Different factors influence small population dynamics and viability, and they can be divided into two categories, deterministic (predictable) and stochastic (random) factors, that operate simultaneously (Hedrick *et al.* 1996; Lande *et al.* 2003; Mace 1989; Reed & Hobbs 2004). Deterministic factors are intrinsic to the population and largely independent *N*, although the Allee effect is the exception to this (Holsinger 2000; Wittmer *et al.* 2005). The Allee effect describes the relationship between any component of individual fitness and the number, or density, of conspecifics, for example mate acquisition, predator-avoidance behaviour, or reduction in inbreeding (Stephens et al. 1999).

Stochasticity includes genetic, demographic and environmental factors, all of which, individually and combined, can determine population viability (Ballou 1992; Frankham 2003; Hedrick *et al.* 1996; Mace 1989; Reed & Hobbs 2004; Snyder *et al.* 1996). The magnitude of stochastic threats depends on population size (Holsinger 2000), although environmental stochasticity exerts a substantial influence over population growth rate

regardless of size (Holsinger 2000; Lande 1993). Environmental stochasticity dominates demographic stochasticity in larger populations (Lande 1988). Populations may also suffer from catastrophic events such as fire, flood, or disease epidemics that rapidly reduce population size (Holsinger 2000). The influence of demographic stochasticity over population viability increases as population size decreases (Ballou *et al.* 2010; Lande 1993). Genetic stochasticity, the changes in genetic variation caused by genetic drift and mutation, can exert a substantial influence over the genetic diversity and persistence, of captive populations (Ballou & Cooper 1992b; Ballou & Lacy 1995). Drift is defined as the loss of alleles due to random fluctuations in gamete sampling from one generation to the next (Höglund 2009; Lacy *et al.* 1995; Pollak *et al*. 2005).

1.6 The genetics of captive populations

Genetic diversity is measured in both individuals and populations, and can be described in terms of allelic diversity and heterozygosity (Ballou & Foose 1996; Ballou *et al*. 2010). Both types of variation are assayed by presumed neutral markers (Amos & Balmford 2001). Allelic diversity is important for a population's long-term ability to adapt to environmental change, and therefore represents evolutionary potential (Ballou *et al.* 2010; Briscoe *et al.* 1992; Frankham 2003; Princée 1995). It is sensitive to population bottlenecks, such as those encountered by the founding of captive populations (Amos & Balmford 2001). Heterozygosity is important for individual health and response to selection (Reed & Frankham 2003). It is described either as observed heterozygosity, which is the proportion of genetic loci for which the average individual in a population is heterozygous, or expected heterozygosity (Höglund 2009; Lacy 1994; Pollak *et al.* 2005). Expected heterozygosity is often referred to as gene diversity (*GD*), as it is within this thesis, and is defined as the probability that two homologous genes randomly drawn from the population are distinct alleles (Höglund 2009; Lacy 1994). It is the mean heterozygosity that would exist in a population if it were in Hardy-Weinberg equilibrium. Furthermore, the rate at which a population responds to selection is related to expected heterozygosity (Lacy 1994).

The amount of genetic diversity in a captive population is determined by its founders (Mace *et al.* 1992). Many captive populations are founded with only a few, possibly related, individuals representing only a small fraction of the genetic diversity of the wild population (Ballou & Lacy 1995; de Boer 1989; Mace 1986; Ralls & Ballou 1992). The founders are often collected from small or declining populations of threatened species

(Conde *et al.* 2011b; Lyles & May 1987; Traylor-Holzer 2011; Williams & Hoffman 2009), and endangered species typically have much lower levels of genetic variation than comparable non-endangered species (Frankham 2005a, 2005b, 2006; Reed & Frankham 2003; Spielman *et al*. 2004). The number of founders, and their relatedness to each other, determines the amount of genetic diversity that can be retained in the captive population (Frankham *et al.* 2010; Mace *et al.* 1992; Senner 1980; Willis & Willis 2010). Loss of genetic variation from a population is a serious threat to its long-term viability (Lacy 1997; Willis 1993).

Small closed populations encounter four types of genetic change in captivity, namely: 1) loss of genetic diversity; 2) accumulation of new mildly deleterious alleles; 3) inbreeding and; 4) genetic adaptation to captivity (Ballou & Lacy 1995; Frankham 1995a; Frankham 2008; Princée 1995).

1.6.1 Loss of genetic diversity through genetic drift

Closed populations lose neutral genetic variation (allelic diversity and heterozygosity) through drift (Ballou & Foose 1996; Reed & Frankham 2003; Wilcken & Lees 1998), at a rate of $1/(2N_e)$, where N_e is the effective population size, but gain it through mutation at a rate of $2N_eu$, where u is the neutral mutation rate (Nunney 2000). The N_e is the size of an idealised population that would give rise to the same variance of gene frequency, or rate of inbreeding, as observed in the actual population under consideration (Frankham 1995b). The smallest N_e where drift is balanced by mutation is thought to be $N_e = 500$ (Lees & Wilcken 2009; Vogler *et al.* 2009). Most captive populations are too small for mutation to have a noticeable effect (Lacy 1987), and population size is limited by available captive breeding space (Vogler *et al.* 2009). Drift overwhelms natural selection in small closed captive populations, and is the dominant force in determining allele frequencies (Höglund 2009; Lacy 2000a; Lande 1995). Genetic drift can be reduced by increasing N_e and extending generation length, because each generation is a genetic sampling of the previous one (Lacy *et al.* 1995; Taylor & Barlow 1995).

1.6.2 Accumulation of new mildly deleterious alleles

As populations become smaller, more genetic variation becomes ‘nearly neutral’, and genetic drift replaces selection (Lacy 1997; Nunney 2000). The consequence of this is that mildly deleterious alleles become selectively neutral, and their fate is then determined by

drift rather than selection. Over sufficiently long periods of time mildly deleterious alleles can accumulate in the population and reduce overall fitness. The accumulation of deleterious variants speeds up as the population size decreases, and these alleles can drift to fixation, resulting in a negative feedback loop. Eventually, this can cause the population to decline to extinction. Such events are termed mutational meltdown (Frankham 2005a; Höglund 2009). Mildly beneficial alleles are also subject to drift as they become 'nearly neutral', and the probability of any allele reaching fixation increases as the population size decreases (Franklin 1980; Lacy 2000a; Nunney 2000; Peterson & McCracken 2005).

1.6.3 Inbreeding

Inbreeding (consanguineous matings) is inevitable in small closed populations (Ballou *et al.* 2010a; de Boer 1989; Höglund 2009; Lacy 1992). It is measured by the inbreeding coefficient *F*, which is defined as the probability that two alleles at any one genetic locus will be identical by descent from common ancestors (Ballou *et al.* 2010a; Lacy 1992, 1995, 1997). The *F* of an offspring is equal to the kinship between its parents (Pollak *et al.* 2005), and is proportional to the loss of heterozygosity in the population (Lacy 1992, 1997). Inbreeding often leads to inbreeding depression, including increased juvenile mortality, decreased longevity, lower reproductive success, greater susceptibility to parasites and disease, and a higher rate of developmental defects (Ballou & Ralls 1982; Frankham 2006; Lacy 1992, 1997; Ralls *et al.* 1980). A minimum short-term effective population size of 50 has been recommended to avoid the immediate deleterious effects of inbreeding in a population (Franklin 1980; Lacy 1994).

Inbreeding depression has been well-documented in experimental, wild, captive, and domestic populations (Boakes *et al.* 2007; Crnokrak & Roff 1999; Höglund 2009; Lacy 1992; Reed & Hobbs 2004), and is more severe under stressful conditions (Crnokrak & Roff 1999; Höglund 2009). In the few species where inbreeding depression has been studied in detail, approximately half of the effects are due to recessive lethal alleles, and the other half due to loss of heterozygote advantage (heterosis) (Höglund 2009; Lacy 1992; 1993a; Ralls *et al.* 1980). Inbreeding depression can have a severe impact on the viability of threatened populations and may increase extinction risk (Frankham 2005a, 2006; Höglund 2009; Reed & Frankham 2003). It has been implicated in the decline or extinction of wild populations of the Florida panther *Puma concolor coryi*, bighorn sheep, heath hen *Tympanuchus cupido cupido***,** and middle spotted woodpecker *Dendrocopos medius*

(Frankham 2006). The impact of inbreeding on the viability of the scimitar-horned oryx EEP population is examined in Chapter Eight of this thesis.

If a population is inbred, or suffering from the effects of inbreeding depression, the immigration of just one unrelated individual can reduce its impact (Frankham 1995a; Höglund 2009). This has been demonstrated for Scandinavian wolves *Canis lupus* that were suffering from increased juvenile mortality and hereditary blindness caused by inbreeding. It has also been observed in greater prairie chickens *Tympanuchus cupido pinnatus* that suffered reduced hatching rates due to inbreeding. In both cases, genetic rescue restored pre-inbreeding growth rates (Höglund 2009). This is only an effective strategy if there are alternative populations in the wild, or captivity, with unrelated individuals. Consequently, it is not an option for many captive populations (Williams & Hoffman 2009).

Inbreeding depression can be environmentally dependent and may be more difficult to detect under benign captive conditions (Charpentier *et al.* 2006; Crnokrak & Roff 1999; Frankham 1995a; Kalinowski & Hedrick 1999). Although Boakes *et al*. (2007) detected inbreeding depression for neonatal survival across 119 captive populations.

Captive populations that have experienced persistent inbreeding over several generations may be less susceptible to inbreeding depression because some lethal and sub-lethal alleles have been removed from the population through purging (Hedrick *et al.* 1996; Leberg & Firmin 2008). It is also possible that some populations may have been purged of deleterious alleles before they were brought into captivity (Boakes *et al.* 2007). However, evidence of purging in captive populations is scarce and its effects are unpredictable, modest, and inefficient at increasing fitness, when it does occur (Boakes *et al.* 2007; Fox *et al*. 2008; Frankham *et al.* 2001; Frankham 2005a; Witzenberger & Hochkirch 2011).

1.6.4 Genetic adaptation to captivity

Genetic adaptation to captivity has been documented for a wide range of taxa, including mammals, birds, fish, plants, insects, and bacteria (Frankham 2005b; Frankham *et al.* 2010; Leus *et al*. 2011b; Montgomery *et al.* 2010; Witzenberger & Hochkirch 2011), and may present a serious problem for populations that are undergoing captive breeding for reintroduction to the wild (Frankham & Loebel 1992; Frankham *et al.* 1986; Leus *et al.* 2011b; Montgomery *et al.* 2010; Robert 2009). When a captive population is isolated the mean phenotype of the population may shift away from that of the wild population. When

individuals are later reintroduced to the wild, they may exhibit a decline in fitness compared to their wild counterparts (Arnold 1995).

A number of factors influence the rate of adaptation to captivity, including the similarity of captive and wild conditions, the number of generations in captivity, the N_e, and the intensity of selection (Ford 2002; Frankham 2008; Haig *et al.* 1990; Leus *et al.* 2011b; Williams & Hoffman 2009). Selection in the captive environment is often unconscious on the part of animal managers, for example more docile animals may reproduce better or are easier to handle, and are more likely to pass their genes onto the next generation (Snyder *et al.* 1996; Williams & Hoffman 2009). Additionally, the benign captive environment relaxes selection and individuals survive and reproduce in captivity that would not have survived in the wild (Hakansson & Jensen 2005; Junhold & Oberwemmer 2011; Robert 2009).

Adaptation to captivity can be minimised by reducing the time populations spend in captivity, immigration from the wild, increasing the generation length, decreasing the N_e, and fragmenting the population, so genetic drift reduces the genetic diversity available for selection in individual populations, whilst theoretically retaining it at the species level (Frankham & Loebel 1992; Frankham 1999, 2008; Lande 1995; Leberg & Firmin 2008; Princée 1995). However, these last two methods present a dichotomy. Decreasing the effective population size reduces adaptation to captivity, and therefore enhances the probability of survival of reintroduced populations. At the same time reducing N_e, either directly or through population fragmentation, increases stochasticity and the extinction risk for small captive populations (Earnhardt 1999; Frankham 2005a; Johnson & Schoen 1994).

The different demographic and genetic factors interact in small, closed, captive populations, leading to greater instability and a further decrease in population size, in turn leading to further demographic and genetic problems (Ballou *et al.* 2010; Lacy 2000a). This process is termed the extinction vortex (Ballou *et al.* 2010; Foose *et al.* 1995; Lacy 2000a).

1.7 Coordinated captive breeding programmes

In order to address these issues, many captive populations are managed in coordinated breeding programmes that are designed to maximise the prospects of the species survival over the long-term within the limited resources available (Mace 1989).

Coordinated breeding programmes involve managing captive species as multi-institutional biological populations. This is usually achieved through regional zoo

associations, for example the North American Association of Zoos and Aquariums (AZA), the European Association of Zoos and Aquaria (EAZA), the Australasian Zoo and Aquarium Association (ZAA) (Ballou *et al.* 2010), and very occasionally populations are managed on a global scale through the World Association of Zoos and Aquariums (WAZA) (Ballou *et al.* 2010). In total, there are over 30 regional and national zoo associations, and some of these administer their own cooperative breeding programmes (BIAZA 2011; Bingaman Lackey 2010). Zoo and aquaria associations include hundreds of member institutions; there are approximately 1300 institutional members of WAZA; 218 members of AZA; 327 EAZA members across 36 countries; and 75 institutional ZAA members in Australia and New Zealand (EAZA 2010b; WAZA 2010; ZAA 2010). Collectively, global zoos and aquaria hold over 10,000 species, and more than 850 of these are managed in coordinated breeding programmes (ISIS 2010). This is only a fraction of the number of species in captivity, but the number of coordinated breeding programmes is slowly increasing. However, the number of threatened species in need of captive breeding is also predicted to rise (Briton *et al.* 1994; Junhold & Oberwemmer 2011), and zoos do not have sufficient space to accommodate viable populations of all the species that are threatened with extinction (Balmford *et al.* 1995; Foose *et al.* 1995; Snyder *et al.* 1996).

The most intensively type of managed coordinated captive breeding programmes in the North American (AZA) region is called the Species Survival Plan (SSP). The equivalent in the European region (EAZA) is called the Europäisches Erhaltungszucht Programm, but it is more commonly referred to as the European Endangered Species Programme (EEP). The Australasian (ZAA) version is known as the Australasian Species Management Program (ASMP). EAZA and AZA also operate less-intensive coordinated breeding programmes called European Studbooks (ESB) and Population Management Plans (PMP), respectively (AZA 2010; EAZA 2010a; ZAA 2010).

The species subject to coordinated captive breeding are separated into Taxon Advisory Groups (TAG) that oversee breeding programme management. TAGs are organised at the family level, for example antelope and giraffe TAG. EAZA has 43 TAGs that are responsible for the management of 176 EEPs and 176 ESBs (Bingaman Lackey 2010; EAZA 2010a). There is a clear taxonomic bias towards intensively managing bird and mammal species (Table 1.1), and 91% of EAZA TAGs are dedicated to these taxa (EAZA 2010a). This is reflective of the species held by zoos around the world (Balmford *et al.* 1996; Conde *et al.* 2011a, 2011b). TAGs are responsible for balancing populations

under their care, so that the optimum number of species can be sustainably managed (Bingaman Lackey 2010).

Table 1.1 AZA and EAZA cooperative breeding programmes for each taxa

	AZA		EAZA	
Taxa	**SSP**	**PMP**	**EEP**	**ESB**
Amphibian	3	4	0	2
Bird	24	162	37	68
Fish	1	10	0	7
Invertebrates	2	1	2	1
Mammal	85	142	130	81
Reptile	11	41	7	17

(AZA 2010; EAZA 2010a; EAZA 2010b; WAZA 2010)

Coordinated captive breeding programmes aim to develop self-sustaining populations that maintain demographic stability, maximise genetic diversity, minimise inbreeding and genetic adaptation to captivity, and provide animals for reintroduction projects (Ballou & Foose 1996; Ballou *et al.* 2010; Frankham *et al.* 1986; Hedrick & Miller 1992; Leus & Traylor-Holzer 2008; Montgomery *et al.* 2010). Captive management is designed to minimise changes in the genetic composition of the population whilst it is in captivity, so it will resemble, as closely as possible, the genetic characteristics of the original founding population (Ballou & Lacy 1995; Ballou & Foose 1996; Ballou *et al.* 2010a; Foose *et al.* 1995; Lacy 1994).

Captive breeding programmes require accurate and current data in a standardised format in order to evaluate the genetic and demographic characteristics of a population, predict future trends, and model the effect of different management strategies (Ballou *et al.* 2010; Bingaman Lackey 2010; Wilcken & Lees 1998). The best source of compiled data is a studbook (Ballou *et al.* 2010). Studbooks contain a complete chronology of the captive population listing information on individual identities, location, sex, parentage, relationships between individuals, cause of death, and birth, translocation, and death dates (Ballou *et al.* 2010; Bingaman Lackey 2010; Lacy *et al.* 1995; Vasarhelyi 2002). Studbooks can be regional or global (international studbooks) in scope. Regional studbooks are managed by the relevant regional zoo association, for example EAZA manage European studbooks (ESB). International studbooks are managed under the auspices of WAZA and the IUCN expressed through the Conservation Breeding Specialist Group (CBSG). Their management is assisted by the International Species Information

System (ISIS), which maintains records for 2.4 million animals from more than 10,000 species (Bingaman Lackey 2010; ISIS 2010).

1.8 Pedigree analysis

Coordinated captive breeding programmes use pedigree analysis as the basis of genetic management for captive populations. Pedigree analysis assumes a starting population where the wild-caught individuals (founders) have no known genetic relationship (Ballou & Ralls 1982; Lacy 1994; Lacy *et al.* 1995). Information is often lacking on the geographic origin and relatedness of the founders (Gautschi *et al.* 2003; Witzenberger & Hochkirch 2011), and whilst the founder assumption of non-relatedness is necessary for pedigree analysis, it may not accurately describe the true relationship between founders, and therefore subsequent generations (Lacy 1994; Vasarhelyi 2002; Willis 1993; Witzenberger & Hochkirch 2011). The founder assumption is rarely tested (Vasarhelyi 2002).

Pedigree analysis involves estimating the relatedness of individuals in the population by tracing the pedigrees back to the founding generation (Lacy *et al.* 1995; Wilcken & Lees 1998). This provides a method of accurately estimating how much gene diversity has been lost between the founders and the living descendant population (Lacy 1995). Pedigree analysis can take several forms including the additive matrix method and gene dropping (Hedrick & Miller 1992). The additive matrix method provides an efficient method for calculating inbreeding and kinship coefficients (Ballou 1983; Boyce 1983; Lacy *et al.* 1995). Gene dropping is more efficient at quantifying founder contribution in the descendant populations in large, complex pedigrees, although a large number of simulations need to be run to ensure precision (Hedrick & Miller 1992).

In gene dropping, each founder's contribution to the living descendant population is obtained through Monte-Carlo simulations (Hedrick & Miller 1992; Lacy *et al.* 1995). Each founder is assigned two unique alleles at a hypothetical locus. These alleles are then passed randomly through the pedigree from one generation to the next, following the rules of Mendelian segregation. At the end of each simulation, every individual in the pedigree has a genotype. The distribution of founder allele probabilities is recorded for each individual in the descendant population and the simulation is repeated thousands of times. Each simulation is independent and therefore represents unlinked selectively neutral loci (Ballou *et al.* 2010; Bingaman Lackey 2010). Gene dropping represents the statistical sampling of one locus thousands of times, or sampling thousands of loci only once

(Caballero & Toro 2000; Haig *et al.* 1990; Hedrick & Miller 1992; Lacy *et al.* 1995; Pollak *et al.* 2005).

A number of software programs have been developed for managing studbook databases and analysing pedigree data for population management. SPARKS (Single Population Analysis and Record Keeping System) (Scobie *et al.* 2004) and PopLink (Faust *et al.* 2011) have been specifically developed by the zoological community as studbook database and analysis software packages (Thompson 2004). PM2000 (Pollak *et al*. 2007) has been developed alongside these to execute detailed genetic and demographic analyses based on pedigree data and life tables, and to model population management options and goals.

1.9 Management of captive populations

Population management attempts to maintain stable population size and structure in order to minimise temporal fluctuations and reduce extinction risk (Ballou & Lacy 1995; Mace 1989). Demographic analyses fundamentally provide managers with the necessary information on how many animals need to breed, and when they need to breed. The results of the genetic analyses provide information on which animals should breed, and with whom, to maximise the retention of genetic diversity, and minimise inbreeding (Wilcken & Lees 1998).

Specific goals and objectives are established for each individual captive breeding programme that specify the preservation of minimum amounts of genetic diversity for a set period of time (Lacy 1995; Ralls & Ballou 1992). Ballou *et al*. (2010) recommend the goal of preserving 90% of founder *GD* for 100-years, as this represents a balance between potentially damaging and an acceptable loss of heterozygosity in a population (Ballou *et al.* 2010). This goal is arbitrary and some programmes are not able to meet it so set alternative goals (Frankham *et al.* 2010; Lande 1995; Leus *et al.* 2011b). For example the scimitar-horned oryx EEP has set a goal of retaining 85% of founder *GD* for 100-years (Gilbert 2010b).

Once the genetic goal has been set, the number of animals needed to meet that goal can be calculated from life tables, data on current genetic diversity levels, and estimates of effective population size (Ballou *et al.* 2010; Lacy & Ballou 2002).

The amount and complexity of data obtained from pedigree analysis can be formidable, and different strategies have been developed to identify and rank individuals according to their genetic importance within the population (Ballou & Lacy 1995).

Strategies to rank genetically important individuals include Founder Importance Coefficient (*FIC*), which provides a simple method of identifying genetically important individuals as defined by founder contribution. Individuals descended from over-represented founders in the living population have a high *FIC* (Ballou & Lacy 1995); and Genome Uniqueness (*GU*), which is the probability that an allele chosen at random from an individual is unique within the population. *GU* is used to identify individuals carrying alleles at a high risk of being lost, but it only measures unique alleles and does not take into account alleles that are simply rare and also at risk of being lost (Ballou & Lacy 1995; Ralls & Ballou 1992); and Mean Kinship (*MK*), which quantifies the relationship between any one individual and all living individuals in the population, and is a measure of the rareness of an individual's alleles in the population.

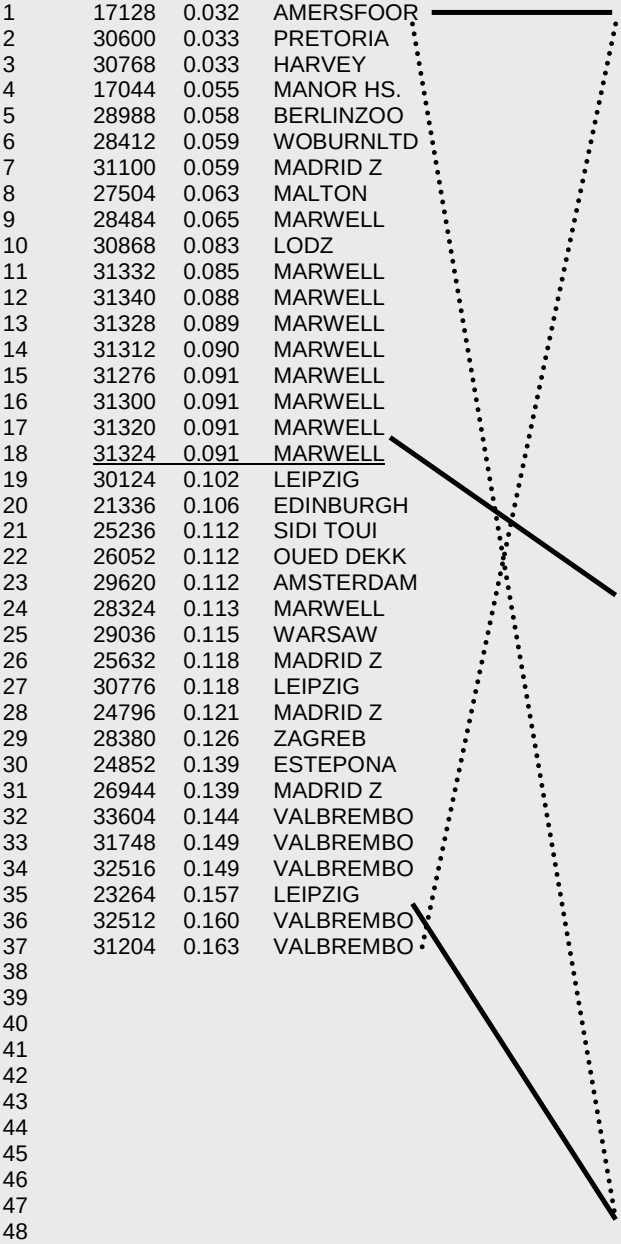
Individuals with low *MK* coefficients (*MKi*), represent genetically important animals (Ballou & Lacy 1995; Ballou *et al.* 2010; Pollak *et al.* 2007; Ralls & Ballou 1992). *MK* is inversely proportional to founder genome equivalents (*FGE*), which describes the combined effect of unequal founder contribution and genetic drift on the genetic diversity of a population (Ballou *et al.* 2010a), and gene diversity (*GD*) (Ballou & Lacy 1995) by $\overline{MK} = 1/(2FGE) = 1 - GD$. So minimising kinship in a population is directly related to maximising *GD* and *FGE*. It also equalises family sizes, and that increases N_e and reduces the loss of *GD* through drift (Ballou & Lacy 1995; Ballou *et al.* 2010; Borlase *et al.* 1993; Frankham 2005b; Ralls & Ballou 1992; Williams & Hoffman 2009).

MK is calculated from pedigree data using the additive relationship matrix method given in Ballou (1983) and Boyce (1983) (Ballou & Lacy 1995; Lacy *et al.* 1995). Ballou & Lacy (1995) compared *FIC*, *GU* and *MK* strategies along with a Maximum Avoidance of Inbreeding (*MAI*) and random mating, and Montgomery *et al.* (1997) compared *MK* and *MAI* strategies with random mating, and both found that the *MK* strategy retained the highest levels of gene and allelic diversity in populations with complex pedigrees and unequal founder representation. Consequently, contemporary population management within the AZA (SSP), EAZA (EEP) and ZAA (ASMP) regions use the *MK* method to assign breeding priority to individuals within a population with a known pedigree (AZA 2004; Wilcken & Lees 1998).

Box 1.1 Population management based on the Mean Kinship strategy

Ranking individuals based on *MKi* provides a rough guide for assigning breeding priority. Individuals in the top half of each list (as indicated by a line), which have a *MKi* below the population mean, should be paired with individuals of similar and low *MKi*. In this example, breeding priority would be given to males ranked 1 – 18 and females ranked 1 – 24. Offspring resulting from these pairs would help equalise founder contribution and retain allelic diversity and expected heterozygosity. Individuals with a *MKi* above the population mean would be prevented from breeding unless all animals were required to breed to ensure demographic stability. In this scenario, males would be paired with females of a similiar *MKi*, as indicated by the solid lines linking pairs. Individuals with large differences between their *MKi* should not be paired, as indicated by the dashed lines. Such pairings would skew the founder representation in the descendant population and any offspring would have both rare and common alleles. It then becomes impossible to increase the frequency of rare alleles in the population without also increasing the frequency of the common ones (AZA 2004; Ballou *et al.* 2010a).

	Males			Females		
Rank	**SB ID**	***MKi***	**Location**	**SB ID**	***MKi***	**Location**
1	17128	0.032	AMERSFOOR	28032	0.029	PRET LICH
2	30600	0.033	PRETORIA	18984	0.031	AMSTERDAM
3	30768	0.033	HARVEY	26576	0.031	PRET LICH
4	17044	0.055	MANOR HS.	13836	0.039	KARLSRUHE
5	28988	0.058	BERLINZOO	16552	0.042	KARLSRUHE
6	28412	0.059	WOBURNLTD	20768	0.045	KARLSRUHE
7	31100	0.059	MADRID Z	25312	0.045	WARSAW
8	27504	0.063	MALTON	21744	0.046	BERLINZOO
9	28484	0.065	MARWELL	27516	0.050	BURFORD
10	30868	0.083	LODZ	20460	0.052	WHIPSNADE
11	31332	0.085	MARWELL	22420	0.053	WHIPSNADE
12	31340	0.088	MARWELL	23268	0.061	MANOR HS.
13	31328	0.089	MARWELL	20248	0.062	BURFORD
14	31312	0.090	MARWELL	15680	0.063	LA PALMYR
15	31276	0.091	MARWELL	24388	0.063	BURFORD
16	31300	0.091	MARWELL	28500	0.065	MALTON
17	31320	0.091	MARWELL	22348	0.067	LONGLEAT
18	31324	0.091	MARWELL	23348	0.068	BURFORD
19	30124	0.102	LEIPZIG	19952	0.072	WHIPSNADE
20	21336	0.106	EDINBURGH	26140	0.072	BERLINZOO
21	25236	0.112	SIDI TOUI	28184	0.072	BERLINZOO
22	26052	0.112	OUED DEKK	30252	0.078	ZAGREB
23	29620	0.112	AMSTERDAM	14752	0.080	DUBBO
24	28324	0.113	MARWELL	14764	0.080	TIPP STAT
25	29036	0.115	WARSAW	30876	0.082	GDANSK
26	25632	0.118	MADRID Z	31268	0.091	BURFORD
27	30776	0.118	LEIPZIG	31288	0.091	PLANCKNDL
28	24796	0.121	MADRID Z	32056	0.091	CHESTER
29	28380	0.126	ZAGREB	23544	0.092	AMSTERDAM
30	24852	0.139	ESTEPONA	31316	0.093	MARWELL
31	26944	0.139	MADRID Z	29692	0.104	AMERSFOOR
32	33604	0.144	VALBREMBO	29044	0.116	BERLINZOO
33	31748	0.149	VALBREMBO	30284	0.118	LEIPZIG
34	32516	0.149	VALBREMBO	30752	0.118	LEIPZIG
35	23264	0.157	LEIPZIG	27144	0.125	VESZPREM
36	32512	0.160	VALBREMBO	29664	0.125	PRAHA
37	31204	0.163	VALBREMBO	27556	0.126	VESZPREM
38				22460	0.129	PRAHA
39				21820	0.131	VALBREMBO
40				29172	0.134	ESTEPONA
41				30016	0.134	ESTEPONA
42				32196	0.136	LA PALMYR
43				24848	0.141	ESTEPONA
44				27820	0.142	ESTEPONA
45				33248	0.144	VALBREMBO
46				33360	0.144	VALBREMBO
47				29388	0.153	VALBREMBO
48				28024	0.154	VALBREMBO



Captive breeding programme managers select breeding pairs from the top of a sorted *MK* list where individuals are ranked from low to high *MKi*. Males with low *MKi* are paired with females of similar and low *MKi*, which rank above the average *MK* for the population, excluding pairings between close relatives to avoid inbreeding. Box 1.1 demonstrates this using data for the scimitar-horned oryx from Chapter Five of this thesis (Ballou & Lacy 1995; Ballou *et al.* 2010; Frankham *et al*. 2010; Lacy 2000b; Vasarhelyi 2002). The impact of selected pairings on the retention of genetic diversity in a population can be modelled in computer programs such as PM2000 (Pollak *et al.* 2007). The amount of genetic diversity maintained after each pairing is defined by genetic descriptors such as average *MK*, *GD*, *FGE* and Gene Value (*GV*), which is *GD* weighted by the reproductive value (*Vx*) (Pollak *et al.* 2007).

The optimal pairings, based on both demographic and genetic criteria, are often modified due to behavioural, veterinary, geographic, social, monetary and political considerations (AZA 2004; Ballou & Cooper 1992b). Modified recommendations are then issued to EEP participants in the form of breeding and transfer recommendations, or a population masterplan (Ballou & Cooper 1992b).

1.10 Incomplete pedigree data

Detailed pedigree analysis including the calculation of kinships, inbreeding coefficients and frequency of founder alleles in the living population are critically dependent on complete pedigrees (Ballou & Lacy 1995; Lacy *et al.* 1995), and the application of population management models are limited by the quality of pedigree data (Ballou & Cooper 1992b; Princée 1995; Russello & Amato 2004; WAZA 2005e). The identity and parentage of every individual since the inception of the captive breeding programme must be known in order to construct complete ancestries for each living animal (Ballou & Cooper 1992b). However, historical record keeping was frequently poor, and pedigree data are often incomplete (Ballou & Cooper 1992b; Ballou & Lacy 1995; Mace *et al.* 1992; Princée 1995). Only 8% of international studbooks for EEP bird, mammal, and reptile species in the 2004/2005 ISIS/WAZA studbook library (ISIS 2006) had complete pedigrees for all the individuals listed. The incompleteness of pedigrees prevents the accurate calculation of demographic and genetic metrics such as inbreeding coefficients (Ballou *et al.* 2010; Lacy 1993a), but it is not known how much pedigree data can be missing before inbreeding coefficients are no longer estimatable. This is examined in detail for the first time in Chapter Four.

Intensive population management cannot be implemented for populations with large proportions of missing pedigree data (Mace & Pemberton 1990; Princée 1995), but management still needs to proceed for captive populations to ensure sustainability (Ballou *et al.* 1995; Princée 1995). To address this, individuals with missing pedigree data may be excluded from population management (Ballou & Cooper 1992b), or included and treated as founders (Ballou & Lacy 1995). If these individual are related to others in the population, then their inclusion could result in inadvertent inbreeding and an unequal founder allele contribution in the descendant population (Ballou & Lacy 1995; Willis 1993). If unrelated to others in the population, their exclusion will result in genetic variation being lost from the population (Willis 1993). Excluding individuals may also reduce the effective population size, and therefore increase the loss of genetic variation through drift (Foose *et al.* 1995; Willis 1993). There is a potential genetic cost to the population in terms of increased inbreeding, and therefore inbreeding depression, or loss of genetic variation for an incorrect decision (Ballou & Lacy 1995; Lacy *et al.* 1995; Willis 1993, 2001).

An alternative approach is to only include the known portion of the pedigree in analyses (Ballou & Lacy 1995). This is the method used by PM2000 for gene drop simulations and the construction of kinship matrices (AZA 2004). It estimates the probabilities that two alleles in two individuals are identical by descent for the known part of the pedigree (Ballou & Lacy 1995). The kinship and inbreeding coefficients calculated for individuals with incomplete pedigrees may be more or less than the values obtained if the pedigrees were complete (Ballou & Lacy 1995). The reliability of genetic parameters calculated from incomplete pedigree data is examined in Chapters Four and Five.

When large amounts of the pedigree data are missing, detailed pedigree analyses are invalid and alternative approaches to population management need to be applied (Ballou & Lacy 1995). AZA and EAZA recommend the creation of analytical studbooks to address the problem of missing pedigree for intensively managed populations (AZA 2004; Willis 2001). Analytical studbooks include assumptions and 'best guesses' of parentage to fill in the gaps in the pedigree data (Lacy *et al.* 1995; Princée 1995). Similarly, assumptions can be made for missing demographic data such as birth dates to enable the calculation of fecundity or mortality rates (Ballou *et al.* 2010; Princée 1995). The analytical studbook data is then imported into pedigree analysis software like PM2000 in place of the true, but incomplete, studbook data.

The founder assumption, missing pedigree data, and cryptic errors in the studbook may result in an incomplete or inaccurate evaluation of genetic diversity in a population, and analytical studbooks only address the issue of missing pedigree data (Ballou & Cooper 1992b; Boakes *et al.* 2007; Signer *et al.* 1994; Witzenberger & Hochkirch 2011). It is not known if the creation of analytical studbooks presents a more valid approach to population management than using true, but incomplete studbooks. A number of studies have evaluated the accuracy of true studbooks using molecular methods, but the comparable accuracy of both the analytical and true studbook is tested for the first time in Chapter Five.

1.11 The role of molecular genetic analysis in captive breeding programmes

Molecular genetic analysis can provide an alternative solution to pedigree-based population management when pedigree data are incomplete (Ballou *et al.* 2010a). A number of molecular techniques are available for evaluating levels of genetic diversity in and between populations, for example multilocus protein electrophoresis (Ritland & Travis 2004; van Kleunen & Ritland 2005), single-copy restriction fragment-length polymorphisms (RFLP) (Avise *et al.* 1995), random amplified polymorphic DNA (RAPD) (Ritland 2005), amplified fragment length polymorphism (AFLP) (Hardy 2003; Ritland 2000, 2005), single nucleotide polymorphisms (SNP) (Santure *et al.* 2010; Slate *et al.* 2009), Mitochondrial DNA (MtDNA) analysis (Hailer & Leonard 2008; van Hooft *et al*. 2003), and analysis of individual hypervariable loci, for example at the Major Histocompatibility Complex (MHC) (Avise *et al.* 1995; Hughes 1991), microsatellites (Armstrong *et al*. 2010), and minisatellites (DNA fingerprinting) (Avise *et al.* 1995).

Hughes (Hughes 1991) advocated using data derived from microsatellites at the MHC, a hypervariable region associated with immune function, as the basis for population management. Molecular methods provide empirical estimates of genetic diversity at a few loci, and pedigree analysis provides a statistical measure of genome-wide diversity. As the aim of captive population management is to preserve as much of the founders' genetic diversity as possible, molecular methods may fail to do this by focusing on only a few loci (Allendorf & Luikart 2007; Ballou *et al.* 2010a; Hedrick & Miller 1992). Historically, population management based on pedigree analysis has been shown to retain more genetic variation than management based on molecular analyses (Haig *et al.* 1990; Gilpin & Wills 1991; Ralls & Ballou 1992; Vrijenhoek & Leberg 1991), although this has not taken missing pedigree data into account.

While molecular genetic analyses may be used to resolve pedigree unknowns, or evaluate the genetic diversity of whole populations, this is often expensive and sample acquisition may be difficult. As a result its widespread application to current captive population management is limited (Ballou *et al.* 2010).

1.12 The sustainability of captive populations

1.12.1 Sustainable captive populations

In terms of simple persistence, a self-sustaining population is one that is able to persist, without supplementation, indefinitely (Lees & Wilcken 2011). It should be large enough to withstand demographic stochasticity and instability, and should endure no net loss of genetic diversity (Ballou & Traylor-Holzer 2011; Lees & Wilcken 2011). The smallest effective population size (N_e) where loss of gene diversity (*GD*) is balanced by mutation is thought to be $N_e = 500$ (Boyce 1992; Lees & Wilcken 2011; Traill *et al.* 2010; Vogler *et al.* 2009; Witzenberger & Hochkirch 2011). This means that populations need to be in the order of several thousand, as N_e is often much smaller than the census *N* (Lees & Wilcken 2011; Reed *et al.* 2003c; Traill *et al.* 2010).

The concept of a minimum viable population size (MVP) is closely aligned with that of a sustainable population, in as much as both concepts are concerned with population persistence over long periods of time, and *N* is a major determinant of persistence (Reed *et al.* 2003c). The minimum viable population (MVP) size is the minimum *N* needed to ensure persistence over a specified period of time for a specific species or population, for example 99% probability of persistence over 100-years (Ballou & Traylor-Holzer 2011; Reed *et al.* 2003c; Shaffer 1981). MVPs can vary between a few hundred and thousands (Lacy 1992), although studies have specified MVPs as low as 20 for the great tit *Parus major* (Saether *et al.* 1998) and as high as 100,000 individuals for the great yellow gentian *Gentiana lutea* (Reed 2005; Traill *et al.* 2010). A self-sustaining population will always be equal to, or above, the MVP because the MVP is limited in time and sustainability is not. The two concepts are complementary because contemporary management is concerned with the minimum viable population size required to ensure a self-sustaining population of a specific species or population under management (Lacy 1992; Lees & Wilcken 2011).

Only 9% of species held in captivity are part of coordinated breeding programmes (ISIS 2010), and management of a captive population does not infer sustainability *per se* (Lees & Wilcken 2009). Sustainability is a serious concern for captive populations (Lees &

Wilcken 2009; Snyder *et al.* 1996) and this is examined in detail in Chapter Seven. Evaluations of Australasian, European, and North American populations under breeding management reveal that large percentages are not genetically sound or self-sustaining (Junhold & Oberwemmer 2011; Leus *et al.* 2011a, 2011b; Long *et al.* 2011; Witzenberger & Hochkirch 2011). A recent study found that 67% of AZA populations had an *N* of less than 100 and a mean N_e of 41 (Witzenberger & Hochkirch 2011). The median population size of 428 AZA managed populations (SSP and PMP) was *N* = 66 (Long *et al.* 2011). Similarly, 36% of European managed mammal and bird populations have populations sizes of less than 50 (Leus *et al.* 2011a). The lack of sustainability does not only apply to regional populations, and only 9% of populations with international studbooks are large enough to be considered self-sustainable (Lees & Wilcken 2011). Such small populations are not viable, and have a high probability of extinction (Bryant *et al.* 1999).

Even those populations that are subject to active population management are not being managed for sustainability. Instead targets, such as the retention of 90% of *GD* for 100-years, specify a tolerable loss of *GD,* which implicitly acknowledges the difficulty of maintaining genetically sustainable populations (Ballou & Traylor-Holzer 2011; Traill *et al.* 2010). Furthermore, many managed populations have a current *GD* below the 90% benchmark (Long *et al.* 2011), and many more are unable to retain 90% of *G*D for 100-years (Frankham *et al.* 2010; Lande 1995; Leus *et al.* 2011b). This issue is explored in Chapter Six, where the impacts of variable demographic and genetic parameters on the retention of gene diversity are examined for the scimitar-horned oryx EEP population (Gilbert 2010b).

In contrast examples of self-sustaining captive populations include the golden lion tamarin. This species was on the brink of extinction in the 1970s, but was established as a sustainable population in captivity, which then provided animals for reintroduction efforts in Brazil (Gippoliti & Carpaneto 1997).

1.12.2 Population size

There are a number of reasons why many captive populations are not self-sustaining. To be genetically and demographically sustainable, captive populations need an N_e of at least 500-5000 (Ballou & Traylor-Holzer 2011; Vogler *et al.* 2009) which translates into actual population sizes of 1700 – 20,000 (Ballou & Traylor-Holzer 2011; Lees & Wilcken 2011). Captive breeding facilities do not have enough space to accommodate large viable populations for all species threatened with extinction, especially large-bodied animals, and

as a consequence many populations are small (Ballou & Cooper 1992b; Ballou & Traylor-Holzer 2011; Lacy 1992; Leus *et al.* 2011b; Snyder *et al.* 1996). One solution to this particular problem is to select representative taxa and reduce the number of species conserved in zoological institutions, providing more space for each remaining species (Ballou & Cooper 1992b).

1.12.3 Financial concerns

Another factor impacting on the sustainability of captive populations is the high cost associated with maintaining large populations of exotic animals outside of their natural habitat (Junhold & Oberwemmer 2011). Financial resources are limited and need to be prioritised at both institutional and regional levels (Balmford *et al.* 1996). This requires a coordinated approach between different breeding programmes, as established in regional collection plans for each taxa (EAZA 2010b).

1.12.4 Effective coordination of programmes

Coordinated intensive management can help populations meet the criteria for sustainable populations, but this requires every institution to implement the management recommendations issued by the programme coordinator (Ballou 1992; Junhold & Oberwemmer 2011). This does not always happen if institutional requirements conflict with population needs (Junhold & Oberwemmer 2011). Additionally, coordinated population management programmes require a coordinator, and coordinators are limited by staff availability (Junhold & Oberwemmer 2011). Consequently, there are a number of programmes which are not currently being actively managed (Espeland 2004). Not all zoos in a region are members of regional coordinated breeding programmes, and population size can be limited by regional association membership (Junhold & Oberwemmer 2011).

1.12.5 Husbandry

Sometimes a lack of sustainability is caused by a fundamental failure of a species to thrive in captivity (Snyder *et al.* 1996). This can be caused by poor reproduction and survivorship, problems with husbandry, adaptation, and disease (Balmford *et al.* 1996; Snyder *et al.* 1996). Reproductive technology is often cited as a solution to a number of problems in captivity, including sustainability, but advances and the application of technology has not been as rapid as predicted (Ballou & Traylor-Holzer 2011).

1.12.6 Population fragmentation

Some captive populations are not sustainable because legislation and disease control measures have resulted in isolated and fragmented populations (Junhold & Oberwemmer 2011). Both EAZA and ZAA have identified that legislative barriers and fragmentation have impacted on the sustainability of captive populations for endangered species in their regions (Hibbard *et al.* 2011; Leus *et al.* 2011a).

Fragmentation occurs in both wild and captive populations, and is a major contributory factor to population extinction (Boyce 1992; Frankham 2010b; Hedrick *et al.* 1996; Henle *et al.* 2004; Price & Gittleman 2007). Population sub-division can have a serious impact on the retention of genetic diversity and the maintenance of demographic stability of both the metapopulation and individual sub-units (Laporte & Charlesworth 2002; Nunney 2000; Wang & Caballero 1999). In particular, population fragmentation, without migration between sub-units, can reduce the N_e of both the metapopulation and the sub-units (Soulé *et al.* 1986; Wang & Caballero 1999), leading to a rapid loss of genetic diversity (Ballou *et al.* 2010a). Chapter Seven tests the impact of simulated population fragmentation on the predicted N_e and retention of genetic diversity for four endangered antelope and gazelle EEP populations. Chapter Eight extends this by examining the impact of fragmentation on the viability of the scimitar-horned oryx EEP population. Whilst the issue of fragmentation has been well-studied for wild and experimental populations, this is the first time the predicted impacts of fragmentation have been tested for real populations. The results have wide-ranging implications for the management of all sub-divided captive populations.

The causes of population sub-division in the wild can be varied, but in captivity the two main reasons are legislative barriers and the high cost of animal transport over long distances (Hibbard *et al.* 2011; Junhold & Oberwemmer 2011; Leus *et al.* 2011a; Mace 1989; Margan *et al.* 1998). In this thesis, these two factors are referred to as economic fragmentation as both have their foundations in financial concern.

As wild populations become increasingly fragmented, they will experience problems comparable to those encountered by captive populations (Mace 1989; Price & Gittleman 2007; Traill *et al.* 2010). In turn, some strategies that have been developed to counteract the negative impacts of captive population sub-division may be applicable to wild populations (Frankham 2010b; Law & Linklater 2007; Mace 1989).

1.13 Thesis rationale

The sustainability of captive populations, and the success of reintroduction efforts, depends on their effective management. Existing population management techniques are inadequate at addressing the challenges faced by modern captive populations. In this thesis, I aim to evaluate the sustainability of captive populations using the management of scimitar-horned oryx as a case study. The specific objectives are; 1) to evaluate the impact of pedigree data quality on the estimation of genetic parameters and the subsequent impact on the management of captive populations; 2) to evaluate the impact of various genetic and demographic parameters on the retention of genetic diversity in captive populations; 3) to quantitatively evaluate the sustainability of captive populations; and 4) to evaluate the impact of fragmentation on population viability and sustainability.

In this thesis, I undertake a series of novel studies that contribute knowledge to the science behind contemporary population management and sustainability. The results are applicable to captive populations in general, but in particular to the scimitar-horned oryx. Each chapter builds on the previous one, with the results of early chapters informing the methodology of later chapters. In Chapter Two, I introduce the scimitar-horned oryx and some of the challenges to conserving the species, and Chapter Three presents some of the general methods used in the thesis. Chapter Four addresses the issue of missing pedigree data in estimating inbreeding coefficients, and Chapter Five evaluates the impact of incomplete pedigree data on population management using established methods. The results presented in Chapters Four and Five inform the methodology of quantifying the retention of genetic diversity in the scimitar-horned oryx EEP (Chapter Six), and the impact of population management and fragmentation on the genetic viability and sustainability of captive populations (Chapters Seven and Eight). Chapter Nine draws together some of the main conclusions of the thesis and makes recommendations for population management and further research.

2.0 Chapter two: an introduction to the scimitar-horned oryx

2.1 Introduction

The scimitar-horned oryx is a migratory arid-adapted antelope that was once abundant and widespread, inhabiting the vast aridland steppes that border the Sahara (Figure 2.1) (Dolan 1966; Gillet 1966; Gordon & Gill 1993; Hufnagl *et al.* 1972; Newby 1978, 1980; Schomber 1963). Over a million scimitar-horned oryx once existed across its historic range, forming aggregations of thousands of animals during their annual migrations (Gillet 1965, 1966; Iyengar *et al.* 2007; Newby 1978; Schomber 1963). Poor-development in the Sahel led to over-grazing and competition with domestic livestock, that combined with over-exploitation, drought and desertification, drove the scimitar-horned oryx to extinction in the wild in the late twentieth century (Table 2.1) (Bassett 1975; Bertram 1988; Devillers & Devillers-Terschuren 2006; Dixon *et al.* 1991; Gordon 1991; IUCN 2010; Moksia *et al.* 2001; Newby 1978, 1980, 1981, 1988, 1990; Newby *et al.* 2004; Schomber 1963). The species now only exists in captive and semi-captive conditions (Gilbert & Woodfine 2004a; IUCN 2010), and its future is dependent on a strategy of intrinsically linked captive breeding and reintroduction to its former range.

2.2 The captive population

The scimitar-horned oryx's demise in the wild coincided with its rise in captivity, and by the end of 2009 the international studbook listed over 1630 oryx in 211 zoological institutions around the world, with an estimated 14,800 additional animals held in private collections in the Middle East and Texas (Figure 2.2 and Table 2.2) (Anderson 2010; McClellan 2010; Newby 2006b; Craig 2008). Approximately 675 of the individuals listed in the international studbook are managed through three regionally coordinated breeding programmes covering the AZA (SSP), EAZA (EEP), and ZAA (ASMP) regions (Gilbert & Woodfine 2004a; Gilbert 2010b; Spevak 2009; Wilkins 2009). Additionally, Japan and China maintain separate national studbooks (Gilbert 2010a). The EEP, established in 1989, is the largest of the three managed populations with approximately 420 individuals, almost twice as large as the SSP population, and 15 times the size of the ASMP population (Gilbert & Woodfine 2004a; Gilbert 2010b; Spevak 2009; Wilkins 2009). The populations in the Middle East and Texas are largely uncatalogued, and are not subject to coordinated population management. The relationship of these animals to the rest of the global

population is unknown. Historically, some of these scimitar-horned oryx have been managed with other antelope species with whom they are known to hybridise. Consequently, it is likely that some hybridisation with addax, Arabian oryx, gemsbok *Oryx gazella gazella*, fringe-eared oryx *Oryx gazella callotis*, and beisa oryx *Oryx gazella beisa* has taken place (Craig 2008; Gilbert & Woodfine 2004a; Newby 2006b). Individuals from these populations should not be included in the formally managed populations, or included in reintroduction projects, until their hybrid status and relatedness to rest of the global population is determined. This process has begun for the Middle Eastern population (Maunder, M. *pers comm.* 2010; Ogden, R. *pers comm.* 2010).

2.3 Reintroduction

A number of reintroduction projects have taken place with oryx released into partially fenced protected areas in their historic range. There are four parks and reserves in Tunisia, two in Morocco, and two in Senegal with semi-captive populations of scimitar-horned oryx (Gilbert & Woodfine 2004a; Woodfine *et al.* 2009). These sites are relatively small with Dghoumes National Park in Tunisia having the largest area at 8,000ha. The largest population of semi-captive oryx consists of 230 individuals and is located in the 2400ha Arrouais Reserve in Souss Massa National Park in Morocco (Müller & Engel 2004; Ouabrou, W. *pers. comm.* 2011). All the other populations have less than 70 individuals each (Table 2.1). The land surrounding all of the parks and reserves is degraded, and there are currently no realistic prospects for oryx to be released from the fenced areas into the surrounding environment (CBSG 2009, 2011; Woodfine *et al.* 2009). As a result, the oryx cannot exhibit their natural behavioural responses, such as migration, to changing climatic conditions (Dolan 1966; Robinson *et al.* 2009; Schomber 1963). To compensate, they are managed to ensure that they have adequate food and water resources throughout the year (Molcanova & Wacher 2010; Woodfine *et al.* 2009).

There are currently no additional identified sites for scimitar-horned oryx reintroductions north of the Sahara, but the prospect of re-establishing free-ranging oryx populations in Chad and Niger has recently been evaluated (CBSG 2009, 2011). Sufficient intact habitat remains in the Ouadi Rimé-Ouadi Achim Reserve in Chad, and the Gadabeji Reserve in Niger to support sustainable free-ranging populations of scimitar-horned oryx. However, hunting and competition with domestic livestock remain problematic at both locations (CBSG 2009, 2011; Mésochina *et al.* 2009; Wacher 2010; Wacher & Newby 2011). Although scimitar-horned oryx have demonstrated considerable potential for rapid

population growth in the past, and appear to readily adapt to novel environments (Dixon *et al.* 1991), the re-establishment of free-ranging populations of oryx south of the Sahara is not a realistic prospect until these threats have been addressed.

2.4 The challenges for scimitar-horned oryx

2.4.1 Sustainability

The scimitar-horned oryx is extinct in the wild, so the species is completely reliant on the management of sustainable captive and semi-captive populations. An estimated 4% of the global population is managed through intensive coordinated captive breeding programmes (Gilbert 2010a) that aim to maximise genetic diversity, maintain demographic stability, and provide animals for reintroduction projects (Ballou *et al.* 2010a). The three managed populations are small and isolated by legislation that prevents the translocation of animals between the regions (DEFRA 2011; Hibbard *et al.* 2011; Leus *et al.* 2011a, 2011ba; Mungall 2004). Aside from the large Texan and Middle Eastern populations, most of the remaining non-managed global population originated from the EEP and SSP, and are genetically related to them. Until the large Texan and Middle Eastern populations are evaluated, and shown to be genetically distinct, there is no alternative source of oryx to supplement the managed populations. Consequently, the managed populations need to be self-sustainable to ensure the persistence of the species. Under these circumstances, it is important to retain as much genetic diversity as possible through effective management, because genetic diversity contributes to sustainability and the success of reintroduction projects (Lacy 1997; Lees & Wilcken 2009; Ralls & Ballou 1992). This is a particular concern in the light of rapid anthropogenic environmental change as populations need to retain evolutionary potential to enable them to respond to novel environmental conditions (Frankham 2008, 2010b; Frankham *et al.* 2010).

A number of factors potentially impact on the sustainability of scimitar-horned oryx including inefficient management, population fragmentation and isolation. Chapters Six, Seven, and Eight examine the impact of these factors on the sustainability of the scimitar-horned oryx EEP population, and other endangered species populations under intensive management.

Effective captive management relies on good quality pedigree data obtained from the international studbook (Ballou *et al.* 2010a), but this is problematic for the scimitar-horned

oryx population for several reasons including an inaccurate founder assumption and missing pedigree data.

2.4.2 The founder assumption

All captive population founders are assumed to be unrelated (Ballou & Ralls 1982; Lacy 1994; Lacy *et al.* 1995), and whilst this may be reasonable for some populations, it is likely to be untrue for the scimitar-horned oryx global population. Most of the founders were caught in two capture operations in Chad in the mid-1960s (Dixon *et al.* 1991). The wild population had already severely declined and contracted, and only a few thousand oryx were thought to be left in Chad (Newby 1988, 2006a). The first capture operation took place in 1963 and procured four animals for the captive population. The second operation took place in 1967 and captured an estimated 44 individuals that were distributed to European, North American and Japanese zoos (Figure 2.3). The number of founders differs between the account given by the animal collector, Van den Brink, and the international studbook (Gilbert 2010a; Van den Brink 1979). This is partially attributable to the fact that records were not kept for the first two years of captivity, and details of births and deaths were only recorded when the animals were later distributed to various zoological institutions (Storm 1986; Lilleor 2002). All of the institutions that received oryx recorded them as founders, even though some were the founders' offspring born in captivity. Consequently, there are more founders recorded in the studbook than were captured from the wild (Gilbert 2010a).

DNA analysis of the EEP population demonstrated that apparently unrelated animals showed higher levels of similarity than would be expected if the founder assumption were accurate, and suggests that the oryx caught in the 1960s were themselves closely related (Dixon *et al.* 1991). This is supported by Van den Brink's (1980) account of the capture operation in Chad in 1967. All of the animals imported from the wild in 1967 were captured in one expedition over a period of two months. Information on the capture location is imprecise, but is described as being in the northern part of Chad in the plane desert (Van den Brink 1980). Newby (2006a) identified this as probably between the Ouadi Achim and the Ouadi Hawach. In 1967, oryx were abundant in this area and it was a key trophy hunting region due to the large number of animals, high quality oryx and excellent terrain for capture operations (Newby 2006a).

Consequently, the founder assumption is likely to be false for the global scimitar-horned oryx population.

2.4.3 Missing studbook data

The founder assumption, missing pedigree data, and cryptic errors in the studbook may result in an incomplete or inaccurate evaluation of genetic diversity in a population (Ballou & Cooper 1992b; Boakes *et al.* 2007; Signer *et al.* 1994; Witzenberger & Hochkirch 2011). The international species studbook is missing 69% of its pedigree data, and this compromises the quality of pedigree analysis, and by extension effective population management. However, it is not known how much pedigree data can be missing before pedigree analyses are invalid. Chapter Four examines this issue further.

The EEP and SSP population managers have created regional analytical studbooks based on the recommendations of AZA and EAZA to compensate for missing pedigree data (AZA 2004; Ballou *et al.* 2010a). Data from these studbooks are analysed in place of data from the true studbooks and breeding and transfer recommendations subsequently made. Although the use of analytical studbooks is widely applied (AZA 2004; Willis 2001), the validity of the approach has not been tested. Chapter Five examines the accuracy of the scimitar-horned oryx true and analytical studbooks in relation to molecular data.

2.5 Scimitar-horned oryx as a case study

The scimitar-horned oryx has become a flagship for Sahelo-Saharan conservation (SCF 2010; CBSG 2009) and an important case study of conservation action. The scimitar-horned oryx was the last large mammal species to go extinct in the wild in the twentieth century (IUCN 2010). The addax and dama gazelle *Nanger dama* are following the same path to extinction in the wild (Newby 2007), and encounter comparable issues in captivity. The scimitar-horned oryx is one of only six recorded large bodied mammal species that have been successfully bred in captivity after their extinction in the wild, and then reintroduced to their former range. The other species are the Arabian oryx, Przewalski's horse, European wisent, Red wolf *Canis rufus* and Père David's deer *Elaphurus davidianus* (IUCN 2008; Stanley-Price 1989; Hu & Jiang 2002; Adams *et al.* 2007; USFWS 2007; van Dierendonck *et al.* 1996; van Dierendonck & Devries 1996; Kleiman 1989).

Research into the scimitar-horned oryx EEP population contributes direct knowledge to the conservation of the species, but the EEP population also presents an ideal case study with which evaluate the sustainability of numerous endangered species populations.

The EEP experiences many of the problems encountered by other intensively managed populations. It has a wide geographical range (Figure 2.4) and is sub-divided by national and international legislation (Gilbert 2010b; Gilbert & Woodfine 2004a; ISIS 2009), as are 33 other intensively managed bovidae species in Europe (EAZA 2010b; ISIS 2010; IUCN 2010). The concerns over the quality of the scimitar-horned oryx pedigree data are experienced by many coordinated captive breeding programmes (ISIS 2006). For example, 83% of international studbooks updated since 2001 and published in the ISIS/WAZA 2006, 2007 and 2008 studbook library database (ISIS 2009) have incomplete pedigree data.

At the same time, the amount of studbook data available for analysis makes it an appropriate study species. The scimitar-horned oryx international studbook is the third largest species studbook on the ISIS/WAZA studbook library database with 7275 listed individuals spanning 136-years (ISIS 2009; Gilbert 2010a). The number of individuals and the depth of the pedigree data in the studbook lend it to detailed analysis. The EEP is also the largest managed antelope population in the European region (Reitkerk & Glatston 2003). Overall, its population is representative of many captive species and it is an important reference population for the intensive management paradigm.

In Chapter Three, I now detail some of the general methods used in this thesis.

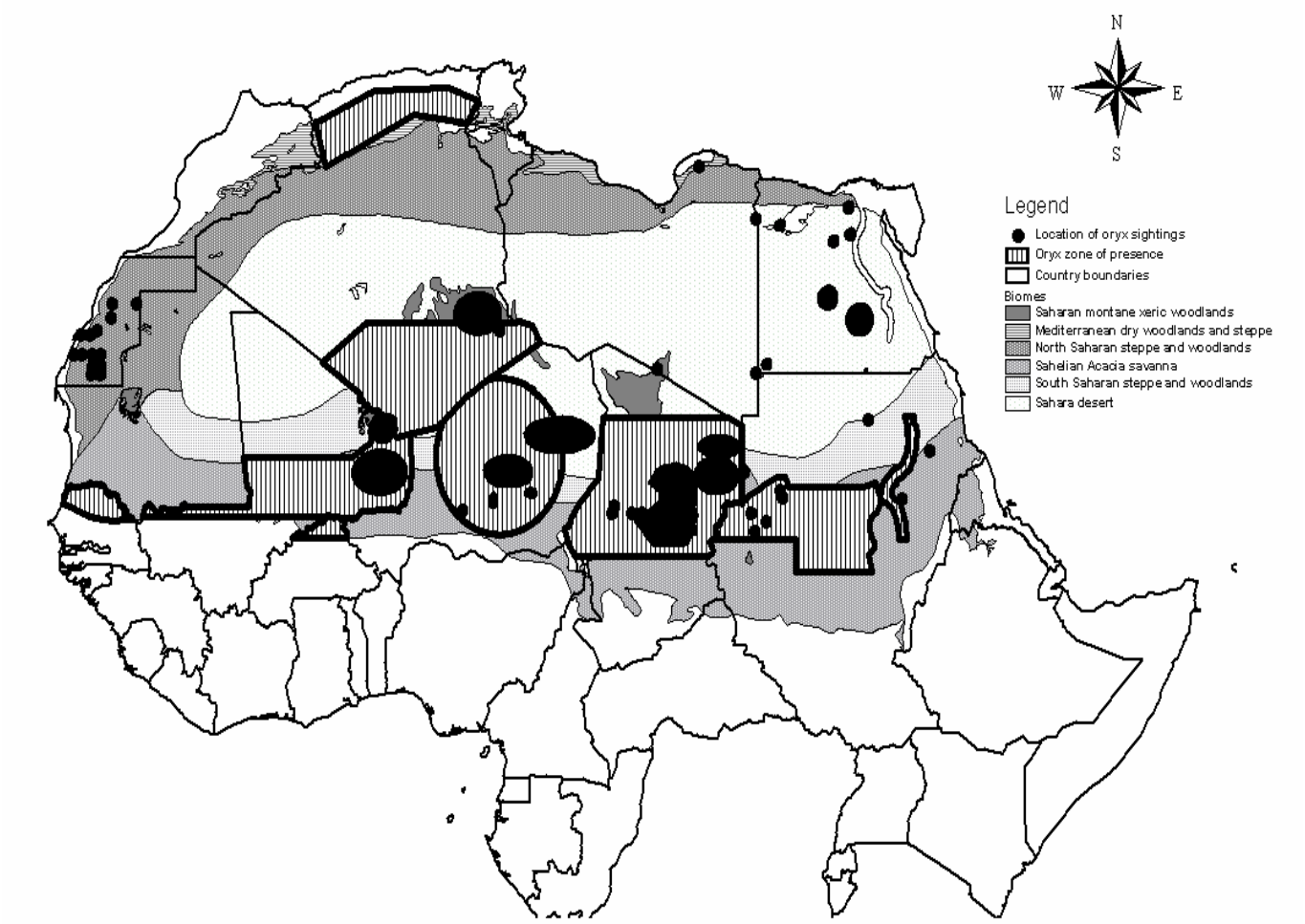


Figure 2.1 Historical distribution map for scimitar-horned oryx based on information in the literature (Barzdo 1982; Devillers & Devillers-Terschuren 2006; East 1999; Hufnagl et al. 1972; IUCN 2008; Lamprey 1975; Loggers, Thevenot & Aulagnier 1992; Newby 1978, 1980, 1981a, 1981b, 1981c, 1982, 1988; Newby *et al*. 2002; Schomber 1963; Wilson 1980).

Table 2.1 The major events in scimitar-horned oryx history

Year	Wild	Captivity	Reintroduction
1830		1832: oryx first recorded in captivity in Europe (origin unknown)	
1840			
1850	1850s: extinct in Egypt & Senegal		
1860			
1870		1876: oryx first recorded in captivity in the USA (origin unknown)	
1880			
1890			
1900	1906: last Tunisian oryx is killed		
1910			
1920			
1930	1932: extinct in Morocco	1931:oryx first recorded in captivity in Australia & Egypt	
1940	1940s: extinct in Libya		
1950	1950s: extinct in Burkina Faso		
1960	1960s: extinct Algeria & Mauritania; 1963: extinct in W. Sahara	1963: 4 founders from Chad 1967: ~44 founders from Chad	
1970	1978: extinct in Sudan	1970: 116 oryx in 28 global institutions	
1980	1980s: extinct in Niger & Chad; 1981: extinct in Mali	1980: 508 oryx in 78 global institutions	1985: 10 oryx to BH
1990		1990: 1114 oryx in 138 global institutions	1995–97: 25 to SM; 1999: 10 to ST & 3 to OD; 1999: 8 to GR
2000	Classified as 'Extinct in the Wild' by the IUCN Red List	2007: 1684 oryx in 203 global institutions	2000–2002: 2 oryx to GR 2007: 9 oryx to Dg
2010		2010: 1689 oryx in 211 global institutions	2010: ~50 in BH, 54 in Dg, ~50 GR, ~30 OD, ~230 SM, ~30 ST

BH: Bou Hedma National Park, Tunisia; **Dg**: Dghoumes National Park, Tunisia; **GR**: Guembeul Reserve, Senegal; **OD**: Oued Dekouk Nature Reserve, Tunisia; **SM**: Souss Massa National Park, Morocco; **ST**: Sidi Toui National Park, Tunisia

References: Bertram 1988; Brice 2006; De Caro 2006; Devillers & Devillers-Terschuren 2006; Dolan 1966; Doppel 2006; Fitzinger 1853; Gilbert & Woodfine 2004a; Gilbert 2008; Gilbert 2010a; Gordon 1991; Hufnagl *et al.* 1972; Lamprey 1975; Loggers *et al*. 1992; Molcanova & Wacher 2010, 2011; Newby 1988; Ouabrou, W. *pers. comm*. 2011; Van den Brink 1979; Woodfine *et al.* 2009; Woodfine, T. *pers. comm*. 2011

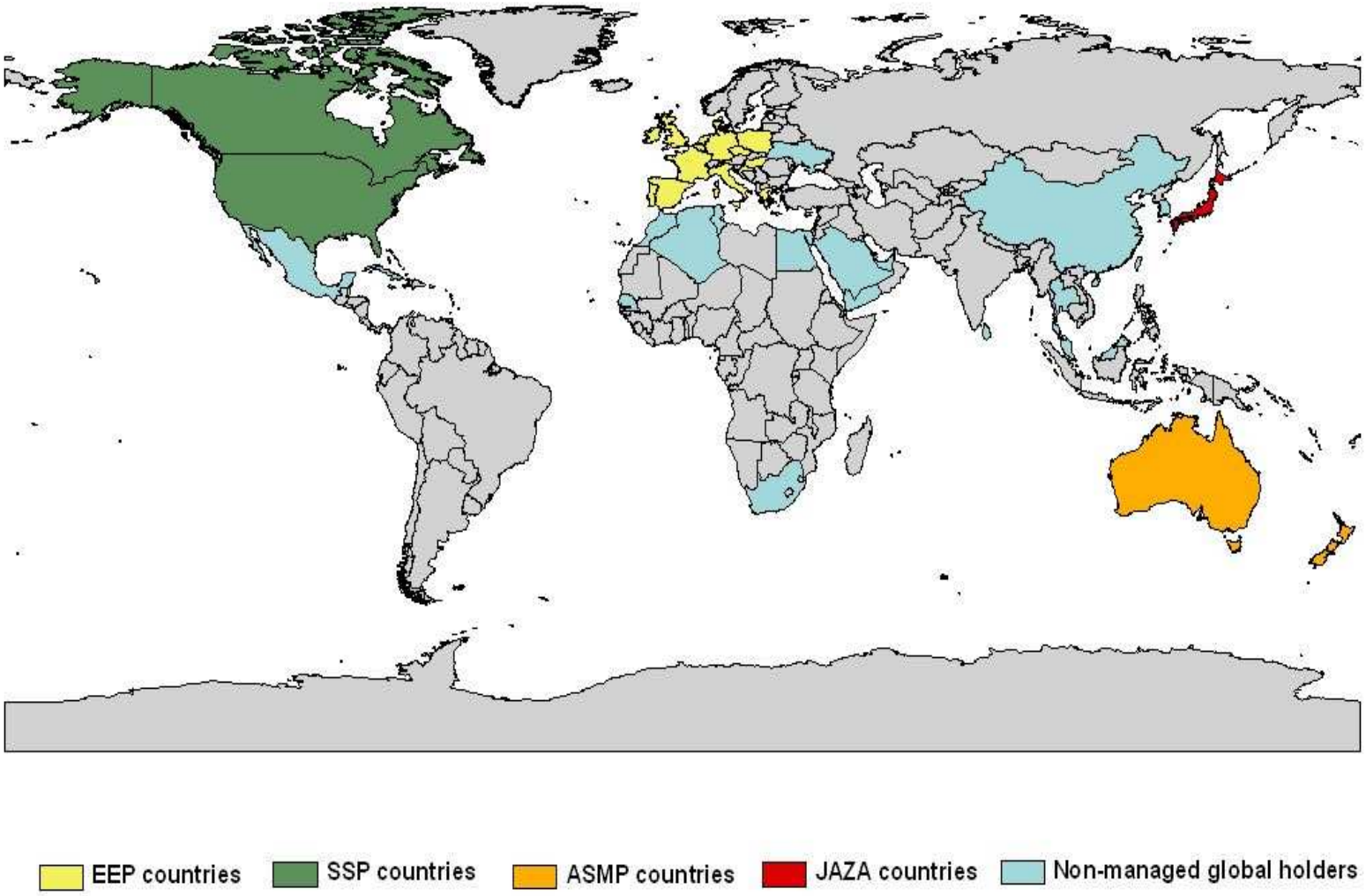


Figure 2.2 The global distribution of scimitar-horned oryx populations. Table 2.2 details the population sizes (Gilbert 2010a)

Table 2.2 Summary of global scimitar-horned oryx populations in 2010

Region / County	Descriptor	Population size	Institutions	Total
North America	Texan ranches	~11,000[1,2]	unknown	11,502
	ISB	285	33	-
	SSP[a]	217	28	-
Mexico	ISB	28	8	28
Cuba	ISB	3	1	3
Europe	ISB	128	21	501
	EEP	428[3]	53	-
Middle East	AWPR	150	1	4177
	AE	3854[4]	2	-
	ISB	118	10	-
	EEP	55	2	-
South Africa	ISB	65	6	65
Northern Africa	Tunisia ISB	17	1	471
	Tunisia FPA	215[5]	4	-
	Morocco ISB	6	1	-
	Morocco FPA	160	2	-
	Algeria ISB	16	1	-
	Egypt ISB	17	5	-
	Senegal FPA	40	2	-
China	ISB	23	5	23
Japan	JAZA / ISB	72	18	18
Singapore	ISB	4	1	4
South Korea	ISB	18	2	18
Sri Lanka	ISB	6	1	6
Thailand	ISB	8	1	8
Australasia	ISB	20	1	20
	ASMP[b]	28	5	28
			Grand Total	**16,927**

Key: [a] defined by AZA filter; [b] defined by ZAA filter; ASMP: Australasian Species Management Plan; AWPR: Al Ain Wildlife Park and Resort; EEP: European Endangered species Programme; FPA: Fenced Protected Area; ISB: International Studbook; JAZA: Japanese Association of Zoos and Aquaria; SSP: Species Survival Plan; AE: United Arab Emirates

References: [1]McClellan (2010); [2]Johnson (2010); [3]Gilbert (2010a); [4]Anderson (2010); [5] Woodfine (2010).

Figure 2.3.1

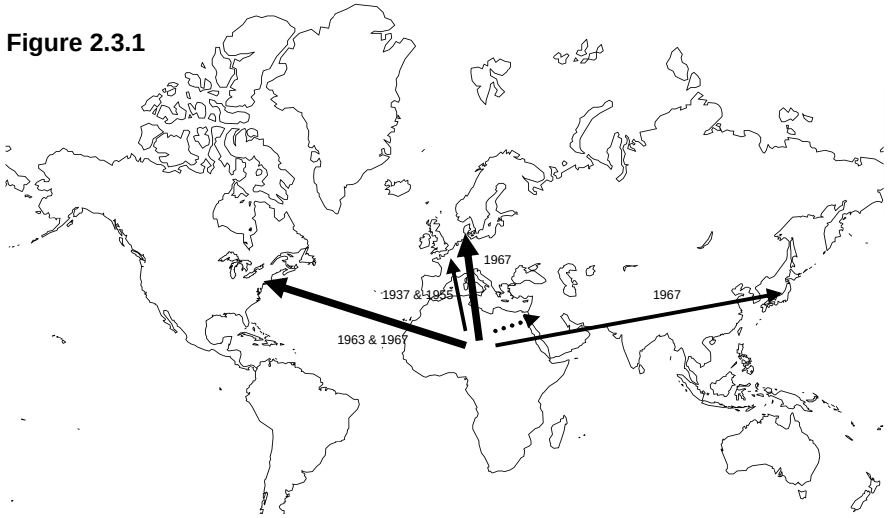


Figure 2.3.2

Figure 2.3.3

Figure 2.3 The distribution of the global founders and their offspring. **Figure 2.3.1** illustrates the location and dates of transfers of the founders. **Figure 2.3.2** illustrates their distribution to institutions after the quarantine period, and **Figure 2.3.3** illustrates the distribution of their descendants to global institutions

Figure 2.4.1

Figure 2.4.2

Figure 2.4 Scimitar-horned oryx EEP institutions in **(Figure 2.4.1**), and outside (**Figure 2.4.2**) the European region. Two institutions, the DDCR and Pretoria Zoo are included on the map in relation to Chapter Five, but are not part of the EEP. Table 2.3 provides a numeric key for the map

Table 2.3 Numeric key for Figures 3.1 and 3.2 with institutional mnemonics. Institutional mnemonics are described in Appendix B

Key	Institution	Key	Institution	Key	Institution	Key	Institution
1	FOTA	17	OBTERRE	33	AMSTERDAM	49	DVURKRALV
2	DUBLIN	18	LA PALMYR	34	AMERSFOOR	50	DEBRECEN
3	MANOR HS.	19	PT ST PER	35	AALBORG	51	VESZPREM
4	MALTON	20	CABOSSE	36	KREFELD	52	ZAGREB
5	KNOWSLEY	21	PLAISANCE	37	KARLSRUHE	53	PUNTAVERD
6	CHESTER	22	LE PAL	38	LEIPZIG	54	BUSSOLENGO
7	BURFORD	23	MONTPELLI	39	BERLIN TP	55	VALBREMBO
8	LONGLEAT	24	PELISSANE	40	GDANSK	56	ATTICAZOO
9	MARWELL	25	CABARCENO	41	PLOCK	57	PRETORIA
10	CHESSINGTON	26	BARCELONA	42	LODZ	58	DDCR
11	WHIPSNADE	27	MADRID Z	43	WARSAW	59	JERUSALEM
12	WOBURNLTD	28	TABERNAS	44	KATOWICE	60	RAMAT GAN
13	GUERNO	29	ESTEPONA	45	OPOLE	61	RABAT
14	BOISSIERE	30	LISBON	46	WROCLAW		
15	LISIEUX Z	31	PLANCKENDI	47	PLZEN		
16	PARIS ZOO	32	AYWAILLE	48	PRAHA		

3.0 Chapter three: general methodology

3.1 Data sources

This thesis predominantly utilised studbook data from the scimitar-horned oryx international studbook. A sub-set of the scimitar-horned oryx studbook data was used for each chapter due to differing data requirements. Appendix B details the institutions whose data were included in the analyses for each chapter, along with the institutional mnemonic.

Much of the thesis focused on the scimitar-horned oryx EEP population. The EEP is distributed over a wide geographical range as detailed in Figure 2.4 and Table 2.3. The Dubai Desert Conservation Reserve (DDCR) and the National Zoological Gardens, Pretoria are included on the map in Figure 2.4 because they provided data and samples for Chapter Five. They are not, nor have ever been, part of the EEP population.

Chapters Four and Seven utilised the data from five and 113 different species and sub-species regional and international studbooks, respectively. The studbooks were either obtained directly from the studbook keepers (Table 3.1), or from the published ISIS/WAZA 2006/2007/2008 studbook library (Table 3.2). The ISIS/WAZA studbook library 2006/2007/2008 contained 1185 studbooks (187 international and 998 regional studbooks) for 787 species, sub-species and hybrids. This represented 99% of all published studbooks and 100% of all international studbooks. The data in the studbooks were provided by 700 studbook keepers from 313 institutions in 45 countries (ISIS 2009).

The general methods detailed in this chapter have an application to Chapters Four, Five, Six, Seven and Eight.

Table 3.1 Studbook data files obtained directly from the studbook keeper. **N**: the number of individuals listed in each studbook

Species	Scientific	SB	SB keeper	N	Chapter
Arabian oryx	*Oryx leucoryx*	ESB	I. Goodwin	1668	7
Grevy's zebra	*Equus grevyi*	ISB	T. Langenhorst	3178	4
Dorcas gazelle	*Gazella dorcas neglecta*	ISB	T. Abaigar	1487	7
Mhorr gazelle	*Nanger dama mhorr*	ISB	G. Espeso	1678	7
Scimitar-horned oryx	*Oryx dammah*	ISB	T. Gilbert	8175	4, 5, 6, 7, 8

Table 3.2 Studbook data file obtained from the ISIS/WAZA studbook library 2006/2007/2008. All the studbooks listed are international studbooks. **N**: the number of individuals listed in each studbook; **Chp**: the chapter number

Species	Scientific name	SB keeper	N	Chp
African wild dog	*Lycaon pictus*	S. Rhodes	3781	4, 7
Alotran gentle lemur	*Hapalemur alaotrensis*	T. Wright	129	7
Amur leopard	*Panthera pardus orientalis*	O. Walters	665	7
Amur tiger	*Panthera tigris altaica*	P. Mueller	4914	4, 7
Arabian leopard	*Panthera pardus nimr*	J. Edmonds	68	7
Arabian oryx	*Oryx leucoryx*	K. Sausman	3572	7
Aruba Island rattlesnake	*Crotalus unicolor*	S. Mays	536	7
Asian small-clawed otter	*Aonyx cinereus*	S. Duncan	3189	7
Aye-aye	*Daubentonia madagascariensis*	T. Wright	104	7
Babirusa	*Babyrousa babyrussa*	T. Kauffels	640	7
Baird's tapir	*Tapirus bairdii*	J. Roman	252	7
Bengal tiger	*Panthera tigris tigris*	P. Müller	1076	7
Black and white ruffed lemur	*Varecia variegata variegata*	I. Porton	2415	7
Black howler monkey	*Alouatta caraya*	K. Harris	592	7
Black lemur	*Eulemur macaco macaco*	I. Porton	1061	7
Black lemur	*Eulemur macaco flavifrons*	I. Porton	177	7
Black lion tamarin	*Leontopithecus chrysopygus*	C. V. Padua	422	7
Black rhinoceros	*Diceros bicornis*	R. Frese	973	7
Black-faced impala	*Aepyceros melampus petersi*	A. Sogorb	223	7
Black-footed cat	*Felis nigripes*	U. Schuerer	586	7
Black-necked crane	*Grus nigricollis*	X. Zhong	423	7
Blue-billed currasow	*Crax alberti*	C. Holmes	55	7
Blyth's trapoan	*Tragopan blythii*	M. Saint Jalme	193	7
Bongo	*Tragelaphus eurycerus isaaci*	L. F. Bosley	2101	7
Bonobo	*Pan paniscus*	Z. Pereboom	399	7
Buff crested bustard	*Lophotis ruficrista*	S. Hallager	344	7
Bush dog	*Speothos venaticus*	R. Dmoch	1438	7
Caracal	*Caracal caracal*	B. Palmer	2312	7
Cheetah	*Acinonyx jubatus*	L. Marker	6692	7
Chinese alligator	*Alligator sinensis*	M. Litton	380	7
Chinese leopard	*Panthera pardus japonensis*	O. Walters	656	7
Congo peafowl	*Afropavo congensis*	S. Vansteenkiste	1243	7
Cotton-top tamarin	*Saguinus oedipus*	H. Colahan	11728	4, 7
Crowned sifaka	*Propithecus verreauxi coronatus*	D. Haring	43	7
Cuvier's gazelle	*Gazella cuvieri*	E. Moreno	1239	7
Diana monkey	*Cercopithecus diana*	G. Catlow	1066	7
Douc langur	*Pygathrix nemaeus*	L. Lippold	449	7
Drill	*Mandrillus leucophaeus*	A. Knieriem	654	7
Fishing cat	*Prionailurus viverrinus*	J. Kinzer	771	7
Fossa	*Cryptoprocta ferox*	A. Winkler	226	7
Giant anteater	*Myrmecophaga tridactyla*	I. Schappert	989	7
Giant eland	*Taurotragus derbianus gigas*	E. Flossic	221	7
Giant otter	*Pteronura brasiliensis*	F. Brandstaetter	440	7
Giant panda	*Ailuropoda melanoleuca*	X. Zhong	739	7
Goeldi's monkey	*Callimico goeldii*	M. Warneke	2712	7
Golden lion tamarin	*Leontopithecus rosalia*	J. Ballou	3727	7
Golden monkey	*Rhinopithecus roxellana*	Z. Yu	431	7
Golden-headed lion tamarin	*Leontopithecus chrysomelas*	P. Galbusera	2396	7
Goodfellow's tree kangaroo	*Dendrolagus goodfellowi*	G. Skipper	209	7
Gordon's wild cat	*Felis silvestris gordoni*	A. Sliwa	211	7
Gorilla	*Gorilla gorilla*	R. Dmoch	2004	7
Great hornbill	*Buceros bicornis*	K. Brouwer	508	7
Grey gentle lemur	*Hapalemur griseus*	D. Roullet	73	7
Grizzled grey tree kangaroo	*Dendrolagus inustus*	M. Rodden	157	7
Hartmann's zebra	*Equus zebra hartmannae*	T. Langenhorst	1383	7
Hooded crane	*Grus monacha*	K. Takami	416	7
Horned Guan	*Oreophasis derbianus*	J. Cornejo	102	7
Indochinese tiger	*Panthera tigris corbetti*	P. Müller	247	7
Kori bustard	*Ardeotis kori*	S. Hallager	679	7

Table 3.2 continued

Species	Scientific name	SB keeper	N	Chp
Lesser bird-of-paradise	*Paradisaea minor*	P. Cooper	142	7
Lion-tailed macaque	*Macaca silenus*	S. Carter	2131	7
Lowland anoa	*Bubalus depressicornis*	G. Noetzold	607	7
Madagascar giant jumping rat	*Hypogeomys antimena*	G. Glendewar	308	7
Malayan tapir	*Tapirus indicus*	S. Prastiti	864	7
Maned wolf	*Chrysocyon brachyurus*	R. Dmoch	3063	7
Maroon-fronted parrot	*Rhynchopsitta pachyrhyncha terrisi*	J. Cornejo	85	7
Matschie's tree kangaroo	*Dendrolagus matschiei*	G. Skipper	483	7
Mauritius pink pigeon	*Columba mayeri*	D. Jeggo	987	7
Mexican grey wolf	*Canis lupus baileyi*	P. Siminski	1151	7
Moloch gibbon	*Hylobates moloch*	L. Cocks	235	7
Muskox	*Ovibos moschatus*	B. Holst	1350	7
Okapi	*Okapia johnstoni*	K. Leus	664	7
One-horned rhinoceros	*Rhinoceros unicornis*	F. von Houwald	406	7
Orangutan	*Pongo pygmaeus*	M. Elder	2668	7
Oriental white stork	*Ciconia boyciana*	H. Ogawa	968	7
Pallas' cat	*Felis manul*	M. Caron	1140	7
Pied tamarin	*Saguinus bicolor*	A. Baker	477	7
Pileated gibbon	*Hylobates pileatus*	R. Zingg	276	7
Polar bear	*Ursus maritimus*	K. Linke	2876	7
Puerto Rican crested toad	*Peltophryne lemur*	E. Gabura	2197	7
Pygmy hippopotamus	*Hexaprotodon liberiensis*	B. Steck	1223	7
Red bird-of-paradise	*Paradisaea rubra*	N. Clum	161	7
Red panda	*Ailurus fulgens refulgens*	A. Glatston	2869	7
Red ruffed lemur	*Varecia rubra*	I. Porton	964	7
Red wolf	*Canis rufus gregoryi*	W. Waddell	1728	7
Red-billed currasow	*Crax blumenbachii*	R. Azeredo	1061	7
Red-crowned crane	*Grus japonensis*	H. Ogawa	1708	7
Sand cat	*Felis margarita*	K. Akers	611	7
Siberian white crane	*Grus leucogeranus*	T. Kashentseva	688	7
Sloth bear	*Melursus ursinus*	J. Kok	587	7
Snow leopard	*Uncia uncia*	L. Blomqvist	2733	7
Somali wild ass	*Equus asinus somalicus*	C. Pohle	341	7
South China tiger	*Panthera tigris amoyensis*	P. Müller	307	7
Southern koala	*Phascolarctos cinereus victor*	S. Vaartjes	633	7
Spectacled bear	*Tremarctos ornatus*	A. Hall	765	7
Spix's macaw	*Cyanopsitta spixii*	R. Watson	117	7
Sri Lankan leopard	*Panthera pardus kotiya*	O. Walters	306	7
Sri Lankan rusty-spotted cat	*Prionailurus rubiginosus phillipsi*	R. Dmoch	180	7
St Vincent parrot	*Amazona guildingii*	D. Woolcock	266	7
Sumatran rhinoceros	*Dicerorhinus sumatrensis*	J. Christman	44	7
Sumatran tiger	*Panthera tigris sumatrae*	P. Müller	1355	7
Vicugna	*Vicugna vicugna*	C. Schmidt	756	7
Vietnamese pheasant	*Lophura hatinhensis*	J. Lévrier	528	7
Wattled crane	*Bugeranus carunculatus*	F. Beall	753	7
Western grey lemur	*Hapalemur occidentalis*	D. Roullet	15	7
White rhinoceros	*Ceratotherium simum simum*	R. Frese	1512	7
White-naped crane	*Grus vipio*	K. Nippashi	1363	7
Yellow-backed duiker	*Cephalophus silvicultor*	L. R. Bachers	317	7

3.2 Data management, analytical and modelling software programs

Studbook databases are maintained in computer programs including PopLink (Faust *et al.* 2011) and SPARKS (Single Population Analysis and Record Keeping System) (Scobie *et al.* 2004). SPARKS was developed, is supported, and is distributed by ISIS (Bingaman Lackey 2010; Thompson 2004). Studbooks databases on the ISIS/WAZA

2006/2007/2008 studbook library are in a SPARKS format. Consequently, the data used in this thesis was held in SPARKS version 1.56 beta (Scobie *et al.* 2004), although a later version v.1.6 beta (Scobie *et al.* 2011) was available at the time of writing. The later version contains extra features which facilitate additional analyses (Ballou *et al.* 2010a). SPARKS can perform basic demographic and genetic analyses, and the data can be exported in specified formats for use in other population analysis computer programs including Lineage (Pollak & Egan 2008b), PM2000 (Pollak *et al.* 2007), PMx (Ballou *et al*. 2011), and VORTEX (Ballou & Lacy 1995; Bingaman Lackey 2010; Lacy & Ballou 2002; Lacy *et al*. 2009; Leus *et al.* 2011b; Scobie *et al.* 2004).

When exporting data for use in other programs, care was taken in setting the export filter conditions (Lacy & Ballou 2002; Thompson 2004). Geographical or institutional filters can be set to select living, or living and dead, individuals located at EEP institutions. Time spans for demographic exports need to be sufficiently large to allow the observation of population trends, but small enough to ensure that data represents contemporary management (assuming past dynamics are used to predict future trends) (Ballou *et al.* 2010a; Lacy & Ballou 2002; Wilcken & Lees 1998). Consequently scimitar-horned oryx data exported from SPARKS for Chapters Four, Five, Six, Seven, and Eight, set filter conditions from 01/01/1990 to the 31/12/2008. The start date was selected because the EEP was established in 1989, and 1990 represents the beginning of contemporary population management for the population (Gilbert & Woodfine 2004a). Data exports for genetic analyses in Chapters Four, Five, Six, Seven, and Eight were filtered to only include living individuals in EEP institutions (where appropriate) on the date that data were current to e.g. 31 December 2008 if all data had been entered into the database up to the end of 2008.

Lineage v1.06 (Pollak & Egan 2008b) is pedigree visualisation and analysis software. It diagrammatically represents extremely complex inter-generational pedigrees. Data are exported from SPARKS using a specific format for Lineage, and imported into Lineage for analysis (Pollak & Egan 2008a; 2008b). This program was predominantly used in Chapter Five to illustrate differences in pedigree structure between the true and analytical studbooks.

PM2000 (Pollak *et al.* 2007) is a Windows-based software program that provides a suite of tools for the genetic and demographic analysis, and management, of pedigreed populations (Lacy & Ballou 2002; Pollak *et al.* 2007). Other pedigree analysis programs are available, for example PyPedal (Boichard 2002; Cole 2007), Pedig (Boichard 2002) and ENDOG (Boichard 2002; Gutierrez & Goyache 2005), but PM2000 was uniquely

designed to analyse both the genetic and demographics of the complex pedigrees generated by exotic species in captivity. Additionally, it is exclusively recommended for the analysis of captive populations by regional zoo associations, and is compatible with studbook data imports from PopLink and SPARKS (AZA 2004; Lacy & Ballou 2002).

Genetic data are exported from SPARKS in exchange.dbf files, and demographic data as separate male (male.prn) and female (female.prn) files. These files are imported into PM2000 to provide the life table and pedigree data for population genetic and demographic analysis, future trend simulations, goal setting, and modelling population management options (Ballou *et al.* 2010a; Lacy & Ballou 2002; Pollak *et al.* 2007; Thompson 2004).

Gene drop simulations are used to estimate a number of population genetic metrics based on the mean retention of founder genetic diversity e.g. gene diversity (*GD*), gene value (*GV*), and founder genome equivalents (*FGE*) (refer to the Glossary for the definitions of genetic terms) (Ballou *et al.* 2010). Two unique alleles are assigned to each founder and Monte-Carlo simulation methods determine the probabilistic transmission of alleles from the founders to the living descendants based on the principles of Mendelian inheritance (Ebert 2008; Lacy 1995; Pollak *et al.* 2007). The number of times this simulation is repeated is user defined, but estimates of retained founder genetic variation are more accurate if a large number of iterations are run (Lacy & Ballou 2002; Pollak *et al.* 2005). Consequently, the default value of 1,000 iterations was changed to 10,000 iterations for gene drop simulations in PM2000 for Chapters Five, Six, and Seven.

PM2000 also uses kinship matrices to estimate inbreeding coefficients for every individual in the population, and kinship coefficients for every pair of animals in the population (Lacy & Ballou 2002). Mean kinships (*MK*), which represent the relatedness of any one individual to the rest of the population, can then be estimated for each individual (Pollak *et al.* 2007). Estimates of inbreeding and kinships derived using kinship matrices in PM2000 were used in Chapters Four, Five, Six and Seven.

Incomplete pedigree data presents a problem for pedigree analysis. PM2000 excludes all animals with unknown parents from genetic analyses and includes only those parts of animals that can be traced back to known founders (Lacy & Ballou 2002). Calculations for inbreeding and kinship coefficients cannot be completed when parentage data are not available. PM2000 uses the known part of the pedigree to replace the missing pedigree when partial pedigree data are available (Pollak *et al.* 2005). The impact of missing pedigree data in the functioning of PM2000 is evaluated in Chapters Four and Five.

PM2000 is distributed by the Chicago Zoological Society (Lacy & Ballou 2002; Pollak *et al.* 2007).

SPARK-Plug version 1.0 (Porter *et al.* 2002) is a data management software package, that enables users of SPARKS to identify missing pedigree data and uncertainties in studbook databases. Users can edit specimen records and create hypothetical ancestors in the form of overlays, which are then applied to studbook databases creating a separate analytical studbook file (AZA 2004; Ballou *et al.* 2010; Porter *et al.* 2002; Wiese & Willis 2000). SPARK-Plug was used to create the analytical studbook for the scimitar-horned oryx EEP population for Chapters Five, Six, Seven and Eight, and the analytical studbook for the Arabian oryx for Chapter Seven. SPARK-Plug was developed by Lincoln Park Zoo, Minnesota Zoo, and AZA, and is available from AZA (Porter *et al.* 2002)

PMx (Ballou *et al.* 2011) was developed in 2011 as the successor to PM2000. In addition to all the analyses that PM2000 performs, it contains additional features which provide alternative approaches to addressing the issue of missing pedigree data. PMx was not used in this thesis, but it is referred to in Chapter Five.

VORTEX version 99.9b (Lacy *et al.* 2009) software is an individual-based simulation model for population viability analysis (Miller & Lacy 2005). It is used exclusively to model fragmentation in the scimitar-horned oryx EEP population in Chapter Eight. VORTEX is owned by, and available from, the Chicago Zoological Society.

I now apply the methods presented here to the following chapters.

4.0 Chapter four: the reliability of inbreeding coefficients derived from incomplete pedigrees

4.1 Abstract

Inbreeding, which may lead to inbreeding depression, has been identified as a problem for small closed populations, including captive populations of endangered species. Individual inbreeding coefficients are calculated from pedigree data, but complete pedigree data are often not available because of incomplete historical records. This impacts on the quality of pedigree based captive population management, and on research into inbreeding depression in pedigreed populations.

The impact of incomplete pedigrees on the reliability of inbreeding coefficients (*F*) has been examined by a number of different studies, but none have quantified how much pedigree data can be missing before *F* is no longer estimatable. For the first time, this chapter aims to determine the threshold completeness for reliably estimating inbreeding coefficients from incomplete pedigrees, and evaluate the impact on population management.

Pedigrees for five species were extracted from their respective studbooks, and portions of the pedigree randomly removed to create hypothetical pedigrees that were 87.5%, 75%, 62.5%, and 50% complete. Inbreeding coefficients were calculated using the additive matrix method. Differences between *F* from complete pedigrees and incomplete pedigrees were tested using a generalised linear model with post-hoc tests. The impact of estimating *F* from incomplete pedigrees on population management was modelled.

Inbreeding coefficients were reliably estimated from incomplete pedigrees when pedigree completeness was at least 62.5% complete for all five species. Furthermore, the impact of overestimating or underestimating *F* had minimal impact on population management decisions until pedigree completeness fell below 62.5%.

Application of the results is discussed, along with study limitations of the research, and recommendations for future research.

4.2 Introduction

The successful breeding of endangered species in captivity requires sound management of genetic resources, to ensure retention of genetic diversity for future evolutionary potential, and to minimise inbreeding and adaptation to captive conditions (Frankham *et al.* 1986; Frankham & Loebel 1992; Hedrick & Miller 1992; Mace 1989; Miller 1994, 1995).

Inbreeding has detrimental impacts on a number of fitness-related traits including neonatal survival, growth, reproduction, longevity and susceptibility to disease (Amos & Balmford 2001; Ralls & Ballou 1983; Ralls *et al.* 1988; Cassinello *et al.* 2001; Cassinello 2005; Frankham 1995c; Marshall *et al.* 2002; Negro & Torres 1999; Vasarhelyi 2002). Fitness declines as inbreeding increases (Ballou 1997) and it can impact severely on the health of small isolated populations (Frankham 1995b). Zoological institutions manage their captive populations to avoid inbreeding, particularly for species that cannot be supplemented with additional founders from the wild. Inbreeding is perhaps the greatest threat to the short-term survival of captive populations (Franklin 1980; Senner 1980; Willis 1993).

Inbreeding can affect individuals and whole populations (Marshall *et al.* 2002), and it can be evaluated through molecular techniques or pedigree analyses (Avise *et al.* 1995; Höglund 2009). Here I measure it with the inbreeding coefficient *F*, which is the probability that the two alleles at a genetic locus in an individual are identical by descent, that is, are derived by replication of a single allele from a common ancestor (AZA 2004; Ballou 1983; Ralls *et al.* 1988). The coefficient ranges in value from zero for a non-inbred individual to unity for a homozygous individual (Ballou 1983; Ralls *et al.* 1988), with inbreeding depression directly related to *F* (Falconer & MacKay 1996). Research into inbreeding depression in scimitar-horned oryx has shown that juvenile mortality increases significantly when inbreeding coefficients exceed 0.125 (Mace 1989). I examine the impact of inbreeding on juvenile mortality in the scimitar-horned oryx EEP population in Chapter Eight. High levels of juvenile mortality can destabilise small populations because it reduces the number of individuals in the first-year age-class, resulting in an aging population (Ballou *et al.* 2010a).

Managers of captive populations principally use pedigree analysis to assign breeding priority (Hedrick & Miller 1992). Two genetic measures are used to determine breeding priority, individual mean kinship coefficients (mk_i), and individual inbreeding coefficients

(*F*) (Ralls & Ballou 1992). Individual inbreeding coefficients can be calculated for a pedigree derived from a studbook using computer programmes such as SPARKS (Scobie *et al.* 2004) and PM2000 (Pollak *et al.* 2005), which use kinship matrices to calculate *F* by Monte-Carlo simulation (Hedrick & Miller 1992). The *F* value is calculated in relation to the founders of the population, which are assumed to be unrelated, using a method given by Ballou (1983). The method assumes neutrality with respect to selection in order to calculate transmission probabilities by Mendelian ratios (Charlesworth & Charlesworth 1987). Calculations of inbreeding coefficients critically depend on a complete knowledge of the pedigree (Ballou & Lacy 1995). When a proportion of the pedigree is missing, inbreeding coefficients may be underestimated (Cassell *et al.* 2003; Hagger 2005; Lutaaya *et al.* 1999) or miscalculated by these methods. Computer software designed to calculate *F* will always return a value of zero when one parent is missing from the pedigree (Lutaaya *et al.* 1999) which can lead to a false impression that the individual in question is estimated to be heterozygous.

Contemporary captive breeding programmes for endangered species universally avoid inbreeding where possible (Kalinowski & Hedrick 1999; Kalinowski *et al.* 2000), to approach the ideal of populations in which all individuals have an inbreeding coefficient of zero. Inbreeding is inevitable in small closed populations (Frankham *et al.* 2010), and when forming breeding pairs the general principle is that the projected inbreeding coefficient of the offspring should not be above the mean kinship (*MK*) value for the population (Wilcken & Lees 1998). Exceptions to this general principle are sometimes made when individuals vary widely in *MK* values. In this instance, population managers may deliberately breed closely related individuals with low *MK*s, in order to produce inbred offspring that can be outbred in the next generation. Their objective is to obtain a more even founder representation and an improved retention of gene diversity (Wilcken & Lees 1998).

Managers need to assess inbreeding, both within a population and at an individual level, in order to maximize genetic diversity within captive breeding programmes. Miscalculation of an individual's *F* may result in pairings that will lead to inbred offspring, thereby reducing the genetic diversity of the population, and potentially resulting in inbreeding depression. Alternatively, two individuals may be incorrectly prevented from breeding together as a result of overestimating the projected inbreeding coefficient of their offspring. If a large proportion of the pedigree is missing, an animal may be excluded from

the breeding programme altogether to avoid possible inbreeding, resulting in a potential loss of genetic diversity (Willis 1993).

A number of studies have evaluated inbreeding depression in domestic and captive populations using pedigree data to calculate the level of inbreeding at both an individual and population level. Some of the studies remove individuals with incomplete pedigrees from the analysis, as the detection of inbreeding depression depends on accurate estimates of inbreeding (Ballou & Ralls 1982; Boichard *et al*. 1997; Cassell *et al.* 2003). This approach ensures that only good quality pedigrees are used, but may result in limited samples sizes for some analyses (Laikre & Ryman 1991). A few studies have gone further by attempting to define the relationship between the quality of pedigree data and the estimate of inbreeding at an individual level (Boichard *et al.* 1997; Cassell *et al.* 2003; Vanraden 1992). These studies have mainly focused on the completeness of the pedigree needed to declare an animal non-inbred, as opposed to determining the pedigree completeness needed to accurately estimate *F*.

In this chapter I assess the validity of inbreeding coefficients calculated from incomplete pedigrees derived from the international studbooks for scimitar-horned oryx, Grevy's zebra *Equus grevyi*, cotton-top tamarins *Saguinus oedipus*, African wild dog *Lycaon pictus* and Amur tiger *Panthera tigris*. These studbooks are excellent models for such a study due to their size, pedigree completeness, depth of pedigree and representation of different taxa and breeding and management systems. The particular objectives of this chapter are; 1) to determine average inbreeding coefficients for pedigrees subjected to incremental removal of information; and 2) to determine the validity of using inbreeding coefficients derived from pedigrees of varying completeness in captive species management and research. The results will be widely applicable for inbreeding research and captive breeding programmes that rely on studbooks with incomplete pedigrees.

4.3 Methodology

4.3.1 Pilot study: scimitar-horned oryx

The initial analyses were carried out on pedigrees derived from the international studbook for scimitar-horned oryx which contained records dating back to 1875. The historical listing from 1st January 1930 to 31st December 2004 was extracted from SPARKS and imported into PM2000 where individual inbreeding coefficients (*F*) were calculated by simulating an additive kinship matrix. All individuals with 100% known ancestry, and with four or more generations, were selected (N = 183) for incremental removal of individuals to model the impact of incompleteness on *F*. Analyses required a minimum of four generations in order to construct hypothetical pedigrees for all the completeness categories. Hypothetical pedigrees with only a small amount of missing pedigree data required the removal of one great-grandparent or two great-great-grandparents. Pedigrees therefore, had to be of sufficient depth to allow this.

The pedigree for each selected individual was extracted from the studbook, and every animal in the final four generations was allocated an identification number between 1 and 15, with 15 being the descendant at the base of the tree for which the inbreeding coefficient was calculated. Individuals were then randomly selected for removal from the pedigree using a random number generator in MS Excel to generate whole random numbers between 0 and 14 (fx = INT (RAND()*15)), with the number selected indicating which individual should be removed from the pedigree. The selected individuals were removed if the resulting known ancestry was 87.5%, 75%, 62.5% or 50% known (Figure 4.1 and Appendix C). In instances where it was not possible to obtain one or more data points for an individual because the resulting known ancestry varied from the four conditions, the individual was removed from the data set, resulting in equal sample sizes for each condition.

The random removal of ancestors was appropriate even though the social behaviour and captive management regime for some species may result in only sires or dams being missing from the pedigree data. An evaluation of the species studbooks on the 2004/2005 ISIS/WAZA Studbook library (ISIS 2006) reveals that 2.5% were missing only sires, 1% were missing only dams, and 17% were missing both parents out of the 679 species listed. The remaining 471 species (69%) had a combination of sires, dams and both parents

missing. Specifically the scimitar-horned oryx studbook had a combination of ancestors missing from incomplete pedigrees.

Once the random ancestors were removed, the incomplete pedigrees were imported into PM2000 and the kinship matrix re-simulated to obtain the *F* for the new pedigree data. Each individual (number 15) yielded five data points, representing each of the levels of completeness. It was noted that when an individual had an *F* of 0.0 at 100% known the *F* did not change regardless of how much ancestry was removed. As this study aims to evaluate the potential changes in *F* as a consequence of varying pedigree completeness, those individuals *F* = 0.0 when pedigrees were complete were removed from the data, resulting in a final sample size of *N* = 167.

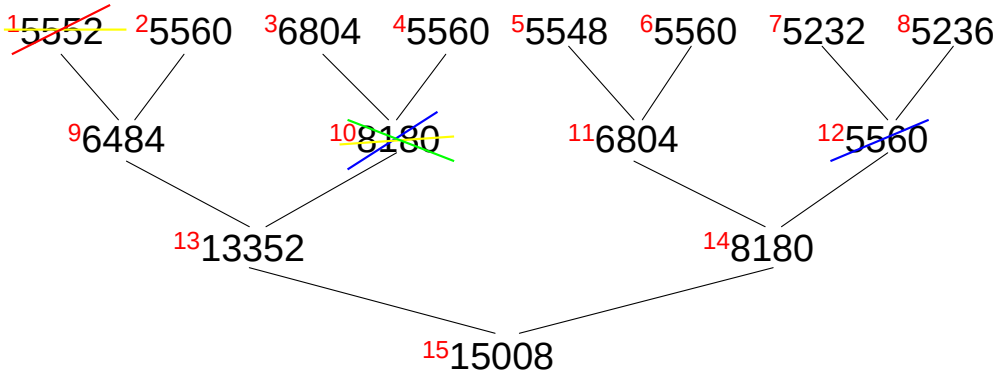


Figure 4.1 An example pedigree for individual 15008 with parts of the pedigree randomly removed to allow creation of hypothetical pedigrees. Number one (5552) was removed (red line) creating a hypothetical pedigree 87.5% complete. Number 10 (8180) was removed (green line) creating a hypothetical pedigree with 75% pedigree completeness. Numbers one (5552) and 10 (8180) were removed yielding 62.5% known ancestry (yellow line). Numbers 10 (8180) and 12 (5560) were removed yielding 50% pedigree completeness (blue lines)

4.3.2 Main study: species and studbook selection

The 20 largest species studbooks (with the most individuals) listed on the ISIS/WAZA 2004/2005 studbook library (ISIS 2006) were reviewed in relation to the pedigree criteria of pedigree depth, completeness and *F* at 100% known. Thirteen of the studbooks had more than 30 individuals (Dytham, 2011) with complete pedigrees of more than four generations and an *F* greater than 0.0001 at 100% known (Table 4.1). Permission

was obtained from the studbook keepers for the use of five of these studbooks in the study: Grevy's zebra, cotton-top tamarins, African wild dogs, Amur tigers and scimitar-horned oryx. These species represent different taxa and are managed in different social groups in captivity. Consequently the five studbooks are good representatives of the different management strategies and provide an overview of the quality of studbook data within the different management systems. The different social management systems are likely to impact on the type of data missing from the pedigrees. For example, offspring born in a harem group (one male and multiple females) should always have a recorded sire, but the dam information may be missing as there is more than one possible dam. In a multi-male and multi-female group the missing parentage information is likely to include both sires and dams.

In contrast to the social organisation in the wild, both scimitar-horned oryx and Grevy's zebra are predominantly maintained in single-sex or harem groups in EEP institutions (Gilbert & Woodfine 2004; Langenhorst 2009; Rademacher & Williams 2000). The cotton-top tamarin has exhibited flexible social behaviour in the wild, resulting in monogamy, polyandry, polygyny and polygynandry, but captive tamarins are normally held in monogamous pairs with their offspring (Carroll 2002). Captive Amur tigers are predominantly held in individual enclosures or in mixed-sex pairs (De Rouck *et al.* 2005), although some EEP institutions hold them in small family groups (Fitzpatrick 2009; Mitchell 2009). The African wild dog represents a different breeding management system in captivity. In the wild this species forms mixed-sex packs headed by an alpha pair who monopolise reproduction (de Villiers *et al*. 2003). This system is mimicked in captivity with males from one litter introduced to females from an unrelated litter, making a new breeding pack where one male and one female establish themselves as the alpha pair. Although, some institutions have bred with just one pair of African wild dog, this is discouraged as offspring have been noted to lack the social skills and experience essential for a cohesive pack (Verberkmoes 2009).

4.3.3 Main study: pedigree analysis

The historical listing from 1st January 1900 to 31st December 2004 was extracted from the SPARKS file for each studbook, and imported into PM2000 where *F* were calculated by simulating a kinship matrix. The studbooks for the cotton-top tamarin,

African wild dog and Amur tiger yielded 300, 187 and 1973 individuals respectively that had 100% known pedigree, four or more generations, and an *F* greater than 0.0001. These three conditions were necessary to ensure adequate completeness and depth of the pedigree to allow the construction of hypothetical pedigrees, and to allow changes in *F* once the parts of the pedigree were removed. It was impractical and unnecessary to include all individuals that fulfilled the criteria for inclusion in the study, so a sub-sample was randomly selected for each species to represent the wider populations. This was achieved by using a random number generator in MS Excel to randomly select individuals for inclusion in the sample for the cotton-top tamarin (fx = INT (RAND()*301), African wild dog (fx = INT (RAND()*188), and Amur tiger (fx = INT (RAND()*1974), so that the resulting sample sizes were *N* = 100 for each studbook. The Grevy's zebra studbook yielded only 50 individuals that fulfilled the selection criteria, so all were included in the study (*N* = 50).

The main study employed the methodology for creating hypothetical pedigrees that was piloted on scimitar-horned oryx. Once the random ancestors were removed, the incomplete pedigrees were imported into PM2000 and the kinship matrix re-simulated to obtain the *F* value for the new pedigree data. Each individual (number 15) yielded five data points, representing each of the levels of completeness.

4.3.4 Statistical analysis

The assumption of normality was not met for the data, but a General Linear Model (GLM) was used to test for differences between the mean inbreeding coefficients derived from the complete (100% known) and the four incomplete pedigrees (87.5%, 75%, 62.5% & 50%) using the model *F* = Completeness + Individual + Completeness*Individual to accommodate the repeated measures on individuals. This was followed by a Tukey HSD post-hoc test to allow estimation of the threshold for reliability of *F* derived from incomplete pedigrees. The results were confirmed by a Kruskal-Wallis test. The statistical analyses were repeated for each species.

4.3.5 Population management thresholds

Decisions in population management are often based on threshold levels, such as the population mean kinship and those identified from research into inbreeding depression. In

this chapter, the threshold levels used were those for non-inbred individuals ($F = 0.00$), the species-specific global mean kinship values, a moderate level of inbreeding ($F = 0.125$), a high level of inbreeding ($F = 0.25$), and a very high level of inbreeding ($F = 0.50$). The mean kinship values for the living global populations were calculated by importing data from each species SPARKS file to PM2000 and simulating kinship matrices. The inbreeding coefficients derived from the incomplete pedigrees were compared to those derived from the 100% known pedigrees to evaluate the impact of missing pedigree data on the threshold values for each species.

Table 4.1 The 20 largest studbooks listed on the ISIS/WAZA 2004/2005 studbook library CD-ROM (ISIS 2006), ranked by total number of individuals in each studbook

Species	Scientific name	Scope	SB N	N*	Earliest date	Permission
Cotton-top tamarin	*Saguinus oedipus*	ISB	9286	300	1893	Yes
Giraffe	*Giraffa camelopardalis*	AZA	7705	481	1824	No
Scimitar-horned oryx	*Oryx dammah*	ISB	7275[a]	167	1875	Yes
Cheetah	*Acinonyx jubatus*	ISB	6275	100	1879	No
Amur tiger	*Panthera tigris altaica*	ISB	4949	1973	1933	Yes
Black-footed ferret	*Mustela nigripes*	AZA	4749	3784	1985	No
Humboldt penguin	*Spheniscus humboldti*	EAZA	4719	0	1947	Yes
Wyoming toad	*Bufo baxteri*	AZA	4452	186	1988	No
Przewalski's horse	*Equus caballus przewalskii*	EAZA	4039	3246	1899	No
African wild dog	*Lycaon pictus*	ISB	3781	187	1902	Yes
Hawaiian goose	*Branta sandvicensis*	AZA	3689	0	1918	No
Jackass penguin	*Spheniscus demersus*	EAZA	3562	0	1961	No
Gold lion tamarin	*Leontopithecus rosalia*	ISB	3414	327	1957	No
Caribbean flamingo	*Phoenicopterus ruber*	AZA	3314	2	1898	No
Ring-tailed lemur	*Lemur catta*	AZA	3241	4	1883	No
Attwater's prairie chicken	*Tympanuchus cupido attwateri*	AZA	3212	1586	1992	No
Grevy's zebra	*Equus grevyi*	ISB	3178[a]	50	1898	Yes
Waldrapp ibis	*Geronticus eremita*	EAZA	3986	0	1948	No
Thomson's gazelle	*Gazella thomsonii*	AZA	2795	0	1966	No
Maned wolf	*Chrysocyon brachyurus*	ISB	2788	573	1933	No

AZA: Association of Zoos and Aquaria (North American region); **EAZA**: European Association of Zoos and Aquaria (European and Middle Eastern regions, and Kazakstan); **ISB**: International (all regions). **SB N**: number of individuals listed in the studbook. **N*** : the number of individuals that fulfilled the criteria for inclusion in the study (individuals with 100% known pedigrees, F>0.0001, and four or more generations) (ISIS 2006; IUCN 2008). [a]: data used from SPARKS file supplied directly by studbook keeper

4.4 Results

4.4.1 Pedigree analysis

Inbreeding coefficients differed between the five conditions for all five species (scimitar-horned oryx $N = 167$, $F_{4,664} = 66.82$, $P < 0.001$; Grevy's zebra $N = 50$, $F_{4,196} = 5.86$, $P < 0.001$; cotton-top tamarin $N = 100$, $F_{4,396} = 17.97$, $P < 0.001$; African wild dog $N = 100$, $F_{4,396} = 45.82$, $P < 0.001$; Amur tigers $N = 100$, $F_{4,396} = 45.55$, $P < 0.001$), and this was confirmed with Kruskal-Wallis tests (scimitar-horned oryx $H_4 = 123.07$, $P < 0.001$; Grevy's zebra $H_4 = 52.72$, $P < 0.001$; cotton-top tamarin $H_4 = 82.07$, $P < 0.001$; African wild dog $H_4 = 60.86$, $P < 0.001$; Amur tigers $H_4 = 124.67$, $P < 0.001$) . Post-hoc Tukey's HSD tests for the GLM with 95% confidence intervals indicated that for all five species the 50% condition had lower F than all the other conditions (62.5%, 75%, 87.5% and 100% known), which did not differ amongst themselves.

Although the assumption of homogenous variances was met for the data sets, the analysis of variance generated a large number of outliers (29% of scimitar-horned oryx, 40% of Grevy's zebra, 27% of cotton-top tamarin, 37% of African wild dog, 28% of Amur tiger, data). The assumption of normality was consequently violated for each species data set and this could not be addressed by transforming the data. The GLM was applied regardless as analysis of variance is more robust to the assumption of normality than to the assumption of homogenous variances (Quinn & Keough 2006), but the results were viewed with caution.

The inbreeding coefficients derived from 100% known pedigrees, hereafter referred to as the control condition, varied between species, with the scimitar-horned oryx having the highest mean F at 0.3278, and the greatest range in F between 0.0078 and 0.6221, and the cotton-top tamarins having the smallest mean F at 0.06, and the smallest range in F between 0.0039 and 0.2813 (Table 4.2, Figure 4.2). A scimitar horned oryx with $F = 0.62$ has a 62% probability that the two alleles at a locus are identical by descent; a cotton-top tamarin with $F = 0.004$ has a <1% probability of homozygous alleles at a genetic locus by descent.

The four experimental conditions (87.5%, 75%, 62.5% and 50% known pedigrees) resulted in an increased range of F for each of the species (Table 4.2), with the exception of the 50% known condition for cotton-top tamarins and Amur tigers. The range for the cotton-top tamarins reduced to $F = 0.00$ - 0.25 for the 50% known condition, and for Amur

tigers the range changed from F = 0.0039 - 0.5989 for the control to F = 0.00 - 0.50 for the 50% known condition.

The 87.5%, 75%, 62.5% and 50% known conditions for the scimitar-horned oryx revealed an increase in the maximum F generated between the experimental conditions and the control of 0.0823, 0.1318, 0.1089 and 0.1592, respectively. A similar increase was observed for the Grevy's zebra where the maximum value for F for the four experimental conditions increased from the control by 0.0208 for 87.5% known and 0.1875 for each of the 75%, 62.5% and 50% known conditions. The cotton-top tamarin data revealed an increase from the control of F = 0.2187 for the 87.5% and 75% known conditions and an increase of 0.0520 for the 62.5% known condition. The African wild dog data also revealed an increased range of F in the experimental conditions when compared to the control, with an increase of F = 0.0208, 0.0625, 0.1042 and 0.1875 for the 87.5%, 75%, 62.5% and 50% known conditions respectively. The Amur tiger data revealed a similar pattern with an increase of F = 0.0215, 0.0906 and 0.0560 for the 87.5%, 75% and 62.5% known conditions, and a decrease in range of 0.0989 for the 50% known condition. The lowest value in the range was reduced to F = 0.0 for all experimental conditions in all species, revealing a reduction of F = 0.0039 for Grevy's zebra, cotton-top tamarins, and Amur tiger, a reduction of F = 0.0078 for the scimitar-horned oryx and a reduction of F = 0.1250 for the African wild dog.

The inbreeding coefficients derived from the incomplete pedigrees increasingly varied as the pedigree became more incomplete for all five species (Figure 4.3). Coefficients derived from pedigrees with 87.5% known ancestry were similar to the coefficients derived from the control. As the level of completeness decreased through to 50% known, the degree of variation increased, and the F derived was no longer comparable to that obtained for the control condition.

As pedigree completeness declined from 100%, the F were generally over – or underestimated when compared to the control for all five species. In the instances where the F varied between the conditions and the control, the scimitar-horned oryx data revealed a mean over-estimation of F for the 87.5%, 75% and 62.5% known experimental conditions, whereas the African wild dog data reveal a trend in underestimating F for the experimental conditions. The remaining three species, the Grevy's zebra, cotton-top tamarins and Amur tigers, indicate variable under– or overestimates, with no discernible pattern, for the different experimental conditions. However, the 50% known condition for all species revealed a notable underestimate of F when compared to the control.

The data for all species, with the exception of the African wild dog, resulted in a greater percentage of individuals with overestimated, rather than underestimated, *F* for the 87.5% known condition (Table 4.3, Figure 4.4). The 75% and 62.5% known experimental conditions showed variable over- and underestimates of *F* across the species and conditions without any discernible pattern. The data for all five species clearly indicated that the 50% known condition was underestimated more than it was overestimated, and moreover, the mean underestimate was considerably larger than the mean overestimate for all species with the exception of the Grevy's zebra

A trend can be observed of increasing average change in *F* (ΔF) as the inbreeding coefficient obtained for the control condition increased through from 0.0039 to 0.6221 for all species and all four experimental conditions (Figure 4.3). This is most apparent in the scimitar-horned oryx and the Amur tiger data (Figures 4.3.1 and 4.3.5) where a greater spread in control inbreeding coefficients were obtained. Despite the lower levels of inbreeding in the remaining species, the same trend was observed for the Grevy's zebra, cotton-top tamarin and African wild dog (Figures 4.3.2, 4.3.3, and 4.3.4). All five species revealed a trend to increasingly overestimate *F* for the 87.5% known condition as the control *F* value increased. The scimitar-horned oryx and cotton-top tamarins showed a trend to increasingly overestimate the 75% known condition *F* as the control *F* value increased. In contrast the Grevy's zebra, African wild dogs and Amur tigers showed a trend of increasingly under-estimating *F* as the control *F* increased for this condition. The 62.5% known condition produced a similar result with three of the species revealing an increasing over-estimation of *F* as the control *F* increased (scimitar-horned oryx, Grevy's zebra and Amur tigers), and two species increasingly underestimated *F* as the control *F* increased (cotton-top tamarins and African wild dogs). The 50% known condition produced consistent results for all five species, clearly showing an increasing under-estimation of *F* as the control *F* increased.

4.4.2 Population management thresholds

The mean kinship values for the global populations of scimitar-horned oryx, Grevy's zebra, cotton-top tamarins, African wild dogs and Amur tigers were 0.0401, 0.0125, 0.0076, 0.0364 and 0.0284, respectively. Table 4.4 shows the percentage of each experimental condition that over- or underestimated *F* when compared to the control so that the stated thresholds were crossed. The exception to this is the underestimate for the 0.00 threshold. In this instance 'underestimate' refers to *F* derived from the experimental

conditions where a value of 0.0 (non-inbred) is returned incorrectly i.e. in the control condition *F* was greater than 0.0 (inbred). It was not possible to overestimate the *F* when *F* for the control is 0.00 as all individuals with and *F* = 0.0 at 100% known were excluded from the study.

It was not possible to underestimate the *F* at the 0.5000 level for the Grevy's zebra, cotton-top tamarin and African wild dog data as the control data had an upper range of *F* = 0.2813, 0.2813 and 0.4375, respectively. Similarly it was not possible overestimate *F* for the African wild dog at the global mean kinship level of 0.0364 as the lower range only extended down as far as *F* = 0.1250.

The percentage of samples for the scimitar-horned oryx that underestimated *F* compared to the control, and by doing so crossed the thresholds, ranged from 0 to 7% for the 87.5%, 75% and 62.5% conditions. Similarly data for the cotton-top tamarins, African wild dogs and Amur tigers show that up to 2%, 10% 12%, respectively, of samples underestimated *F* for the 87.5%, 75% and 62.5% known conditions so that a threshold was crossed. The exceptions to this are the underestimates that returned a value of *F* = 0.00 incorrectly (when *F* > 0.0001 in the control). This varied between 1% for the 87.5% condition for Amur tigers to 35% for the 62.5% condition for cotton-top tamarins. The Grevy's zebra data indicated that *F* was underestimated to a larger extent than for the other species with up to 34% of the data returning an underestimate of *F* that crossed a threshold, and up to 42% of the data returned an *F* of 0.00 incorrectly when compared to the control. The 50% known condition returned the highest percentage of data that crossed thresholds for all of the species, with between two and 86% of the data under-estimating *F* to the extent that it crossed a threshold.

In comparison 1 – 28% of samples across all five species and conditions overestimated *F* sufficiently to cross a threshold. The scimitar-horned oryx data crossed the 0.50 threshold 12 - 28% of the time, but all other species crossed this threshold a maximum of 13% of the time. When inbreeding coefficients were high for the complete pedigrees, the degree of variance for the experimental condition pedigrees increased, resulting in a sufficient change in *F* that it crossed the higher thresholds. Moreover, most of the inbreeding co-efficient values that crossed the thresholds had a similar value, and a very small change was sufficient to push them over the threshold value.

Table 4.2 Descriptive statistics for each of the five species for the five different experimental conditions

	Pedigree completeness category				
	100%	**87.5%**	**75%**	**62.5%**	**50%**
Scimitar-horned oryx					
Range (minimum *F*)	0.0078	0.0000	0.0000	0.0000	0.0000
Range (maximum *F*)	0.6221	0.7044	0.7539	0.7310	0.7813
Grevy's zebra					
Range (minimum *F*)	0.0039	0.0000	0.0000	0.0000	0.0000
Range (maximum *F*)	0.3125	0.3333	0.5000	0.5000	0.5000
Cotton-top tamarins					
Range (minimum *F*)	0.0039	0.0000	0.0000	0.0000	0.0000
Range (maximum *F*)	0.2813	0.3750	0.5000	0.3333	0.2500
African wild dog					
Range (minimum *F*)	0.1250	0.0833	0.0000	0.0000	0.0000
Range (maximum *F*)	0.4375	0.4583	0.5000	0.5417	0.6250
Amur tiger					
Range (minimum *F*)	0.0039	0.0000	0.0000	0.0000	0.0000
Range (maximum *F*)	0.5989	0.6204	0.6895	0.6549	0.5000

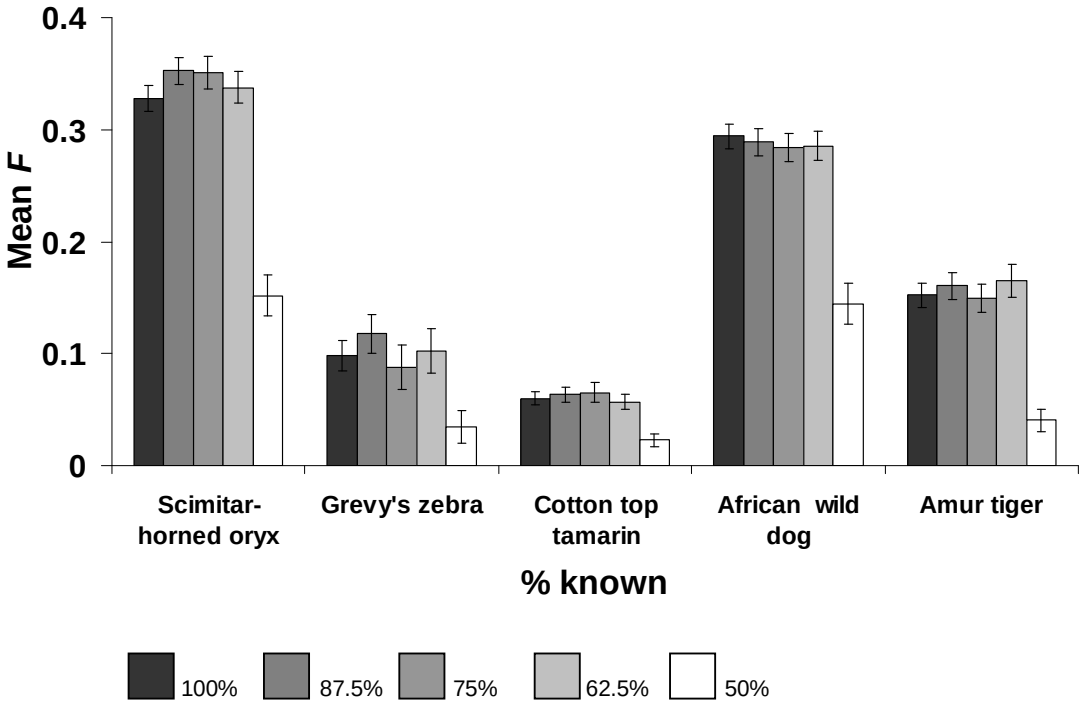


Figure 4.2 The mean inbreeding coefficients derived for pedigrees that are 100%, 87.5%, 75%, 62.5% and 50% complete for each species. The error bars indicate the standard error of the mean for each condition

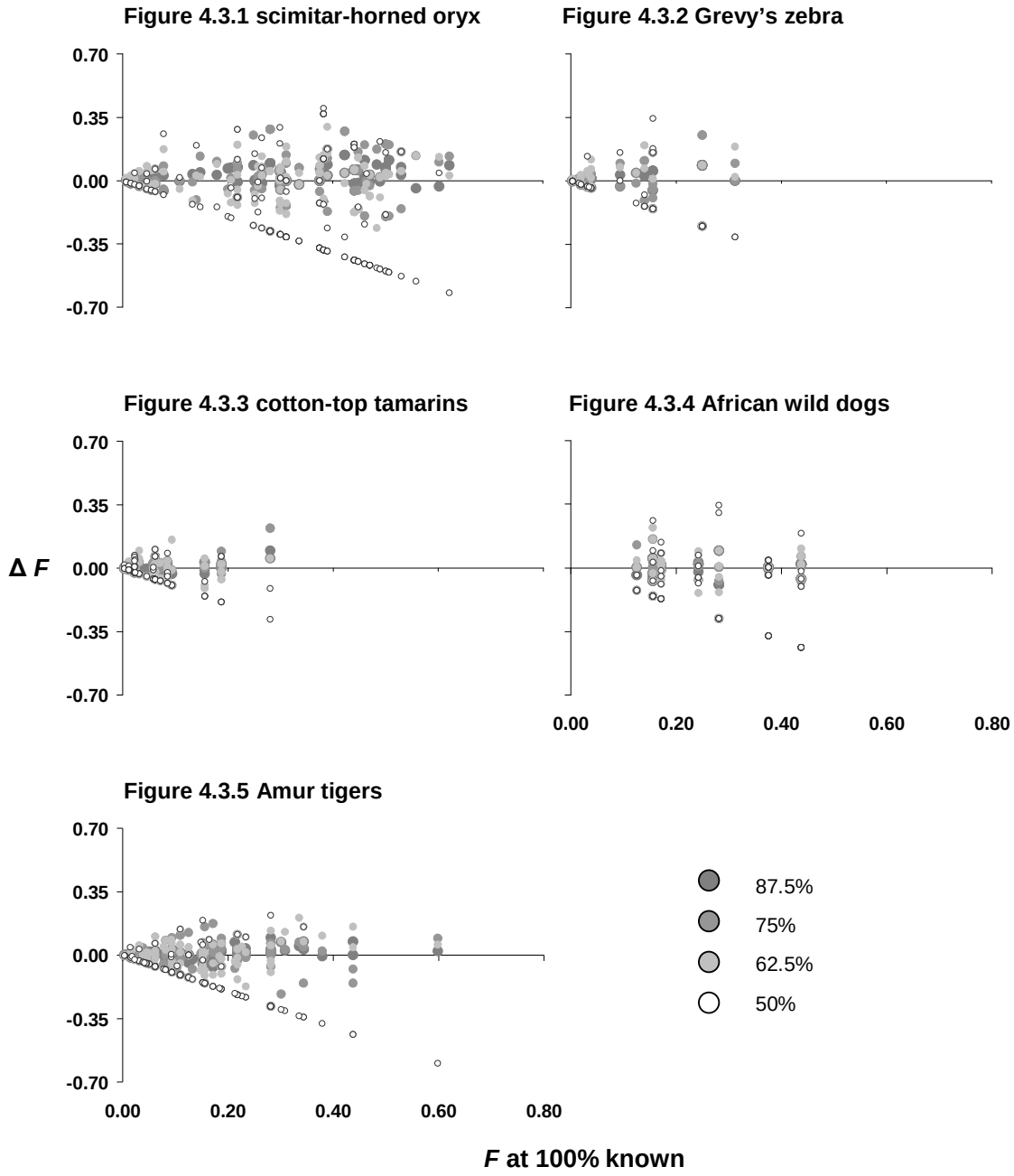


Figure 4.3 Change in *F* from the 100% known category for each individual for the five species: scimitar-horned oryx, Grevy's zebra, cotton-top tamarins, African wild dogs and Amur tigers. The 87.5%, 75% and 50% known conditions indicate an increasing trend of over-estimating *F* compared to the 100% known condition, whereas the 62.5% known condition reveals a slight trend of under-estimating *F* as the *F* value increases

Table 4.3 Mean and total over- and underestimate of *F* for the four experimental conditions in comparison with control

	Pedigree completeness category							
	87.5%		75%		62.5%		50%	
	N	***F***	***N***	***F***	***N***	***F***	***N***	***F***
Scimitar-horned oryx								
Mean overestimate	98	0.0509	91	0.1008	103	0.0811	40	0.1818
Mean underestimate	45	0.0188	60	0.0880	62	0.1077	124	0.2956
Same value as control	24	-	16	-	2	-	3	-
Grevy's zebra								
Mean overestimate	39	0.0313	20	0.0765	23	0.0665	5	0.1937
Mean underestimate	9	0.0263	29	0.0702	27	0.0681	44	0.0942
Same value as control	2	-	1	-	0	-	1	-
Cotton-top tamarin								
Mean overestimate	59	0.0140	40	0.0383	46	0.0329	18	0.0429
Mean underestimate	35	0.0147	47	0.0218	54	0.0339	82	0.0548
Same value as control	6	-	13	-	0	-	0	-
African wild dog								
Mean overestimate	30	0.0300	23	0.0407	32	0.0669	18	0.1047
Mean underestimate	34	0.0415	34	0.0579	34	0.0880	75	0.2246
Same value as control	36	-	43	-	34	-	7	-
Amur tiger								
Mean overestimate	59	0.0245	43	0.0501	57	0.0600	13	0.0980
Mean underestimate	34	0.0189	43	0.0570	40	0.0535	84	0.1485
Same value as control	7	-	14	-	3	-	3	-

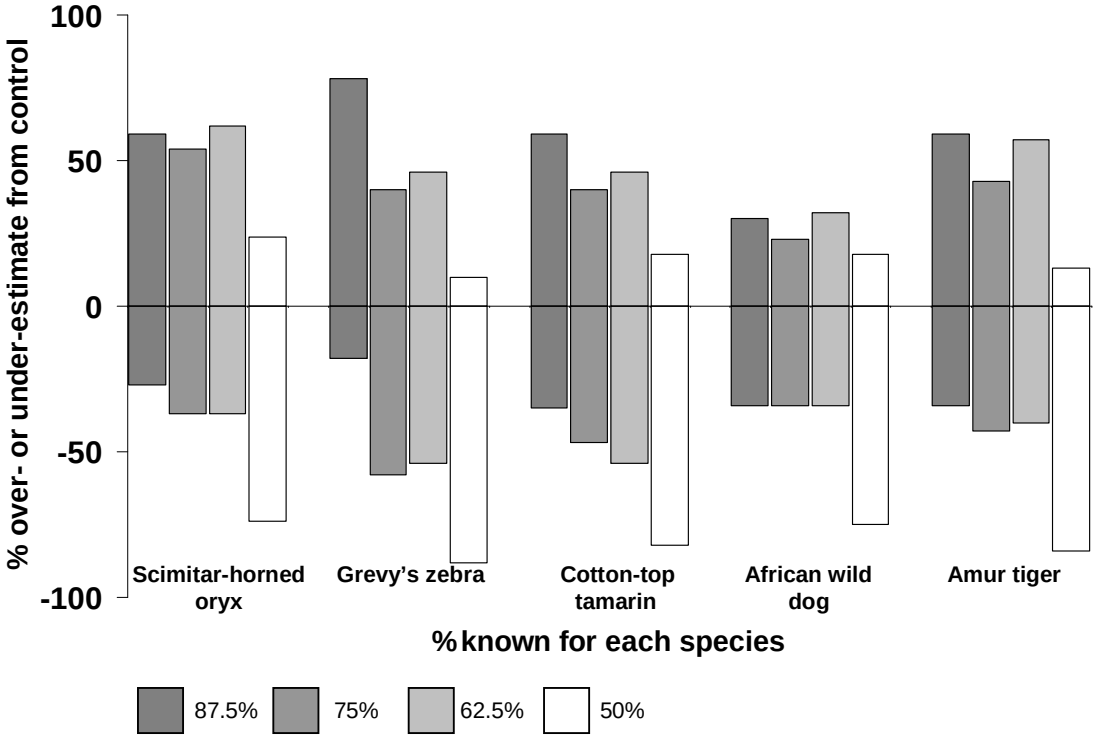


Figure 4.4 Percentage of over- and under-estimation of *F* for each condition compared to the *F* derived from the control. All four conditions, 87.5%, 75%, 62.5% and 50% known are more likely to overestimate *F* than underestimate *F*

Table 4.4 Percentage of the data for each category that over- and underestimate *F* for six threshold levels in comparison with the *F* obtained for the control

		Pedigree completeness category			
		87.5%	**75%**	**62.5%**	**50%**
Scimitar-horned oryx					
0.0000 level	Underestimate	2	5	5	62
0.0401 level	Overestimate	1	2	2	1
	Underestimate	1	4	4	56
0.1250 level	Overestimate	0	1	2	2
	Underestimate	0	1	3	53
0.2500 level	Overestimate	4	5	4	3
	Underestimate	1	6	7	53
0.5000 level	Overestimate	12	28	23	14
	Underestimate	0	2	1	6
Grevy's zebra					
0.0000 level	Underestimate	10	34	42	86
0.0125 level	Overestimate	0	0	0	0
	Underestimate	6	30	34	68
0.1250 level	Overestimate	2	2	6	4
	Underestimate	4	24	16	36
0.2500 level	Overestimate	0	6	4	6
	Underestimate	0	8	2	18
0.5000 level	Overestimate	0	6	2	0
	Underestimate	N/A	N/A	N/A	N/A
Cotton-top tamarins					
0.0000 level	Underestimate	6	26	34	76
0.0076 level	Overestimate	0	1	1	3
	Underestimate	0	0	0	2
0.1250 level	Overestimate	0	8	6	4
	Underestimate	0	0	2	9
0.2500 level	Overestimate	0	0	1	3
	Underestimate	0	0	0	2
0.5000 level	Overestimate	0	2	0	0
	Underestimate	N/A	N/A	N/A	N/A
African wild dogs					
0.0000 level	Underestimate	0	5	5	56
0.0364 level	Overestimate	N/A	N/A	N/A	N/A
	Underestimate	0	5	5	56
0.1250 level	Overestimate	0	0	0	0
	Underestimate	6	5	10	58
0.2500 level	Overestimate	4	5	6	7
	Underestimate	5	2	4	31
0.5000 level	Overestimate	0	2	4	2
	Underestimate	N/A	N/A	N/A	N/A
Amur tigers					
0.0000 level	Underestimate	1	9	10	79
0.0284 level	Overestimate	0	3	1	1
	Underestimate	1	12	8	73
0.1250 level	Overestimate	4	6	13	2
	Underestimate	6	6	10	43
0.2500 level	Overestimate	6	6	9	5
	Underestimate	0	4	2	14
0.5000 level	Overestimate	1	0	4	2
	Underestimate	0	0	0	1

4.5 Discussion

Inbreeding depression is widely recognised as a problem in captive populations (Brown & Brown 1998; Ralls *et al.* 1979, 1980, 1986, 1988; Ralls & Ballou 1982), and the evaluation of inbreeding in a population, whether for research purposes or population management, often relies on good quality pedigree data. Missing pedigree data reduces the reliability of inbreeding coefficients (Lutaaya *et al.* 1999). A number of studies have examined the impact of pedigree completeness and depth on estimated genetic parameters, revealing that complete pedigrees are preferable when seeking reliable estimates of genetic diversity (Boichard *et al.* 1997; Cassell *et al.* 2003; Hagger 2005). Cassell *et al*. (2003) demonstrated that almost no inbreeding was detected when pedigree completeness was less than 30%, but did not establish what level of completeness was needed for the estimation of reliable inbreeding coefficients. Similarly Boichard *et al*. (1997) evaluated the impact of incomplete pedigrees on the effective population size (N_e) and F of a model population and found that incomplete pedigrees significantly increased the estimated N_e and F, with an increase in N_e as the pedigree became increasingly incomplete. They concluded that the level of inbreeding was only reliably estimated when the pedigree information was complete. Whilst these previous studies have evaluated the impact of incomplete pedigrees on the estimation of inbreeding coefficients, they did not evaluate the level of completeness on the reliability of the F calculated from incomplete pedigrees.

When pedigree completeness fell below 62.5%, the inbreeding coefficient was no longer estimatable. At 50% complete there was a strong tendency to over- or underestimate F. A further analysis was carried out to examine if this trend continued when pedigree completeness fell below 50% known (to 37% known) for the scimitar-horned oryx. The trend of over- and under-estimating the F increased with decreasing pedigree completeness. When pedigree completeness declined below 62.5%, the inbreeding coefficient was no longer estimatable, and at 37% complete, the inbreeding coefficient had no resemblance to the F obtained from complete pedigrees.

All five species yielded the same result with regards to the impact of pedigree completeness on inbreeding coefficients i.e. F are reliable if estimated from pedigrees that are more than 62.5% complete. The results are therefore not taxa, social system, or management specific. Rather they apply to incomplete pedigrees regardless of species or social system in captivity. Consequently, the results can be extrapolated to other taxa not examined here.

Historically, good quality records for individual animals were rarely kept, resulting in studbooks containing many animals of unknown ancestry (Ralls *et al.* 1980; Willis 1993) and missing important life-history data. As a result, lack of pedigree data have either limited sample sizes for some studies on inbreeding depression in captive populations (Kalinowski *et al*. 1999; Laikre & Ryman 1991), or led authors to evaluate inbreeding depression using incomplete pedigrees without taking the incompleteness of the pedigree into account (Hoogland 1992; Keller 1998). This has led to a call for more standardised methods of estimating rates of inbreeding from incomplete pedigrees (Marshall *et al.* 2002), and recommendations that more detailed pedigrees need to be used to increase the robustness of research into inbreeding depression (Overall *et al.* 2005). The results in this chapter support the argument that more complete pedigrees should be used for research into inbreeding (at least 62.5% complete).

The lack of complete historical data for captive populations is a widespread problem for population management and research. The 2004/2005 ISIS/WAZA Studbook Library (ISIS 2006) contains European and international studbooks for 133 EEP bird, mammal and reptile species. Only ten of these species programmes have complete pedigrees for all individuals listed in the studbooks, and sixty of the studbooks have individuals listed with incomplete pedigrees between 62.5% and 99.99% known (ISIS 2006). For example, the international studbook for the Hartmann's mountain zebra (N = 346) records that 76.5% of its pedigree is known but only 43% (N = 148) individuals have a complete pedigree recorded. However 33% (N = 115) of the population have 62.5% or more of the pedigree recorded, increasing the number of animals which can be included in inbreeding analyses. The scimitar-horned oryx EEP lists only 4% (N = 20) of the population as having complete pedigrees, but the number of individuals included in analyses can be increased to 34% (N = 161) when individuals with 62.5% or more complete pedigrees are included. It is especially important for scimitar-horned oryx to obtain an accurate indication of inbreeding as research has shown that inbreeding coefficients above 0.125 have a detrimental impact on fitness factors such as juvenile survival (Mace 1989). Consequently, it is desirable to manage the population to maintain inbreeding coefficients below this level. It is difficult to achieve this with incomplete pedigree data, but the inclusion of individuals that have up to 62.5% pedigree completeness will allow for a more accurate and intensive management approach, as well as gaining additional information for studies on inbreeding depression (Chapter Eight). Whilst 62.5% pedigree completeness was the

threshold for reliability, the trend of over- or under-estimating inbreeding coefficients, means that complete pedigrees should be used for research whenever possible.

The results presented here also have implications for the management of captive populations. Population management decisions, specifically the pairing of animals for reproduction, are based on a number of different factors, and the projected *F* of potential offspring is only one of the considerations. Using the recommended threshold level of population mean kinship (Wilcken & Lees 1998), it was possible to retrospectively evaluate the impact of over- or under-estimation of *F* on the management of the current EEP population of scimitar-horned oryx. One percent of pedigrees in the 87.5% condition, and 2% in each of the 75% and 62.5% conditions overestimated the inbreeding coefficient to such a degree that they crossed the mean kinship threshold. The consequence for the EEP population is that one to two percent of individuals with 99.99-62.5% known pedigrees would have projected inbreeding coefficients overestimated to such a degree that the parents of the individual would not have been paired. If these individuals had complete pedigree data, the inbreeding coefficients would be below the threshold and breeding would go ahead. Conversely the parents of one to four percent of individuals in the EEP population with more 62.5% known pedigrees would be allowed to breed due to underestimated projected inbreeding coefficients of the offspring.

Whilst some degree of inbreeding is inevitable in small closed populations (Frankham *et al.* 2010), the ideal scenario is that all individuals in a population have inbreeding coefficients of 0.0. Population managers aim to keep the level of inbreeding at $F = 0.0$ if at all possible. All of the experimental pedigree conditions for all the species returned an $F = 0.0$ incorrectly for a varying percentage of the samples when compared to the *F* derived from the complete pedigree data. When applying this to the scimitar-horned oryx EEP, it meant that two to five percent of the individuals in the population with pedigree completeness between 99.99% and 62.5% were considered non-inbred, whereas if the pedigrees had been complete they would probably have had an inbreeding coefficient above 0.0. Only a small percentage of individuals crossed the lower thresholds, but there would still be a predicted impact on the genetic management of the population. In a reasonably large population, the prevention of two individuals from breeding due to inbreeding constraints may not have a large impact on the retention of overall genetic diversity, but this impact will increase as the size of the population decreases. In very small populations, the pairing of certain individuals may be essential if mean kinship is to be minimised and allelic diversity retained, even if this results in an offspring with an

undesirably high inbreeding coefficient. In principle, inbred offspring with low mean kinships can then be paired with unrelated individuals (with low mean kinships), and the resulting offspring will have an acceptable inbreeding coefficient (0.0 or below the population mean kinship) (Wilcken & Lees 1998).

Some studies have attempted to resolve the issue of pedigree ambiguity and improve population management by utilising molecular genetic techniques to supplement their knowledge and fill the gaps in pedigrees (Jones *et al.* 2002; Russello & Amato 2004). Whilst this approach may provide valuable information, the associated costs and levels of expertise are not achievable for the majority of captive breeding programme managers (Ballou *et al.* 2010a). The results of this study detail how much data can be missing from a pedigree before the *F* is no longer estimatable, and provides practical and usable guidance on the use of incomplete pedigrees to researchers and population managers.

This research used five levels of pedigree completeness to examine the impact of missing pedigree data on estimation of inbreeding coefficients. The approach taken was to remove ancestors from the pedigree until pedigree completeness fitted into one of the five pedigree completeness categories. Examination of any studbook will show that inbreeding coefficient data are continuous and not categorical. The experimental design does not account for pedigree completeness between categories, and consequently it is not known at what point the *F* stops being estimatable between 62.5% and 50%.

This chapter examined the impact of inbreeding coefficients on research into inbreeding depression and population management. These results have a direct application to Chapters Five, Seven, and Eight of this thesis, and further research into inbreeding depression in pedigreed populations. The application for population management would benefit from being combined with research into the impact of incomplete pedigrees on the estimation of other genetic parameters, specifically mean kinship coefficients. This would provide a comprehensive threshold for pedigree completeness for population management.

Ideally all studbooks for captive populations should contain a full historical data set for every individual in the population. In many instances, the reality falls short of this expectation and alternative solutions need to be found to guide captive management programmes and evaluate the impacts of inbreeding. The results presented here will help inform research into inbreeding depression in captive populations by increasing sample size. It will also assist population managers in making more informed decisions for captive species that have incomplete studbooks.

In this chapter I established that there is a pedigree completeness threshold (62.5% complete) for estimating inbreeding coefficients. Studbooks with less pedigree completeness need alternative strategies for managing populations. AZA, EAZA and ZAA recommend that analytical studbooks, which fill in the gaps in the pedigree with ‘best guesses’, are created to address this issue (AZA 2004). I now examine the recommended approach of creating analytical studbooks, and test the validity of their assumptions (Chapter Five).

I also apply the results of this chapter in examining the sustainability of captive population for multiple species in Chapter Seven, and in evaluating inbreeding depression for the scimitar-horned oryx EEP population in Chapter Eight.

5.0 Chapter five: small population management and the analysis of studbook data

5.1 Abstract

Small closed populations, such as those found in captivity, risk losing genetic diversity through genetic drift and inbreeding. Contemporary population management aims to reduce this by issuing breeding recommendations based on mean kinship coefficients derived from studbook data. The effectiveness of population management is compromised because many studbooks have missing pedigree data. To address this, regional zoo associations recommend creating an analytical studbook that fills in the gaps in the pedigree with assumptions and 'best guesses'. However, the effectiveness of this approach has not been tested. For the first time, this chapter used molecular markers to evaluate the comparative accuracy of true and analytical studbooks, using the scimitar-horned oryx international studbook as a case study. This studbook is particularly appropriate because it has a large data set that is missing a substantial amount of its pedigree (69%), and is representative of many other studbooks.

There was a positive relationship between the true and analytical studbooks, regardless of pedigree completeness (r^2 = 74.7%, $P < 0.001$). There was no relationship between either the true or analytical studbook and the molecular data until original pedigree completeness reached 87.5% (r^2 = 51.84%, P = 0.029). However, sample size was too small at this level of pedigree completeness to be conclusive (N = 9). The impact of using molecular, true and analytical studbook data for population management decisions was evaluated in both a true studbook and analytical studbook framework. Overall, the analytical studbook performed better, indicating that it is appropriate to use analytical studbooks for population management. The results highlight the importance of complete pedigree data for populations under intensive management. When pedigree data are largely missing, molecular analyses may provide an alternative approach to preserving genetic diversity in captive populations. Limitations of the study are discussed along with recommendations for future research.

5.2 Introduction

The long-term survival of a population depends on the retention of genetic diversity, both in terms of heterozygosity and allelic diversity (Earnhardt 1999; Frankham 1995a, 2005a; Lacy *et al.* 1995). Genetic variation enables populations to retain evolutionary potential for adaptation to both short- and long-term changing environmental conditions (Ballou *et al.* 2010a; Frankham *et al.* 2002; Lacy *et al.* 1995; Lande 1995). Populations with reduced genetic diversity generally have reduced fitness (Avise *et al.* 1995; Ballou *et al.* 2010a; Frankham *et al.* 2002; Lacy *et al.* 1995).

Endangered species with small or declining populations often have lower levels of genetic diversity than related, non-endangered species with large population sizes (Frankham 2003; Frankham *et al.* 2002). Frankham *et al.* (1992a) reviewed the genetic diversity of endangered bird, mammal, fish, insect and plant species and found that 84% had lower levels of genetic diversity than related non-endangered species.

Small populations have an increased risk of extinction because of stochasticity, and the smaller the population, the greater the risks posed by stochasticity (Foose *et al.* 1995; Frankham *et al.* 2010). Small populations lose heritable genetic diversity at a rate that is inversely proportional to the effective population size (N_e), and so tend to lose genetic diversity at a faster rate than large populations (Ballou 1992; Ballou & Cooper 1992b; Foose *et al.* 1995; Höglund 2009). Consequently, small populations are often characterised by reduced allelic diversity, reduced heterozygosity, and increased inbreeding (Frankham *et al.* 2002).

Captive populations are usually small and closed (Ballou & Cooper 1992b; Ballou & Lacy 1995; Mace 1989). Small closed populations will inevitably lose genetic diversity because each generation is a genetic sample of the previous one, and some of the variation present in the founders will be lost in each generation through genetic drift (Frankham 2005a; Mace 1989; Lacy *et al.* 1995). Reduced genetic diversity increases the risk of population extinction and reduces the likelihood of the species being successfully reintroduced to the wild (Arnold 1995; Ballou & Lacy 1995; Ford 2002; Frankham 1995a, 2008; Frankham *et al.* 2002; Princée 1995; Robert 2009). This chapter aims to evaluate the retention of genetic diversity in a small closed captive population which provides animals for reintroduction projects.

Contemporary population management aims to address these threats by implementing strategies for the long-term retention of genetic variation in terms of gene

diversity (expected heterozygosity) and allelic diversity under selective neutrality (Arnold 1995; Ballou *et al.* 1995; Ballou & Lacy 1995; Mace 1989; Ralls & Ballou 1992; Willis & Willis 2010).

Genetic variation can be measured through molecular techniques or pedigree analysis (Ballou *et al.* 2010a; Frankham *et al.* 2002), and coordinated captive breeding programmes use pedigree data as documented in the studbook as the basis for captive population management (Ballou & Lacy 1995; Vasarhelyi 2002).

Pedigree analyses estimate the founder contribution, the allele probability distributions, and the degree of inbreeding for each individual in the living population (Ballou & Lacy 1995) using computer simulations of the stochastic process of Mendelian transmission of alleles through the pedigree (Ballou & Lacy 1995; Lacy *et al.* 1995). Such analyses make several assumptions, most notably that the population founders are all unrelated to each other (Ballou & Cooper 1992b; Willis & Willis 2010) and that the population is in Hardy-Weinberg equilibrium (Lacy *et al.* 1995; Ralls & Ballou 1992).

The amount and complexity of pedigree data in large populations can be formidable, and different strategies have been developed to identify and rank individuals according to their genetic importance within the population (Ballou & Lacy 1995). The mean kinship strategy has been shown to be the most effective at retaining gene and allelic diversity in populations with complex pedigrees and unequal founder representation (Ballou & Lacy 1995; Montgomery *et al.* 1997). Consequently, contemporary population management within the AZA (SSP), EAZA (EEP) and ZAA (ASMP) regions use the *MK* method to assign breeding priority to individuals within a population with a known pedigree (AZA 2004; Lees & Wilcken 1998).

This method of population management is reliant on complete and accurate pedigree data. When pedigree data are missing or inaccurate, pedigree analyses and effective population management are compromised, as the exact nature of the relationship between individuals in the population cannot be determined (Ballou & Cooper 1992b; Lacy *et al.* 1995; Princée 1995; Ralls & Ballou 1992; Russello & Amato 2004). In such cases the kinship and inbreeding coefficients calculated may be more or less than the values derived if the pedigree were complete (Ballou & Lacy 1995). Many captive populations are missing full ancestry data, either because records were not kept during the early stages of captivity, or because it has not been possible to assign parentage due to multiple breeders in group living-species where individuals are not easily identifiable (Ballou & Lacy 1995; Ballou *et al.* 2010a; Frankham *et al.* 2002; Lacy *et al.* 1995; Princée 1995). Additionally,

pedigree data may contain cryptic errors which may never be detected, for example the misidentification of parents (Ballou & Cooper 1992b; Princée 1995).

Population management needs to continue in spite of missing or poor-quality pedigree data (Ballou *et al.* 1995), and population managers have four options in addressing this issue: 1) exclude individuals with unknown, or partially unknown, ancestry from the population (Ballou & Lacy 1995; Lacy *et al.* 1995; Willis 1993); 2) treat the individuals with unknown ancestry as founders (Willis 1993); 3) include individuals with unknown ancestry in the population, but use only the known portion of the pedigree in the analyses (Ballou & Lacy 1995); and 4) include all individuals and fill the gaps in the pedigree with parentage assumptions and hypothetical lineages (AZA 2004; Princée 1995). Each approach has its flaws. Excluding individuals with unknown ancestry from the population will result in the loss of genetic diversity if these individuals are unrelated to others in the population, or are from under-represented founders (Ballou & Lacy 1995; Willis 1993). Willis (1993) established that the loss of genetic diversity from excluding founders was greater than the loss of genetic diversity from including related animals, as if they were founders. However, treating individuals with unknown ancestry as founders may result in increased levels of inbreeding causing inbreeding depression (Ballou & Lacy 1995; Willis 1993). Excluding individuals with incomplete ancestry, or treating them as founders, may be appropriate if there are only a few individuals lacking pedigree data (Ballou *et al.* 2010b). Many captive populations are missing large portions of their pedigree data (ISIS 2009), and so these approaches would have a large impact on the quality of the genetic analyses.

The alternative strategy of including individuals with incomplete ancestries, by considering only the known portion of the pedigree in the analyses, may result in over- or under-estimates of genetic variation (Ballou & Lacy 1995). This is only practical if large percentages of the ancestry are known, for example more than 80% (Ballou *et al.* 2010). The results from Chapter Four indicated that when more than 37.5% of pedigree data were missing, the genetic values generated were not be representative of those that would be derived if the pedigree data were complete.

AZA and EAZA advocate the fourth option of filling gaps in pedigree data with assumptions and hypothetical lineages (AZA 2004). Most of the assumptions made are for missing parentage data where the most likely sire or dam is selected from potential parents. If no one individual is more likely to be a parent than any other individual, hypothetical parents are created that are an amalgamation of all potential parents (AZA 2004; Ballou *et*

al. 2010a). This approach resolves the problem of unknown ancestry confounding the genetic analyses, but the assumptions made may be erroneous (Lacy *et al.* 1995; Princée). These assumptions are used to fill gaps in pedigree data in true studbooks, thereby creating analytical studbooks. The creation of analytical studbooks to estimate genetic variation is widely practised by population managers. However, there is little evidence to support the assumption that this is a more appropriate technique for managing captive populations with incomplete pedigree data, than analysing only the known portion of the pedigree in true studbooks.

Molecular genetic analysis can provide an answer to this problem, in as much as it provides an independent measure of genetic variation that is not reliant on the accurate recording of pedigree data. Estimated genetic variation derived from true and analytical studbooks can be compared against an empirical estimate of absolute levels of genetic variation obtained from molecular analyses (Ballou *et al.* 2010a).

A number of molecular techniques are available for evaluating levels of genetic diversity in and between populations (Armstrong *et al* 2010; Avise *et al.* 1995; Ballou *et al.* 2010a; Hailer & Leonard 2008; Hardy 2003; Ritland 2000, 2005; Ritland & Travis, 2004; Santure *et al.* 2010; Slate *et al.* 2009; van Hooft *et al.* 2003; van Kleunen & Ritland 2005). The DNA used in molecular studies can be extracted from various sample types such as blood, organ and skeletal muscle tissue, and faeces (Beja-Pereira *et al.* 2009; Ruiz-Gonzalez *et al.* 2008). Tissue and blood samples are a good source of quality DNA (Beja-Pereira *et al.* 2009; Milligan 1998), but collection of these samples often involves restraining, sedating or anaesthetising animals which presents a risk to the animal (Fowler 1995; Soto-Calderon *et al.* 2009). Conversely the collection of faecal samples is non-invasive, but DNA extracted from faecal samples may be degraded due to environmental, dietary or technical factors, and the DNA yield may be low (Beja-Pereira *et al.* 2009; Brinkman *et al.* 2010; Idaghdour *et al.* 2003; Luikart *et al.* 2008, Soto-Calderon *et al.* 2009; Zhang *et al*. 1991).

Although a large number of molecular techniques are available, some are more appropriate than others in evaluating genetic diversity in populations. Campos *et al*. (2006), Hughes (1991), and Zhang *et al*. (1991) have all advocated the use of the MHC for evaluating genetic diversity within populations because it has extraordinary levels of genetic variation (Zhang *et al.* 1991). The genes at the MHC are thought to be important for parasite and pathogen resistance (Zhang *et al.* 2006) and are maintained by selection (Allendorf & Luikart 2007; Frankham *et al*. 2010). Conversely, pedigree analysis begins

with the assumption of selective neutrality of genetic variation (Lacy *et al.* 1995). The measures of genetic variation and relatedness derived from the MHC are therefore not directly comparable to those derived from pedigree analysis, as selection pressure acts as a confounding factor. Almost all current population genetic studies using molecular markers choose co-dominant multi-allelic microsatellites or bi-allelic SNPs due to their high levels of polymorphism (Weir *et al*. 2006), although the use of SNPs is still not common for studying the genetic diversity of non-model organisms (Höglund 2009). These techniques provide more information for more precise estimates of relatedness than techniques such as RAPDs and AFLPs, which use dominant markers (Wang 2004).

Microsatellites are selectively neutral markers (Höglund 2009) that are often used for population studies on wild, captive and reintroduced populations as they can be extremely variable showing high levels of polymorphism within individuals and populations (Allendorf & Luikart 2007a; Frankham *et al*. 2010). A search on ISI Web of Knowledge using filter criteria 'microsatellites* captive breeding' returned 70 hits for 65 species, across invertebrates (2), lizards (2), amphibians (1), chelonians (4), fish (13), birds (11) and mammals (32) including three species of Sahelo-Saharan gazelles (Ruiz-Lopez *et al.* 2009) (Appendix D).

Microsatellites have been specifically used to evaluate the accuracy and validity of studbooks and pedigrees by comparing estimates of relatedness and inbreeding derived from pedigree data with estimates of relatedness and heterozygosity obtained from microsatellite analyses (Armstrong *et al.* 2010; Bink *et al.* 2008; Coltman 2005; Ivy *et al.* 2009; Leroy *et al.* 2009; Nielsen et al., 2007; Ruiz-Lopez *et al.* 2009; Toro *et al.* 2002; Wisely *et al.* 2003). Many of these studies have used 'method of moment' estimators to provide estimates of relatedness coefficients for individuals (Csilléry *et al.* 2006b; Pino-Querido *et al.* 2010; Russello & Amato 2004; Toro *et al.* 2002; van Hooft *et al.* 2003). These multi-loci estimators express relatedness on a continuous scale (Oliehock *et al.* 2006) in a similar way to the estimates of relatedness (mean kinship) derived from pedigree data. The moments estimators developed by Queller and Goodnight (1989), Li *et al*. (1989), Lynch and Ritland (1993), and Wang (1999) have become the most commonly used as they can estimate relatedness with relatively few markers (5-20) (Csilléry *et al.* 2006b). A number of studies have compared the performance of these estimators, and whilst their accuracy depends on the population under investigation, van de Casteele *et al*. (2006b), van Hooft *et al*. (2006a), Russello and Amato (2003), and Csilléry *et al*. (2004) found that the Lynch-Ritland estimator (*LR*) performed best.

In this chapter, I evaluate the accuracy of the true and analytical scimitar-horned oryx studbooks by comparing measures of relatedness derived from microsatellite analyses against those derived from the two studbooks. This will determine which studbook contains pedigree data most appropriate for the captive management of scimitar-horned oryx. The specific objectives are; to 1) determine if the true studbook is an accurate record of the genetic diversity of the captive population; and 2) to determine whether the analytical or true studbook should be used as the basis for management decisions. Whilst this chapter specifically refers to the scimitar-horned oryx studbooks, the results will apply more widely to all captive breeding programmes that rely on studbooks with missing pedigree data. If the analytical studbook proves to be the most accurate technique available at present for managing captive populations with incomplete pedigrees, it will validate current approaches to endangered species management in zoological institutions. However, the approach may need to be reviewed if the analytical studbook for scimitar-horned oryx proves to be less accurate than the true studbook.

5.3 Methodology

5.3.1 Samples

A total of 168 faecal, blood, and tissue biopsy samples were obtained for the purpose of extracting DNA for microsatellite analysis. Samples were collected from captive animals held in various zoological institutions in Europe, Dubai and South Africa. Three to six fresh (<12h) faecal pellets were collected from the ground and placed in 50ml tubes containing c.30g silica gel (Type III indicating, Sigma) with a small piece of filter paper separating the faecal material from the silica gel (Wasser *et al.* 1997). The tubes were then held at ambient temperature for several weeks prior to being stored long term at 4°C. Blood, tissue and skin samples were collected by zoo veterinarians when animals received veterinary treatment or were restrained for health checks, transport or routine ear-tagging. Blood samples were shipped within 24h for processing, or held at -20°C and shipped frozen. All tissue samples were placed in 100% alcohol and shipped at ambient temperature. One-hundred and seven faecal and 61 blood, tissue, and skin samples were obtained from oryx in 27 institutions in 14 countries in Europe, Israel, South Africa and the United Arab Emirates. Two of the skin/blood samples supplied by Marwell Wildlife came from animals transported to Australia in 1987. One-hundred and eleven samples were from individually identifiable oryx, but six of these were duplicates, thereby reducing the total number of individually identifiable samples to 105 (Table 5.1). Errors in the amplification of faecal DNA samples reduced the number of viable samples to 85.

5.3.2 DNA extraction

DNA extraction took place in a dedicated area using the QIAamp® DNA stool minikit (Qiagen) according to manufacturer's instructions but with the modifications detailed in Iyengar *et al.* (2007) (Appendix E and F). DNA was extracted from blood using the protocol described in Bruford *et al.* (1998), and from skin biopsies using a standard phenol–chloroform protocol (Bruford *et al.* 1998; Milligan 1998).

5.3.3 Microsatellite analyses

A set of 13 polymorphic microsatellite loci were used to evaluate genetic diversity in scimitar-horned oryx. Six of the microsatellite loci were successfully used in a previous study on scimitar-horned oryx (MAF46, MAF50, OarFCB304, OarAE119, OarCP26, RBP3) (Iyengar *et al.* 2007), the remaining seven (D5S2, RT5, RT6, 11HDZ550,

BMC3224, BMS4008, OARFCB48) were identified from candidate microsatellite loci in the literature (Alpers *et al.* 2004; Buchanan *et al.* 1994; Hawkins *et al.* 1995; Huebinger *et al.* 2002; Maddox 2001; Toldo *et al.* 1993; Wilson *et al.* 1997). PCR amplifications were carried out in 10 µl volumes (9 µl Abgene thermo-start *Taq* polymerase (1.5 mM $MgCl_2$), 0.2 µl of each primer (20 mM) and 0.6 µl of DNA template (~20 ng μl^{-1}). Amplification conditions were as follows: Initial denaturation at 95^{o}C for 15mins, followed by 35 cycles at 94^{o}C for 1min, 55 ^{o}C for 1min and 72^{o}C for 1 min. There was a final extension of 72^{o}C for 5 minutes. For faecal DNA samples, a multi-tube approach was employed to minimize the effects of allelic drop-out. Each sample was amplified and genotyped independently at each locus two times in the case of heterozygote genotypes and four times in the case of homozygote genotypes. Two separate poolplexes ((MAF46, MAF50, Oar FCB304, OarAE119, OarCP26, RBP3) and (D5S2, RT5, RT6, 11HDZ550, BM3224, BM4004, OAR48)) were created by mixing PCR product from each locus in equal proportions and subsequently combining 3 µl of the mixture with 7 µl HiDi formamide and 0.3 µl LIZ 500 size standard (Applied Biosystems). The poolplexes were subject to capillary electrophoresis on an ABI 3130xl genetic analyser. Alleles were sized relative to the size standard, scored using Genemapper v4.0 Software (Applied Biosystems) and manually checked. Both positive and negative controls were run alongside the samples.

Microsatellite data were examined for departures from the Hardy-Weinberg equilibrium to test for selective neutrality using GENALEX 6.3 (Peakall & Smouse 2006), before calculating allelic diversity and allelic richness. Pairwise estimates of the coefficient of relatedness (*LR*) for all individuals were calculated using the Lynch & Ritland (1999) method within GENALEX 6.3.

5.3.4 Analytical studbook

The analytical studbook was constructed based on the principles established by AZA (2004) whereby gaps in the pedigree data were filled with assumptions made from reviewing the true studbook (Appendix G). The decision-making process used in the assignment of parentage assumptions to fill gaps in pedigree data is summarised in Figure 5.1. The assumptions were entered into an overlay file in the computer program SPARK-plug (Porter *et al.* 2002) and merged with the true studbook database to create a new analytical studbook database in SPARKS (Scobie *et al.* 2004). The resulting analytical studbook database contained 100% known pedigree data.

Table 5.1 Details of all the biological samples received from zoological institutions including those not used in the final analyses due to fragmentation of DNA, duplication, non-amplification, or because they were received from anonymous or unidentifiable individuals. Please see Appendix B for institutional mnemonics

Institution	Number of samples	Sample type	Samples included in the analysis
AALBORG	6	Faeces	0
AMERSFOOR	1	Blood	1
AMSTERDAM	6	Faeces	4
BERLINZOO	6	Faeces	5
BURFORD	6	Faeces	5
CHARD	1	Blood	0
CHESTER	16	Faeces	0
DDCR	6	Faeces	0
EDINBURGH	5	Faeces	0
ESTEPONA	13	Blood/skin	8
JERUSALEM	1	Faeces	0
KARLSRUHE	5	Faeces	4
LA PALMYR	4	Blood	4
LE PAL	4	Faeces	0
LEIPZIG	5	Blood/faeces/skin	5
LISBON	2	Faeces	0
MADRID Z	2	Skin	1
MANOR HS.	3	Faeces	2
MARWELL	33	Blood/faeces/skin	18
MONTPELLI	2	Faeces	0
PRAHA	8	Blood/faeces	4
PRETORIA	5	Faeces	4
VALBREMBO	12	Blood	10
WARSAW	4	Faeces	4
WHIPSNADE	5	Faeces	3
WOBURNLTD	4	Faeces	1
ZAGREB	6	Faeces	2

5.3.5 Study population

An artificial study population (N = 85) was created in PM2000 (Pollak *et al.* 2007) using a sub-set of individuals from the true international studbook. A second population was created with the same individuals, but based on data from the analytical international studbook. Individuals were included in these two populations if they had yielded viable DNA, and pairwise estimates of relatedness (*LR*) had been obtained from molecular analyses (N = 85).

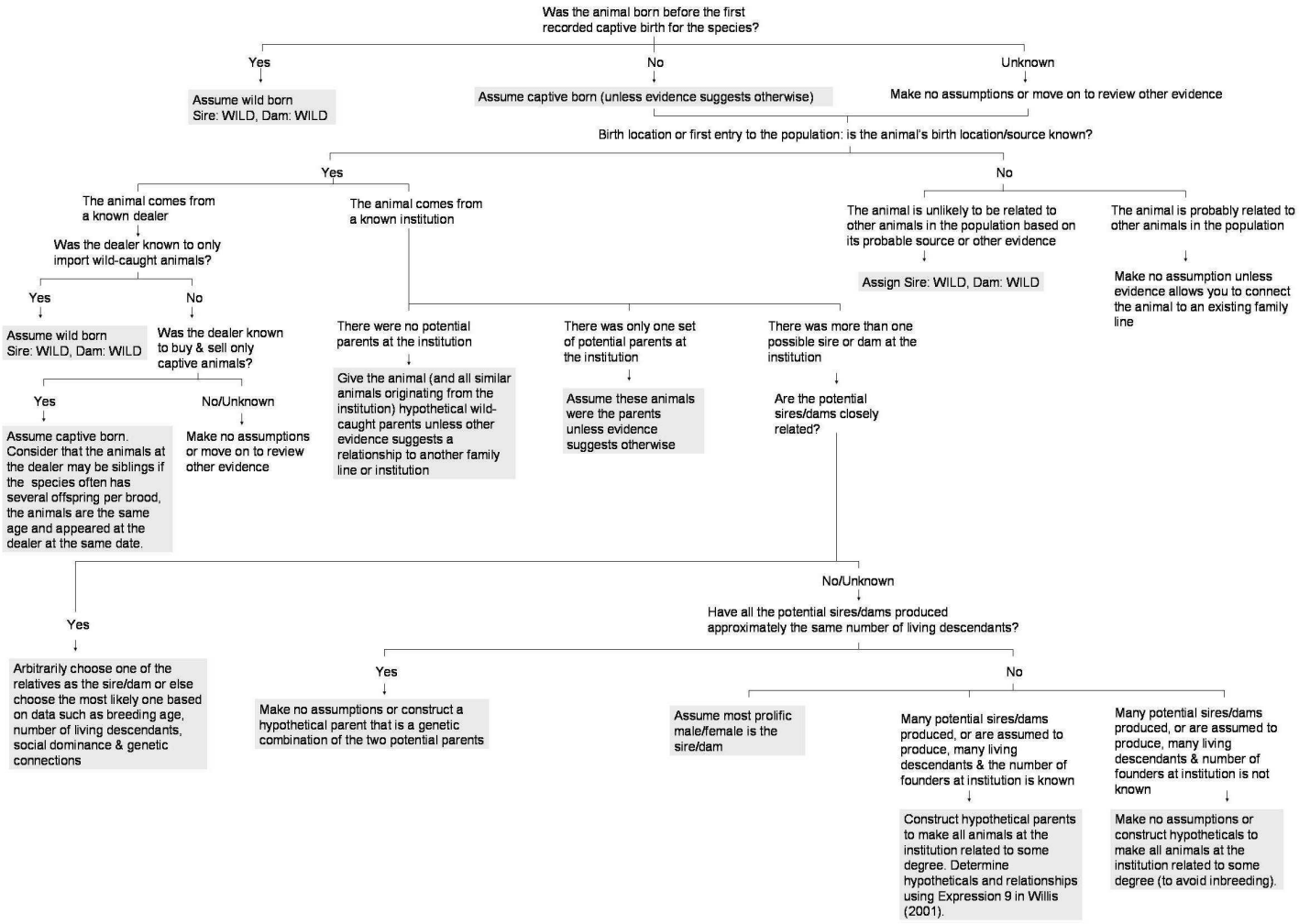


Figure 5.1 A decision tree for making parentage, birth date and birth type assumptions, and creating hypothetical animals in an analytical studbook. This diagram is based on guidance issued by AZA (AZA, 2004) for creating analytical studbooks. The grey areas indicate assumptions

5.3.6 Mean kinship coefficients (mk_i)

Pairwise coefficients of relatedness (mk_i) were calculated from the pedigree data by exporting demographic and genetic data from the true scimitar-horned oryx international studbook database in SPARKS to PM2000. Filter conditions for the data export were 'all living oryx between 01/01/1980 and the 04/04/2007'. The 85 individuals comprising the study population were selected for inclusion in the simulation, a gene drop analysis with 10,000 iterations was run and kinship matrices for the sample population were simulated using the additive matrix method given in Ballou (1983). Mk_i were calculated for every individual in the study population ('all data' $N = 85$). This was repeated a further five times to filter out the effects of missing pedigree data on the results, but with the exclusion of all individuals with more than 37.5%, 50%, 62.5%, 75% and 87.5% pedigree completeness resulting in sample sizes of $N = 59$, 46, 36, 25 and 9 respectively.

The methodology was then repeated exporting data with the same filter conditions from the analytical studbook and running the same six simulations in PM2000 to obtain comparable mk_i.

Regression analyses were applied to the data to test for a relationship between the mk_i derived from true and analytical studbooks. Correlations then tested the relationship between *LR* and mk_i derived from the true and analytical studbooks. Data residuals were not all normally distributed, and sample size ranged from $N = 9$ to 85 violating the assumptions for a Pearson product-moment correlation. Furthermore, a rank-order analysis was appropriate to these data because population management decisions are primarily based on ranking individuals using mk_i. Individuals with 0% known pedigree in the 'all data' category were removed from regression and correlation analyses because the default mk_i of $mk_i = 0.5$ biased the data. This resulted in a sample size of $N = 72$ for the 'all data' category.

5.3.7 Impact on population management

The impact of managing the study population based on *LR*, mk_i derived from the true studbook, and mk_i derived from the analytical studbook was evaluated by separating males ($N = 37$) and females ($N = 48$) and ranking individuals of each sex from low to high *LR* and mk_i values. This resulted in six listings, two (one male and one female) for the molecular data (*LR)*, two for the true studbook (mk_i), and two for the analytical studbook (mk_i).

The top 10 males and females for each of the three data sets were paired in PM2000 using the previously exported true studbook as a framework. One offspring was created per pair (equivalent to a growth rate of 12%) and the resulting changes in Gene Diversity (*GD*), Gene Value (*GV*), Founder Genome Equivalents (*FGE*) and average Mean Kinship (*MK*) were calculated. The process was repeated pairing the top 18 males and females which represented all of the above-average genetically important males and the top 18 females (representing a growth rate of 21%). The same methodology was repeated using the analytical studbook as a framework for both the top 10 and top 18 pairs.

5.3.8 Random mating

The retention of genetic diversity for population management strategies based on relatedness values was compared against a baseline model where breeding pairs were selected at random. This random model represents a population with the same annual growth rate as the true mk_i, analytical mk_i and *LR* breeding models but without any genetic management. A random number generator was used in excel (fx = *INT* (*RAN*D()*85) to generate a list of 10, and then 18, males and females. These animals were paired in PM2000 using the previously exported true studbook as a framework. One offspring was created per pair and the resulting changes in *GD*, *GV*, *FGE* and *MK* were calculated. This was repeated with the analytical studbook as a framework, and with same list of males and females. This entire methodology was repeated 29 times resulting in 30 repeats of each simulation. A paired t-test was used to evaluate differences between the true and analytical studbooks in *GD*, *GV*, *FGE*, *MK* and the change in value (Δ) of each of these measures from the baseline (a simulation with no breeding pairs).

5.4 Results

5.4.1 Analytical studbook

The true studbook was the basis for the analytical studbook. The gaps in the pedigree were filled by 447 parentage, birth date and gender assumptions for 297 individuals. This increased the amount of pedigree known for the study population from 49.7% (31.2% in the entire studbook) to 100% known (62.3% in the entire studbook) (Appendix G). Figure 5.2 is a visualisation of the pedigrees from the true (Figure 5.2.1) and analytical (Figure 5.2.2) studbooks for the study population using the computer program 'Lineage' (Pollak & Egan 2008) to demonstrate the changes in pedigree structure and composition between the data sets. Figure 5.2 illustrates the complexity of the pedigrees, and the inter-generational nature of the study population. Whilst both the true and analytical populations had extremely complex pedigrees, Figure 5.2 shows that the analytical study population had more ancestors (N = 217) recorded than the true studbook (N = 174). Inbreeding in the population resulted in some ancestors appearing several times in the pedigree of individual animals (Figure 5.3). The relationships between individuals also changed as the gaps in the pedigree were filled in, and relationships defined. Additionally, the mean number of generations in the study population increased from 11 in the true studbook to 15 in the analytical studbook.

Figure 5.3 further demonstrates the differences between the true and analytical studbook using individual studbook number 32056 as an example. The pedigree extracted from the true studbook (Figure 5.3.1) illustrates that 13 out of the 30 ancestors recorded were founders. Only 10 of these denoted founders were true founders, the remaining three 12056, 5840 and 5896 are animals whose parentage was unknown, and in this instance were designated as founders. An additional two ancestors (studbook numbers 6072 and 13532) had an unknown sire and 25252 has an unknown dam. The missing pedigree data means that only 40% of the pedigree was known for this individual in the true studbook. Conversely the same individual had 47 ancestors, of which 16 were founders, in the analytical studbook and had 100% known pedigree. The analytical studbook produced a more complex pedigree for 32056 than the true studbook, and showed approximately 11 ancestral generations since the population was founded, compared to eight for the true studbook. However, ancestral inter-generational breeding means that this was only a rough estimate.

Figure 5.2.1

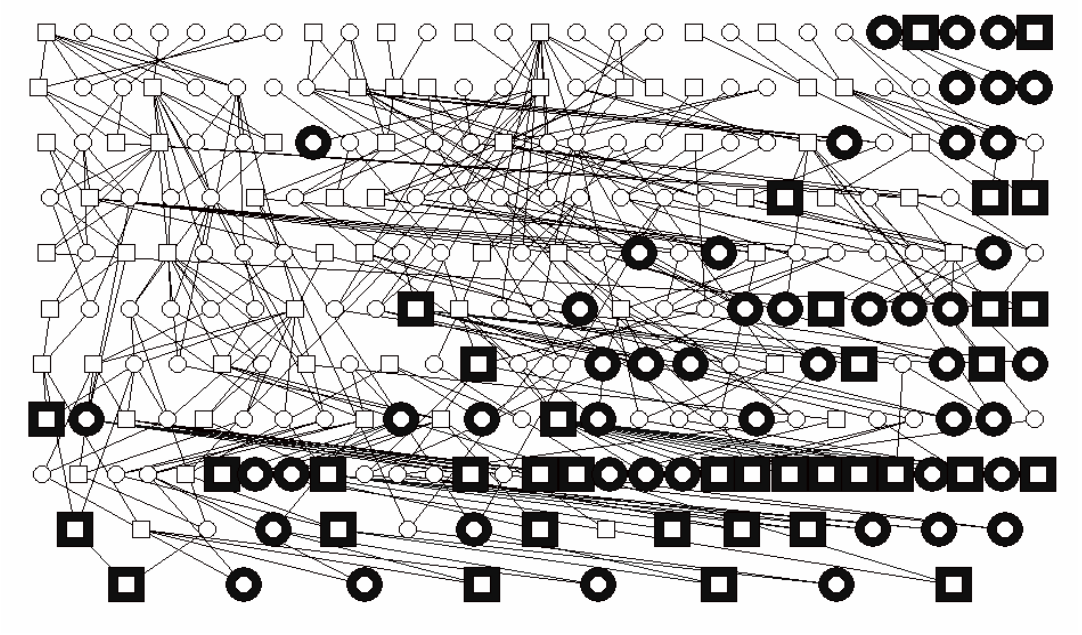


Figure 5.2.2

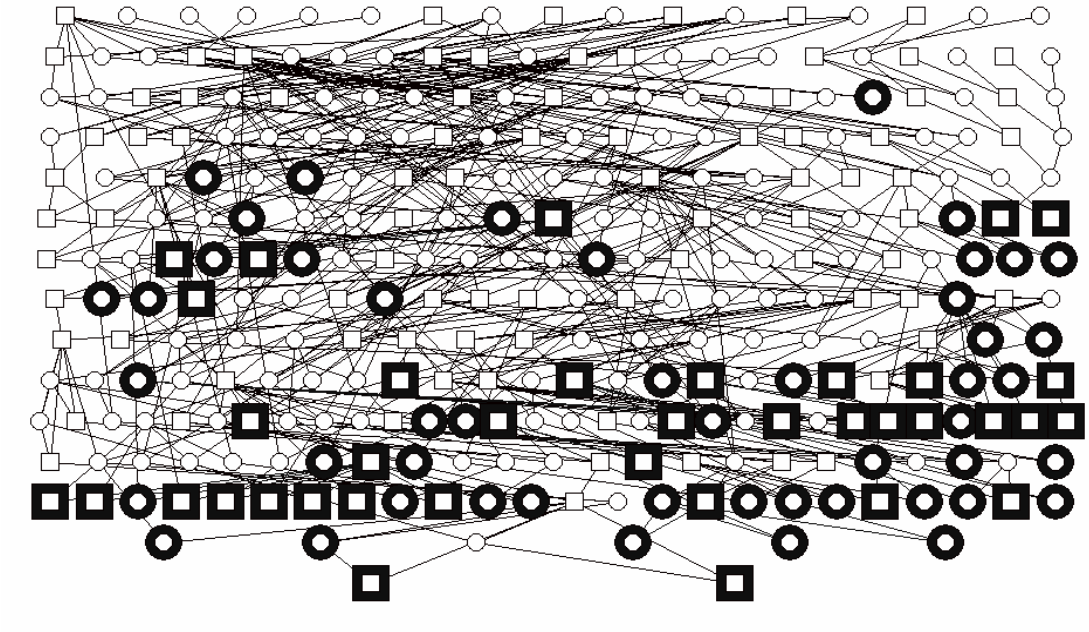


Figure 5.2 **Figure 5.2.1** illustrates the pedigree for the study population extracted from the true studbook, and **Figure 5.2.2** illustrates the pedigree for the same individuals extracted from the analytical studbook. The individuals in the study (*N* = 85) are indicated by the heavy black outlines, with males denoted by squares and females by circles. The individuals in grey are ancestors and represent the historical pedigree of the study population. The lines connect parent to offspring, and the horizontal arrangement of individuals provides an approximation for the number of generations of the captive population

Figure 5.3.1

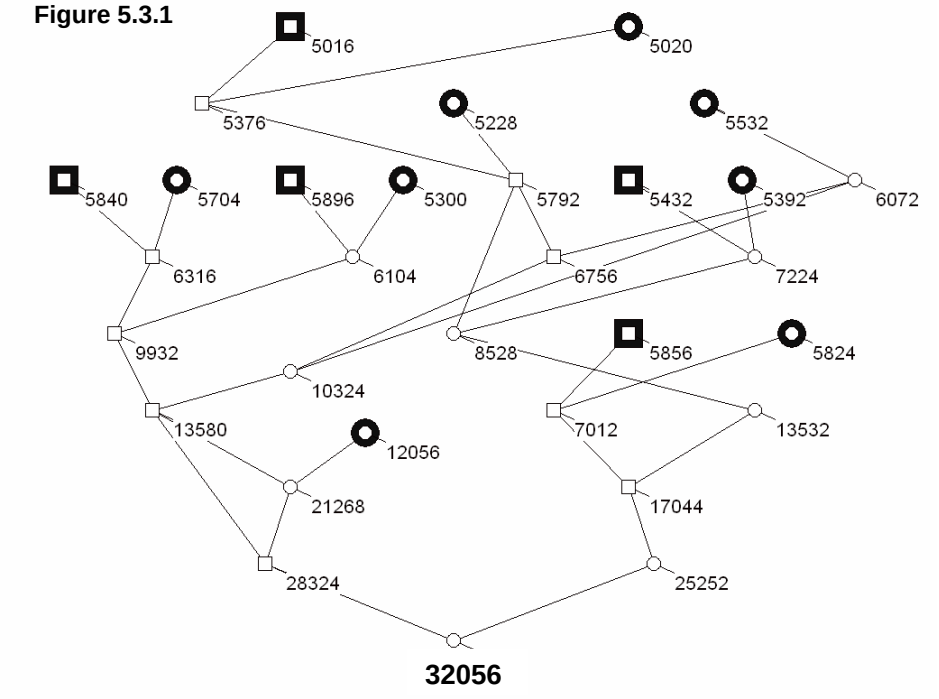


Figure 5.3.2

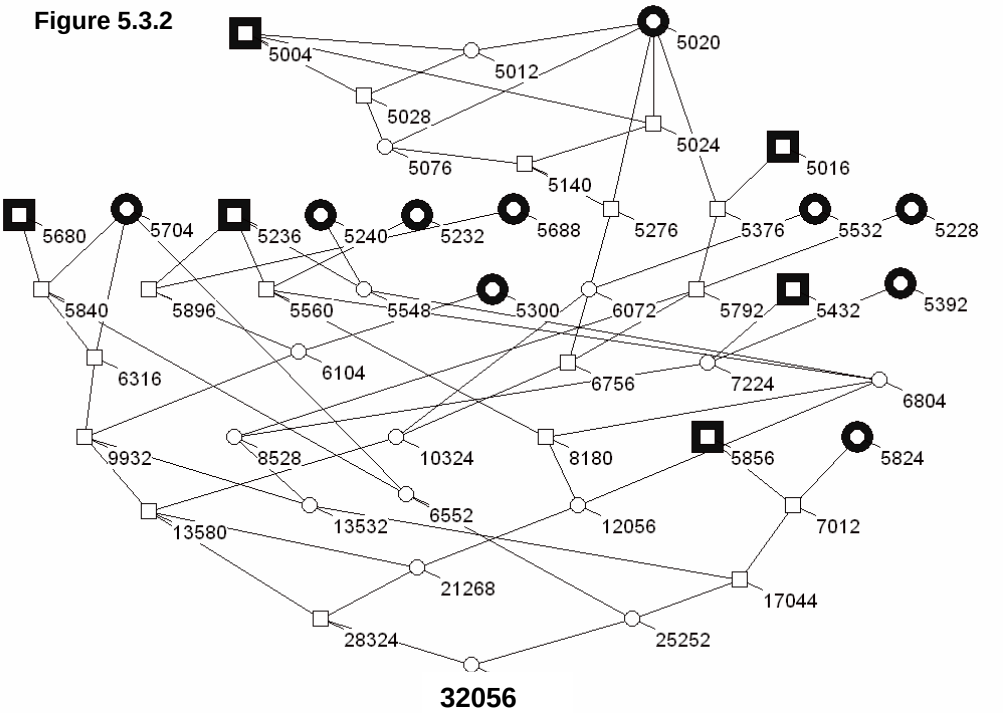


Figure 5.3 The pedigree for individual 32056 extracted from the true studbook (**Figure 5.3.1)** and the analytical studbook (**Figure 5.3.2**) and visualised using the Lineage program. Males are denoted by a square, females by a circle and founders by a thick black outline

5.4.2 Evaluation of studbooks using molecular markers

Mean kinship coefficients derived from the true and analytical studbooks were plotted against each other (Figure 5.4 and Appendix H) and tested for a relationship. All variations of pedigree completeness revealed a relationship between the true and analytical studbooks (Table 5.2). The average mk_i for the analytical data set (N = 72) was 0.093 compared to 0.102 obtained for the true studbook. In total 72% of the population returned a higher mk_i for the true studbook than the analytical studbook data, even after the individuals with 0% known pedigree (and a default mk_i of 0.5) were removed. Moreover, standard deviations in *MK* were greater for the true studbook than the analytical studbook for all data categories except the >75% known sample (Table 5.3).

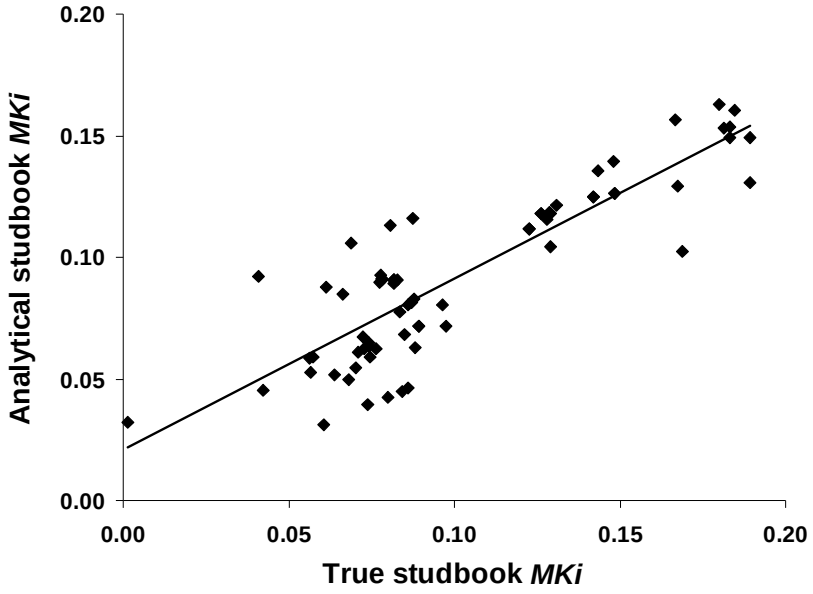


Figure 5.4 The graph represents the mk_i derived from the true and analytical studbooks for the 'all data' category minus the individuals with 0% known pedigree (N = 72)

Table 5.2 Pedigree completeness categories with associated P and r^2 values and sample sizes

Category	*P*	r^2	*N*
All data	< 0.001	74.7%	72
>37.5% known	< 0.001	78.6%	59
>50% known	< 0.001	91.5%	46
>62.5% known	< 0.001	93.4%	36
>75% known	< 0.001	94.8%	25
>87.5% known	< 0.001	98.3%	9

Table 5.3 Summary statistics for mk_i derived from the true and analytical studbooks

True Studbook						
Data	**All data N= 72**	**>37.5% N=59**	**>50% N=46**	**>62.% N=36**	**>75% N=25**	**>87.5% N=9**
Mean	0.1020	0.1070	0.1205	0.1408	0.1427	0.1699
SD	0.0421	0.0449	0.0501	0.0514	0.0469	0.0445

Analytical Studbook						
Data	**All data N=72**	**>37.5% N=59**	**>50% N=46**	**>62.% N=36**	**>75% N=25**	**>87.5% N=9**
Mean	0.0928	0.0982	0.1082	0.1322	0.1387	0.1682
SD	0.0342	0.0340	0.0436	0.0504	0.0508	0.0390

Mean kinship coefficients derived from both studbooks were plotted against the *LR* values to test for a relationship between each studbook and the molecular data (Figure 5.5). A weak relationship existed between the *LR* values and the true studbook mk_i for >37.5% known but there was no relationship between *LR* and the analytical studbook mk_i for this data set, or the 'all data', >50% known, >62.5% known, and 75% known categories. It was only when pedigree completeness exceeded 87.5% that a positive relationship between the analytical studbook mk_i and the *LR* values was evident. No relationship existed between the *LR* relatedness values and the true studbook mk_i (Table 5.4, Figure 5.6).

Table 5.4 Results of the correlation between the Lynch-Ritland molecular data and the mean kinship data derived from the true and analytical studbooks

Category	Comparison	*P*	*r*	r^2	*N*
All data	*LR* v true studbook	0.410	-0.099	0.009%	72
	LR v analytical studbook	0.272	0.131	0.011%	72
>37.5% known	*LR* v true studbook	0.029	-0.284	8.07%	59
	LR v analytical studbook	0.404	-0.222	1.23	59
>50% known	*LR* v true studbook	0.163	-0.209	4.37%	46
	LR v analytical studbook	0.153	-0.214	4.58%	46
>62.5% known	*LR* v true studbook	0.211	-0.214	4.58%	36
	LR v analytical studbook	0.161	-0.239	5.71%	36
>75% known	*LR* v true studbook	0.299	-0.216	4.67%	25
	LR v analytical studbook	0.193	-0.270	7.29%	25
>87.5% known	*LR* v true studbook	0.097	0.586	34.34%	9
	LR v analytical studbook	0.029	0.720	51.84%	9

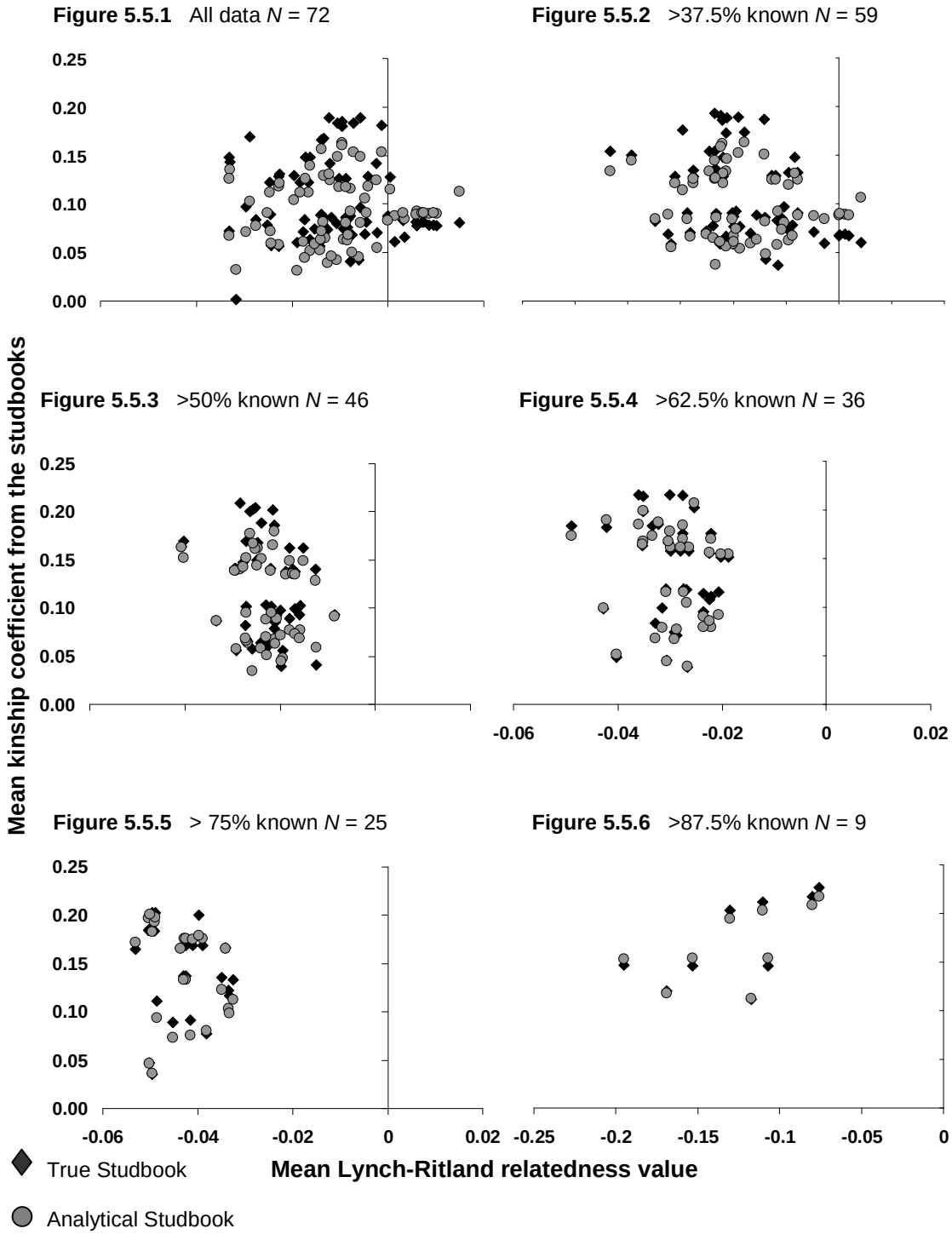


Figure 5.5 The mk_i derived from the true and analytical studbooks plotted against the mean Lynch-Ritland relatedness value (*LR*) for the six data categories

5.4.3 Random mating

Random mating simulated the same annual population growth as the study population under the analytical mk_i, true mk_i and molecular *LR* models, but without any genetic management. It provided a baseline measure of the retention of genetic diversity in a breeding non-managed population with which to compare the retention of genetic diversity under the true mk_i, analytical mk_i and *LR* breeding strategies.

Random mating resulted in a decrease in the retention of mean *GD* in both the true and analytical populations after 10 and 18 breeding pairs had been created and produced one offspring each (Figure 5.7 and Table 5.5). The difference in *GD* between the true and analytical studbooks after 10 pairs is a reflection of the differences in the starting *GD* between the two studbooks and is not a result of creating the breeding pairs. However, randomly creating 18 pairs does result in a discernable change (Δ) in the retention of *GD* from the starting baseline value (Table 5.5).

A similar result was obtained for differences in *GV* between the two studbooks after the creation of 10 and 18 breeding pairs (Table 5.5).

The retention of genetic diversity as represented by *FGE* differed between the true and analytical studbooks after the creation of both 10 and 18 breeding pairs. Moreover this difference was a reflection of the changes (Δ) in *FGE* as each pair was created and not because of the different baseline *FGE* values between the studbooks. Similarly average *MK* and the change (Δ) in *MK* differed between the true and analytical studbooks after the creation of 10 and 18 breeding pairs (Table 5.5 and Figure 5.7).

Table 5.5 The results of the paired *t*-tests for eight genetic metrics caused by the creation of random pairs in the true and analytical studbooks

	10 breeding pairs		**18 breeding pairs**	
	t_{29}	*P*	t_{29}	*P*
GD	48.82	< 0.001	51.34	< 0.001
ΔGD	1.99	0.056	3.59	< 0.001
GV	67.55	< 0.001	64.45	< 0.001
ΔGV	0.88	0.384	-3.44	0.002
FGE	59.69	< 0.001	57.41	< 0.001
ΔFGE	2.53	0.017	2.16	0.039
MK	50.89	< 0.001	51.4	< 0.001
ΔMK	3.28	0.0032	3.59	< 0.001

5.4.4 Impact on population management

The individuals included in the top 10 and top 18 listing of genetically important individuals vary between the three data sets (Table 5.6 and Figure 5.6). The *LR* and the analytical studbook mk_i listings share only three females with the same rank (studbook ID 28032, 26576 and 23268 ranked at one, two and 12 respectively, indicated in light grey on Table 5.6). The analytical and true studbooks share only two males with the same rank (studbook ID 17128 and 27504, ranked at one and eight respectively, indicated in dark grey on Table 5.6). There are no shared rankings between *LR* and the true studbook mk_i.

Population Managers assign breeding priority using ranked mk_i, but adjust it depending on the *F* of future offspring and practicality. As a result there may be differences in ranks and mk_i between paired males and females. Individuals will still be paired as long as males and females are of similar rank and their mk_i are below the population mean. Table 5.7 illustrates how many males and females with below average mk_i would be assigned breeding priority if pairs with below average *LR* were selected for breeding. The table shows that the analytical studbook mk_i are more closely aligned to the molecular *LR* data than the true studbook mk_i, with the analytical mk_i and *LR* sharing 67% of breeding females and 50% of breeding males. In comparison only 39% of breeding females and 22% of breeding males are shared between the true studbook mk_i and the molecular *LR* data.

The impact of assigning breeding priority to individuals using only *MKi* or *LR* on *GD*, *GV*, *FGE* and average *MK* in the study population was quantified by creating breeding pairs between the top 10 and 18 males and females in PM2000 using the true and analytical studbook data as a framework. Change in the genetic diversity of the study population was modelled after each pair produced one offspring (Figures 5.8 and 5.9).

Selecting breeding pairs based on true mk_i, analytical mk_i and *LR* resulted in an increase in all measures of genetic diversity (*GD, GV, FGE* and average *MK*) in the analytical studbook framework after 10 and 18 pairs. Random mating reduced all measures of genetic diversity except *GV*, which showed a small increase of 0.001 after 10 pairs and 0.002 after 18 pairs in the analytical studbook framework. Breeding pairs selected using the analytical mk_i resulted in a greater increase in genetic diversity (*GD, GV, FGE* and average *MK*) after 10 and 18 pairs than those selected using true mk_i and *LR* when modelled in the analytical studbook framework (Figures 5.8 and 5.9).

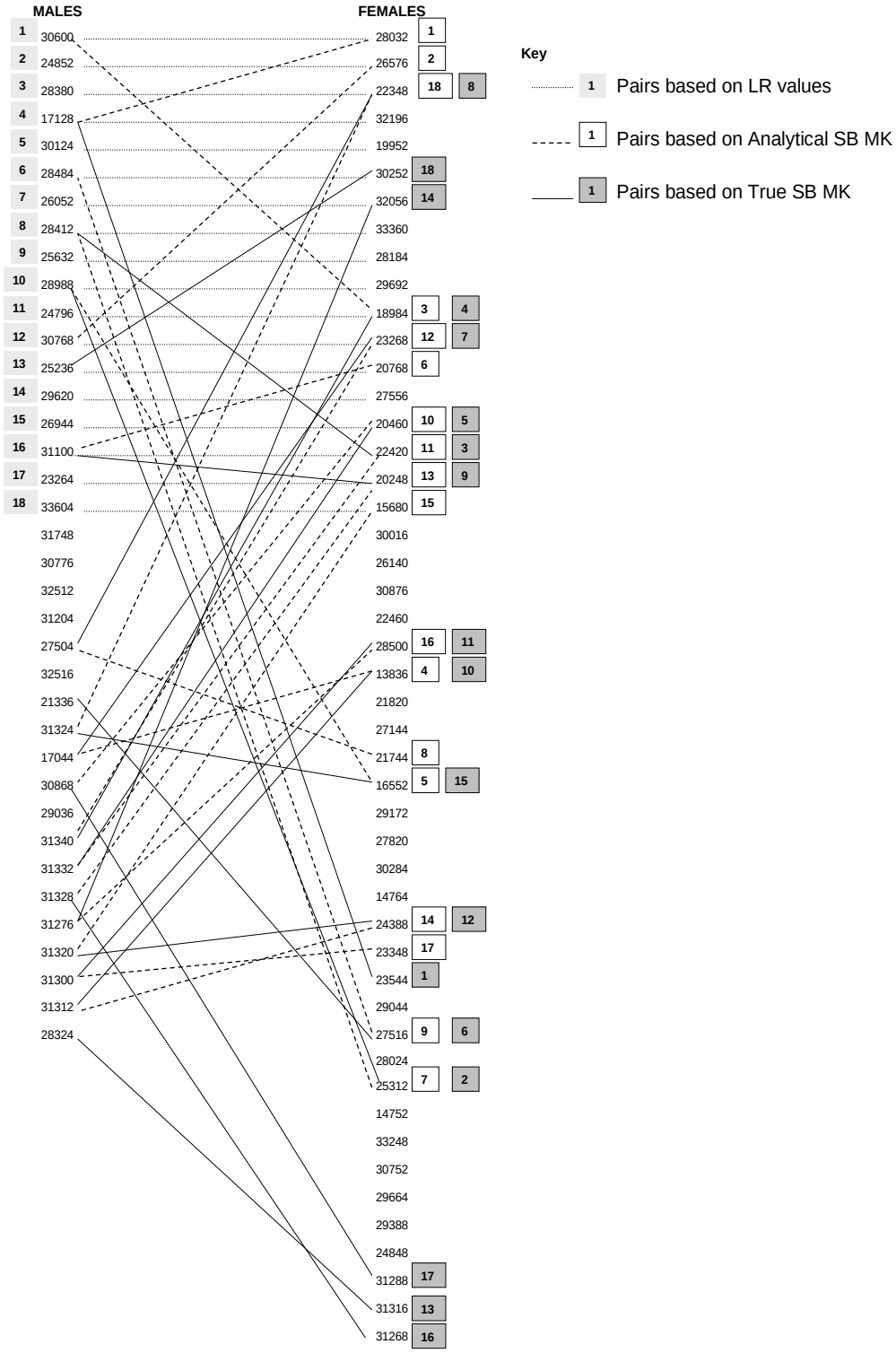


Figure 5.6 Top 18 breeding pairs based on the molecular *LR* values and true and analytical studbook mk_i

Table 5.6 The top 18 breeding females and males for each data set. Individuals are ranked by gender from low to high *LR*

	Females						Males					
	Molecular		Analytical SB		True SB		Molecular		Analytical SB		True SB	
Rank	SB ID	*LR*	SB ID	mk_i	SB ID	mk_i	SB ID	*LR*	SB ID	mk_i	SB ID	mk_i
1	28032	-0.0516	28032	0.0290	23544	0.0410	30600	-0.0365	17128	0.0323	17128	0.0015
2	26576	-0.0433	26576	0.0311	25312	0.0422	24852	-0.0359	30768	0.0325	28988	0.0562
3	22348	-0.0331	18984	0.0313	22420	0.0565	28380	-0.0331	30600	0.0333	28412	0.0575
4	32196	-0.0330	13836	0.0395	18984	0.0607	17128	-0.0317	17044	0.0545	31340	0.0612
5	19952	-0.0296	16552	0.0424	20460	0.0637	30124	-0.0288	28988	0.0583	31332	0.0662
6	30252	-0.0275	20768	0.0447	27516	0.0682	28484	-0.0256	31100	0.0588	21336	0.0688
7	32056	-0.0251	25312	0.0454	23268	0.0710	26052	-0.0247	28412	0.0592	17044	0.0703
8	33360	-0.0247	21744	0.0464	22348	0.0723	28412	-0.0244	27504	0.0633	27504	0.0743
9	28184	-0.0245	27516	0.0500	20248	0.0729	25632	-0.0228	28484	0.0655	31100	0.0745
10	29692	-0.0195	20460	0.0519	13836	0.0739	28988	-0.0228	30868	0.0831	31312	0.0773
11	18984	-0.0190	22420	0.0527	28500	0.0743	24796	-0.0226	31332	0.0849	31300	0.0782
12	23268	-0.0176	23268	0.0610	24388	0.0763	30768	-0.0190	31340	0.0877	31320	0.0782
13	20768	-0.0174	20248	0.0624	31316	0.0779	25236	-0.0183	31328	0.0894	28324	0.0806
14	27556	-0.0173	24388	0.0626	32056	0.0782	29620	-0.0165	31312	0.0899	31276	0.0817
15	20460	-0.0163	15680	0.0631	16552	0.0799	26944	-0.0163	31320	0.0905	31324	0.0817
16	22420	-0.0142	28500	0.0648	31268	0.0817	31100	-0.0153	31276	0.0907	31328	0.0817
17	20248	-0.0140	22348	0.0675	31288	0.0828	23264	-0.0139	31324	0.0907	30868	0.0877
18	15680	-0.0140	23348	0.0683	30252	0.0836	33604	-0.0112	31300	0.0911	25236	0.1226

Table 5.7 Individuals with an *LR* below the population mean are ranked from low to high *LR*. The individuals highlighted in grey in the ASB and TSB columns have a mk_i above the population mean

Females				Males			
SB ID	LR rank	ASB rank	TSB rank	SB ID	LR rank	ASB rank	TSB rank
28032	1	1	44.5	30600	1	3	35
26576	2	2	44.5	24852	2	30	35
22348	3	17	8	28380	3	29	26
32196	4	42	35	17128	4	1	1
19952	5	19	29	30124	5	19	28
30252	6	22	18	28484	6	9	35
32056	7	28	14	26052	7	22	19
33360	8	45.5	44.5	28412	8	7	3
28184	9	20.5	26.5	25632	9	27	23
29692	10	31	32	28988	10	5	2
18984	11	3	4	24796	11	28	24
23268	12	12	7	30768	12	2	35
20768	13	6	19	25236	13	22	19
27556	14	37	36	29620	14	22	19
20460	15	10	5	26944	15	31	25
22420	16	11	3	31100	16	6	9
20248	17	13	9	23264	17	35	27
15680	18	15	25	33604	18	32	35
30016	19	40.5	44.5				
26140	20	20.5	26.5				
30876	21	25	23				
22460	22	38	37				
28500	23	16	11				
13836	24	4	10				

Only the pairings selected using the true and analytical mk_i methods resulted in increased genetic diversity for all four measures (*GD, GV, FGE* and *MK*) in the true studbook framework. Genetic diversity was reduced after 10 and 18 pairs for breeding pairs selected by random mating and the *LR* method. After 10 pairs, the true mk_i method resulted in a greater increase in genetic diversity than selecting pairs based on the analytical *MK* method, but after 18 pairs there was little difference (*GV* Δ+0.001), no difference (*GD* and *MK*) or a small decrease (*FGE* Δ-0.01) in genetic diversity between the true *MK* method and analytical *MK* method when modelled in the true studbook framework.

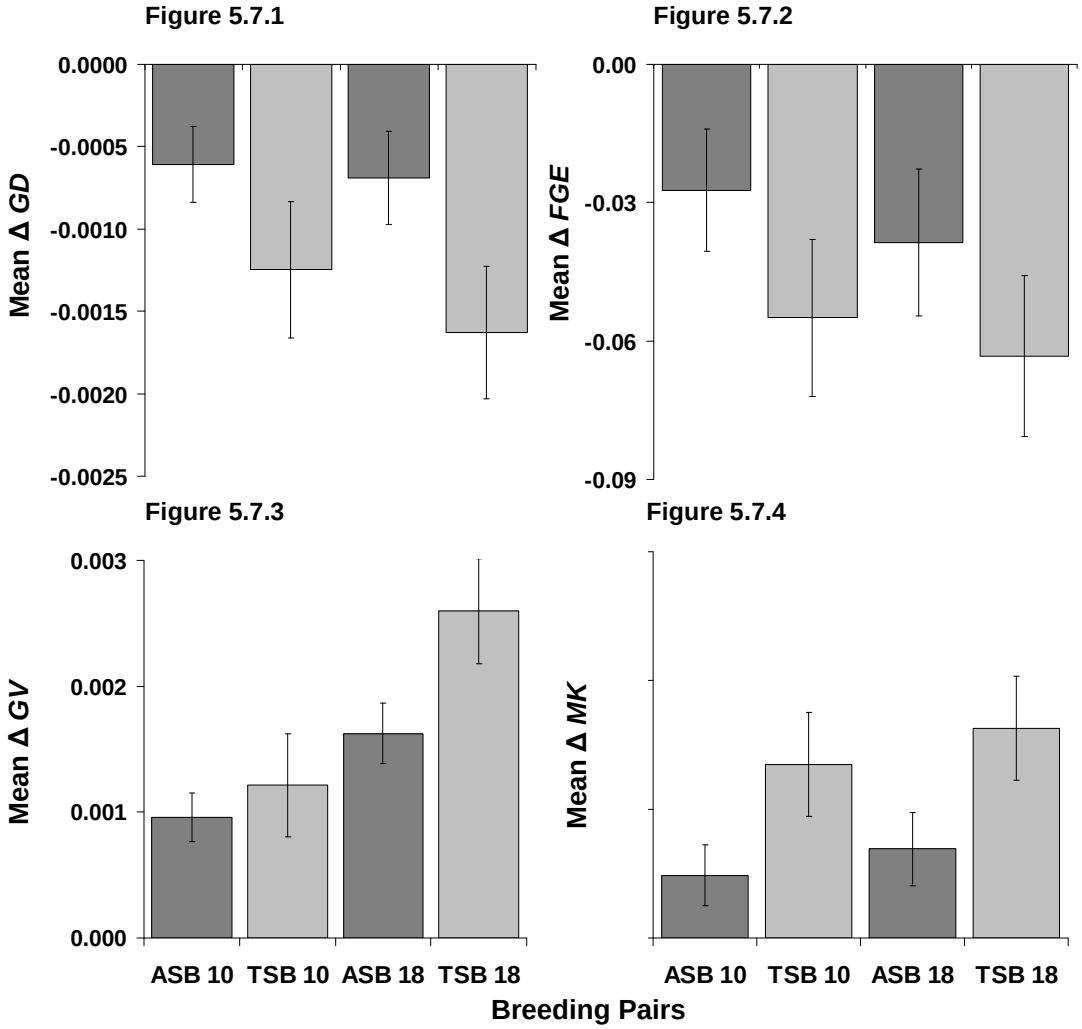


Figure 5.7 Mean change in *GD* (**Figure 5.7.1**), *FGE* (**Figure 5.7.2**), *GV* (**Figure 5.7.3**), and *MK* (**Figure 5.7.4**) after 10 randomly selected pairs and 18 randomly selected pairs were created in the analytical (ASB) and true studbook (TSB) frameworks in PM2000

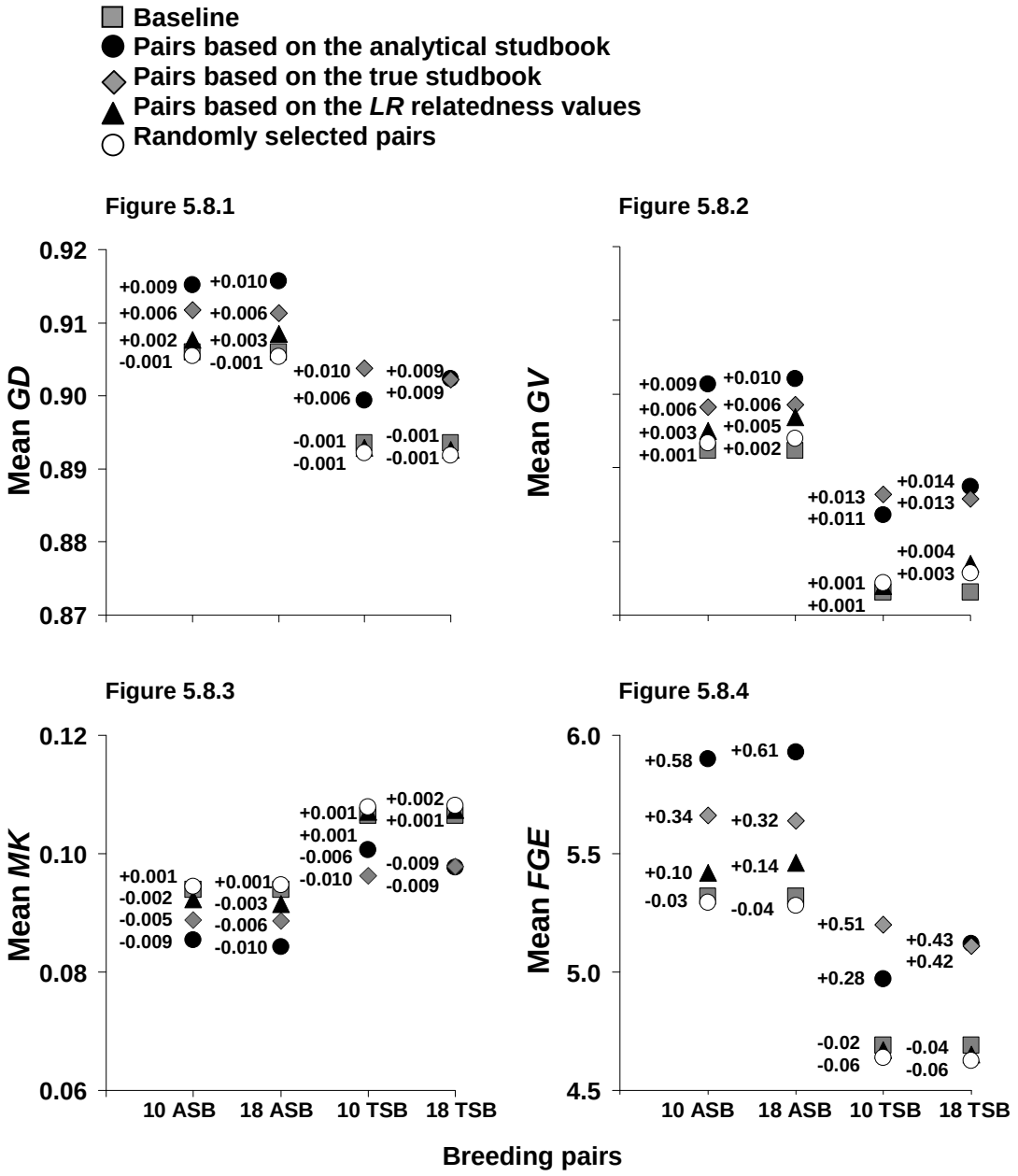


Figure 5.8 Mean final (cumulative) *GD, GV, FGE* and average *MK* in the study population after the top 10 and 18 individuals of each gender have been paired and have produced one offspring. The pairings were modelled in PM2000 (Pollak *et al.* 2007) using data exported from the true and analytical studbooks. The data points on the graphs represent the change in value (Δ) between the baseline and final pairing. The numbers on the *x*-axis represent the number of pairs for the analytical studbook (ASB) and true studbook (TSB)

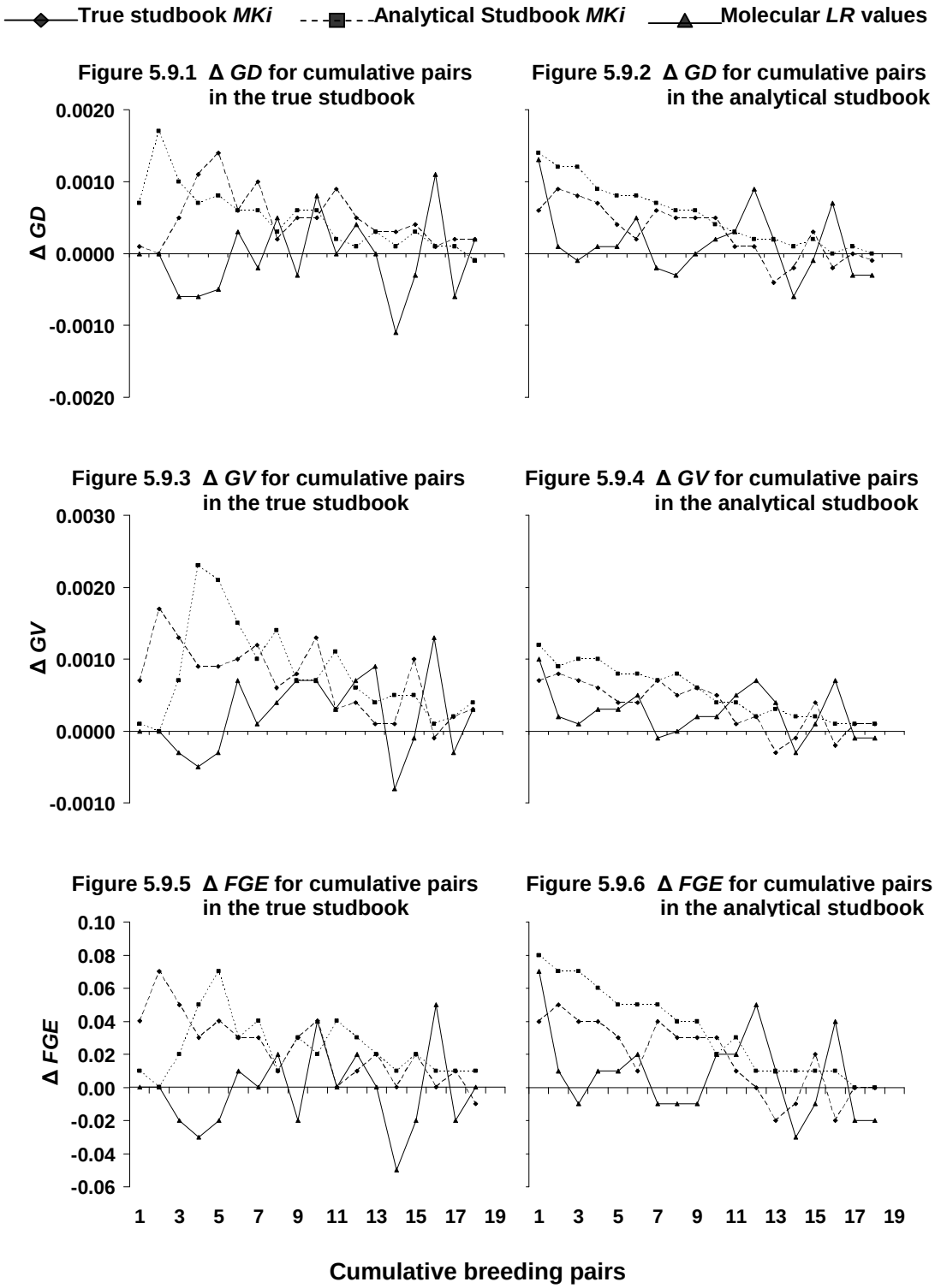


Figure 5.9 The cumulative change (Δ) in genetic diversity, as represented by *GD*, *GV* and *FGE* , when males and females are paired based based true studbook mk_i, analytical studbook mk_i and molecular *LR*. Cumulative changes in genetic diversity are shown for breeding pairs modelled in the true and analytical studbooks

5.5 Discussion

There was a clear relationship between the true and analytical studbooks for all data completeness categories. This was to be expected to some extent as the analytical studbook was based on the true studbook, but the amount of missing pedigree data in the true studbook could have severely reduced the strength of the relationship between the two. The 11 individuals recorded in the true studbook that had 0% pedigree completeness, and were therefore given default mean kinship coefficients of $mk_i = 0.5$, had a large impact on the concordance between the two studbooks. The default value of $mk_i = 0.5$ has historically been assigned to individuals with no pedigrees because PM2000 has no data with which to fill in the gaps. The alternative is to remove these individuals from the managed population altogether as their contribution cannot be evaluated. This approach is predicted to reduce gene diversity if they are largely unrelated to other individuals in the population (Willis 1993, 2001).

There was no evidence of any concordance between the true or analytical studbooks and the molecular data until original pedigree completeness reached 87.5% known. At that point a relationship was only observable between the molecular data and the analytical studbook. It should be noted that sample size was reduced to $N = 9$ for this category, resulting in some ambiguity as to whether this was a genuine relationship or an artefact of small sample size. There was no relationship between the true studbook with 87.5% pedigree completeness and the molecular data, indicating that the missing pedigree still had a notable impact on concordance even though only 12.5% was missing. The implication of these results is that analytical studbooks, with their potentially incorrect assumptions, provide a better data source for statistical genetic analyses than true studbooks with their incomplete pedigrees.

It is interesting to note that the assumptions used to fill in the gaps in the pedigree when original pedigree completeness was less than 87.5% known, did not bring the molecular data and the analytical studbook into accord. Consequently, whilst analytical studbooks have role to play in completing pedigree data for genetic analyses, they may only be valid when original pedigree data is at least 87.5% complete.

There was one anomaly in the data set. A negative relationship was detected by statistical analyses between the true studbook and the molecular data for the >37.5% pedigree completeness category. The r^2 value was $r^2 = 8\%$. The cause of this result is

unknown, but it is unlikely to be reflective of a genuine relationship, because r^2 is small, and negative instead of positive.

Evaluation of the impact of population management decisions based on molecular, true studbook, and analytical studbook breeding priority rankings, revealed a greater concordance between the analytical studbook and the molecular data than between the true studbook and the molecular data. Furthermore, there was a greater consistency between true *MK*, analytical *MK* and *LR* relatedness values when the breeding pairs were modelled in the analytical studbook framework compared to the true studbook framework. However, true studbook, analytical studbook data, and molecular data, performed better in retaining genetic diversity, than did random mating. The lack of concordance between the molecular data and the studbooks resulted in a lower retention of genetic diversity when pairs were constructed using molecular *LR* values in the studbook frameworks, than when they were paired using studbook mk_i. This should not be taken as evidence that using molecular data to create breeding pairs is less effective than using studbook data. These results are merely an artefact of using a studbook framework to evaluate management decisions, and the lack of concordance between the studbook and molecular data.

Molecular analyses provide empirical estimates of absolute genetic diversity for only a few loci, whereas pedigree-based methods provide statistical estimates of mean genome-wide diversity relative to the founding population (Ballou *et al.* 2010a). As a result, some degree of variation between the studbooks mk_i and the molecular relatedness was expected. Previous combined molecular and studbook analyses on Przewalski's horse, Arabian oryx, Bali starling *Leucopsar rothschildi* and waldrapp ibis *Geronticus eremite* have found errors in their studbooks (Boakes *et al.* 2007; Signer *et al.* 1994; Witzenberger & Hochkirch 2011). In contrast other authors have reported a good, but imperfect, correlation between relatedness data derived from molecular analyses and data derived from studbooks, for example the Persian wild ass *Equus hemionus onager* and the black-footed ferret (Nielsen *et al.* 2007; Wisely *et al.* 2003).

There are three possible sources of error for a lack of concordance between the scimitar-horned oryx studbooks and the molecular data: 1) missing pedigree data; 2) the founder assumption, and; 3) cryptic errors where individuals have been mis-identified (Nielsen *et al.* 2007; Signer *et al.* 1994). The greater, but imperfect, concordance between the analytical studbook and the molecular data when original pedigree completeness reached 87.5% suggests that missing pedigree data were important in determining concordance between studbook and molecular data (Nielsen *et al.* 2007). However, the

methods used to catch the population founders in Chad in the mid-1960s (Chapter Two), and poor historical records mean that the founder assumption is likely to be inaccurate for this species (Gilbert & Woodfine 2004a; Mace *et al.* 1992). The fact that scimitar-horned oryx are group-living, do not have a unique pelage patterns, and easily lose identifying ear tags, means that cryptic errors in the studbook are also likely (Gilbert & Woodfine 2004a; Leus *et al.* 2011b).

The premise for these analyses is that the molecular relatedness data are an empirical, independent, and unbiased measure of absolute genetic diversity (Ballou *et al.* 2010a). They are therefore the most appropriate data with which to evaluate the accuracy of studbooks (Witzenberger & Hochkirch 2011). However, 40% of the DNA samples included in the final analyses originated from faecal material, and ungulate faecal samples can yield poor quality DNA (Maudet *et al.* 2004a). Allelic dropout, false alleles, and low DNA yields have been reported for molecular genetic studies using DNA from ungulate faecal samples (Maudet *et al.* 2004b). DNA from scimitar-horned oryx faecal samples had to be processed multiple times to ensure accuracy (Iyengar *et al.* 2007; Ogden, R. *pers. comm.* 2010). Whilst every precaution was taken to ensure the molecular analysis results were accurate, blood and tissue samples yielded better quality DNA, and would have been preferable if it had been possible to source a sufficient number of samples.

An insufficient number of microsatellite markers may result in an unreliable estimate of relatedness (Csilléry *et al.* 2006; Garant and Kruuk 2005; Slate *et al.* 2009; Weir *et al.* 2006). Genetic diversity was evaluated using only 13 microsatellite loci, and then the results extrapolated across the genome. Increasing the number of markers is likely to provide more accurate estimates of relatedness (Santure *et al.* 2010). However, Nielsen *et al.* (2007) found that 12 microsatellite markers were sufficient for evaluating studbook accuracy of the endangered Persian wild ass, and Wisely *et al.* (2007) used only five when evaluating the black-footed ferret studbook.

Some institutions provided more samples than others, for example Marwell Wildlife supplied 18 of the 85 samples included in the final analyses. These were from closely related individuals, and consequently had high relatedness values and mean kinship coefficients, thereby raising the mean kinship coefficients of the data set. The large number of related individuals meant that the genetic distances between individuals was smaller, and may have caused biases in both the studbook and molecular data. If would have been preferable to obtain samples that were representative of the whole EEP population, rather than a particular sub-set, for example the UK population.

The small sample size for some pedigree completeness categories (>87.5% known, *N* = 9), compromised the authority of the results. The samples were collected opportunistically due to ethical considerations, but targeting specific individuals with more than 87.5% pedigree completeness could have increased the sample size in that category. It should be noted that only 7% of living individuals listed in the international studbook have more than 87.5% pedigree completeness (Gilbert 2010). Increasing the sample size in this category would have required targeted individuals being sedated or anaesthetised for the sole purpose of collecting biological samples.

This study would benefit from being extended to include more individuals with complete, nor near compete (>87.5% known), pedigrees. The small sample size (*N* = 9) for the 87.5% pedigree completeness category casts doubt on whether the relationship between the molecular data and the studbook data is valid. Increasing the sample size for this category would address this limitation.

Eliminating the data derived from faecal samples would remove concerns over sample quality, resulting in more robust molecular data. Similarly, elimination of some of the samples from over-represented families, would ensure a more accurate representation of genetic diversity of the captive population, and would remove concerns of closely related individuals biasing the data. This would necessitate the collection of additional blood and tissue samples to ensure an adequate sample size.

The recent development of Single Nucleotide Polymorphisms (SNP) for use in wildlife biology addresses the concerns of using only a few molecular (microsatellite) loci. SNPs are likely to provide more accurate estimates of relatedness between individuals because hundreds of markers are used in each analysis, in comparison with 13 microsatellite loci used for the analyses in this chapter. SNPs have not yet been identified for scimitar-horned oryx, or many endangered species, but this should be a priority for future genetic analyses for the species (Ogden, R. *pers. comm.* 2010), including extensions to this study.

The evaluation of both true and analytical studbook accuracy would benefit from being extended to include other taxa with differing social structures, and studbook completeness. Comparisons of molecular and studbook data could then be used to evaluate studbook accuracy and quantify different sources of error. Species such as the Grevy's zebra have unique stripe patterns resulting in every individual being identifiable from birth. Consequently, the international studbook for this species has more than 99.5% pedigree completeness (Langenhorst, 2011). In this case, errors due to missing pedigree data are not

relevant. Similarly, the bongo EEP has 100% known pedigree, individuals are easily identifiable, and the capture method for the original population founders means that the founder assumption is likely to be accurate (Veasey, J., *pers. comm.* 2010). If analyses revealed any lack of concordance between molecular and studbook data, cryptic errors are likely to be the major contributory factor.

The newly developed version of SPARKS (version 1.6 beta) (Scobie *et al.* 2011), and PMx (Ballou *et al.* 2011), the replacement for PM2000, allows multiple parentage to be added as percentages based on probable sires or dams, for example three possible males (A, B and C) could be the sire of one individual. The dominant male in the group (A) is the most likely sire, but the other two males (B and C) cannot be completely excluded. Under these circumstances, the true studbook records the identity of the sire as unknown. Historically, the analytical studbook would have recorded male A as the sire. The new feature in SPARKS 1.6 beta allows the sire to be recorded as 50% dominant male (A), 25% male B, and 25% male C. The program then incorporates the corresponding percentages of all the males' pedigree into the analyses. As usage of SPARKS 1.6 beta and PMx increases, this feature is likely to be used more widely by population managers in replacement of traditional analytical studbooks. However, no research has been carried out to evaluate if this is a more accurate approach to resolving the issue of pedigree completeness than the construction of traditional analytical studbooks. It would be greatly beneficial to include a comparison of the new SPARKS 1.6 beta and PMx approach to missing pedigree data, alongside the results of this chapter.

Some true studbooks have proven to be accurate when compared against molecular data (Nielsen *et al*. 2007), but this was not the case for the scimitar-horned oryx international studbook. This is largely due to substantial amounts of missing pedigree data, but cryptic errors and the founder assumption may also contribute. The analytical studbook provided a more robust data source for statistical genetic analyses for population management than the pedigree-deficient true studbook, when small amounts of pedigree data were incomplete. Its value decreased when large proportions of the pedigree were missing. Molecular analysis of captive populations can provide an alternative approach to assigning breeding priority to individuals in the population, but it is expensive, time-consuming, and there are ethical implications to sample collection (Ballou *et al.* 2010a). Additionally, new techniques need to be developed and applied to ensure that molecular data are representative of genome-wide diversity, and not just a small number of loci. Alternatively, the new features in SPARKS 1.6 beta and PMx may provide a more accurate

solution to the issue of missing pedigree data, but they won't address the problems caused by the founder assumption, or cryptic errors in studbooks.

Despite the limitations of these analyses, there are advantages to using analytical studbooks for the analysis of captive populations where pedigree data are missing. I now use this approach to examine scimitar-horned oryx population dynamics in Chapter Six, scimitar-horned oryx and Arabian oryx population sustainability in Chapter Seven, and scimitar-horned oryx EEP population viability in Chapter Eight.

6.0 Chapter six: the retention of gene diversity in the scimitar-horned oryx EEP population

6.1 Abstract

Coordinated captive breeding programmes aim to maximise the retention of genetic diversity, and often set a goal of retaining 90% of founder gene diversity (*GD*) for 100- or 200-years. Many programmes are unable to meet this goal including the scimitar-horned oryx EEP population. A number of different genetic and demographic factors influence the retention of *GD* including population size (*N*), population growth rate (*r*), generation length (*T*) and the effective population size (N_e).

This chapter aims to evaluate the impact of *N, r, T* and N_e on the retention of gene diversity in the scimitar-horned oryx EEP population in relation to the established goals of retaining 90% of *GD* for 100- and 200-years.

A series of population simulations were run to determine the individual, and then the simultaneous, impact of differing *r, T* and N_e values on the retention of *GD* in the scimitar-horned oryx population. The impact of differing population growth rates on the selection of breeding pairs was then quantified.

Increasing *N* improved the retention of *GD*, but could not meet the 90/100 or 90/200 goals. Increasing *r, T* and N_e individually and combined, could attain the goals, but parameter values were beyond the boundaries of practical application to the EEP population. Increased population growth rate did not maximise retention of *GD*, and the generation length could not be extended beyond current parameters due to mean longevity. Consequently, N_e was the most important factor governing retention of *GD* in the population. Current management of the scimitar-horned oryx EEP population needs to be reoriented in order to maximise N_e, specifically size of polyganous groups need to be reduce to equalise family size.

Limitations of the study are discussed along with recommendations for population management and future research.

6.2 Introduction

Genetic diversity can be rapidly lost from small closed captive populations (Haig *et al.* 1990), through inbreeding, selection and genetic drift (Frankham 2003, 2006; Princée 1995). Much of the population's original genetic variation will be eroded after a number of generations in captivity (Mace 1986, 1989; Ryman & Laikre 1991). There has been much debate on the causes and processes that drive small populations to extinction, but it is now generally accepted that loss of genetic diversity increases extinction risk (Frankham 1995a, 2005a, Frankham 2006).

Coordinated captive breeding programmes aim to preserve as much of the founders' gene diversity (expected heterozygosity) as possible (Ballou & Lacy 1995; Ballou *et al.* 2010a; Lees & Wilcken 2009) to: 1) ensure long-term evolutionary potential (Leus & Traylor-Holzer 2008); 2) to minimise inbreeding (observed heterozygosity) and associated inbreeding depression (Ballou *et al.* 2010a; Hedrick & Miller 1992; Miller & Hedrick 1993; Ralls & Ballou 1992); and 3) to minimise adaptation to captive conditions (Frankham & Loebel 1992; Lacy 1994; Montgomery *et al.* 2010; WAZA 2005e). Whilst all three aims are important to population viability, it is the first aim of retaining founder gene diversity (*GD*) that forms the backbone of captive population management. This chapter aims to evaluate the impact of various genetic and demographic factors on the retention of gene diversity in captive populations, using the scimitar-horned oryx as a model.

Many breeding programmes set goals for maintaining prescribed levels of founder *GD* over specified periods of time (Lacy 1995; Ralls & Ballou 1992). The genetic goals will vary depending on the purpose and duration of the breeding programme, and the characteristics of the population in question (Lacy & Ballou 2002; Wiese & Willis 2000). A general strategy for endangered species in captivity is to preserve 90% of the founders' *GD* for 200-years (Ballou *et al.* 2010a; Hedrick & Miller 1992; Lees & Wilcken 2009; Ralls & Ballou 1992). The 90/200 rule originated from an assumption that human population growth and development would stabilise, or decline, in the next 150-200 years, releasing suitable habitat for the reintroduction of endangered species (Frankham 1999; Lacy 1994; Vogler *et al.* 2009). At the same time it is envisaged that technological developments in assisted reproduction and cryopreservation techniques will complement living populations in zoological facilities (Lees & Wilcken 2009). Consequently, the 200 year time frame represents a reasonable expectation of how long populations will need to

be maintained in captivity. The 90% retention of *GD* is an intuitive balance between potentially damaging and an acceptable loss of heterozygosity in a population (Ballou *et al.* 2010a).

Once the genetic goal has been set, the number of animals needed to meet that goal can be calculated from life tables, data on current genetic diversity levels, and estimates of effective population size (Ballou *et al.* 2010a; Lacy & Ballou 2002). The 90/200 rule has often been modified to a less demanding 90/100 rule as a 100-year time frame results in smaller and more manageable, but still viable, target population sizes (Ballou *et al.* 2010a; Mace & Lande 1991; Ralls & Ballou 1992; WAZA 2005e). Additionally, 100-years may be the longest duration that legislative systems are capable of effectively operating over (Mace & Lande 1991). Although the 90/100 rule is arbitrary, it does provide quantitative guidance enabling the development of specific population management goals (Ballou & Cooper 1992b).

The 90/100 rule is often recommended as a management goal for captive populations (Ballou *et al.* 2010a), but many programmes are not able to meet this, or other demographic and genetic goals (Frankham *et al.* 2010; Lande 1995; Leus *et al.* 2011b). For example, the black-footed ferret *Mustela nigripes* SSP has set a goal of retaining 80% of the founders' gene diversity for 25-years (Wisely *et al.* 2003), and the Arabian oryx *Oryx leucoryx* EEP has set a goal of retaining 82% of founder gene diversity for 100-years (Gilbert 2009a). The 2010 scimitar-horned oryx EEP breeding and transfer plan stated that the population has retained 91% of the founders' gene diversity, but it would not be possible to retain 90% of *GD* for 100-years based on existing population parameters. Consequently, the EEP has set a realistic goal of retaining 85% of founder *GD* for 100-years (Gilbert 2010b).

A number of different genetic and demographic factors directly influence the retention of *GD* (Lacy & Ballou 2002). Individual processes acting alone can pose a threat to small populations, but they become a more significant contributor to population instability and decline when they combine and act synergistically with other demographic or genetic processes (Ballou 1992; Lacy 2000a).

The key factors that influence retention of *GD* by affecting the rate of genetic drift in captive populations are: 1) generation length (*T*) (Taylor & Barlow 1995); 2) population growth rate per capita (*r*) (Taylor & Barlow 1995); 3) population size (*N*); and 4) the effective population size (N_e) (de Boer 1989; Willis & Willis 2010).

The generation length, growth rate, population size, and the effective population size are all integral population parameters that can be partially manipulated through management in order to increase retention of founder *GD* (AZA 2004; Ballou *et al.* 2010a).

The long-term sustainable population growth rate (*r*) has a critical effect on extinction dynamics. If *r* is positive, then the population will increase in size (within a given carrying capacity), and if it is negative over the long-term, the population will decline to extinction (Holsinger 2000; Lande 1993; Thompson 2004). However, *r* is also closely tied to the rate at which genetic diversity is lost (Thompson 2004). *GD* is lost when growth rates are slow, because small populations lose genetic diversity more rapidly than large populations (Ballou *et al.* 2010a). Consequently rapid population growth to the carrying capacity may help to maintain *GD* (Ballou *et al.* 2010a; Lees & Wilcken 2009; Thompson 2004).

Generation length (*T*) is the mean age of both male and female reproduction (Ballou *et al.* 2010a; Pollak *et al.* 2005). Genetic variation is lost with each generation; therefore increasing *T* preserves more *GD* over time by reducing the number of generations (Amos & Balmford 2001; Mace 1986; Wilcken & Lees 1998). Also, the rate of genetic adaptation to captivity is inversely proportional to *T* , and so increasing *T* slows maladaptation (Frankham & Loebel 1992; Frankham *et al.* 2010).

The mean *T* can be manipulated by delaying the age of first reproduction for animals approaching reproductive maturity, and breeding from older individuals (Bishop *et al.* 2009; Frankham & Loebel 1992; Mace 1989). Theoretically, this approach will increase *T*, but in practice it may be difficult to achieve (Frankham 1995a). If such population management measures can be successfully implemented, then sufficient levels of *GD* can be maintained in smaller populations (Ballou *et al.* 2010a; WAZA 2005e). Reproductive technology such as cryopreservation of embryos and gametes can assist with this for some species (Ballou *et al.* 2010a; Frankham 2005b). Both *r* and *T* are linked to the rate of accumulation of inbreeding in a population, as well as the retention of *GD* (Gage 1995).

Loss of *GD* through genetic drift is a problem for small isolated populations, as exemplified by endangered species in captivity (Earnhardt 1999; Lacy 1993a; Pollak *et al.* 2005; Wilcken & Lees 1998). Drift is particularly noticeable in small populations as the random fluctuations that result from gamete sampling have a larger impact (Franklin 1980; Höglund 2009; Pollak *et al.* 2005). Deleterious alleles are kept at low frequency in large populations because of the balance between mutation and natural selection. Selection is less effective in small populations, and mildly deleterious alleles become selectively

neutral. Consequently, their fate is determined by genetic drift and some of these alleles increase in frequency reducing individual fitness. Over long time spans, these alleles can drift to fixation (100% frequency) in the population reducing population fitness (Frankham 1999, 2005a; Höglund 2009; Lacy 1987; Peterson & McCracken 2005). The chance of this happening increases with decreasing population size (Peterson & McCracken 2005; Wayne & Miyamoto 2006).

Additionally, inbreeding is inevitable in small closed populations, and this may result in lower overall population fitness (Lacy 1992; Reed 2005).

Genetic diversity is retained for longer in large populations (Frankham 1995a; Thompson 2004), but it is the effective population size (N_e) that determines exactly how much variation can be preserved from one generation to the next (Frankham 1995a, 1995b; Wang & Caballero 1999). The N_e has various related definitions (Wang & Caballero 1999), and there are a number of different ways to calculate it (Boyce 1992; Nunney & Elam 1994), involving both demographic (Blackwell & Doerr 1995; Engen *et al.* 2010; Kaeuffer *et al.* 2007) and genetic methods (Nunney 2000). The variance effective size is the version most commonly used in relation to captive breeding programmes (Wang & Caballero 1999). The variance N_e is the size of an idealised population which would have the same genetic variance, and is influenced by genetic drift at the same rate, as the real population (Lacy 1995; Nunney 2000; Wright 1931). The population management software program, PM2000 (Pollak *et al.* 2007) uses demographic models based on the number of breeding males and females to calculate the variance effective size (Ballou *et al.* 2010a; Bishop *et al.* 2009). This approach is valid because whilst N_e is defined in terms of the rate of genetic change, it is operationally demographic in nature (Soulé *et al.* 1986). The variance N_e method is particularly useful to conservation managers because historical trends can be used to predict future N_e (Ballou *et al.* 2010a; Lacy & Ballou 2002; Nunney 2000).

Closed populations lose neutral gene diversity at a rate of $1/(2N_e)$ per generation due to drift (Ardren & Kapuscinski 2003; Matocq 2004; Nunney 1993), and gain variation at a rate of $2N_eu$ through mutation (where u is the mutation rate) (Nunney 2000). So, the smaller the N_e, the faster the rate of loss of *GD* through drift, the slower the rate of increase in *GD* through mutation, and over the long-term, the greater the total loss of genetic diversity (Ballou *et al.* 2010a). A small N_e reduces the efficiency of selection (Gompper *et al*. 1997; Wang & Caballero 1999; Yi & Streelman 2005), and this may cause the fixation of deleterious alleles through drift and result in eventual mutational meltdown

(Charlesworth 2002; Soulé *et al.* 1986; Waples 2010). However, the weak natural selection in small N_e also has the effect of reducing adaptation to captivity (Charlesworth 2009). The effective population size (the inbreeding N_e) also summaries the extent of inbreeding in a population (Keller *et al*. 2005; Wang & Caballero 1999), and populations with small N_e may have lower fitness and be more susceptible to extinction (Ardren & Kapuscinski 2003; Frankham 2005b; Jehle *et al.* 2001; Reed 2005; Tallmon *et al.* 2004; Wang 1996). There is a difference between variance N_e, which predicts *GD*, and inbreeding N_e, which predicts observed heterozygosity, but the two are the same in panmictic populations of constant size (Pollak *et al.* 2005; Wang 1996).

There is no consensus on how large an N_e needs to be to ensure population viability (Nunney 2000), and the minimum N_e probably varies between species (Miller & Waits 2003). Franklin (1980) suggested a minimum short-term effective size of approximately 50 individuals to avoid the deleterious effects of inbreeding, and a long-term N_e of 500 where drift balances mutation in order to retain evolutionary potential. Other authors have suggested alternative figures of N_e>200 to maintain fitness (Reed *et al.* 2003c), and 500-1000 (Franklin & Frankham 1998) and 1000-5000 (Lynch & Lande 1998) to retain long-term genetic variation (Frankham 1995a).

The concept of N_e is based on an ideal theoretical population that does not experience overlapping generations, temporal fluctuations in size, selection or migration, and in which all individuals are asexual (equal sex ratio) and have an equal probability of contributing progeny to the next generation (equal family sizes) (Ballou *et al.* 2010a; Wilcken & Lees 1998). Real populations differ considerably from this concept, and so the N_e is usually considerably smaller than the census population (*N*) (Ballou *et al.* 2010a; Charlesworth 2002, 2009; Shrimpton & Heath 2003; Waples 2010; Wilcken & Lees 1998).

Ratios of effective population size to census size N_e/N vary depending on the species biology, but N_e/N will typically be in the range of 0.10-0.75 (Frankham 1995b; Franklin & Frankham 1998; Lees & Wilcken 2009; Nunney 2000; Reed *et al.* 2003b, 2003c). However, ratios as low as 10^{-6} have been reported for Pacific oysters *Crassostrea gigas* (Frankham 1995b) and 0.028 for wild Amur tigers *Panthera tigris altaica (*Alasaad *et al. 2011),* and as high as 0.83 for prairie dogs *Cynomys ludocicianus* (Frankham 1995b).

One consequence of low N_e/N ratios is that a population of several thousand may be needed to achieve an N_e of 500 over the long-term (Nunney 2000; Reed *et al.* 2003c). This presents a problem for captive breeding programmes as space is limited, and so many threatened species have population sizes that are too small to avoid inbreeding and loss of

genetic diversity (Vogler *et al.* 2009). Smaller census population sizes will be needed to maintain viable captive populations if larger N_e/N ratios, and therefore larger N_e, can be achieved (Lees & Wilcken 2009). This can be accomplished through population management (AZA 2004; Frankham 2006), as indicated by a study of 17 captive populations which showed that management resulted in a mean N_e/N of 0.26 (Lees & Wilcken 2009). The current management approach of minimising kinship is an effective way of maximising a population's N_e because it is equivalent to equalising family sizes (Borlase *et al.* 1993; Frankham 2006, 2008).

This chapter aims to evaluate the impact of various genetic and demographic parameters on the retention of gene diversity in the scimitar-horned oryx EEP population. The specific objectives are to (1) evaluate the current demographic and genetic status of the scimitar-horned oryx EEP population, and (2) to evaluate the impact of manipulating *T*, *r*, *N*, and N_e on the retention of *GD* in relation to the established goals of maintaining 90% of *GD* for 100 and 200-years. Whilst this chapter specifically refers to the scimitar-horned oryx EEP population, the results provide a reference for managing demographic, genetic and social parameters in respect to minimising the loss of *GD* in small populations.

6.3 Methodology

6.3.1 The scimitar-horned oryx EEP population

The analytical studbook was found to be more accurate than the true studbook in Chapter Five. Consequently, it was used as the data source for the analyses in this chapter. Data were exported from SPARKS v1.56beta (Scobie *et al.* 2004) using the demographic filter dates of 01/01/1990 to 31/12/2009, and a geographic filter to select animals located in scimitar-horned oryx EEP institutions. Genetic filters were set for animals living on the 31/12/2009 in scimitar-horned oryx EEP institutions.

Demographic and genetic data were imported into PM2000 v1.213 (Pollak *et al.* 2007). The date set for calculations was 31/12/2009. Data were filtered in PM2000 by removing those animals that were owned by, but not located at, EEP institutions. Additionally, those animals that were permanently sterilised or castrated before the 31/12/2009 were removed from the data set, but those that were castrated after this date were included, thereby resulting in a population size of $N = 423$. Demographic analyses based on life table data were then completed, kinship matrices simulated using the additive matrix method (Ballou 1983), and a gene drop analysis with 10,000 iterations was run. A number of demographic and genetic metrics were extracted from the analyses (Table 6.1) to provide baseline values for the analytical EEP population. Effective population size was calculated using a demographic model based on the number of breeders in the EEP population (Ballou *et al.* 2010a).

Table 6.1 Demographic and genetic metrics obtained from analysis of the scimitar-horned oryx EEP population

Metric	Abbreviation
Demographic	
Census population size (number of individuals)	*N*
Generation length (in years)	*T*
Population growth rate (per capita)	*r*
Genetic	
Founders (number of individuals)	-
% pedigree complete	% known
Gene diversity	*GD*
Gene value	*GV*
Founder genome equivalents	*FGE*
Mean inbreeding coefficient	*F*
Average mean kinship coefficient	*MK*
Effective population size (historical & current)	N_e
Effective to census population size ratio	N_e/N
GD that can be maintained for 100 & 200-years	-
Years that 90% of *GD* can be maintained for	-

6.3.2 Increasing retention of gene diversity in the scimitar-horned oryx EEP population

The populations size (*N*) was increased to a maximum allowable population size (carrying capacity) of 10,000,000 to model the effect of increasing *N* whilst maintaining all other baseline parameters (N_e = 79.5, N_e/N = 0.1883, *r* = 1.074, *T* = 6.73, no new founders). The maximum amount of *GD* retained and the length of time 90% of *GD* could be retained for, was modelled using 100- and 200-year timespans.

Simulations were run for a sequence of alternative genetic goals, given by successive increments of 0.01 in the ratio of effective to census population size (N_e/N) as a result of incrementing N_e from N_e/N = 0.01 up to N_e/N = 1.0, with a maximum allowable population size of *N* = 10,000,000 individuals. This population size is unachievable for most captive populations. It was included in the model to remove the limiting effect of carrying capacity on population dynamics. All other population metrics remained the same as the baseline values. The impact on retention of *GD* was assessed in terms of the percentage *GD* that could be retained over 100- and 200-years. The maximum allowable population size was then decreased to calculate the minimum *N* needed to retain 90% of GD for 100 and 200-years for each N_e/N ratio. The process was repeated with a maximum allowable population size of *N* = 423, representing a 0% increase in carrying capacity (*K*) (and therefore a 0% increase in *N*). Retention of *GD* was assessed by the length of time (in years) that 90% of *GD* could be retained in the population.

This process was repeated for generation length (*T*) where *T* was increased in increments of 0.10 from *T* = 0.10 to *T* = 20.0, and for maximum potential population growth rate (*r*) where *r* was increased in increments of 0.01 (1% growth rate) from *r* = 0.0 (0% growth rate) up to *r* = 1.0 (100% growth rate). As an increasing growth rate is dependent on *K* and *N*, simulations were not run for models which restricted *K* and *N* to the current carrying capacity (*N* = 423).

It is theoretically possible to manipulate more than one parameter in a captive population to increase the retention of *GD*, whilst maintaining a population size of *N* = 423 ± 5. A Latin square design (Guichon & Doncaster 2008) was used to conduct cross-factored manipulations of N_e and *T*, where N_e was increased in increments of 0.01 from N_e = 0.01 to N_e = 1.0, and *T* was increased from *T* = 0.1 to *T* = 20.0 in increments of 0.1. The impact on *GD* was modelled over 100-year and 200-year periods. Cross-factored

manipulations were not conducted in conjunction with *r* because an increase in growth rate required a corresponding increase in *K* and *N,* which were modelled as static at 423 for these simulations.

6.3.3 Increasing the maximum allowable growth rate to maintain gene diversity

Birth data from 01/01/1990 to 31/12/2008 were extracted from the scimitar-horned oryx true studbook for each EEP institution. The true studbook was used because it does not contain assumptions on parentage which alter the breeding success rate. The breeding success of female scimitar-horned oryx, as defined by the production of one live offspring, was calculated by comparing number of females producing an offspring against number of females in a breeding situation (held in a mixed herd or harem group with a breeding male). This provided the data to calculate the probability of breeding success when any one adult male (> 4 years) and any one female adult (> 3 years) scimitar-horned oryx were paired ($\bar{x}$ = 0.64 $\sigma \pm$ 0.11).

The increase in population size per annum over a 100-year period (2009-2108) was calculated for a starting population of *N* = 423 with increasing *r* in increments of 0.01 from *r* = 0.00 to *r* = 0.21 (analysis of life table data between 1990 and 2008 showed a maximum annual growth rate of *r* = 0.20). From this, the number of births and pairs per annum needed to meet the growth rate were calculated for each *r*, and for each probability of breeding success (0.53, 0.64, and 0.75 representing ($\bar{x}$ = 0.64 $\sigma \pm$ 0.11)).

Contemporary population management theory dictates that only those individuals of above average *MK* (genetically important) should breed (chapter five), and increasing *r* results in the pairing of increasing numbers of females in the population. The current EEP population was used as a baseline model to calculate the number of breeding pairs needed to meet the annual population growth rates of 0-21% for breeding success probabilities of 0.53, 0.64 and 0.75. The number of genetically unimportant females needed to form breeding pairs to meet *r* was calculated as the difference between the number of pairs needed and the number of genetically important females in the population.

This method was then applied to a model of sustained population growth over 100-year period. The difference between the number of genetically important females and the number of females needed to form breeding pairs in each year, and for each *r,* were recorded and a mean calculated over the 100-year period for each probability of breeding success. Data residuals were not normally distributed and sample size was *N* = 22,

therefore a non-parametric Kruskal-Wallis test was applied to the data to test for differences between the mean number of genetically unimportant females paired for each probability of breeding success.

The impact of breeding the entire population, both genetically important and unimportant animals, on the genetic diversity of the current EEP was assessed by creating breeding pairs using a static *MK* list. The individuals with the lowest mean kinships were paired first, with each male paired with three females due to an unequal sex ratio. A total of 300 pairs were created. Each pair was then assigned one surviving offspring, and the impact on *GD*, *GV*, *FGE* and *MK* was modelled.

6.4 Results

6.4.1 The scimitar-horned oryx EEP population

The scimitar-horned oryx EEP population had 118 males and 305 females totalling 423 individuals in 52 institutions in 16 countries in Europe and the Middle East. The N_e was considerably smaller than the census size at $N_e = 80$ with a ratio of $N_e/N = 0.19$, based on 24 male and 116 female breeders. In order to achieve an effective population size of 500, the scimitar-horned oryx EEP population would require a census population size of 2,655.

The historical N_e was smaller at $N_e = 38$ calculated over 5.9 generations in captivity. Current *GD* was over the 90% goal at 91%, but most population founders were only captured in the mid-1960s meaning that the European captive population has lost nearly 9% of original *GD* in only 45 - 47 years. Deterministic projections of *GD* loss in the scimitar-horned oryx EEP showed that only 83% of founder *GD* can be retained for 100-years (15 generations), or 90% of founder *GD* can be retained for a further 14 years, or 2.1 generations (59 - 61 years in total, or 8.8 generations). The amount of *GD* that could be retained for 200-years (30 generations) from the start of the captive population was reduced to 75% of founder *GD* (Table 6.2).

The number of founder genome equivalents in the EEP population was 5.7. It is also worth noting that the mean inbreeding coefficient for the whole EEP is $F = 0.1813$, which is over twice as much as the average mean kinship for the population $MK = 0.0872$ (Table 6.2).

Table 6.2 Population parameters for the scimitar-horned oryx EEP population

Parameter	Abbreviation	Value
Census population size	*N*	423
Founders	-	35
% pedigree known	% known	100
Gene diversity	GD	0.9128
Gene value	GV	0.9095
Founder genome equivalents	FGE	5.73
Mean inbreeding	F	0.1813
Average mean kinship	MK	0.0872
Historical effective population size	N_e	38.32
Current effective population size	N_e	79.54
Current effective to census population size ratio	N_e/N	0.1883
Generation length in years	*T*	6.73
Population growth rate	*r*	0.074
GD that can be maintained for 100-years	-	82.77%
GD that can be maintained for 200-years	-	74.91%
Length of time that 90% of GD can be maintained for	-	14 years

6.4.2 Increasing retention of gene diversity in the scimitar-horned oryx EEP population

Increasing the maximum allowable population size (carrying capacity) up to a total size of N = 10,000,000, whilst maintaining all other current population parameters (N_e=80, N_e/N = 0.19, r = 0.074, T = 6.7), could not attain the goal of preserving 90% of *GD* for 100 or 200-years (Table 6.3).

Independently increasing the values of all three parameters (r, N_e, and T) met the goal of retaining 90% of *GD* for 100- and 200-years, assuming no limitation on population size (N) (Figure 6.1). The minimum values needed to meet the goals required large population sizes (Table 6.3), but the simulations were highly sensitive to increases in parameter values when parameter values were small. For example, the population size needed to meet the 100 and 200-year goals approximately halved between growth rates of 0.08 to 0.09, between N_e of 0.19 to 0.20, and between T of 6.8 and 6.9 years. The required N continued to decline as the three parameters increased, but at a diminishing rate. Once r, N_e and T reached approximately 0.30, 0.40, and 11.0 years, respectively, increases in parameter value resulted in minimal decreases in the N needed to meet the goals. Similarly, the amount of *GD* retained increased rapidly at low parameter values, but the rate of increase declined as parameter value increased (Figure 6.1). In an idealised population where N_e/N = 1.0, the population sizes needed to retain 90% of *GD* for 100 and 200-years were N = 535 and N = 1112, respectively i.e. effective population sizes of N_e= 535 and N_e = 1112 were required to meet the 90/100 and 90/200 goals. So, the larger the N_e, the smaller the population needed for sustainability.

Increasing r, N_e, and T had a notable impact on the retention of *GD* when the parameter values were low, as observed in the scimitar-horned oryx EEP population. Increasing these values was predicted to substantially improve retention of gene diversity in the scimitar-horned oryx EEP.

The goal of preserving 90% of *GD* for 100-years was theoretically attainable whilst maintaining a zero population growth (N = 423 ± 5) when more than one parameter was manipulated at any one time. However, this was only possible by simultaneously increasing N_e/N and T (Figure 6.2). In this instance the lowest N_e/N needed to meet the goal of retaining 90% GD for 100-years for a population of N = 423 was N_e/N = 0.42 with T = 20 years, and a N_e/N = 0.77 with T = 20 years was required to meet the goal for 200-years. Alternatively, in an idealised population where N_e/N = 1.00, the generation length

still needed to be 8.4 years for the 100-year goal, and 15.3-years for the 200-year goal, both of which were notably above the current generation length of T = 6.7 years. Increasing paired r and T values, or paired r and N_e, values could not meet the goal without providing the additional carrying capacity that increasing r required.

Table 6.3 The minimum parameters (N, N_e/N, r and T) needed to retain 90% of wild *GD* for 100 and 200-years, assuming all other parameters are equal to the current population parameters (N = 423, N_e/N = 0.19, *GD* = 91.28%, r = 0.074, T = 6.7) with the exception of K (maximum of 10,000,000)

Retention	*N*	N_e	*r*	*T* (years)
100-years	Not possible. Only 89.99% of *GD* can be retained for 100-years or 90% for 73 years. An *N* of N=15,278 was needed to retain 89.9% of *GD* for 100-years	0.19 N = 19,136	0.08 N = 17,559	6.8 N = 54,582
200-years	Not possible, only 89.99% of *GD* can be retained for 200-years or 90% for 73 years. An *N* of *N*=50,353 was needed to retain 89.9% of GD for 200-years	0.19 N = 66,817	0.08 N = 55,726	6.8 N = 282,735

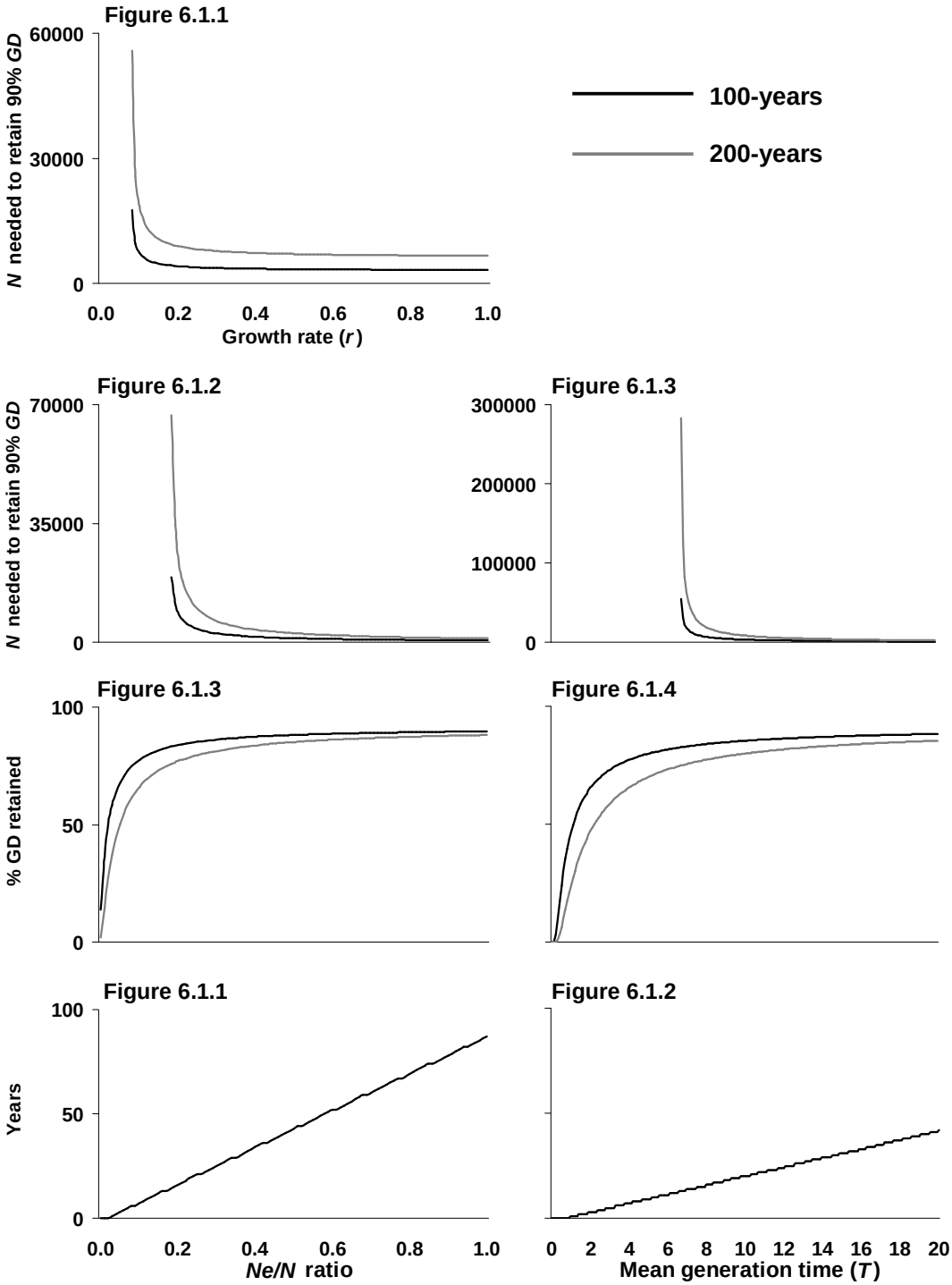


Figure 6.1 The impact of maximum allowable *r*, mean *T* and N_e/N on retention of *GD* in the scimitar-horned oryx EEP population. Retention of *GD* is described by the *N* needed to retain 90% of *GD* for 100 and 200-years when **6.1.1**) *r* is increased, **6.1.2**) when N_e/N is increased, and **6.1.3**) when *T* is increased. Retention of *GD* is described by the percentage of *GD* retained after 100 and 200-years when *N* = 423 when **6.1.4**) N_e/N is increased and **6.1.5**) when *T* is increased. Figures **6.1.6** and **6.1.7** show the length of time in years that the population can retain 90% of *GD* with increasing N_e/N and *T* respectively, whilst maintaining a population size of *N* = 423

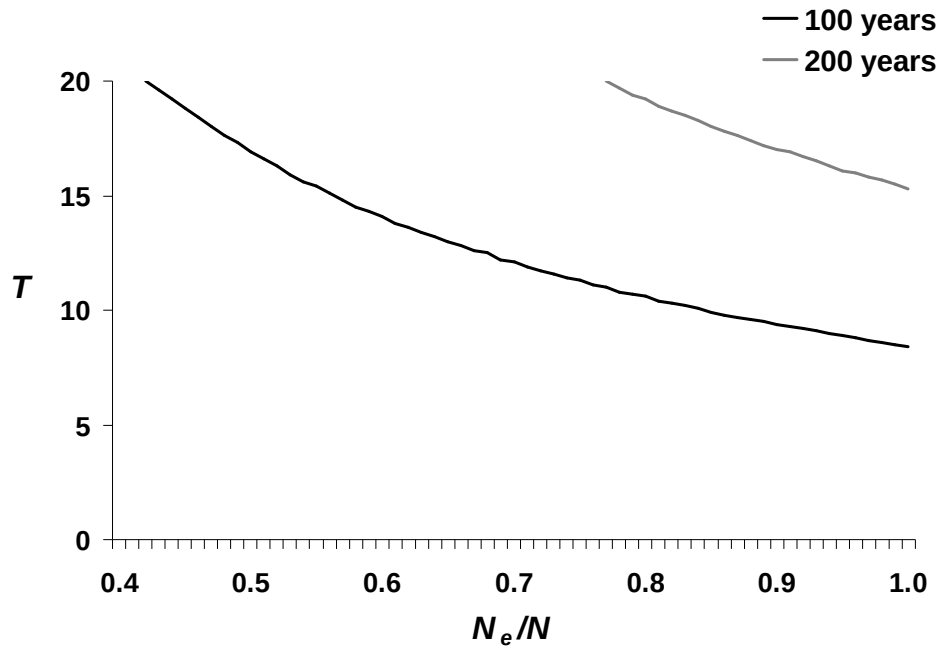


Figure 6.2 The combined effect of increasing N_e/N and T on the retention of 90% of GD for 100-years assuming a maximum census population size of 423 ± 5

6.4.3 Increasing the maximum allowable growth rate (*r*) to maintain gene diversity

A review of historical studbook data revealed that a mean of 64% plus or minus a standard deviation of 10.78 of adult females (>3 years) produce an offspring when held in a breeding group, resulting in a probability of 0.64 ± 0.11 for any one adult female breeding when held with an adult male (>4 years) (a variance between 0.53 and 0.75). Currently the number of adult females (N_F), including females provided with temporary contraception, in the EEP is $N_F = 229$, so if all these females were placed in a breeding situation then the maximum number of females breeding (N_{FB}) in a year would be $N_{FB} = 229*0.64 = 147$. If each breeding female produced one surviving offspring (surviving to recruitment age) and there was no mortality in the population, the population could grow by a maximum of 35% or $r = 0.35$ per annum. It is highly unlikely that these two assumptions could be met, and a review of the EEP population growth between 1990 and 2008 reveals a mean annual growth rate of 7.4% with a maximum of 20% for males and 15% for females.

Contemporary population management uses the *MK* method pairs the most genetically important individuals in order to meet the demographic requirements (*r*) of the

population. When reproductive success fell below 100%, more individuals were paired to achieve the same *r*. Some of these individuals had high *MK* coefficients, and were considered to be genetically unimportant (Table 6.4). As *r* increased, and predicted reproductive success decreased, the number of pairs required to meet the prescribed *r* increased. When the annual growth rate increased above 18%, and the lowest probability (0.53) of breeding success was realised, the number of females needed to form breeding pairs exceeded the number of adult females currently in the EEP population. When the higher probabilities of breeding success were attained (0.64 and 0.75), the number of genetically unimportant females forming breeding pairs decreased.

Figure 6.3 illustrates this effect over a 100-year period. The percentage of genetically unimportant females breeding increased as the population growth rate increased for the different breeding success probabilities (H_2 =13.5, P = 0.001). At a continuous annual growth rate of 5% over a 100-year period and a breeding success probability of 0.53, 95% of pairs consisted of genetically important females and 5% consisted of genetically unimportant females. The proportion of pairs containing genetically unimportant females increased as the growth rate increased until r = 0.21 (21%) when 49% of pairs contained genetically unimportant females. When reproductive success increased to a probability of 0.64 and 0.75 success, the percentage of genetically unimportant pairs was reduced at the same annual growth rates e.g. pairs did not contain genetically unimportant females until the growth rate is 8% per annum for a 0.64 breeding success probability, and 12% annual growth rate for a 0.75 breeding success probability.

Retention of genetic diversity, as represented by *GD*, *GV*, *FGE* and average *MK*, were impacted when genetically unimportant females were used to form breeding pairs to meet the demographic requirements i.e. growth rate of the population (Figure 6.4). Projected retention of genetic diversity for all four parameters began to decline when more than 150 breeding pairs were formed. Despite this, final *GD*, *GV* and *FGE* (after 300 pairs were formed and produced one offspring each) were higher than starting genetic diversity (GD = Δ +0.0013, GV = Δ +0.0022, and FGE = Δ +0.09), and *MK* was lower than the starting value (Δ -0.0013). However, when breeding was halted after 150 pairs, thereby only pairing the most genetically important animals, *GD*, *GV* and *FGE* increased by Δ +0.0099, Δ +0.0094, and Δ +0.74 respectively, and *MK* reduced by Δ -0.0099.

Table 6.4 The number of genetically unimportant females paired with increasing projected annual growth rates for the current EEP population

Growth rate	Probability of breeding success		
%	0.53	0.64	0.75
0	0	0	0
1	0	0	0
2	0	0	0
3	0	0	0
4	4	0	0
5	13	0	0
6	22	0	0
7	34	3	0
7.4[§]	37	5	0
8	44	11	0
9	53	19	0
10	62	27	2
11	74	36	10
12	84	44	16
13	93	52	23
14	102	60	30
15	111	67	36
16	123	77	45
17	133	85	51
18	142	93	58
19	151*	101	65
20	163*	110	73
21	172*	118	79

[§]current annual growth rate; *exceeds the number of females available

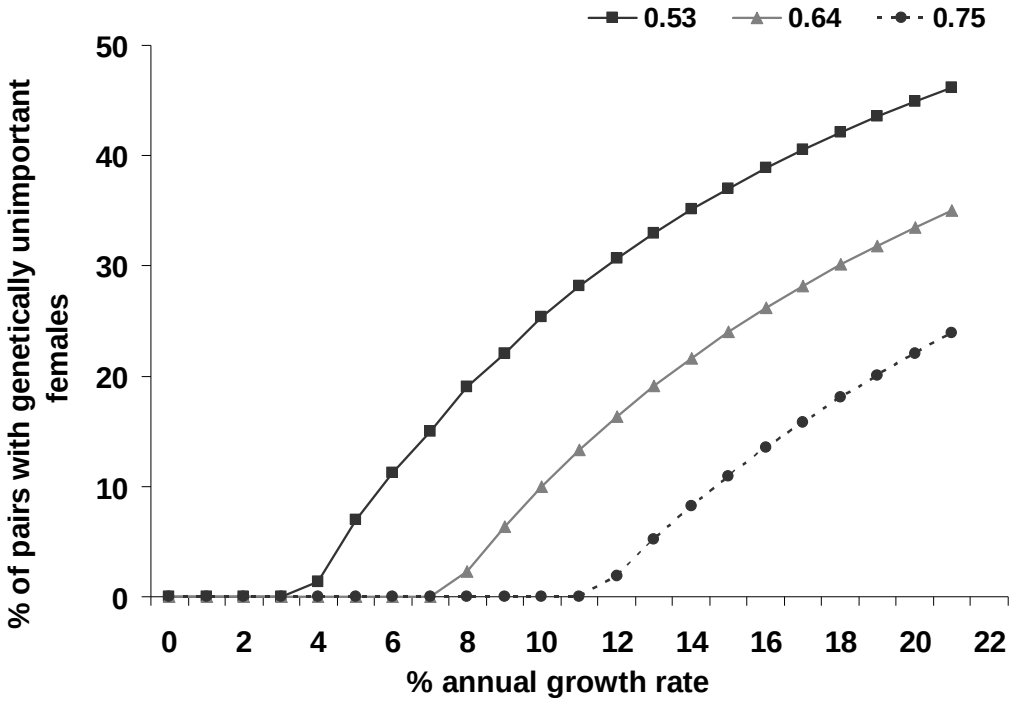


Figure 6.3 Breeding pairs with genetically unimportant females as a percentage of total pairs. Growth rate is modelled as constant over 100-year period. Data are for populations with 0.53, 0.64 and 0.75 breeding success probabilities

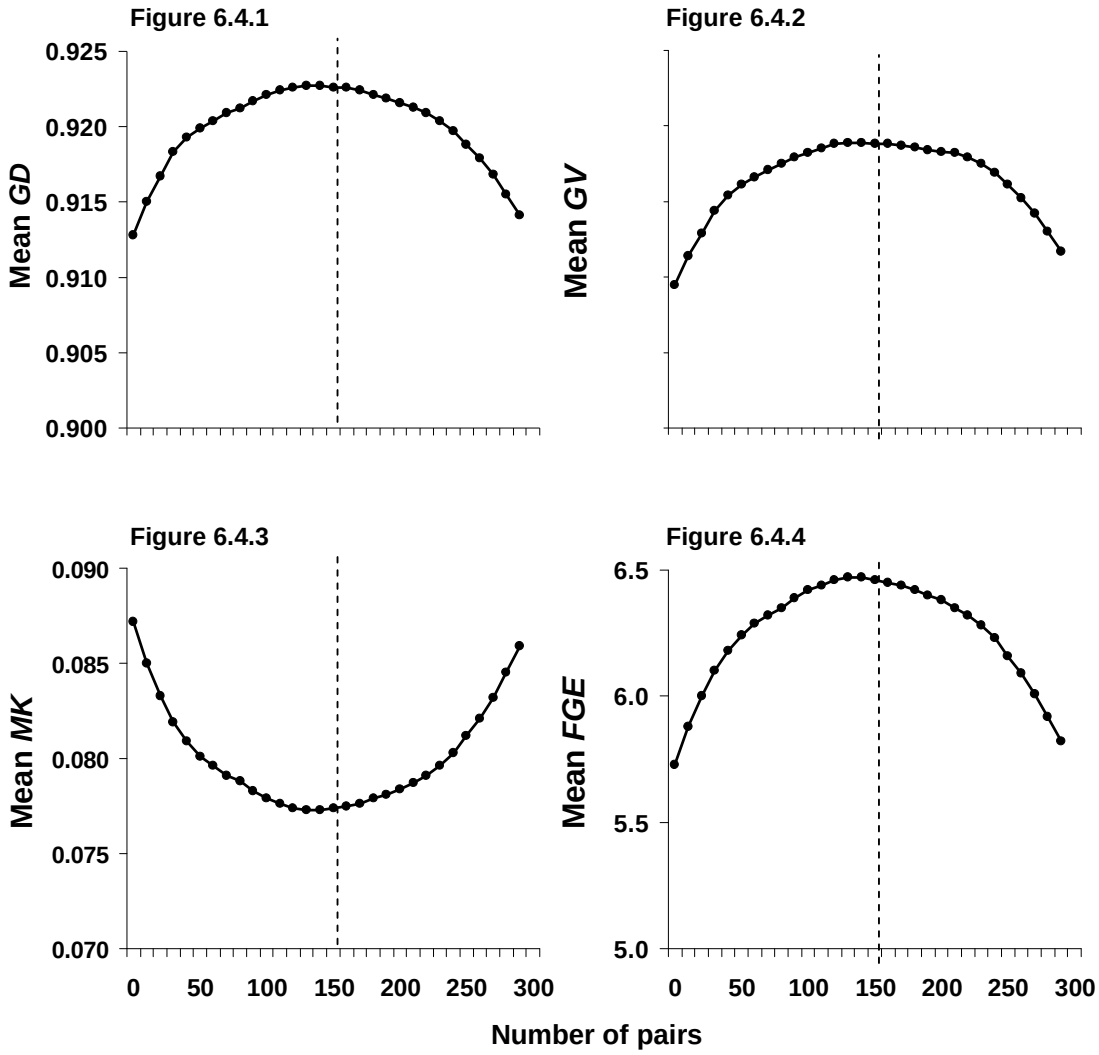


Figure 6.4 The modelled impact of reproduction (one live offspring per pair) on the retention of genetic diversity as represented by gene diversity (**6.4.1**), gene value (**6.4.2**), mean kinship (**6.4.3**) and founder genome equivalents (**6.4.4**). All four measures of genetic diversity began to decline after 150 breeding pairs were formed (as represented by the dashed line)

6.5 Discussion

The scimitar-horned oryx EEP population cannot meet the 90/100 and 90/200 goals without manipulation of demographic and genetic variables. The population had an effective population size larger than the minimum recommended for short-term avoidance of inbreeding, but smaller than N_e recommended for the long-term preservation of evolutionary potential (Frankham 1995a; Franklin 1980). Consequently, the population is likely to have reduced evolutionary potential which may impact on reintroduction success (Wisely *et al*. 2003).

The EEP would require a census population size of 2,655 individuals in order to achieve an N_e of 500, although this is based on the assumption that the N_e/N ratio remained unchanged. This assumption is not always met in real populations, and N_e/N may decrease as population size increases (Pray *et al.* 1996; Shrimpton & Heath 2003).

Increasing the population size alone did not meet the 90/100 and 90/200 goals, but it did improve the retention of *GD* in the population. It was only possible to meet the goals by simultaneously increasing two of *r*, *T*, N_e and *N*. Even a small increase in any of *r*, *T* or N_e resulted in the 90/100 and 90/200 goals being achieved, but the associated increases in population size were prohibitive. When parameter values were substantially increased, the associated *N* were reduced, but still remained large. For example, increasing the N_e/N ratio to 1.0 met the 90/100 goal with a population size increase of 100 individuals. Increasing the actual EEP population to $N = 535$ would impact on captive populations of other antelope species because of competition for space (Vogler *et al.* 2009), particularly other endangered desert antelope such as the addax and the Arabian oryx (Reitkerk & Glatston 2003). These two species already have much smaller EEP population sizes than the scimitar-horned oryx at 225 and 153, respectively, and decreasing them further would decrease their viability (Engel *pers. comm.*, 2011, Goodwin *pers. comm.*, 2011). The goals could be met without increasing population size by simultaneously increasing the N_e/N ratio and *T*, but an N_e/N of 1.0 and a *T* of 8.4 years, or N_e/N of 0.42 and a *T* of 20 years were required to meet the conservative 90/100 goal, and this is not a realistic prospect for the scimitar-horned oryx EEP population.

An idealised population of $N_e = 1.0$ assumes hermaphroditic individuals, equal family sizes, non-overlapping generations, stable population sizes, and no population subdivision (Ballou *et al.* 2010a; Briton *et al.* 1994; Charlesworth 2009; Höglund 2009;

Mace & Lande 1991; Wright 1931). The scimitar-horned oryx EEP population cannot meet these assumptions. Consequently, N_e will always be smaller than N.

The N_e is less than N when the sex ratio among breeders departs from parity, as occurs in polyganous mating systems (Briton *et al.* 1994; Frankham 1995b; Franklin 1980; Höglund 2009; Mace 1989; Nunney 1993; Rourke *et al.* 2009; Soulé 1980). Free-ranging scimitar-horned oryx formed herds with approximately equal sex ratios prior to their extinction in the wild (Newby 1974). In contrast, they are predominantly managed in polyganous groups in the EEP, with one male to several females in order to remove aggression between adult males when constrained by space (Engel 2004; Gilbert 2010a, 2010b). There is no variance in sex ratio at birth (Engel 2004; Gilbert 2010a; Wakefield & Engel 2004) and housing surplus males is a concern for the EEP. These males are either castrated, held in the two small single sex groups located in the EEP, or removed from the EEP population. This has resulted in a skewed sex ratio of approximately 1:3 males to females (Gilbert 2010a).

The reduction in N_e due to biased sex ratios can be understood in terms of the variance in reproductive success. Males have a high reproductive success relative to females in polyganous systems, so overall variance increases as the sex ratio bias increases (Nunney 1993). The 2010 EEP breeding and transfer plan recommended that 26 males and 114 females were paired for breeding, but harem sizes varied from one male with two females to one male with 20 females (Gilbert 2010b). As a result, there is also variance in reproductive success between males. A male with a larger harem makes a greater gametic contribution to the population than a male with a small harem, and this also increases variance (Basset *et al*. 2001; Frankham 1995b; Pearse & Anderson 2009). If the reproductive success of males and females is not equal between lineages, founder representation (family size) will vary, and this also reduces N_e (Ballou *et al.* 2010a; Frankham 1995b; Leus *et al.* 2011b; Mace 1989; Princée 1995). For example Matocq (2004) found that variation in family size reduced the N_e/N of woodrats *Neotoma macrotis* by approximately 54%.

The N_e of a population is also affected by fluctuating population size over a number of generations (Frankham 1995b; Leus *et al.* 2011b; Olsen & Klemetsdal 2010; Shrimpton & Heath 2003). Fluctuations are common in animal populations, and are caused by demographic and environmental stochasticity, and catastrophes (Frankham 1995b; Woodworth *et al.* 1994). The SHO EEP has recovered from a bottleneck caused when the captive population was founded in the mid-1960s, and in 2004 it numbered nearly 500

individuals. Such founding events, with subsequent growth, result in a reduced N_e compared to a population that has always remained at its present size (Charlesworth 2009). Since 2004 the SHO population has decreased (Gilbert 2010a). As the population is relatively small, it is subject to stochasticity, and carrying capacity has changed as institutions have joined and left the EEP. These processes have contributed towards a low N_e/N ratio for the species (Soulé *et al.* 1986). Similarly, the scimitar-horned oryx EEP population confounds the assumption of non-overlapping generations, and consequently the N_e is further reduced (Bishop *et al.* 2009; Leus *et al.* 2011b).

The scimitar-horned oryx EEP is not an unusual case and many species have comparable social systems, both in the wild and captivity, with overlapping generations, fluctuating population sizes and unequal family sizes (Basset *et al.* 2001; Soulé 1980).

Whilst it is not possible to obtain an N_e/N ratio of 1.0, it may be feasible to increase the ratio for the scimitar-horned oryx EEP population. Mace (1986) records the N_e/N ratio of captive scimitar-horned oryx as 0.20, and Frankham (1995b) reports N_e/N ratios for other herbivores at 0.23 for elk, 0.50 for wildebeest *Connochaetes taurinus*, and 0.44 for bighorn sheep. A study of captive populations by Lees and Wilcken (2009) revealed a mean N_e/N of 0.26 across 17 populations, which was lower than the mean of 0.34 reported by Frankham (1995b). However Frankham used 192 estimates of N_e/N across 102 model, wild, and captive species (including human) to calculate the mean ratio (Frankham 1995b). The estimates illustrate that higher N_e/N ratios are obtainable for captive populations.

Increasing the generation length also reduced the census size needed to meet the goals. The mean T can be manipulated by delaying the age of first reproduction for animals approaching reproductive maturity, and breeding from older individuals (Frankham & Loebel 1992; Mace 1989). Whilst this approach will theoretically increase T, in practice it may be difficult to achieve (Frankham 1995a). Firstly, it may not be possible to delay reproduction in young animals due to holding space restrictions. Secondly, many EEP institutions have expressed concern in delaying reproduction because of the fear that it causes early reproductive senescence. Finally, older animals may be less fecund (Gilbert 2010a; Mace 1989). Critically, mean longevity for EEP SHO males and females is 3.7 and 7.0 years, respectively, although the international studbook records SHO living to 29 years (Gilbert 2010a). Furthermore, the mean age of last reproduction is 8.4 and 9.2 years for males and females, respectively (Gilbert 2010a, 2010b), so T is restricted by both reproductive and actual lifespans and cannot be substantially increased above that observed in the EEP population. It should be noted that these data reflect historical demography, and

changes in management could alter future trends. If appropriate population management measures could be successfully implemented, then *T* could be extended (Gilbert 2010b; WAZA 2005e).

Reproductive technology such as cryopreservation of embryos and gametes can also extend *T* (Frankham 2005b; Gilbert 2010b). Much work has already been accomplished in this area for scimitar-horned oryx (Roth *et al.* 1999; Morrow & Monfort 1998; Morrow *et al*. 1999; Pope *et al.* 1991), although it is not currently applied to population management of the species. In theory, generation lengths can be extended indefinitely using cryopreservation techniques (Mace 1989). These management techniques may be able to increase scimitar-horned oryx *T* to some extent, but unless they are applied across the whole population, they will have a limited impact on extending generation length.

Contemporary population management uses the mean kinship (*MK*) method to maximise the retention of genetic diversity by compensating for past management which resulted in unequal family sizes (Ballou *et al.* 2010a; Frankham 2006, 2008). The *MK* method pairs the most genetically important individuals in order to meet the demographic requirements (*r*) of the population. When reproductive success fell below 100%, more individuals were paired to achieve the same *r*, and some of these had high *MK* coefficients (from over-represented families and so considered genetically unimportant). Although more *GD* is theoretically retained at increased growth rates, the results of the breeding pair indicated that *r* should not increase above 3-4% for reproductive success rates of 0.53, 7% for success rates of 0.64, and 11% for success rates of 0.75. When growth rates exceeded these values, overall retention of genetic diversity decreased. An increase in reproductive success would reduce the number of genetically unimportant females in breeding pairs, equalise family sizes, and increase retention of *GD* (Ballou *et al.* 2010a; Brown *et al.* 2005).

Reproductive success is a key factor in determining the impact of population management and growth on the retention of *GD*. The impact of reproductive success on the retention of genetic diversity should be extended to other species to provide a comprehensive evaluation of this factor. Research is also recommended to determine which variables influence reproductive success for captive scimitar-horned oryx in order to improve the retention of genetic diversity for this species.

Rapid population growth is sometimes an appropriate strategy when a population is in the growth phase of captive propagation, but once a population has reached capacity, as the scimitar-horned oryx EEP population has, the focus should be on genetic management

as long as demographic stability is maintained (Ballou *et al.* 2010a). Consequently, increasing *r* is not a viable strategy for conserving *GD* in the scimitar-horned oryx EEP population.

In this chapter I used individual and Latin square models to evaluate the impact of differing parameter values on the retention of gene diversity. Consequently, a maximum of two parameters were altered at any one time. The simulations were also restricted by the programming within PM2000. For example, the loss of *GD* could be simulated under various scenarios, but the impact of mutation was not modelled even in population sizes where mutation would occur. This may have led to an underestimate of genetic diversity at large population sizes.

It is not possible for the scimitar-horned oryx EEP to meet even the conservation 90/100 goal, unless substantial changes are made to the captive management of the species. Increasing the growth rate and generation length are not realistic options unless additional capacity is made available and reproductive technology is widely applied in conjunction with traditional population management techniques (Ballou & Cooper 1992b; Ballou *et al.* 2010a; Williams & Hoffman 2009).

The current N_e/N, and consequently the current N_e, is too small to allow retention of sufficient gene diversity. The N_e/N could be increased by changing from a herd structure to breeding pairs, but this would be in conflict with the social behaviour of the species (Briton *et al.* 1994; Frankham 1995a; Gilbert & Woodfine 2004a). Alternatively, reducing harem size, rotating males more frequently, and using cryopreservation and artificial insemination techniques to equalise male reproductive success and sex ratios would increase the N_e/N (Ballou *et al.* 2010a; Briton *et al.* 1994; Lande 1995; Mace 1986; Waples 2010). However, altering social structures and rotating males more frequently may be disruptive to the social structure in hierarchical and herd species like the scimitar-horned oryx (Ballou & Cooper 1992b; Mace 1989), and would increase the cost of maintaining the population. An alternative strategy, would be to allow breeding across all founder lineages and then to cull within families to equalise family sizes, although this has legal and ethical implications (Franklin 1980). Additional research into the precise effects of polygany and harem size on predicted N_e and the retention of *GD* would help to refine management recommendations.

Other populations have also experienced challenges in meeting the 90/100 or 90/200 goals. For example, Bishop *et al.* (2009) found that the Nile crocodile *Crocodylus niloticus* population could only retain 90% of *GD* for 100-years if the N_e were to increase and remain at >150, or >250 for 200-years.

Ideally, captive populations should endure no net loss of genetic diversity, but this is not possible for many species (Vogler *et al.* 2009), and so the 90/100 and 90/200 goals represents a compromise between what is ideal and what is realistic (Ballou *et al.* 2010a; Vogler *et al.* 2009). Despite this, it is still not achievable for many captive breeding programmes (Bishop *et al.* 2009; Gilbert 2009a, 2009b; Wisely *et al.* 2003). It is unknown how much genetic diversity can be lost before a species loses its ability to respond to environmental change (Frankham *et al.* 2010). The goal of retaining 90% of *GD* for either 100 or 200-years is arbitrary (Ballou & Cooper 1992b; Ballou *et al.* 2010a), and so the validity of such a goal is therefore questionable. It provides a quantitative guideline to develop management objectives (Ballou & Cooper 1992b) but at a timescale beyond the influence and responsibility of population managers. Consequently, more modest goals on shorter timescales may have a greater impact on retention of gene diversity because objectives would remain under the control of individual population managers.

The results presented in this chapter clearly demonstrate the importance of N_e on the retention of *GD*, and present an argument for changing the current management of the scimitar-horned oryx EEP population in order to increase N_e. The inability of the population to meet even the modest goal of 90% retention of founder *GD* for 100-years by increasing *r*, *T* or N_e within practical boundaries, questions the validity and applicability of the goal.

I now proceed to model the N_e and predicted loss of *GD* in relation to population sustainability for the scimitar-horned oryx, and other antelope and gazelle EEP populations, in Chapter Seven.

7.0 Chapter seven: the sustainability of captive populations

7.1 Abstract

Successful reintroduction projects depend on self-sustaining captive populations that are demographically stable and genetically diverse. Sustainability is a serious concern for captive populations as many have small census, and effective, population sizes. The problems associated with small populations are compounded by economic fragmentation and isolation of population sub-units.

This chapter aims to evaluate the impact of economic fragmentation on the sustainability of four threatened antelope and gazelle EEP populations; the scimitar-horned oryx, Arabian oryx, mhorr gazelle and dorcas gazelle. It then examines the sustainability of regional and global population populations of 111 different taxa.

Fragmenting the scimitar-horned oryx, Arabian oryx, mhorr gazelle and dorcas gazelle populations into unequal sized isolated sub-units reduced the effective population size, increased the loss of gene diversity, and increased the mean inbreeding of the sub-units and the metapopulation. Increasing fragmentation led to a reduction in predicted levels of genetic diversity after 100- and 200-years. Population sub-division may lead to substantial declines in genetic diversity in captive populations.

Examination of regional and global captive populations revealed that effective population sizes (N_e) and the ratio of effective to census population sizes (N_e/N) varied between taxa. Additionally, intensively managed regional populations, such as EEPs and SSPs, had higher N_e/N ratios than global populations for the same species. Despite this, many regional populations had effective population sizes below the minimum N_e needed for short-term viability ($N_e = 50$), and no regional or global population had an N_e needed for self-sustainability ($N_e = 500$).

The limitations of the study are discussed along with recommendations for further research.

7.2 Introduction

Successful reintroduction projects depend on self-sustaining captive populations that are genetically diverse and demographically robust to provide animals for release (Ballou 1992; Gusset & Dick 2011; Kleiman 1989). Self-sustainable populations should be able to persist without supplementation in perpetuity, and endure no net loss of genetic diversity (Ballou & Traylor-Holzer 2011; Lees & Wilcken 2011; Redford *et al.* 2011). Genetic diversity can be rapidly lost from small closed captive populations through genetic drift (Frankham 2003, 2006; Haig *et al.* 1990; Princée 1995).

Genetically viable populations are those large enough to retain a substantial proportion of genetic variation to avoid mutational meltdown and inbreeding, and adapt to future environmental change (Lacy 1993a; Traill *et al.* 2010). Franklin (1980) recommended a minimum short-term effective population size (N_e) of 50 to provide short-term genetic viability and avoid the immediate deleterious effects of inbreeding. Closed populations lose neutral gene diversity at a rate of $1/(2N_e)$ per generation due to drift (Ardren & Kapuscinski 2003; Matocq 2004; Nunney 1993), and gain variation at a rate of $2N_eu$ through mutation (where u is the mutation rate) (Nunney 2000). So, the smaller the N_e, the faster the rate of loss of *GD* through drift, the slower the rate of increase in *GD* through mutation, and over the long-term, the greater the total loss of genetic diversity (Ballou *et al.* 2010a). This chapter aims to evaluate the implications of small N_e on the retention of genetic diversity in four captive populations.

The smallest effective N_e which suffers no net loss of genetic diversity (i.e. where drift is balanced by mutation) is thought to be $N_e = 500$ (Boyce 1992; Lees & Wilcken 2011; Traill *et al.* 2010; Witzenberger & Hochkirch 2011; Vogler *et al.* 2009), although this varies between species (Miller & Waits 2003). Several authors have suggested that larger N_e, up to 5000, are required for the long-term retention of genetic diversity (Ballou & Traylor-Holzer 2011; Vogler *et al.* 2009). N_e is often much smaller than the census N (Chapter Six, Lees & Wilcken 2011; Traill *et al.* 2010; Reed *et al.* 2003c), and N_e/N ratios are typically in the range of 0.10-0.75 (Frankham 1995b; Franklin & Frankham 1998; Reed *et al.* 2003c; Reed *et al.* 2003b; Lees & Wilcken 2009; Nunney 2000). This translates into census population sizes of several thousand individuals (Ballou & Traylor-Holzer 2011; Lees & Wilcken 2011; Nunney 2000; Reed *et al.* 2003c).

Sustainability is a serious concern for captive populations (Lees & Wilcken 2009; Snyder *et al.* 1996). Evaluations of Australasian, European, and North American

populations under breeding management reveal that a large proportion are not genetically sound, and are too small to be self-sustaining (Junhold & Oberwemmer 2011; Leus *et al.* 2011a, 2011b; Long *et al.* 2011; Witzenberger & Hochkirch 2011). As a result they are vulnerable to extinction (Bryant *et al.* 1999).

Even those populations that are subject to active population management are not being managed for sustainability. Instead targets, such as the retention of 90% of *GD* for 100-years, specify a tolerable loss of *GD*, which implicitly acknowledges the difficulty of maintaining genetically sustainable populations (Ballou & Traylor-Holzer 2011; Traill *et al.* 2010). Numerous managed populations are unable to retain 90% of *GD* for 100-years (Frankham *et al.* 2010; Lande 1995; Leus *et al.* 2011b), and some already have *GD* below the 90% benchmark (Long *et al.* 2011).

There are a number of reasons why captive populations are not self-sustaining. To be genetically and demographically sustainable, captive populations need an N_e of at least 500-5000 (Ballou & Traylor-Holzer 2011; Vogler *et al.* 2009) which translates into actual population sizes of 1700 – 20,000 (Ballou & Traylor-Holzer 2011; Lees & Wilcken 2011). Captive breeding facilities do not have enough space to accommodate large viable populations for all species threatened with extinction, especially large-bodied animals, and as a consequence many populations are smaller than the minimum sustainable size (Ballou & Cooper 1992b; Ballou & Traylor-Holzer 2011; Lacy 1992; Leus *et al.* 2011b; Snyder *et al.* 1996).

Some captive populations are not sustainable because legislation and disease control measures have resulted in isolated and fragmented populations (Junhold & Oberwemmer 2011). Both EAZA and ZAA have identified that legislative barriers and fragmentation have impacted on the sustainability of captive populations for endangered species (Hibbard *et al.* 2011; Leus *et al.* 2011a). This chapter aims to evaluate the impact of population fragmentation on the sustainability of regionally coordinated breeding programmes.

Fragmentation occurs in both wild and captive populations, and is a major contributory factor to population extinction (Boyce 1992; Frankham 2010b; Hedrick *et al.* 1996; Henle *et al.* 2004; Price & Gittleman 2007). Population sub-division can have a serious impact on the retention of genetic diversity and the maintenance of demographic stability of both the metapopulation and individual sub-units (Laporte & Charlesworth 2002; Nunney 2000; Wang & Caballero 1999). In particular, population fragmentation, with no migration between sub-units, can reduce the N_e of both the metapopulation and the

sub-units (Soulé *et al.* 1986; Wang & Caballero 1999), leading to a rapid loss of genetic diversity (Ballou *et al.* 2010a).

The causes of population sub-division in the wild can be varied, but in captivity the two main reasons are legislative barriers, often in place to protect the economically important agricultural industry, and the high cost of animal transport over long distances (Hibbard *et al.* 2011; Junhold & Oberwemmer 2011; Leus *et al.* 2011a; Mace 1989; Margan *et al.* 1998).

Concerns over avian influenza in Europe have resulted in an EU import ban on all bird species, and transport restrictions have been implemented for bovidae in response to disease outbreaks (Leus *et al.* 2011a). For example there is currently no mechanism to allow the import of animals from non-EU countries into the EU unless Directive 2004/68/EC is fully enforced, and it is currently not possible to fully enforce it (DEFRA 2011). Historically, disease outbreaks within the EU have resulted in individual countries or regions being isolated. Specifically, the bovine spongiform encephalopathy (BSE) epidemic in the 1980s and 1990s, and the foot and mouth disease (FMD) outbreaks in the UK in 2001 and 2007, restricted animal transfers between the UK and the rest of the EU (Boden 2001; DEFRA 2010; Leus *et al.* 2011a; VLA 2011). More recently, outbreaks of six different bluetongue (BT) serotypes within the EU have limited the movement of bovidae between bluetongue serotype zones (DEFRA 2011). Individual countries or institutions may also specify their own import restrictions to control disease, for example import bans on animals that originate from regions, or collections, with World Organisation for Animal Health (OIE) listed diseases (Appendix I) (DEFRA 2010; OIE 2011).

Despite the sub-division of captive populations, most actively managed populations (EEPs and SSPs), are generally regarded as a single panmictic population, or a metapopulation with unrestricted gene-flow between the sub-units or demes (Kaumanns *et al.* 2008; Margan *et al.* 1998). This is not an accurate representation of the EEP or SSP populations. For example, the scimitar-horned oryx EEP population is spread across 16 different countries including two that are outside the EU (Croatia and Israel), and five different European bluetongue serotype zones (Figure 7.1). There is unequal dispersal between countries and bluetongue zones (Gilbert 2010a), and EEP breeding and transfer recommendations have had to be amended to incorporate legislative barriers to migration (Gilbert 2010b; Junhold & Oberwemmer 2011). Consequently, the EEP population can be

considered a metapopulation made up of a number of unequal sized sub-units or demes, rather than a single panmictic population.

The issues associated with population fragmentation in the scimitar-horned oryx EEP population apply also to other captive populations. In particular, those species that are covered by European legislation aimed at the agricultural industries, for example the bovidae family. Thirty-three out of 34 cattle, antelope, giraffe, sheep and goat EEP and ESB species have populations that extend over multiple countries and multiple bluetongue zones (Table 7.1). Sixteen of these species are included on the IUCN Red List of threatened species (IUCN 2010), and so the ESBs and EEPs contribute to assurance populations for the species (EAZA 2010b; WAZA 2005a).

The issues associated with fragmented populations extend beyond regional coordinated breeding programmes. There has been a growing recognition that regional populations are not self-sustainable and that inter-regional or global management is required for long-term species conservation (Lees & Wilcken 2009; WAZA 2005e; Wood *et al.* 2008). However, the legislative and cost barriers that cause the fragmentation of regional populations also apply to global populations. This chapter evaluates the implications of global population fragmentation on 111 taxa.

This chapter aims to evaluate the impact of population fragmentation on the sustainability of captive populations using the scimitar-horned oryx, Arabian oryx, dorcas gazelle, and mhorr gazelle EEP populations as examples. The specific objectives are: 1) evaluate the genetic impact of economic fragmentation on the scimitar-horned oryx EEP population, using EU legislation as a framework; 2) Determine if the genetic impact of economic fragmentation applies to additional endangered species in captivity; 3) Evaluate the sustainability of regional and global captive populations for a wide range of taxa.

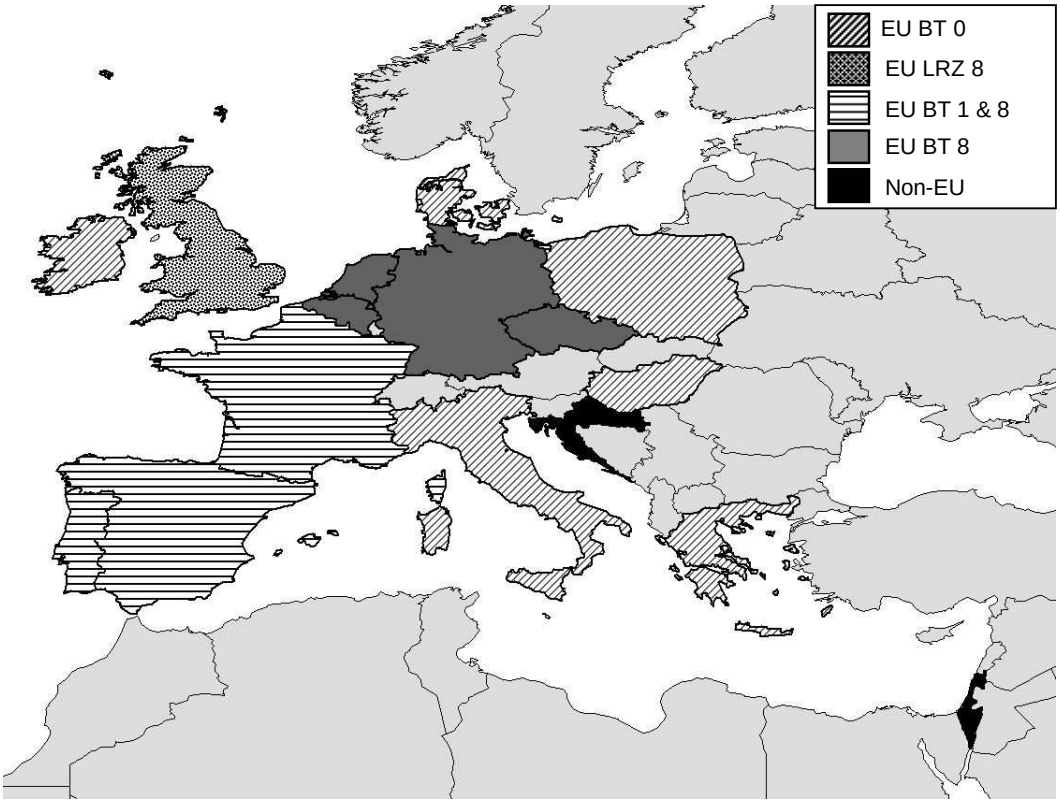


Figure 7.1 Map of the scimitar-horned oryx EEP region: EU with no bluetongue (EU no BT); EU bluetongue lower risk zone serotype 8 (EU BT LRZ 8); EU bluetongue serotype 1 and 8 (EU BT 1 & 8); EU bluetongue serotype 8 (EU BT 8), and; non-EU. The light grey countries do not have scimitar-horned oryx or are not part of the EEP (DEFRA 2010; Gilbert 2010a)

Table 7.1 A list of bovidae EEPs and ESBs whose populations cover multiple countries and multiple bluetongue zones (EAZA 2010b; ISIS 2010; IUCN 2010). Please refer to the Acronyms on page three for an explanation of the country codes

Species	Countries	IUCN status
Cattle & camelid TAG		
African buffalo *Syncerus caffer*	AE, DE, ES, GB	LC
Anoa *Bubalus depressicornis*	BE, CZ, DE, FR, GB, HU, NL, PL	EN
Banteng *Bos javanicus*	DE, FR, GB, IT, NL, PL	EN
European bison *Bison bonasus*	AT, BE, CH, CZ, DE, DK, ES, FR, GB, HU, IT, LT, NL, PL, RU, SE, SK, UA	VU
Gaur *Bos gaurus*	DE, EE, FR, GB	VU
Antelope and giraffe TAG		
Addax *Addax nasomaculatus*	AE, CZ, DE, FR, GB, HR, HU, IL, IT, NL, PL, PT, QA, SE, SK	CR
Arabian oryx *Oryx leucoryx*	AE, AT, BE, BG, CH, CZ, DE, ES, FR, GB, IL, PT, QA	VU
Blue duiker *Cephalophus monticola*	DE, ES, FR, NL	LC
Bontebok *Damaliscus dorcas*	AE, ES	LC
Dorcas gazelle *Gazella dorcas neglecta*	DE, ES, FR, GB	VU
Mhorr gazelle *Nanger dama mhorr*	DE, ES, FR, HU, NL	CR
Eastern bongo *Tragelaphus eurycerus isaaci*	AE, BE, CZ, DE, DK, ES, FR, GB, IE, NL, PL, PT, SE, SK	CR
Giraffe *Giraffa camelopardalis*	AE, AT, BE, CH, CZ, DE, DK, ES, FR, GB, GR, HU, IE, IL, IT, KZ, LT, LV, NL, NO, PL, PT, SE, SI, SK, RU, UA	LC
Greater kudu *Tragelaphus strepsiceros*	CZ, DE, DK, EE, FR, GE, GB, HU, NL, PL, RU, SK	LC
Kirk's dik dik *Madoqua kirkii*	AE, BE, DE, ES, FR, GB, GE, IL, NL, PL, SK	LC
Lechwe *Kobus leche*	AT, BE, DE, FR, GB, PL, SE	LC
Lesser kudu *Tragelaphus imberbis*	CH, DE, FR, GB, PL	NT
Lowland nyala *Tragelaphus angasii*	AE, AT, CZ, DE, FR, GB, IL, IT, KZ, NL, PL, PT	LC
Nile lechwe *Kobus megaceros*	CZ, DE, FR, GB, IT, PL	EN
Okapi *Okapia johnstoni*	BE, CH, FR, DE, DK, GB, NL, PT	NT
Roan antelope *Hippotragus equinus*	AE, AT, DE, FR, GB, HU, IT, NL, PT	LC
Sable antelope *Hippotragus niger*	CZ, DE, DK, ES, FR, GB, NL, SE, RU	LC
Scimitar-horned oryx *Oryx dammah*	BE, CZ, DE, DK, ES, FR, HR, GB, GR, HU, IE, IL, IT, NL, PL, PT	EW
Western sitatunga *Tragelaphus spekii gratus*	AT, BE, CZ, DE, DK, ES, FR, GB, GR, HR, HU, IT, NL, PL, PT, SK	LC

Table 7.1 continued

Species	Countries	IUCN status
Sheep and goat TAG		
Blue sheep *Pseudois nayaur*	CZ, DE, EE, FR, GB, RU	LC
Chinese goral *Naemorhedus caudatus*	CZ, DE, EE, FR, GB, PL	VU
East Caucasian tur *Capra cylindricornis*	DE, EE, FR, GE, RU	NT
Japanese serow *Naemorhedus crispus*	AT, CZ, DE, GB,	LC
Muskox *Ovibos moschatus*	CZ, FI, FR, LT, NL, NO, RU	LC
Nubian ibex *Capra nubiana*	AE, CH, EE, FR, IL, QA	VU
Takin *Budorcas taxicolor*	BE, CZ, DE, DK, EE, FI, FR, GB, HU, LV, PL, SK, RU	VU
Turkmenian markhor *Capra falconeri heptneri*	AE, CZ, DE, EE, FI, FR, GB, IT, KZ, LV, MD, PL, RU, SE, SK, UA	EN
West Caucasian tur *Capra caucasica*	CZ, DE, EE, FR, SK,	EN

7.3 Methodology

7.3.1 Fragmentation of the scimitar-horned oryx EEP population

At the time of analysis, the scimitar-horned oryx EEP population was fragmented into unequal sized sub-units based on EU membership, bluetongue zones, and individual countries (Figure 7.1). Figure 7.2 illustrates allowable inter-bluetongue-zone transfers, with associated effective population sizes, within the scimitar-horned oryx EEP population as of March 2011.

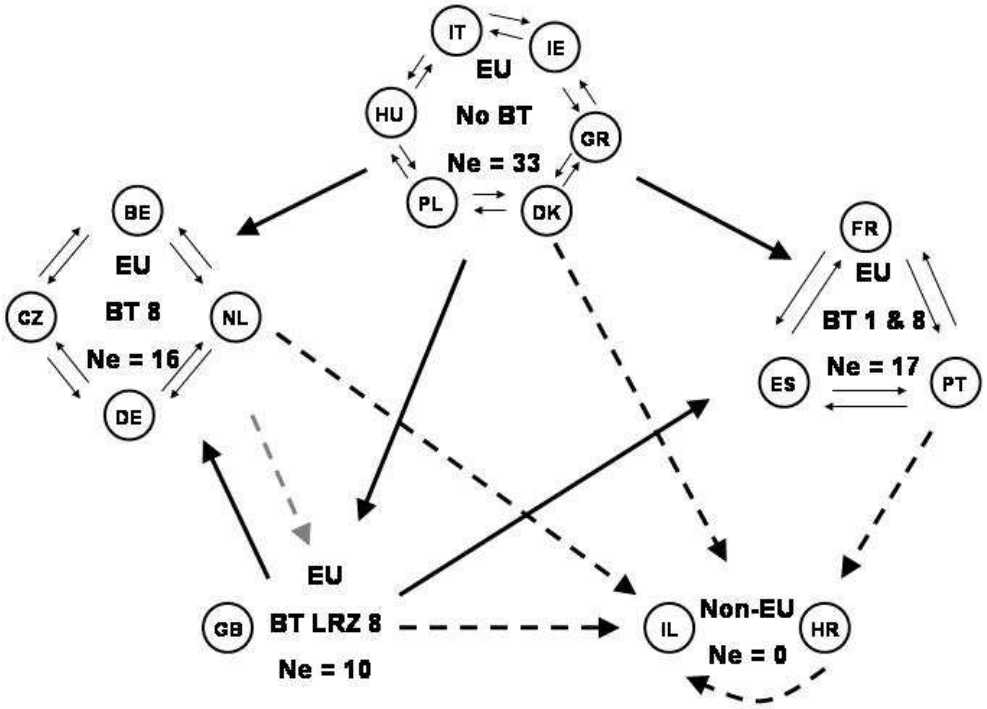


Figure 7.2 Allowable transfers between the five bluetongue zones and EU countries in the scimitar-horned oryx EEP. Corresponding N_e for each bluetongue sub-unit are included. Arrows indicate direction of animal transfers currently possible within the EEP population. The grey arrow between BT8 and BT LRZ8 indicates that transfers may be possible but are only considered on an individual basis by DEFRA

Abbreviations for each sub-unit of the metapopulation are listed in Table 7.2. The metapopulation subunits varied in size depending on the criteria used to fragment the EEP (Table 7.3).

In order to evaluate the genetic consequences of population fragmentation, both the metapopulation and each sub-unit needed to be examined (Nunney 2000). The international studbook for scimitar-horned oryx was the source of quantitative data on

international transfers between metapopulation sub-units of EU and non-EU countries, UK and continental EU countries, and bluetongue zones, and between individual countries to quantify transfers between metapopulation sub-units.

Table 7.2 Abbreviations for populations and population sub-units

Population	Abbreviation
International studbook population (global population)	ISB
European Endangered species Programme population	EEP
European Studbook population	ESB
Species Survival Plan population	SSP
Population Management Plan population	PMP
European Union countries	EU
Non-European Union countries	Non-EU
Bluetongue free zone	No BT
Bluetongue serotype 8 zone	BT 8
Bluetongue serotype 1 and 8 zone	BT 1 & 8
Bluetongue serotype 8 low-risk zone	BT LRZ 8
United Arab Emirates (UAE)	AE
Austria	AT
Belgium	BE
Bulgaria	BG
Switzerland	CH
Czech Republic	CZ
Germany	DE
Denmark	DK
Spain	ES
France	FR
United Kingdom	GB
Greece	GR
Croatia	HR
Hungary	HU
Ireland	IE
Israel	IL
Italy	IT
Netherlands	NL
Poland	PL
Portugal	PT
Qatar	QA

Data exports from SPARKS (Scobie *et al.* 2004) to PM2000 (Pollak *et al.* 2007) used the same filter conditions as the data export in Chapter Six with a further filter that removed all castrated or permanently sterilised animals (regardless of the date) to make the population dynamics more representative of the current population. This resulted in a population size of $N = 390$. Demographic analyses based on life-table data were then completed, kinship matrices simulated using the additive matrix method (Ballou 1983), and a gene drop analysis with 10,000 iterations was run (Pollak *et al.* 2007). Genetic metrics for *GD*, *MK*, *F*, current and historical N_e, N_e/N ratio, and founder allele retention

were extracted from the analyses to provide comparative values for the whole EEP population. This process was then repeated for each metapopulation sub-unit as listed in Table 7.3.

Table 7.3 Metapopulation structures for the fragmented scimitar-horned oryx EEP population. The different models represent a nested hierarchy of increasing fragmentation. **N**: population size

Metapopulation	Countries	N
1. Whole EEP	BE, CZ, DE, DK, ES, FR, HR, GB, GR, HU, IE, IL, IT, NL, PL, PT	390
2. European Union		
EU	BE, CZ, DE, DK, ES, FR, GB, GR, HU, IE, IT, NL, PL, PT	342
Non-EU	HR, IL	48
3. UK isolation		
EU excluding UK	BE, CZ, DE, DK, ES, FR, GR, HU, IE, IT, NL, PL, PT	272
UK	GB	70
Non-EU	HR, IL	48
4. Bluetongue zones		
EU no BT	DK, GR, HU, IE, IT, PL	104
EU BT 1 & 8	ES, FR, PT	107
EU BT 8	BE, CZ, DK, NL	61
EU BT LRZ 8	GB	70
Non-EU	HR, IL	48
5. Countries		
BE	Belgium	11
CZ	Czech Republic	9
DE	Germany	23
DK	Denmark	21
ES	Spain	28
FR	France	76
GB	United Kingdom	70
GR	Greece	12
HR	Croatia	7
HU	Hungary	2
IE	Ireland	19
IL	Israel	41
IT	Italy	21
NL	Netherlands	18
PL	Poland	29
PT	Portugal	3

7.3.2 Retention of genetic diversity in a fragmented population

Gene diversity is lost from a closed population at a rate of $1/(2N_e)$ per generation (Ballou *et al.* 2010a). The equation $GD\ lost_t = 1/(2N_e)$ was applied to theoretical populations of five different sizes (N = 423, N = 1000, N = 5,000, N = 10,000 and N = 50,000) with N_e/N ratios increasing in increments of 0.01 from N_e/N = 0.01 to an ideal population of N_e/N = 1.00 in order to map the combined effects of population size and N_e/N

ratio on the per generation loss of *GD*. Gene diversity lost per generation, current *GD,* current N_e/N ratios and mean *F* were then modelled for each metapopulation sub-unit. Subsequently, current *GD* as a proportion of original founder *GD* was modelled against predicted *GD* at 100 and 200-years after the population was founded, using the amount of *GD* lost per generation as a factor for each metapopulation sub-unit.

The theoretical impact of sustained small N_e on the very long-term decline of *GD* (expected heterozygosity) can be modelled for each sub-unit of the metapopulation using the equation $H_t/ H_0 = 1 – [1/(2N_e)]^t$ (Frankham *et al.* 2010) in which H_t/ H_0 represents the predicted heterozygosity as a proportion of founder heterozygosity at generation *t*. This equation was applied to various effective population sizes derived from the metapopulation sub-units to evaluate the impact of sustained small N_e on loss of heterozygosity after 5 – 100 generations.

Each metapopulation sub-unit has its own founders, and whilst some of these were shared between sub-units, others were unique to a particular deme. Founder allele retention in the descendant population was derived using gene drop simulations with 10,000 iterations for the current EEP, the EU, non-EU, and bluetongue zones BT 0, BT 8, BT 1 & 8 and BT LRZ 8 sub-units. Data residuals were not normally distributed and did not respond to transformation, so a Wilcoxan signed rank test was applied to paired EU and non-EU data, and a Kruskal-Wallis test was applied to the bluetongue zone sub-units to evaluate differences in allele retention between demes.

The associations between mean current N_e and extant *GD,* mean historical N_e and extant *GD*, and *N* and N_e were evaluated using Spearman's rank correlation as data residuals were not normally distributed and did not respond to transformation. Bluetongue zone 'BT LRZ8' was removed as it was a duplicate of the 'GB' sub-unit resulting in *N*=23.

The difference between mean *F* and average *MK* in each sub-unit and a contiguous EEP population was quantified in order to evaluate the impact of population fragmentation on those genetic parameters.

7.3.3 Arabian oryx, and mhorr and dorcas gazelle EEP populations

Permission to analyse the fragmentation in EEP populations was obtained from the EEP coordinators for four species of aridland antelope and gazelle: Arabian oryx *Oryx leucoryx*, Mhorr gazelle *Dama gazella mhorr*, Sarahawi dorcas gazelle *Gazella dorcas neglecta* and Cuvier's gazelle *Gazella cuvieri.* All four species are included on the IUCN Red List of threatened species (IUCN 2010), they are subject to European movement

restrictions for bovidae, and the captive populations are important assurance populations in their own right as well as being a source of animals for reintroduction projects. Consequently, it is important that these populations are managed for the long-term retention of genetic diversity and demographic stability.

The SPARKS data sets for Arabian oryx, and mhorr, Cuvier's and dorcas gazelle were obtained directly from the respective EEP coordinators to ensure current data were used. Data sets were up to date until 31/12/2010, 10/03/2011, 14/01/2008 and 31/12/2010, respectively. An analytical SPARKS data set was used for the European Arabian oryx population instead of the true studbook, as used for the other three species, because the true Arabian oryx studbook had a pedigree completeness for EEP institutions of only 44%. Replacing the true Arabian oryx studbook with the analytical studbook increased pedigree completeness to 68% (100% for European institutions, and 49% for Middle Eastern institutions). Assumptions to fill in missing pedigree data had not been made for many Middle Eastern animals because of a lack of information on which to base the assumptions (Chapter Five). Pedigree completeness for the EEP populations was 99%, 100% and 100% for mhorr, Cuvier's and dorcas gazelles respectively.

Demographic data were exported from each studbook data set in SPARKS v.1.56 for animals living in EAZA institutions between 01/01/1990 and 31/12/2010. Genetic data were exported for animals living on the 'current to' date in EAZA institutions. The demographic and genetic files were imported into PM2000 v.1.213. The date set for calculations was the 'current to' date for each programme. Data were filtered in PM2000 by removing those animals that were owned by, but not located at, EEP institutions, and those animals that were permanently sterilised or castrated. Sample sizes were $N = 182$, $N = 178$, $N = 121$, $N = 208$ for Arabian oryx, and mhorr, Cuvier's and dorcas gazelle, respectively.

Demographic analyses based on life-table data were completed, kinship matrices simulated using the additive matrix method, and gene drop analyses with 10,000 iterations were run for each population. This process was then repeated for each metapopulation sub-unit for each species as listed in Tables 7.4, 7.5 and 7.6 to obtain comparative genetic metrics (*GD*, N_e, N_e/N, *MK*, *F*). The Cuvier's gazelle data were then removed from the analyses as the whole EEP population for Cuvier's gazelle was held in three institutions in Spain, and therefore not subject to population fragmentation.

The rate of *GD* lost per generation was calculated for each metapopulation sub-unit for each species using the equation $GD\ lost_t = 1/(2N_e)$.

Table 7.4 Metapopulation structure with associated population sizes for the Arabian oryx. The different models represent a nested hierarchy of increasing fragmentation.*Switzerland is not part of the EU, but reciprocal agreements mean that there are no barriers to the transport of exotic bovidae between the EU and Switzerland. **N**: population size

Metapopulation	Countries	*N*
1. Whole EEP	AE, AT, BE, BG, CH, CZ, DE, ES, FR, GB, IL, PT, QA	182
2. European Union		
EU	AT, BE, BG, CH*, CZ, DE, ES, FR, GB, PT	68
non-EU	AE, IL, QA	114
3. UK isolation		
EU excl. UK	AT, BE, BG, CH*, CZ, DE, ES, FR, PT	61
UK	GB	7
Non-EU	AE, IL, QA	114
4. Bluetongue zones		
EU no BT	AT, BG	5
EU BT 1 & 8	ES, FR, PT	30
EU BT 8	BE, CH*, CZ, DE	25
EU BT LRZ 8	GB	7
Non-EU	AE, IL, QA	114
5. Countries		
AE	UAE	66
AT	Austria	1
BE	Belgium	7
BG	Bulgaria	5
CH	Switzerland	6
CZ	Czech Republic	3
DE	Germany	9
ES	Spain	1
FR	France	20
GB	United Kingdom	7
IL	Israel	10
PT	Portugal	9
QA	Qatar	38

Table 7.5 Metapopulation structure with associated population sizes for the mhorr gazelle. The different models represent a nested hierarchy of increasing fragmentation. **N**: population size

Metapopulation	Countries	*N*
1. Whole EEP	DE, ES, FR, HU, NL	178
2. European Union		
EU	DE, ES, FR, HU, NL	178
3. UK isolation		
EU excl. UK	DE, ES, FR, HU, NL	178
UK	-	0
Non-EU	-	0
4. Bluetongue zones		
EU no BT	HU	6
EU BT 1 & 8	ES, FR	122
EU BT 8	DE, NL	50
5. Countries		
DE	DE	39
ES	ES	115
FR	FR	7
HU	HU	6
NL	NL	11

Table 7.6 Metapopulation structure with associated population sizes for the Sarahawi dorcas gazelle. The different models represent a nested hierarchy of increasing fragmentation. **N**: population size

Metapopulation	Countries	*N*
1. Whole EEP	DE, ES, FR, GB	208
2. European Union		
EU	DE, ES, FR, GB	208
3. UK isolation		
EU excl. UK	DE, ES, FR	196
UK	GB	12
4. Bluetongue zones		
EU BT 1 & 8	ES, FR	183
EU BT 8	DE	13
EU BT LRZ 8	GB	12
5. Countries		
DE	DE	13
ES	ES	179
FR	FR	4

7.3.4 Global captive populations

The ISIS/WAZA studbook library database version 2006/2007/2008 (ISIS 2009) listed 194 individual taxa in international studbooks. Studbooks were selected if they were active (non-archived), had a 'current to' date from 2001 onwards, and had a SPARKS data file available. Duplicate studbooks were removed, for example the grey gentle lemur *Hapalemur griseus* had two studbooks, one that contained the species and the other than contained hybrids and animals of unknown origin. The species studbook was included, and the studbook containing the hybrids was rejected. Data from AZA (AZA 2010) and EAZA (EAZA 2010b) were used to select international studbooks for species with at least one formal coordinated captive breeding programme: Species Survival Programmes (SSP) and Population Management Plans (PMP) for AZA populations, and EEPs and European Studbook Programmes (ESB) for EAZA populations. The Australasian Zoo and Aquarium Association (ZAA) ASMP programmes were not included as data on programmes were not readily available. In total 121 international studbooks were included. The size of the living population in each studbook ranged from $N = 1$ individual for the Didem sifaka *Propithecus diadema*, to $N = 2706$ individuals for the cotton-top tamarin *Saguinus Oedipus*.

Demographic and genetic data for each studbook were exported from SPARKS and imported into PM2000. The date set for calculations was the 'current to' date for each studbook. Demographic and genetic analyses were carried out to obtain the N_e and N_e/N ratios for each international studbook population. Data were then exported again from SPARKS using filter files to remove data from non-AZA and non-EAZA institutions from the analyses, to obtain N_e and N_e/N values for regional managed populations.

Only 21 (17%) of the studbooks had 100% known pedigree, and eight studbooks had less than 20% pedigree completeness. As large amounts of missing data impacts on genetic and demographic analyses (Chapter Four), any studbook or regional population with less than 62.5% pedigree completeness was removed from analyses. This resulted in 104 international studbook populations and 132 regional (EEP, ESB, SSP and PMP) populations.

The populations were then separated into taxonomic groups according to the AZA and EAZA taxon advisory groups (TAG), which oversee management of coordinated captive breeding programmes (Appendix J). The mean N_e and N_e/N were calculated for

global populations (ISB) and regional (AZA and EAZA) populations for each taxonomic group.

The impact of regionally managed populations on N_e and N_e/N in comparison to (mostly) unmanaged ISB populations was assessed by comparing the N_e and N_e/N derived from the ISB population with those derived from AZA and EAZA populations for each species. Species that had an ISB population with >62.5% pedigree completeness, and at least one AZA or EAZA programme population with >62.5% pedigree completeness were selected, resulting in $N = 86$. Differences between the N_e and N_e/N ratios of global and regional populations were calculated.

Differences between AZA and EAZA regional populations in comparison to ISB populations were evaluated by selecting species with an ISB population, an SSP/PMP population, and an EEP/ESB population. Species were included if all three data sets had more than 62.5% pedigree completeness, resulting in $N = 41$. Differences in N_e/N between global populations and each regional population were compared using a paired t-test. Data for N_e were not normally distributed and were transformed using Johnson transformation. Paired t-tests were then applied to ISB versus EAZA population, and ISB versus AZA populations.

7.4 Results

7.4.1 Fragmented populations

Before the bluetongue outbreak in Europe (2000-2006 inclusive) 58% of male and 61% of female scimitar-horned oryx transfers occurred within the same bluetongue zone. From 2007 to 2010 inclusive 67% of male and 78% of female transfers took place between institutions within the same bluetongue zone. There have been no transfers from Europe to Israel (non-EU) since 1973, and no recorded transfers in the history of the studbook from Israel to Europe. Similarly oryx were transferred from the EU to Croatia (non-EU) in 2000 and 2003, but there have been no recorded transfers from Croatia to any institutions within the EU.

Gene diversity is lost at a rate of $1/(2\ N_e)$ and a predicted loss of 0.61% of *GD* per generation was calculated for the whole EEP population. If the historical EEP N_e (N_e = 38) had been the same as the current N_e (N_e = 80) then it would have been possible to retain a maximum of 91.23% of the founders' *GD* for 100-years (assuming a *T* of 6.7 years (Chapter Six), and discrete generations resulting in 15 generations per 100-years). The small historical N_e resulted in an accelerated loss of *GD*, totalling 8.8% loss of *GD* since the founders were captured in the 1964 and 1967 (Gilbert 2010a). The *GD* observed in the EEP population at the time of writing (91.2%) was the same as the projected level of *GD* at filial generation 15 in a model where historical N_e equalled current N_e (N_e = 80).

When the scimitar-horned oryx EEP population was modelled as fragmented, the sum of the effective population sizes for each sub-unit was less than that of the contiguous EEP population (Figures 7.3, 7.4 and 7.5). As a result *GD* was predicted to erode faster in all of the fragmentation scenarios, than the whole EEP. The cumulative N_e was the same for the EU, UK and non-EU model and the bluetongue model (N_e = 76), which were only slightly smaller than the EU and non-EU model at N_e = 77. However, *GD* loss per generation is related to individual sub-unit N_e not the cumulative metapopulation N_e, so *GD* will be lost at a faster rate in the bluetongue model than the EU, UK and non-EU model. Sub-dividing the EEP population into country sub-units resulted in a cumulative N_e of 58, but five sub-units had an N_e of 0.0, so all *GD* would be lost in one generation from those demes. This level of fragmentation was predicted to result in a rapid decrease in gene diversity.

Sub-units with smaller effective population sizes (e.g. BT LRZ 8, N_e = 16, 5% *GD* lost generation^{-1}) were predicted to lose *GD* at a faster rate than population fragments with a larger effective population size (e.g. no BT, N_e = 33, 1.5% *GD* loss generation^{-1}). All sub-units were predicted to lose *GD* at a much faster rate than the contiguous EEP population (N_e = 80, 0.6% *GD* lost generation^{-1} Figures 7.3 and 7.4). Some fragments e.g. the non-EU sub-unit, had an N_e = 0, and were predicted to lose all *GD* within one generation. When the EEP population was fragmented into individual countries with no migration between sub-units, five countries had an N_e = 0, three countries had the largest effective population sizes of N_e = 10, and the remainder varied between N_e = 2 and 9. Consequently predicted *GD* lost generation^{-1} per country varied between 5% and 100% (Figure 7.3).

The ratio of N_e/N varied between population sub-units ($\bar{x}$ = 0.17, σ = 0.13). The whole EEP population had an N_e/N = 0.19, but individual country ratios varied between N_e/N = 0.0 and 0.48, and bluetongue zone population sub-units varied between N_e/N = 0.0 and 0.31 (Figure 7.3). Census population size was clearly associated with N_e (r_{22} = 0.703, P < 0.001).

The scimitar-horned oryx EEP population had a current effective population size of N_e = 80. After 50 generations $H_t/ H_0 = 1 – [1/(2*80)]^{50}$ = 0.72, which equated to a 28% loss of heterozygosity from the population, and 47% loss of heterozygosity after 100 generations. Heterozygosity decreased more rapidly with decreasing N_e, to the point where almost all heterozygosity was predicted to be lost in a 100 generations for populations with an N_e of 10 or less (e.g. BT LRZ8 and non-EU sub-units) (Figure 7.5). Effective population sizes of N_e = 16, as found in the BT 8 zone, were predicted to retain only 53% of heterozygosity after 20 generations and 4% of heterozygosity after 100 generations. As the N_e increased, so did the amount of heterozygosity retained until the effective population size reached N_e = 80 for the entire EEP population, when the model predicted that 53% heterozygosity will be retained after 100 generations (Figure 7.5).

Figure 7.6 demonstrated that a fragmented EEP population can maintain much less founder *GD* over 100- and 200-years than a larger contiguous EEP population. Current *GD* and current N_e, and current *GD* and historical N_e in each fragment were correlated (r_{22} = 0.715, P < 0.001 and r_{22} = 0.951, P < 0.001 respectively) (Figure 7.3) with more *GD* retained in sub-units with higher current and historical effective population sizes.

Founder allele retention within the contiguous EEP was uneven with very low levels of retention for some founders, for example founder studbook numbers 5060 and 5064

(0.0017 and 0.0061 founder allele retention, respectively) and high levels of retention for others e.g. 5236 and 5704 (0.9835 and 0.9633 respectively) (Figure 7.7). Furthermore, allele distribution across the EEP, and therefore between sub-units, was uneven resulting in some founders alleles being present in only a few sub-units. There was a difference in allele retention between the EU and non-EU sub-units ($Z_{35} = 573$, $P < 0.001$). Most (69%) of the founder alleles were retained in both the EU and non-EU populations to differing extents, but 10 founders had no alleles retained in the non-EU population, and one founder (5684) only had alleles retained in the non-EU population (Figure 7.7.1). This variance in allele retention between founders was mirrored when the EEP population was fragmented into the different bluetongue zone sub-units ($H_4 = 15.38$, $P = 0.004$) with alleles from two founders (5060 and 5064) only found in BT 1 & 8, and others only retained in small frequencies in several populations, for example 5692 found in no-BT (0.0549), BT 1 & 8 (0.0131) and BT LRZ 8 (0.0669). In contrast, some founders, for example studbook number 5236 had a high allele retention in all sub-units (Figure 7.7.2). The uneven founder allele retention and distribution between sub-units means that any sub-unit extinction will result in some founder alleles being lost from the EEP population.

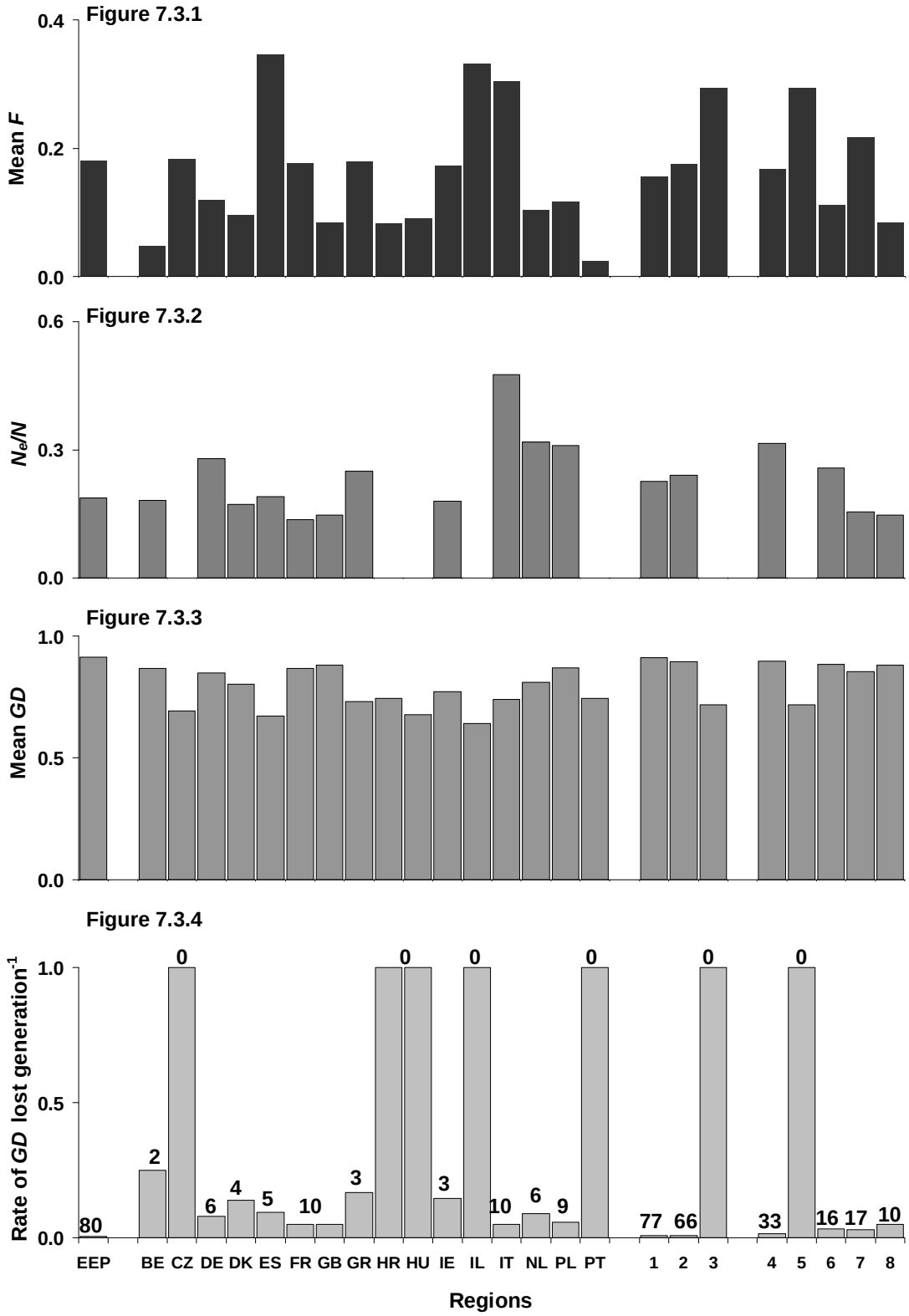


Figure 7.3 Genetic diversity of the fragmented scimitar-horned oryx EEP population as represented by: the mean *F* (**7.3.1**), the N_e/N ratio (**7.3.2**), mean *GD* (**7.3.3**), and rate of *GD* lost per generation (**7.3.4**). The N_e of each population is annotated on **7.3.4**. Sub-units: EU (1), EU excluding the UK (2), non-EU (3), no BT (4), non-EU (5), BT 8 (6), BT 1 & 8 (7), and LRZ 8 (8)

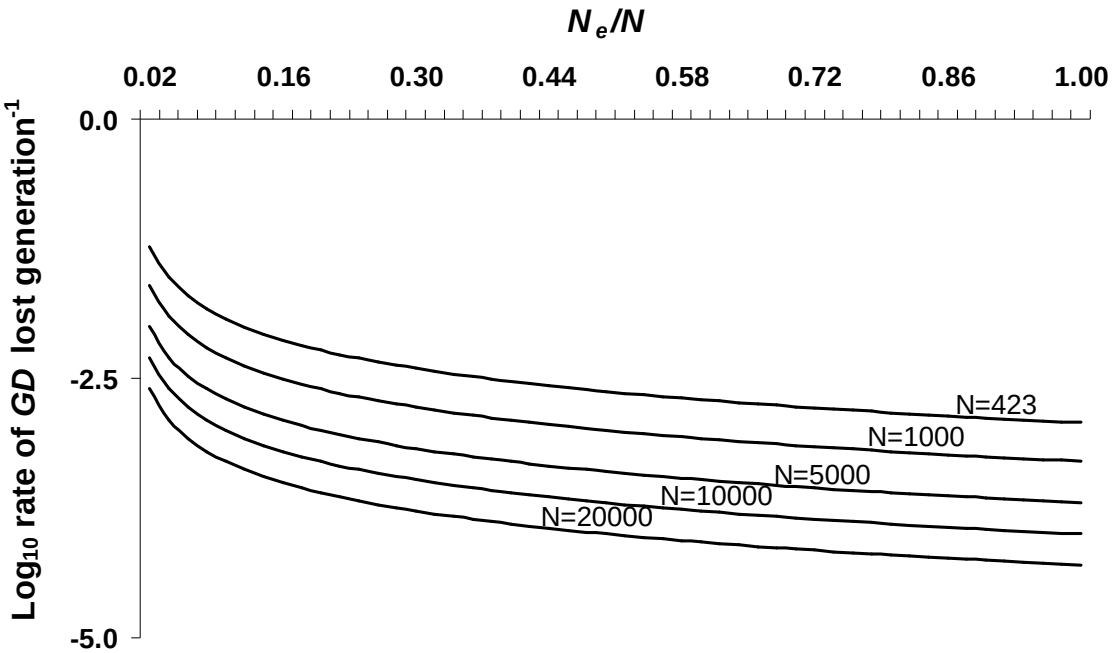


Figure 7.4 Log_{10} rate of *GD* loss per generation with changing N_e/N ratios for five different population sizes; N = 423, N = 1000, N = 5000, N = 10,000 and N = 20,000

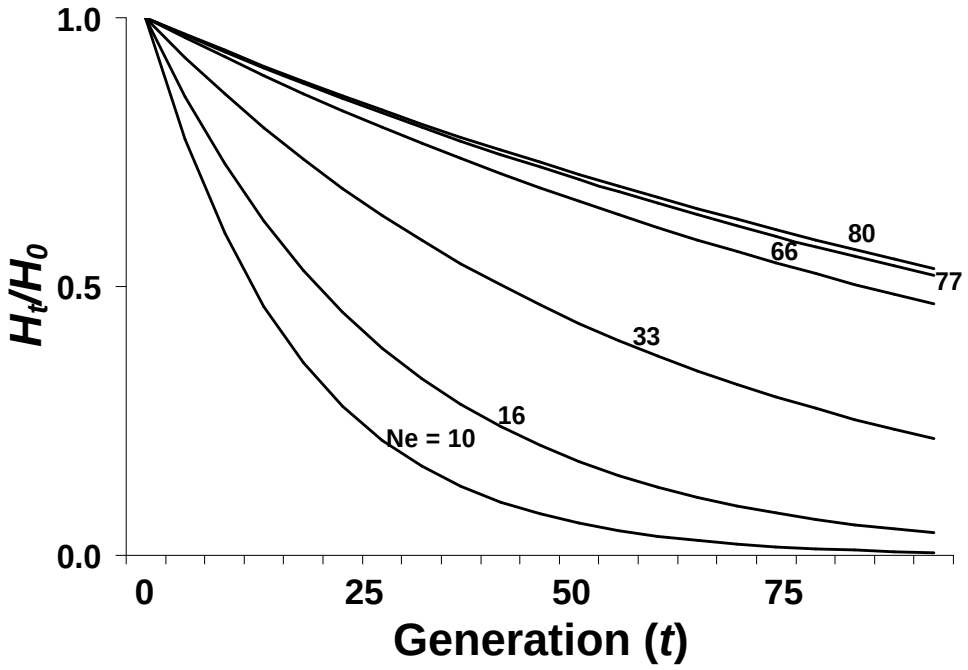


Figure 7.5 The impact of sustained small effective population size on the retention of heterozygosity in the scimitar-horned oryx EEP population after 100 generations. The effective population sizes modelled are based on sub-unit N_e

The mean inbreeding coefficient (mean *F*) varied between population sub-units ($\bar{x}$ = 0.1690, σ = 0.0898), with Israel (Δ + 0.1501), Italy (Δ + 0.1222), Spain (Δ + 0.1653), non-EU (Δ + 0.1134) and BT 1 & 8 (Δ + 0.0359) having higher mean *F* than the EEP (Figures 7.3 and 7.8). When the average *MK* was compared against mean *F* for the population sub-units France (Δ + 0.0.0447), Italy (Δ + 0.0.0443), Spain (Δ + 0.0.0172), the EU (Δ + 0.0672), the EU excluding the UK (Δ + 0.0.0679), no-BT (Δ + 0.0649), non-EU (Δ + 0.0123), and BT 1 & 8 (Δ + 0.0692) all had mean inbreeding coefficients that were greater than the average *MK* for those sub-units.

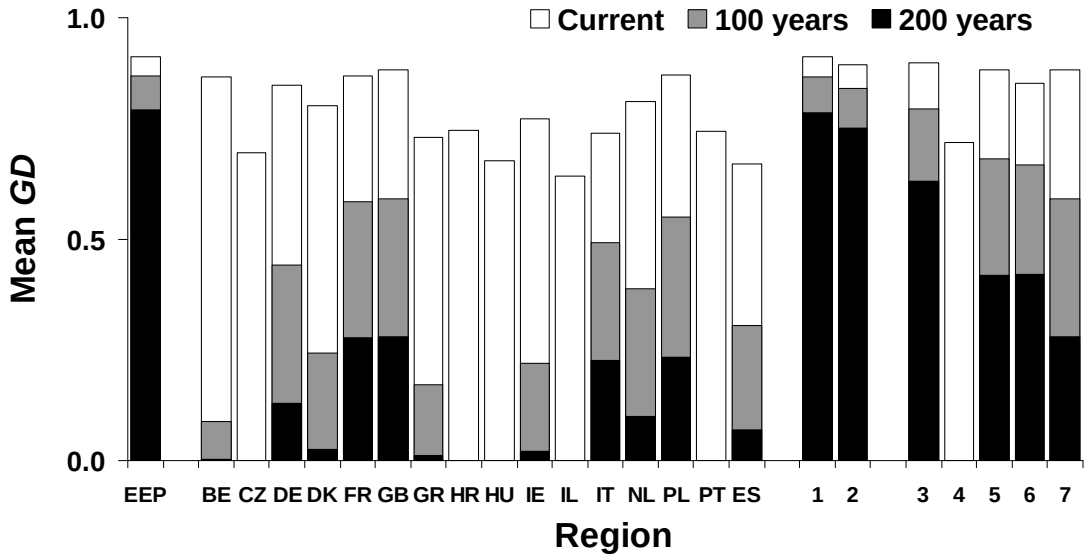


Figure 7.6 Current gene diversity, projected GD 100 and 200-years after the current population was founded (eight generations and 54 years, and 23 generations and 154 years from the present respectively) in each region. Sub-units: EU (**1**), EU excluding the UK (**2**), no BT (**3**), non-EU (**4**), EU BT 8 (**5**), EU BT 1 & 8 (**6**), and EULRZ 8 (**7**)

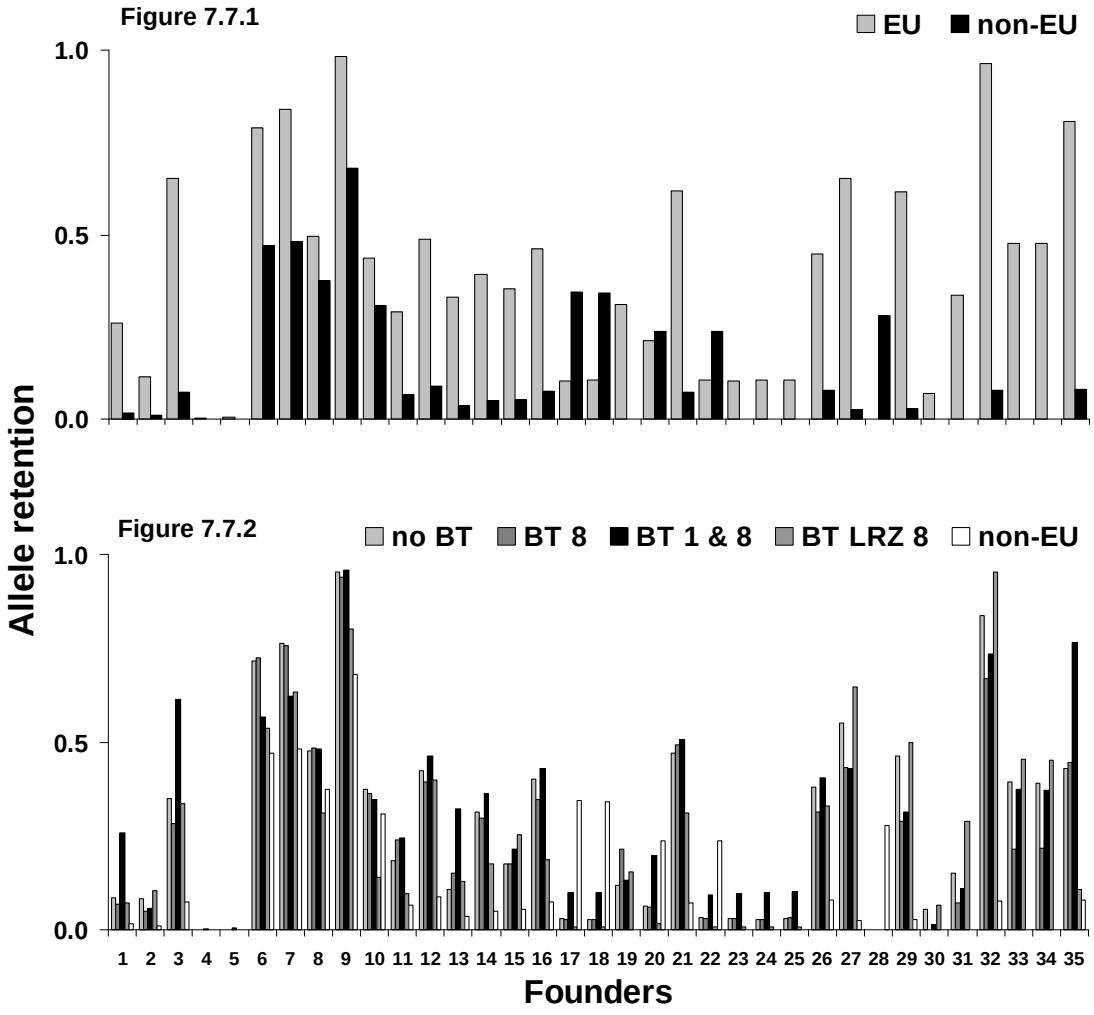


Figure 7.7 Founder allele retention in the living descendant population of the scimitar-horned oryx EEP population in EU and non-EU countries (**Figure 7.7.1**) and in each bluetongue zone (**Figure 7.7.2**)

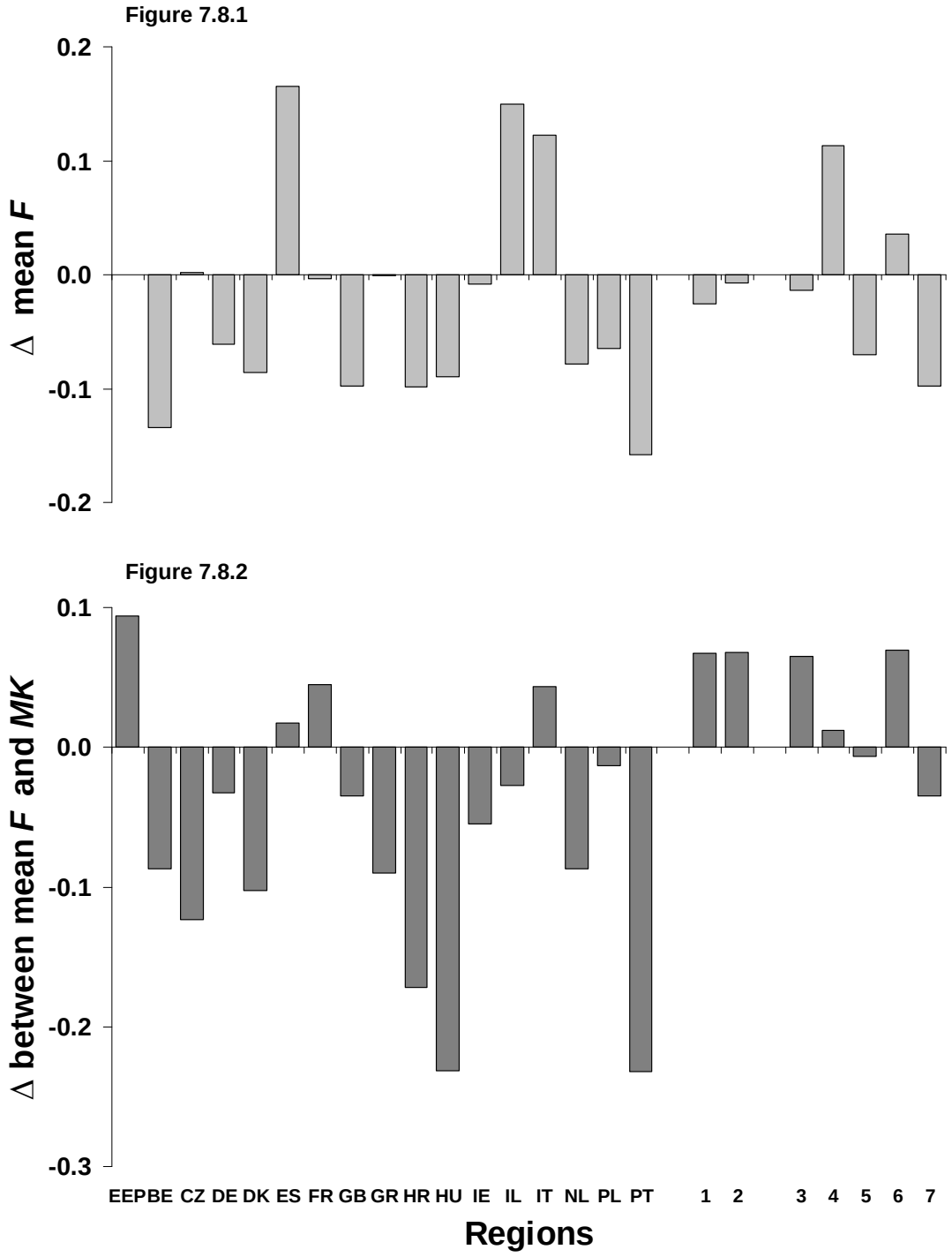


Figure 7.8 The difference in mean *F* between each population sub-unit and the contiguous EEP population (**Figure 7.8.1**), and the difference between the mean *F* and the average *MK* for each population sub-unit (**Figure 7.8.2**). Sub-units: EU (**1**), EU excluding the UK (**2**), no BT (**3**), non-EU (**4**), BT 8 (**5**), BT 1 & 8 (**6**), and BT LRZ 8 (**7**)

7.4.2 Arabian oryx, and mhorr and dorcas gazelle EEP populations

Fragmentation potentially affects all captive populations, and Figure 7.9 illustrates the impact of fragmentation on effective population sizes and the predicted rate of *GD* lost per generation for the Arabian oryx, mhorr gazelle and dorcas gazelle EEP populations when they were modelled as fragmented EU and non-EU countries, bluetongue zones, and individual country sub-units. The Arabian oryx contiguous EEP population had an $N_e = 29$ with a loss of 0.017 *GD* per generation, but N_e varied between zero and nine when split into individual countries (with a corresponding loss of 1.0 - 0.053 *GD* lost generation^{-1}), and from $N_e = 0$ - 13 when separated into the different bluetongue sub-units (with a corresponding loss of 1.0 – 0.038 *GD* per generation). A similar trend was observed for the mhorr and dorcas gazelle EEP populations which had $N_e = 49$ and 56 respectively. The N_e substantially decreased when the populations were separated into individual countries or bluetongue zone sub-units, with a corresponding increase in *GD* lost per generation.

7.4.3 Global captive populations

The mean N_e for global populations across all taxa was $N_e = 58$, and the mean N_e/N for the same populations was $N_e/N = 0.25$. In comparison the mean N_e for regionally coordinated programmes across all taxa was $N_e = 26$, and the mean N_e/N for the same populations was $N_e/N = 0.29$ (Table 7.7, Figure 7.10).

A total of 63% of species with EEP and/or SSP coordinated breeding programmes had an N_e/N ratio higher than that of the global populations. When ESBs and PMPs were included, this increased to 66% (Figure 7.11). There were regional differences in the difference between the N_e/N ratios of the managed regional populations and the global (ISB) population. The European EEPs and ESBs showed a mean difference of + 0.0256 between the managed populations and the ISB populations ($T_{66} = 2.43$, $P = 0.018$), but there was no difference between the ISB and SSP/PMP populations ($T_{55} = 0.98$, $P = 0.329$). There was no difference in the N_e between the regional programmes and the ISB populations (EEP/ESB $T_{66} = -0.73$, $P = 0.466$; SSP/PMP $T_{55} = -1.00$, $P = 0.322$).

The difference (Δ) in the N_e/N ratio between regional programmes and the ISB population varied across the taxa (Figure 7.11), for example Amphibians had the smallest difference in N_e/N (-0.0004) and Xenarthra had the largest difference in N_e/N (+0.1128). However, both amphibians and Xenarthra are represented by only one species. The remaining taxa have a $\Delta N_e/N$ ratio that varies between 0.0027 and 0.0787.

The N_e/N of paired EEP/ESB and SSP/PMP populations differed with a mean of N_e/N = 0.31 for the European populations and a mean of N_e/N = 0.28 for the North American populations (T_{40} = 2.13, P = 0.040). In total, 71% of European coordinated captive breeding programmes had higher N_e/N ratios than the matched North American programmes. There was no difference between the mean N_e of paired EEP/ESB and SSP/PMP populations (T_{40} = 1.15, P = 0.256).

Most of the effective population sizes of both EEP and SSP populations are below the N_e = 50 recommended for short-term retention of genetic diversity. In fact only 18 regional populations (14%) have an N_e greater than 50. In contrast, 43 (41%) of global (ISB) populations have an N_e > 50. No regional or ISB population has an effective population size of N_e = 500. A substantial increase in population size would be required to meet an N_e = 500, and even an N_e = 50, based on current N_e/N ratios (Figures 7.12 and 7.13).

Census population size would need to increase by a mean of 71 for EEP populations, and 145 for SSP populations to meet the N_e = 50 goal, based on current N_e/N ratios. Global populations had a mean N_e in excess of 50 even though 59% of populations have an N_e < 50. Population size would need to increase by a mean of 1686 for EEP populations, 2328 for SSP populations and 2111 for global populations to ensure an N_e= 500, based on current N_e/N ratios. It should be noted that the census population size needed to obtain N_e = 500 varies considerably between taxa (Figure 7.13).

Most regional populations had a larger N_e/N ratio than global populations (Figure 7.11). When the maximum N_e/N ratio observed for the species was applied to ISB populations, the N needed to meet the N_e= 50 goal was reduced by a mean of 10%. When the same ratio was applied to ISB populations in relation to the N_e= 500 goal, the N needed to meet the N_e = 500 goal was reduced by a mean of 11% from the census sizes in Figure 7.12. Despite this, census population sizes for N_e = 50 still varied between 71 and 1,603, and the N needed for N_e = 500 ranged from 709 to 16,026.

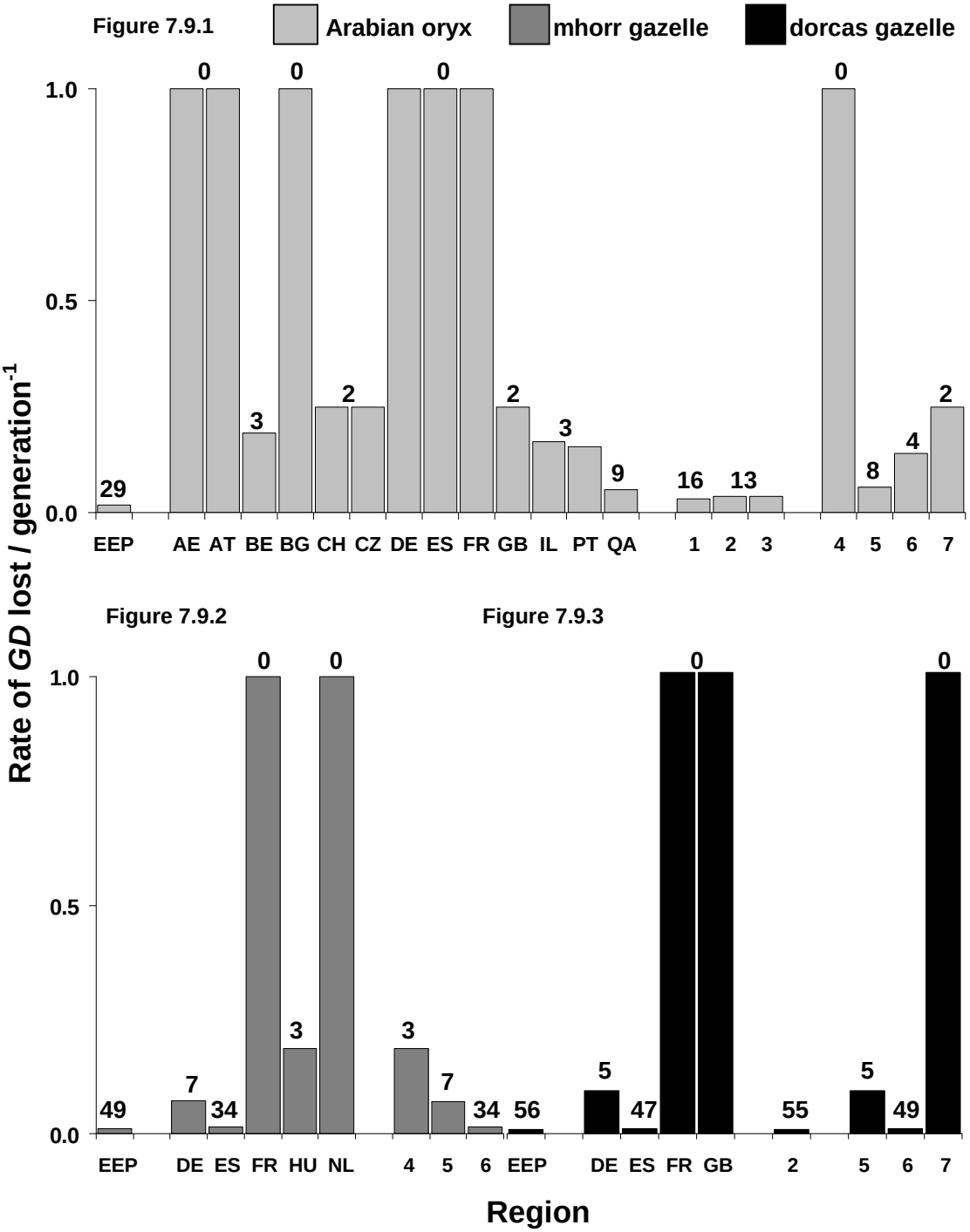


Figure 7.9 Rate of *GD* lost per generation in fragmented EEP populations for Arabian oryx **(Figure 7.9.1)**, mhorr gazelle **(Figure 7.9.2)**, and dorcas gazelle **(Figure 7.9.3).** The effective population size for each sub-unit is floated above the graph. Sub-units: EU (1), EU excluding the UK (2), non-EU (3), no BT (4), BT 8 (5), BT 1 & 8 (6), and BT LRZ 8 (7)

Figure 7.10.1

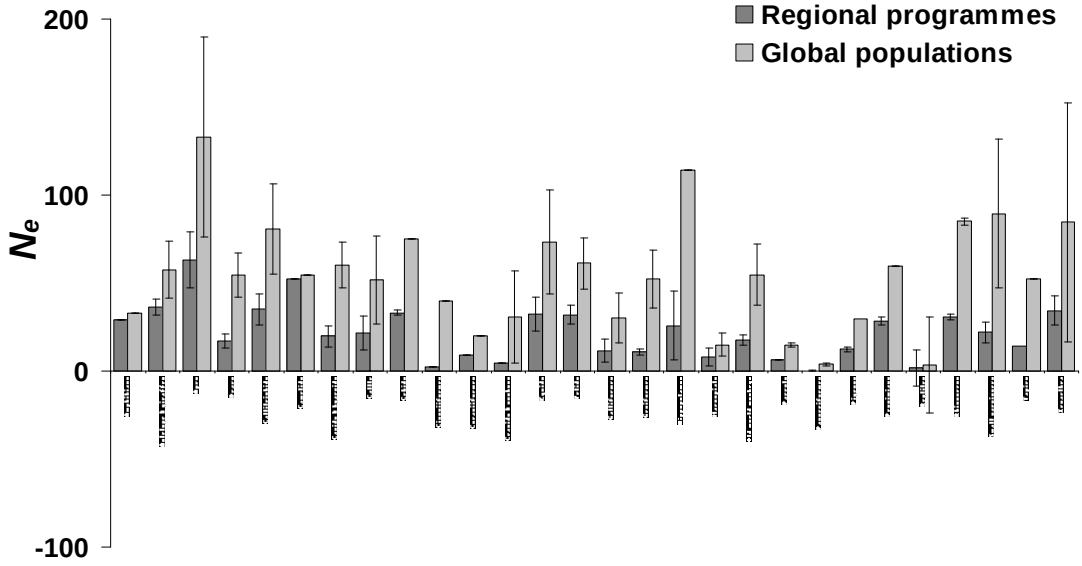


Figure 7.10.2

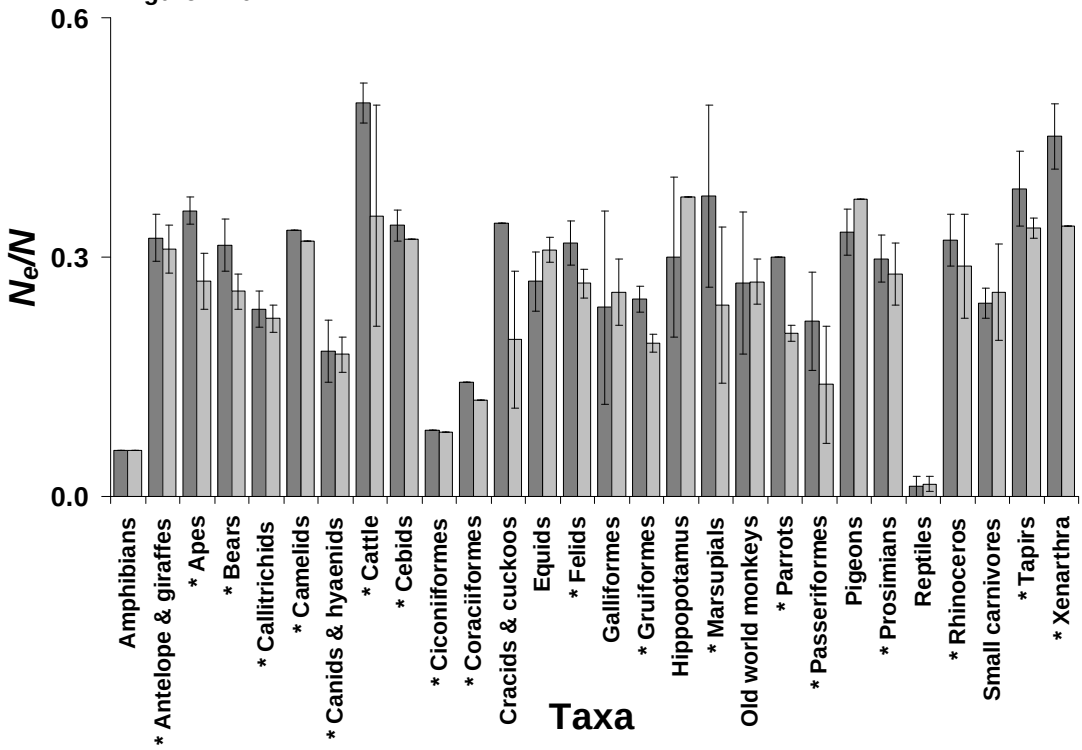


Figure 7.10 The N_e (**Figure 7.10.1**) and N_e/N (**Figure 7.10.2**) for 111 international studbook species grouped into taxa as specified by the regional taxon advisory groups (TAGs). Only studbooks with more than 62.5% pedigree completeness were included. Taxa highlighted with '*' had a larger N_e/N ratio for regional breeding programme populations than global populations. See Table 7.9 in Appendix B for a full list of species in each TAG

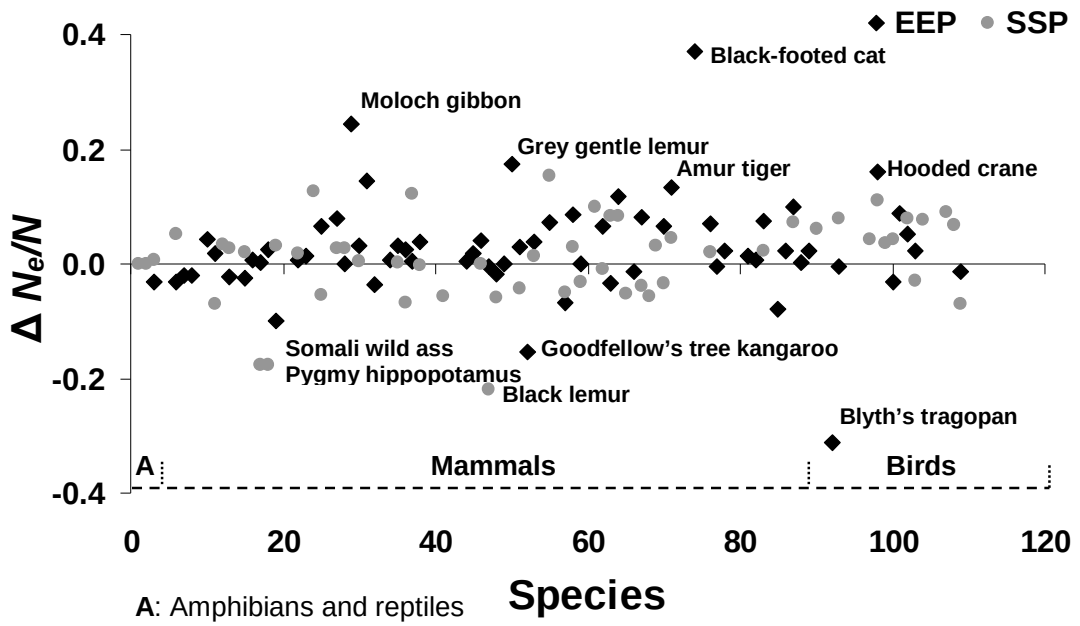


Figure 7.11 The difference in N_e/N ratios between the international studbook population and regional cooperative breeding programmes (EEP and SSP) for 84 species for amphibians, mammals and birds. The numbers on the x-axis correspond to species identification numbers, grouped into taxa as specified by the regional TAGs. **Species' numbers:** Amphibians **(1)**; Reptiles **(2-3)**; antelope and giraffes **(4-12)**; cattle (**13-14)**; equids **(15-17)**; hippopotamus **18**; rhinoceros **(19-22)**; camelids **23**; tapirs **(24-25)**; apes **(27-31)**; callitrichids **(32-37)**; cebids **(38)**; old world monkeys **(39-43)**; prosimians **(44-52)**; marsupials (**53-56)**; xenarthra **(57)**; small carnivores **(58-61)**; bears **(63-66)**; canids and hyaenids **(67-71)**; felids **(72-89)**; ciconiiformes **(90)**; coraciiformes **(91)**; cracids and cuckoos **(92-93)**; galliformes **(94-97)**; gruiformes **(98-105)**; parrots **(106-108)**; passeriformes **(109-110)**; pigeons **(111)** (See Table 7.9 in Appendix B for full species listing)

Table 7.7 Descriptive statistics for N_e and N_e/N ratios for global populations and regional coordinated captive breeding programmes for species with ISBs. Only those populations with more than 62.5% known pedigree were included

	Regional programmes	Global population
Mean N_e	26.2	57.5
Min. N_e	0.00 *	0.00 §
Max. N_e	116.02 (black-&-white ruffed lemur)	290.41 (orang-utan)
Mean N_e/N	0.28	0.25
Min. N_e/N	0.00	0.00
Max. N_e/N	0.705 (black-footed cat)	0.491 (lowland anoa)

*Three species have $N_e = 0.00$; Aruba Island rattlesnake *Crotalus durissus unicolor*, grizzled grey tree kangaroo *Dendrolagus inustus* and Western grey lemur *Hapalemur occidentalis*. §Three species have $N_e = 0.00$; Aruba Island rattlesnake, Blyth's tragopan *Tragopan blythii* and Chinese alligator *Alligator sinensis*

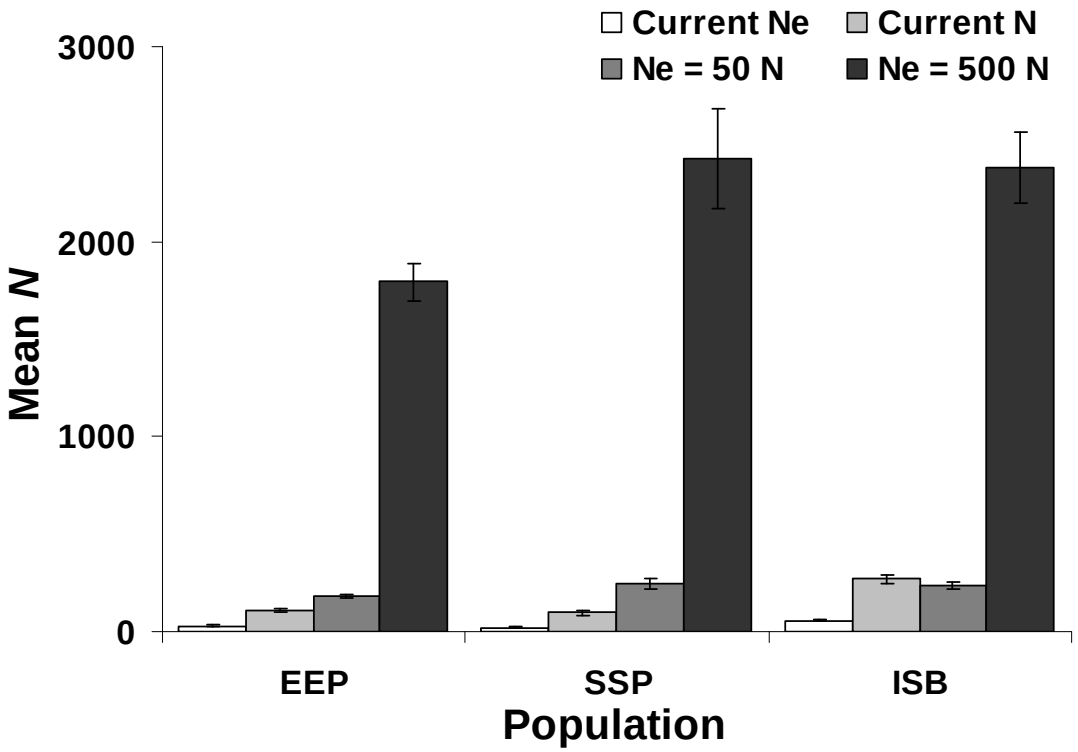
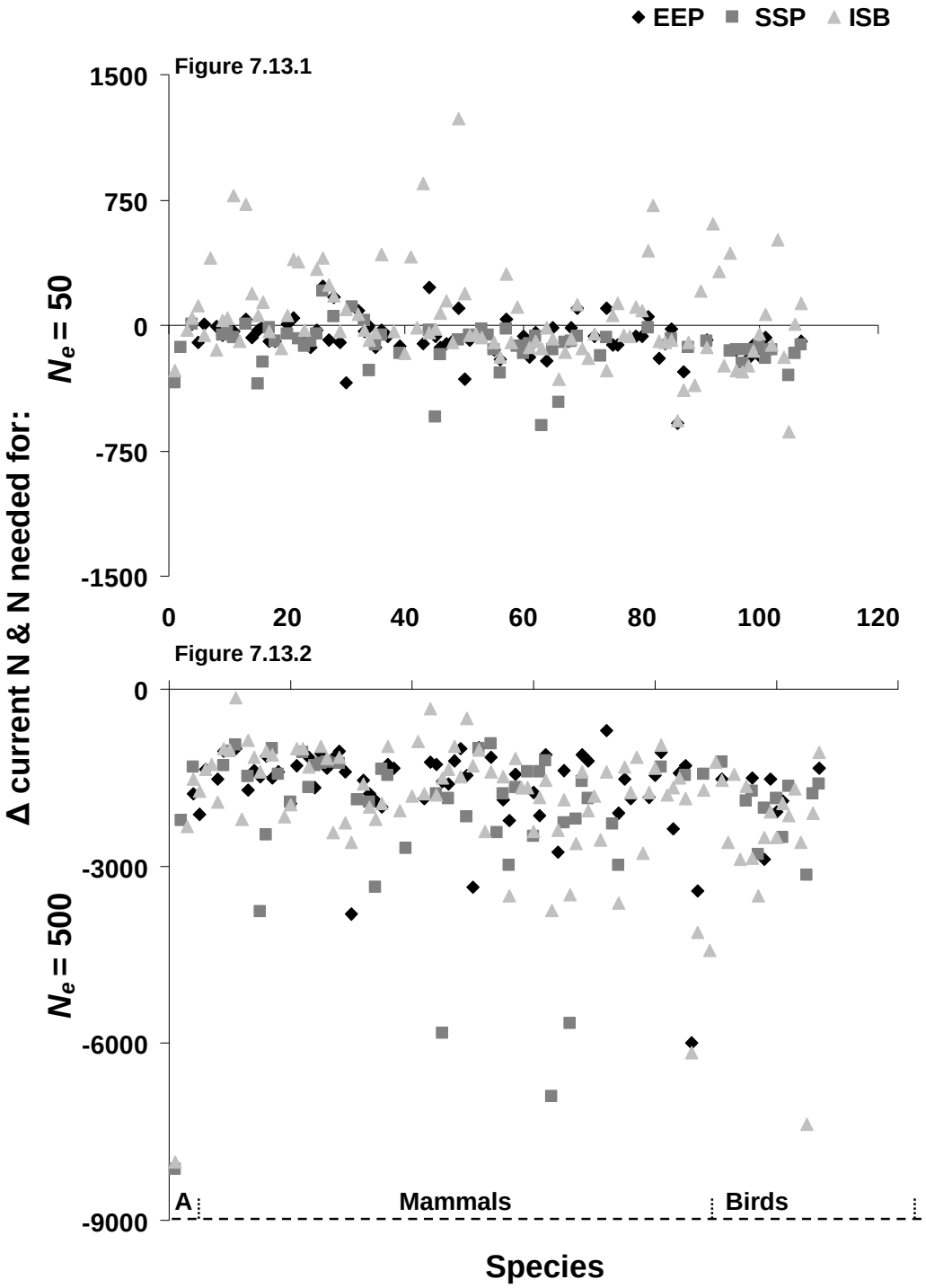


Figure 7.12 The current census and effective population sizes of EEP, SSP, and ISB populations, and the census population sizes needed to attain effective population sizes of $N_e = 50$ and $N_e = 500$ based on current N_e/N ratios



A: Amphibian and reptile

Figure 7.13 The difference between the census population size in the ISB and the *N* needed for effective population sizes of N_e = 50 (**Figure 7.13.1**), and N_e = 500 (**Figure 7.13.2**) based on current N_e/N ratios. The species numbers correspond to the taxa listed in table 7.9, Appendix B

7.5 Discussion

The sustainability of captive populations is a serious concern for population managers. Captive populations cannot be considered ‘assurance populations’ or an ‘insurance policy’ if populations are not self-sustainable in perpetuity.

Fragmentation of populations, even when sub-divided into only two unequal sized sub-units, was predicted to reduce the cumulative effective population size of the metapopulation, resulting in sub-units and a metapopulation that were predicted to lose *GD* at a faster rate that the contiguous EEP population for all four species of antelope and gazelle. Further examination of the scimitar-horned oryx EEP population revealed fragmentation would result in increased inbreeding and unequal founder allele retention across the fragmented EEP population.

Migration is often restricted due to legislation designed to protect the agricultural industry from disease transmission (DEFRA 2011). This legislation applies to exotic species because they are part of the same families as agricultural livestock, for example antelope are bovids, as are cattle (DEFRA 2010). There have been historical incidences of exotic species introducing diseases to naïve domestic populations. In 1987 zebra were translocated from Africa to Spain resulting in the emergence of African horse sickness in the Iberian Peninsula (Gummow 2010; Rodriguez *et al*. 1992). Concern has also been expressed about the disease transmission between zoological institutions for some captive populations (Ryan & Thompson 2001). However, the movement of animals between zoological collections is accompanied by stringent veterinary procedures, including disease testing, vaccination, and quarantine. Additionally, most exotic animals will not have any contact with agricultural livestock, so the risk of disease transmission between them is likely to be minimal. As economic fragmentation poses a substantial risk to the sustainability of captive populations, there is a strong argument for populations of endangered species managed through coordinated captive breeding programmes to be exempt from some of the legislation as long as stringent veterinary procedures are adhered to. At the very least, the costs and benefits of applying agricultural legislation to endangered species need to be carefully examined.

The sub-division of regional populations due to economic and legislative factors is likely to reduce genetic viability and sustainability. If population fragments are small and isolated, as observed in the scimitar-horned oryx, Arabian oryx, mhorr gazelle and dorcas gazelle EEP population models, demographic stochasticity, as well as drift, will increase

extinction risk for individual sub-units (Ballou & Foose 1996). Extending regional populations to a global level will not address the issue of sustainability, as many global populations are too small to ensure persistence indefinitely. Effective population management can increase the N_e, and therefore genetic viability, of populations, as evidenced by higher N_e/N ratios in the regionally managed populations than the global populations. It is interesting to note that the European EEP/ESB programmes performed better in terms of N_e/N ratios than the North American SSP/PMP programmes. This is not due to different taxa being managed in different regions as the paired species EEP/SSP analysis revealed a difference between the regional ratios with 71% of European programmes yielding higher N_e/N ratios than their North American counterparts. The cause of this difference is unknown and warrants further investigation. It is also worth noting that the global and regional data are not independent as regional programme data are a sub-set of the global data. If independent data were used, the differences between regional and global populations would be more pronounced.

Despite the limitations, the analyses reveal a difference in global and regional N_e/N ratios, consequently I recommend that population management is coordinated at a global scale in order to move towards population sustainability. This strategy has been previously recommended by WAZA (2005e), Iyengar *et al*. (2007), Wood *et al*. (2008) and Lees and Wilcken (2009), although this is the first time it has been based on quantitative analysis of the sustainability multiple taxa.

Even this is unlikely to result in sustainable global captive populations unless the ratio of N_e to N can be greatly increased, with a simultaneous increase in census population size. Currently, only 13% of regional populations and 37% of global populations have an N_e of 50 or more, and no population has an N_e of 500. Captive breeding space is limited, so the establishment of self-sustainable populations will require a reorientation of priorities for zoological institutions, so that either fewer species in larger numbers are held by individual institutions for public display, or more space is made available outside of traditional zoos for the breeding of endangered species. There is already precedence for this for some ungulate species, for example, there are an estimated 3,800 and 11,000 scimitar-horned oryx in private collections and ranches in the Middle East and Texas, respectively (Anderson 2010; Johnson 2010; McClellan 2010). These animals are largely unrecorded, and are not currently included in any regional or global breeding programme, but they demonstrate the potential of managing the captive breeding of endangered species beyond the existing zoo-model. If population management plans were extended further to

include semi-captive, reintroduced, and wild populations, captive populations could constitute an important component of an integrated conservation plan for each species. Under this model, the effective population size of a species may exceed the size necessary for long-term evolutionary potential and sustainability. However, the issue of population sub-division reducing the effectiveness of population management may still apply unless the barriers to migration can be reduced or removed.

The evaluation of regional and global populations utilised data from published international studbooks on the ISIS/WAZA 2006/2007/2008 studbook library database (ISIS 2009). International studbooks were only included if they had been updated to the end of 2001 onwards, and whilst 93% of studbooks included in the study were updated from 2006 onwards, data were not current at the time of writing. The evaluation of the sustainability of regional and global programmes would benefit from more current data to ensure that it represented contemporary management and populations.

Additionally standardised filter files were used to extract data for the regional AZA and EAZA programmes. If an institution was an AZA or EAZA member and held the relevant species, then their data were included in the analyses. Whilst this approach is likely to be representative of current populations, some AZA/EAZA institutions may have been excluded from some breeding programmes because of historical non-compliance. Alternatively, non-AZA/EAZA institutions may be included in some breeding programmes because they have either genetically important animals or much-needed space. For example the scimitar-horned oryx EEP includes two non-EAZA institutions. In either case the institutions and their animals, would have been either incorrectly included or incorrectly excluded from the analysis of the regional breeding programme populations.

Some regional breeding programmes maintain separate studbooks that contain more accurate and current data than the international studbook. An example of this is the Arabian oryx where the regional studbook contains more current data from more European and Middle Eastern institutions that the international studbook (ISIS 2009). The Arabian oryx EEP bases population management recommendations on data analysis of the regional studbook, rather than the international studbook (Gilbert 2009a; ISIS 2009). Consequently, restricting data to the international studbooks as the basis of comparisons between regional and global populations may not accurately represent all the populations for each species.

Despite the limitations of using ISBs from the ISIS/WAZA studbook library database, the use of international studbooks provided data quality consistency across all three populations, and eliminated studbook quality as a bias in the analyses.

Studbooks were only included if global or regional populations had more then 62.5% pedigree completeness to modify the impact of pedigree completeness on estimation of N_e. However, the inclusion of some studbooks with incomplete pedigree data (>62.5% complete) may have resulted in over- or underestimated N_e or N_e/N as estimates were based on the numbers of breeders in the population. Only 19% of international studbooks used in the analyses contained 100% known pedigree data.

Research into the fragmentation of endangered species in captivity would benefit from being extended to include other taxa, such as cattle, sheep and goat, and bird species, whose population management has been affected by EU legislation. If comparable results are obtained for those taxa, it would a present a strong argument to re-evaluate EU legislation and contemporary population management strategies.

The results presented in this chapter demonstrate that captive populations are currently not sustainable, and are therefore not fulfilling the role of 'assurance populations'. Population fragmentation is predicted to increase the rate of loss of *GD* per generation, and reduce the long-term genetic viability of regionally managed populations. Contiguous regional populations had a higher N_e/N ratio than ISB populations, suggesting that active population management can improve the genetic viability of captive populations. I therefore recommend that a global overview is taken for the management of captive populations. However, to ensure self-sustainability the traditional zoo-model may need to be replaced by integrated conservation plans that combine the management of captive, semi-captive, and wild populations of each species.

I now examine the viability of one captive population, the scimitar-horned oryx EEP, in Chapter Eight under the fragmentation models presented here.

8.0 Chapter eight: viability of the scimitar-horned oryx EEP population

8.1 Abstract

Small closed populations are vulnerable to extinction because genetic, demographic and environmental stochasticity have a large impact on them. This increases when populations are fragmented into small sub-units. This chapter used population viability analysis (PVA) to evaluate the impact of five levels of fragmentation on the viability of the EEP scimitar-horned oryx population, in relation to the goal of 99% probability of population persistence for 100-years. Time to extinction, remnant genetic diversity, and population size of extant populations were also tested.

Inbreeding, in the form of lethal equivalents (*LE*), was incorporated into the model and sensitivity tested to determine if variations in *LE* impacted on the population predictions. The results of the sensitivity testing are discussed in relation to the widespread use of default values for *LE* in PVAs. Carrying capacity (*K*) was also sensitivity tested to determine the minimum viable population size (MVP_K) for the population.

Any fragmentation of the population resulted in the viability goal of 99% probability of population persistence for 100-years not being attained. As fragmentation increased, genetic diversity, time to extinction, and the size of the extant population decreased. Migration was modelled between sub-units, and the optimum amount of migration for each model varied between 1 – 4% depending on the fragmentation model. No amount of migration could achieve panmixia and compensate for population fragmentation.

The limitations of the methods used in this chapter are discussed, along with recommendations made for population management and further research.

8.2 Introduction

Small closed populations, such as those found in captivity, are more vulnerable to extinction than large populations, because catastrophes and environmental, demographic and genetic stochasticity have a greater impact on them (Ballou & Cooper 1992b; Lacy 2000a; Lande 1993; Lande *et al.* 2003; Reed & Hobbs 2004; Traill *et al.* 2010). These populations may also suffer from Allee effects, inbreeding depression, and loss of genetic diversity causing a reduction in the population's ability to adapt to environmental change (Frankham *et al.* 2010; Holsinger 2000; Lande 1995; Wilcken & Lees 1998; Wittmer *et al.* 2005). The demographic and genetic factors interact synergistically leading to further reductions in population size, which in turn causes more genetic and demographic problems. This process is termed the 'extinction vortex' (Ballou *et al.* 2010a; Foose *et al.* 1995; Lacy 2000a). When small populations are also isolated, and migration cannot alleviate the negative effects of demographic instability or genetic impoverishment (Lacy 1993a; Lucentini *et al.* 2009), their eventual fate is nearly always extinction (Ballou & Ralls 1982; Ballou & Foose 1996; Senner 1980).

The demographic and genetic pressures on small closed populations are increased further when those populations are fragmented into small sub-units, as captive populations often are (Ballou & Foose 1996; Henle *et al.* 2004; Junhold & Oberwemmer 2011; Lacy 1997; Chapter Seven). However, the genetic effects of population sub-division are multiple and complex (Frankham *et al.* 2010; Nunney 2000; Robert 2009). This chapter aims to evaluate the impact of multiple genetic and demographic factors on the population viability of the scimitar-horned oryx EEP.

In theory, complete population sub-division should increase the retention of alleles and *GD* across the metapopulation relative to a panmictic population, because genetic stochasticity will cause different alleles to drift to fixation in different population sub-units (Brito 2009; Leus *et al.* 2011b; Margan *et al.* 1998; Wang & Caballero 1999). Whilst each population sub-unit is expected to lose variability rapidly resulting in lower *GD* and fewer alleles (Redford *et al.* 2011), genetic diversity is retained across the metapopulation in the form of between sub-unit variation, and so is protected from further decay. As a result, the levels of genetic variation contained within a fragmented metapopulation are expected to be greater than those retained in a panmictic population of the same size (Brito 2009; Lacy 1994; Lacy & Lindenmayer 1995; Lande 1995). At the same time, rare alleles will be present in much higher frequencies in the sub-units, where they exist, than they would have

been in a panmictic population, and this helps protect them from the effects of drift (Lacy 1987). Population sub-division is reversible up to the point that one sub-unit goes extinct, but there is no way to recover lost alleles if a captive population remains panmictic (Lacy 1987).

Population fragmentation has also been shown to lead to increased population fitness in some instances, because the proportion of recessive lethal alleles fixed at the metapopulation level is reduced compared to an individual sub-unit (Brito 2009). At the same time, selection against homozygotes for recessive lethal alleles rapidly reduces their representation in the population (i.e. they are purged from the population) (Brito 2009; Lacy & Lindenmayer 1995; Leus *et al.* 2011b; Robert 2009).

When a population is sub-divided into independent units for a sufficient period of time, the sub-units evolve independently and local adaptation drives genetic differentiation between the sub-populations (Franklin 1980; Höglund 2009; Lacy 1987; Robert 2009). Environmental heterogeneity across the metapopulation increases local adaptation, which results in greater overall genetic diversity (Chesser *et al.* 1980; Lacy 1987, 1994; Nunney 2000; Reed *et al.* 2003a). Local adaptation in sub-units leads to less efficient selection in a fragmented population than in a panmictic population of the same size. Consequently, adaptation of the metapopulation to the captive environment is reduced (Frankham 2008; Margan *et al.* 1998; Montgomery *et al.* 2010; Robert 2009). Drift overwhelms selection in small populations, so this process only applies if sub-units are large enough to negate the effects of drift (Frankham *et al.* 2010; Williams & Hoffman 2009). Adaptation is minimised if populations are so small that drift becomes the dominant factor influencing genetic variation (Frankham 2005b; Frankham *et al.* 2010; Lacy 1987; Leus *et al.* 2011b).

Sub-division may also reduce the impact of catastrophes on the population. It is likely that catastrophic events will affect only a few sub-units or a small geographic area, and therefore the risk of extinction to the entire metapopulation is lower than if the population was panmictic (Margan *et al.* 1998; McCarthy & Lindenmayer 2000; Nunney 2000; Reed 2004). Consequently, dispersal of a population over a large area protects the metapopulation from epidemic disease and other catastrophes (de Boer 1989; Lacy 1994).

The maintenance of higher levels of genetic variation through population sub-division critically depends on the demographic stability of the sub-units. Smaller sub-units will experience greater demographic stochasticity and reduced growth (Brito 2009; Lacy & Lindenmayer 1995; Robert 2009). This can result in a greater loss of both gene and allelic diversity in sub-divided populations (Brito 2009; Lacy & Lindenmayer 1995). Models and

experimental studies that show increased genetic diversity for fragmented populations are based on the assumption that sub-units are large, equal, and constant, in size (Lacy 2000a; Montgomery *et al.* 2010; Wang & Caballero 1999). These assumptions are rarely met for real populations (Brito 2009; Lacy & Lindenmayer 1995; Wang & Caballero 1999; Chapter seven). Furthermore, such models are based on replicate sub-populations, rather than sub-units derived from different founders (Ballou & Lacy 1995; Frankham 2008). Any advantages of population sub-division are reversed if population sub-units go extinct (Hedrick & Miller 1992; Margan *et al.* 1998; Lacy & Lindenmayer 1995; Leus *et al.* 2011b).

Dividing the population into small isolated sub-units leads to inbreeding which may lead to inbreeding depression (Benedick *et al.* 2007; Frankham 2003; Lacy 1994; Tallmon *et al.* 2004). Depression is often expressed as increased juvenile mortality, decreased longevity, and reduced reproductive success (Frankham *et al.* 2010). Consequently, increased inbreeding depression contributes to an increased probability of metapopulation extinction (Fox *et al.* 2008). Some studies have shown that increased inbreeding leads to improved population fitness because of purging of deleterious alleles from the population. However, evidence of purging in captive populations suggests that it is inefficient when it occurs (Boakes *et al.* 2007; Frankham 2005a; Larsen *et al.* 2011; Witzenberger & Hochkirch 2011), and is not a recommended strategy for population management (Fox *et al.* 2008; Kalinowski *et al.* 2000; Willis & Wiese 1997).

Theoretically, the effects of genetic drift and inbreeding can be countered by occasional migration (one migrant every generation) between sub-units (Brito 2009; Frankham *et al.* 2011; Lacy 1987; Margan *et al.* 1998), although small populations, or polyganous species, will require greater rates of migration to offset inbreeding (Brito 2009; Briton *et al.* 1994; Lacy & Lindenmayer 1995; Williams & Hoffman 2009). The costs and benefits of population sub-division disappear if migrants are exchanged more frequently because the metapopulation will approach panmixia (de Boer 1989; Lacy 1987; Williams & Hoffman 2009). However, studies with real populations have shown that metapopulations with migration still suffer from the effects of small population size because of demographic instability in the sub-units (Brito 2009; Lacy & Lindenmayer 1995).

In conclusion, sub-dividing a natural or captive population will probably lead to reduced genetic diversity and a higher risk of extinction because of increased stochasticity (Höglund 2009; Mace & Purvis 2008; McCarthy & Lindenmayer 2000; Reed 2004; Wang

& Caballero 1999). There may be a substantial time delay between fragmentation and an increased extinction risk from genetic impoverishment (Benedick *et al.* 2007). Consequently, populations should only be divided if each sub-unit is large enough to avoid the effects of loss of genetic diversity, stochasticity, and inbreeding (de Boer 1989; Boyce 1992).

The synergistic demographic and genetic interactions in a population can be modelled to provide an evaluation of the quantitative risk of population extinction (Brook *et al.* 1997; Coulson *et al.* 2001; Lacy 1993b; Lacy 2000a; Reed *et al.* 2003c). This process, known as population viability analysis (PVA), combines stochastic and deterministic factors in a mathematical model and is evaluated by running computer simulations (Shaffer 1981). The combination of stochastic and deterministic forces makes PVAs more accurate than predictions based on life table analysis alone (Brook *et al.* 1997; Hedrick *et al.* 1996; Lacy 1993b; Possingham *et al.* 1993; Ralls *et al*. 2002).

PVAs have been run on over 140 species (Boyce 1992; Patterson & Murray 2008; Reed *et al.* 2003c; Traill *et al.* 2010) from a variety of taxa (Reed *et al.* 2003c). They not only predict the risk of extinction, but also predict population dynamics prior to extinction (McCarthy *et al.* 2001).

PVAs have been used to fulfil a number of objectives including: 1) evaluating the minimum viable population size (MVP) required for population persistence; 2) determining which variables and assumptions have the largest impact on population dynamics and persistence; 3) evaluating a suite of management options on population viability; 4) evaluating the impact of population sub-division and migration on the probability of population extinction; and 5) determining what factors influence patch occupancy in a metapopulation (Boyce 1992; Brook *et al.* 1999; Coulson *et al.* 2001; Lacy 1993b; McCarthy *et al.* 1995; Traill *et al.* 2010).

The minimum viable population size is defined as the smallest number of individuals needed for a population to persist. It is a useful benchmark for population management (Lacy 1992; Mace 1989; Serfass *et al.* 1993) as N is a major determinant of population persistence (Reed *et al.* 2003c). MVP is usually quantified as a specific, but arbitrary, probability of population persistence for a specified period of time e.g. 99% probability of population persistence for 100-years (Albers 1989; Foose *et al.* 1995; Reed *et al.* 2003c; Traill *et al.* 2010). PVAs have been widely used to determine MVP, and estimates vary depending on the specific definition of MVP, the underlying assumptions, and the species

or population concerned (Lacy 1992; Traill *et al.* 2010). Despite this, MVPs typically number in the thousands (Reed *et al.* 2003c; Traill *et al.* 2010).

PVAs have also been used to identify key factors that influence population persistence (McCarthy *et al.* 1995; Ralls *et al.* 2002). This type of sensitivity testing not only highlights those parameters that have the greatest influence on population viability, but they can also indicate which parameters need additional study to be more accurate (Kohmann *et al.* 2005; Lande *et al.* 2003; McCarthy *et al.* 1995).

PVAs are an important management tool because quantitative pre-extinction population dynamics and extinction probabilities can be used to evaluate the efficacy of various management options (Fieberg & Ellner 2000; Lacy 1993b; Possingham *et al.* 1993; Traill *et al.* 2010; Ralls *et al.* 2002). In such cases, PVA is used as a relative, rather than absolute, predictor of the consequences of management (Ball *et al.* 2003; Frankham 2010b).

Population sub-division, with and without subsequent migration, has been incorporated into a number of different PVAs (Boyce 1992; Foose *et al.* 1995; Reed 2004; Lacy & Lindenmayer 1995; Lindenmayer *et al.* 2000). Models have tested the impact of different fragmentation scenarios and varying dispersal rates between population sub-units (Lacy & Lindenmayer 1995; Reed 2004). Some of this work has been carried out in conjunction with evaluations of optimal patch size and patch occupancy in a fragmented landscape (Ball *et al.* 2003; Hedrick & Miller 1992; Lindenmayer *et al.* 2000).

Numerous computer programs are available for conducting PVAs including VORTEX (Lacy *et al.* 2009), ALEX (Possingham *et al.* 1992), GAPPS (Harris, Metzger & Bevin 1986) and RAMAS/Space (Akçakaya & Ferson 1992). VORTEX has been used by the IUCN/SSC Conservation Breeding Specialist Group (CBSG) for many endangered species PVAs (Lacy 1993b; Reed 2004), and is ideally suited to small isolated populations that are subjected to stochasticity and inbreeding depression (Brito 2009; Brook *et al.* 1997; Possingham *et al.* 1993; Valera *et al.* 2005). Furthermore, VORTEX is more suitable for small captive population PVAs than other computer programs in 15 out of 19 considerations listed in the VORTEX manual (Miller & Lacy 2005).

VORTEX uses a Monte Carlo simulation algorithm to examine the effects of carrying capacity, catastrophes, demographic, environmental and genetic deterministic and stochastic variables and processes, and the interactions between them (Brito 2009; Lacy 1993b; Lacy & Lindenmayer 1995; Leus & Traylor-Holzer 2008; Possingham *et al.* 1993; Reed 2004). VORTEX can simulate the multiple and interacting events that determine the

persistence of populations by randomly sampling from defined binomial probability distributions (Caughley 1994; Lacy 1993b). It models population processes as discrete, sequential events, with probabilistic outcomes (Lacy 1993b; Ralls *et al.* 2002).

VORTEX also allows population sub-division with user-defined migration between the sub-units (Lacy 1993b). Models can include factors such as density-dependent reproduction, migration, harvesting, supplementation, and mortality (Kohmann *et al.* 2005). Density-dependence has been shown to have an important influence over population persistence (Brook *et al.* 1997; Reed *et al.* 2003c), and some form should be included in PVA models. In its simplest form density-dependent mortality is incorporated into PVAs when *N* exceeds the user-defined carrying capacity because VORTEX truncates the population by removing animals from all age and sex classes (Kohmann *et al.* 2005; Lacy 1993b; Reed *et al.* 2003c; Reed 2004).

There is compelling evidence that loss of genetic diversity increases the susceptibility of small populations to extinction (Boyce 1992; Frankham 2003; Frankham 2005a; Frankham 2006; Frankham 2010b; Robert 2006), yet only 60% of published PVAs include genetic effects in their models (Traill *et al.* 2010). Changes in genetic diversity can be evaluated because VORTEX assigns two alleles at one genetic locus to each founder (therefore representing unlinked loci). The progression of alleles through the pedigree is tracked by randomly sampling one allele from each parent to be transmitted to each offspring, in effect a gene drop simulation of genetic transmission through the pedigree is performed (Lacy 1993b; Lacy & Lindenmayer 1995). This means that the interactions between demographic and environmental stochasticity can be synergistically modelled along with the effects of inbreeding and reduced genetic variability (Lacy 1997; Lacy 2000a; Lande *et al.* 2003; Robert 2006).

The inclusion of inbreeding depression has a substantial impact on median times to extinction for many taxa (Frankham 2010a), but it is often only represented in PVAs as an increase in juvenile mortality in the form of lethal equivalents (*LE*) per diploid (Lacy 1993b; Leus & Traylor-Holzer 2008; Reed *et al.* 2003c; Traill *et al.* 2010). Lethal equivalents are defined as the number of recessive alleles that would depress fitness to the extent observed in the population (Leus & Traylor-Holzer 2008). CBSG use a default value of 3.14 *LE* per diploid individual (Bingaman Lackey 2010), which is the mean number of *LE* in 40 captive mammalian populations (Ralls *et al.* 1988). However, limitations of the methodology used to calculate this means that it is likely to be a substantial underestimate (Frankham 2010b; Ralls *et al.* 1988; Reed 2004), and more realistic estimates of *LE* should

be used in PVA models (approximately 12 *LE* for wild populations) (Brook *et al.* 2002; Frankham 2003; Frankham 2005a; Frankham 2010b). Models can also include user-defined purging of some recessive deleterious alleles from the population (Boakes *et al.* 2007; Reed *et al.* 2003c; Reed 2004). Inbreeding often impacts other fitness measures, such as reduced reproduction and longevity, and increased susceptibility to disease. VORTEX does not model these effects (Lacy & Lindenmayer 1995).

Usually VORTEX makes the assumption that founders are all unrelated and non-inbred, which underestimates existing or pending genetic problems in a population that have already lost a substantial amount of variation (Lacy 2000a). However, studbook files can be imported into VORTEX to form the founding population, so that the model is based on a real, rather than an ideal, population (Miller & Lacy 2005). VORTEX can also mimic current captive population management strategies by pairing individuals using mean kinship coefficients within specified inbreeding coefficient limits (Ballou & Lacy 1995; Kalinowski & Hedrick 1999).

Each scenario is run hundreds of times to allow for mean measures of population viability including the probability of extinction, final population size, growth rates, gene diversity and inbreeding (Lacy & Lindenmayer 1995; Leus & Traylor-Holzer 2008).

Population viability analysis is a useful tool, but it is an inexact science and no model is a perfect reflection of reality (McCarthy *et al.* 2001; Reed *et al.* 2003c). PVA should only be used if there is sufficient quality data to build a model, and there is a specific objective that can be met by using PVA (Ralls *et al.* 2002).

PVAs have often been criticised because data were either not available, not of sufficient quality to precisely estimate population parameters, or collected over too-short time spans to allow a reliable representation of the population (Boyce 1992; Ellner *et al.* 2002; Engen & Saether 2000; Patterson & Murray 2008; Reed *et al.* 2003c). Studbooks provide a unique resource to parameterise population models because they contain precise life history data on the entire captive population. In the case of the scimitar-horned oryx, the studbook dates back to 1875, and contains 8354 listed individuals (Gilbert 2010a). Even if the data were restricted to the EEP region from the founding of the EEP, the studbook nevertheless provides 19 years of individual data for 1970 individuals (Gilbert 2010a). The extent of these data allows precise estimates of model parameters (Boyce 1992; Conde *et al.* 2011a; Coulson *et al.* 2001; Lacy 2000a; Lande *et al.* 2003).

An important aspect of the PVA process is model validation (Boyce 1992; McCarthy & Broome 2000). Uncertainty is a concern in the evaluation of model predictions, and it is

important to assess the sensitivity of the model to variations in input parameters (Boyce 1992; Kohmann *et al.* 2005; Lande *et al.* 2003; Possingham *et al.* 1993). However, models should also be validated independently (Patterson & Murray 2008). This is problematic if all the available data have been used to construct the model (Ellner *et al.* 2002; McCarthy *et al.* 2001). Some authors have split the available data and used half to estimate parameters for the model, and half to test its accuracy in order to avoid circularity (Brook *et al.* 1999; Coulson *et al.* 2001).

PVAs have also been criticised because they assume that future trends will reflect historical population dynamics (Patterson & Murray 2008), and this may not be the case (Coulson *et al.* 2001).

Most PVAs do not include interactions with other species and ecosystems, and consequently are not realistic characterisations of most natural populations (Boyce 1992; Lande *et al.* 2003). However, captive populations are removed from these interactions, so single-species analyses are appropriate in this case.

PVAs do not predict what will happen to a population, they simply forecast the likely effects of those factors that are included in a model (Lacy 1993b; Lacy 2000a). Some parameters may be estimated, or not included at all (Ludwig 1999; Traill *et al.* 2010), and VORTEX does not model all possible population dynamics (Lacy 1993b). Consequently, PVAs may over- or underestimate population viability (Lacy 2000a).

Despite the criticisms, PVAs can be extremely precise and accurate in their predictions of population viability when good quality data are used to determine the input parameters (Brook *et al.* 1999; Clark *et al.* 1991; Lindenmayer *et al.* 2003; Reed *et al.* 2003c; Traill *et al.* 2010). They are particularly useful in evaluating different management options, as relative predictions are generated, rather than absolute forecasts for a population (Brook *et al.* 1999).

This chapter aims to evaluate the pre-extinction population dynamics and the probability of extinction of the scimitar-horned oryx EEP under various scenarios. The EEP is the largest intensively managed scimitar-horned oryx population in the world (Gilbert 2010b; Spevak 2009; Wilkins 2009), and it is central to the persistence of the species. Assessing and understanding its viability is vital in effectively managing the captive population and reintroducing it back to its historic range. The scimitar-horned oryx is also representative of many other captive ungulate species in terms of geographic distribution and population fragmentation. Consequently, the results will help guide the management of other endangered species in captivity.

Its specific objectives are: 1) to test the sensitivity of the model to different genetic management strategies, varying annual female reproduction, and increasing levels of inbreeding as represented by lethal equivalents; 2) to establish the MVP needed to attain a 99% probability of population persistence for 100-years; 3) to evaluate the impact of population sub-division under five fragmentation scenarios on population viability; 4) establish the optimal level of migration between population sub-units in a fragmented scimitar-horned EEP population.

8.3 Methodology

8.3.1 Model parameters

8.3.1.1 Model definitions

The baseline model was created in VORTEX v. 9.99b (Lacy *et al*. 2009) using the parameters specified in Table 8.1. The baseline model was modified to allow population sub-division. The simulations were run 500 times for each model in order to obtain a rigorous description of the population's behaviour under deterministic and stochastic influences (Miller & Lacy 2005). A time period of 100-years was chosen over which to run the simulations. A one hundred year period represents approximately 15 non-overlapping generations for the scimitar-horned oryx, and is ten times as long as the mean lifespan (Chapter Six). Restricting the analyses to 100-years provides enough time for stochastic processes to influence the population dynamics for this species, but keeps it within a realistic management time-frame.

The definition of population extinction for the simulation was 'complete extinction' when no individuals in the population remained, or 'functional extinction' when only individuals from one sex remained after 100-years.

8.3.1.2 Inbreeding

Inbreeding depression was defined by juvenile mortality (individuals that died within one year of birth) and longevity in the EEP population of scimitar-horned oryx. The impact of inbreeding on reproduction was not evaluated as breeding is controlled through EEP recommendations. Differences in fitness may not be discernable under these non-competitive captive conditions (Miller & Hedrick 1993). Data were extracted from the studbook for all animals that died between 1990 and 2008 in the EEP. All individuals that had less than 62.5% known pedigree (Chapter Four), that had unknown lifespans due to missing birth or death dates, were of unknown gender, and were euthanased for management reasons were removed from the analyses. This resulted in a sample size of $N = 405$ for the impact of inbreeding on longevity and a sample size of $N = 335$ for the impact of inbreeding on juvenile mortality.

Longevity data did not have normally distributed residuals and did not respond to transformation, so a Spearman rank-order correlation tested the relationship between inbreeding coefficient and longevity.

In order to evaluate the impact of inbreeding on juvenile mortality, the proportion of the analysed population that died within the first year of life (365 days) was calculated for six inbreeding coefficients ± 0.01: 0.0, 0.125, 0.25, 0.375, 0.5, and 0.625. Sample sizes were N = 65, 5, 17, 21, 15 and 5, respectively. Data residuals were not normally distributed and were transformed using an arcsine square root transformation. A Pearson's product-moment correlation was applied to the data, and then applied again after the F = 0.0 category was removed. The number of lethal equivalents per diploid individual was calculated by plotting the juvenile mortality data on the x-axis and fitting a best-fit line to it. The mean number of lethal equivalents per gamete is close to the line (*b*). In diploid individuals the number of lethal equivalents is twice that of *b* (Ralls *et al.* 1988).

The results of the inbreeding depression analyses were incorporated into the PVA model, with 50% of *LE* due to recessive alleles. Restricting dominant *LE* to 50% allowed some lethal alleles to be purged, but did not facilitate a complete purge (Reed 2004). Evidence of purging in captive populations suggests that it is inefficient when it occurs (Boakes *et al.* 2007; Brito 2009; Frankham 2005a; Larsen *et al.* 2011). Estimates of lethal equivalents for captive scimitar-horned oryx differed between those calculated from the studbook and those reported by Ralls *et al*. (1988), and so this factor was selected for sensitivity testing in the PVA.

8.3.1.3 Reproduction

Scimitar-horned oryx have a polyganous breeding system both in captivity, where oryx are usually held in harem groups with one male and 1-40 females (Gilbert 2010a), and in the wild where mixed sex herds are dominated by one breeding male (Gilbert & Woodfine 2004a). Consequently a polyganous breeding system was included in the PVA model.

Studbook data on all individuals born between 1990 and 2008 (N = 1924) were exported from the international studbook and the population means, medians and standard deviation was calculated for age at first reproduction, age at last reproduction, and longevity for males and females. Whisker box plots were used to remove outliers so the calculations were not biased by extreme variables. Post-reproductive lifespans tend to be inconsistently calculated and are inherently biased (Levitis & Bingaman Lackey 2011), and so the integer median reproductive values were used to define the reproductive and longevity parameters for the population viability model (Miller & Lacy 2005).

The number of broods per year, the distribution of broods, the maximum number of progeny per brood, and the proportion of progeny born into different litter sizes was calculated from the exported studbook data, as was the mean number of breeding adult males and females to form the input parameters for the PVA. Density dependent reproduction was included in the model as reproduction is at least partially controlled through EEP breeding recommendations that pair more individuals at lower population densities, and fewer as the population approaches capacity. The impact of density dependent in the population was represented by: = [$\bar{x}\,F_P$ – {($\bar{x}\,F_P$ - $\bar{x}\,F_R$)*((N/K)^1)}]*(N/(0+N)), where $\bar{x}\,F_R$ represents the mean number of females reproducing in the population as the population approaches capacity, and $\bar{x}\,F_P$ represents the mean percentage of adult females that produce an offspring when paired with an adult breeding male i.e. potential reproduction. The mean number of adult females that reproduced was calculated from studbook data, and the mean percentage of females that reproduced when placed in a breeding situation was derived from the calculations in chapter six. An Allee effect was not included in the function i.e. the Allee effect was modelled as zero, as SH oryx do not require a minimum herd size to breed, and mate acquisition is not a limiting factor as they are polyganous species (Stephens *et al.* 1999).

Environmental stochasticity has a major influence on population viability in both large and small populations (Lande 1993; Robert 2006; Reed 2004), but dominates demographic stochasticity in populations of N = 100 or more (Lande 1988). Consequently, it was important that variance due to environmental stochasticity was included in the model. The proportion of total variance (environmental and demographic variation) for breeding females attributable to environmental variance is explained by: $\sigma_{EV} = \sqrt{\sigma^2_{EV}} = \sqrt{\sigma^2_{TOT} - \overline{\sigma^2_{DS}}}$ where σ^2_{TOT} is the total variance across the data, and $\overline{\sigma^2_{DS}}$ is the mean binomial variance across female breeding rates (Miller & Lacy 2005). The variance attributable to demographic stochasticity (σ_{DS}) is explained by: $\sigma_{DS} = \sqrt{\hat{p}\hat{q}/n-1}$ where $\hat{p}$ is the proportion of observations in a category, and $\hat{q}$ is the reciprocal $(1-\hat{p})$ (Miller & Lacy 2005).

Mean annual adult male and female reproduction rates were plotted and quadratic trend models fitted to the data using time series analysis to evaluate changing trends in male and female reproduction over time (Chatfield 2004). Sensitivity testing was applied to the percentage of adult females breeding to evaluate the impact of increased

reproduction on population viability and retention of genetic diversity (Kohmann *et al.* 2005).

8.3.1.4 Mortality

Age specific mean mortality rates for male and female scimitar-horned oryx in the EEP were calculated for juvenile, sub-adult and adult age classes from data extracted from the studbook (all EEP deaths between 1990 and 2008). The variance in mean mortality attributable to environmental variance was calculated using the same method as the environmental variance estimation for reproduction (Appendix K).

8.3.1.5 Environmental concordance between survival and reproduction

Data on oryx mortalities between 1990 and 2008 were extracted from the international studbook and circumstances of death relating to environmental factors analysed (N = 1367). Environmental conditions do not vary between the different stages of the lifecycle for scimitar-horned oryx i.e. they are either held in the same facility all of their lives, or transferred to other captive facilities with comparable husbandry standards. As a result the PVA model included concordance between survival and reproduction (Miller & Lacy 2005).

8.3.1.6 Environmental correlation among populations

All EAZA institutions have to meet a minimum standard of animal husbandry and welfare (EAZA 2004), and the husbandry guidelines for scimitar-horned oryx (Gilbert & Woodfine 2004a) have been distributed to all EEP participants. Consequently, husbandry standards should be comparable across the EEP resulting in some measure of environmental correlation between different institutions. However, the EEP covers 52 institutions in 16 countries, and some of the effects of environmental and climatic variation between metapopulation sub-units may be evident. As a result an environmental correlation among populations of 50% was included for the metapopulation models.

8.3.1.7 Catastrophes

Catastrophes are extreme forms of environmental variation that result in sudden large changes in reproduction and/or survival (Miller & Lacy 2005; Robert 2006). Data on scimitar-horned oryx that reproduced and died in the EEP between 1990 and 2008 (Gilbert 2010a) were analysed for evidence of catastrophic population declines caused by reproduction, mortality or external factors. In this instance evidence for catastrophic events was defined as demographic rates different to those described by normal levels of

variation. Specifically, catastrophic rates were at least two standard deviations from the mean value (Miller & Lacy 2005). Evidence of two types of catastrophic events was found within the studbook data, both of which effectively impacted survival. The impact of the catastrophes, defined by severity and frequency, were calculated for each type of catastrophe. The resulting data were then inputted into the PVA model.

8.3.1.8 Dispersal

Data on all transfers between institutions within the EEP between 1990 and 2008 were extracted from the studbook. Mean and median age at first and last transport, and post-transport survival rates for males and females were calculated. Dispersal rates for males and females between the EU, non-EU countries, the UK, each bluetongue zone, and each country were calculated based on historical transfers. Differences between male and female dispersal rates were examined using a Wilcoxan signed rank test as data were not normally distributed and did not respond to transformation. The dispersal rates were used to specify dispersal between population sub-units in the fragmented EEP PVA models.

8.3.1.9 Carrying capacity

The scimitar-horned oryx EEP has the largest antelope population in Europe (ISIS 2010), and there are no plans to increase the number of EEP participants as this would negatively impact on the captive breeding programmes for other important aridland antelope, for example the addax and Arabian oryx. Consequently, carrying capacity of the EEP population is predicted to remain static for the foreseeable future (Gilbert 2010b). As a result carrying capacity was modelled at the 2010 *K*.

Quasi-density-dependence was included in the model as VORTEX truncates the population size when it exceeds the user-defined carrying capacity (Kohmann *et al.* 2005; Reed *et al.* 2003c; Reed 2004).

8.3.1.10 Population harvest

The EEP population is a source of animals for reintroduction projects in North Africa (Gilbert 2010a; Woodfine *et al.* 2009), and removal of individuals for reintroduction was modelled as a regular harvest from the population. The international studbook was analysed to ascertain the mean number, and the demographic composition, of individuals removed from the EEP population for past reintroduction events. Future reintroductions are likely to take place at lower frequency than has been observed in the past, with the aim of genetic augmentation of existing populations rather than the creation of new ones. There is

currently a lack of genuine opportunity for future reintroductions throughout most of the species' historical range and this limits the number and scope of new projects (Chapter Two). Additionally, other source populations are available. As a result of these considerations, the frequency of population harvests for reintroduction purposes was reduced from historical levels. Individuals will not be supplied for reintroduction if the EEP population is far below the carrying capacity. Consequently, harvest was modified with the density-dependent function = (N/K) > 0.80, so that animals would not be removed if the population fell below 80% of carrying capacity.

8.3.1.11 Population supplementation

The scimitar-horned oryx is extinct in the wild (IUCN 2010) and so captive populations cannot be supplemented by wild-caught individuals. Unrelated animals are known to exist in the North American SSP population (Gilbert 2010a; Iyengar *et al.* 2007), and thought to exist in the Japanese and Middle Eastern populations (Nishiki 1992; Soulé *et al.* 1986); Ogden, R. pers. comm., 2010), but the transfer of exotic bovids into the European Union is extremely difficult due to legislation aimed at the agricultural industry (DEFRA 2010; DEFRA 2011). As a result the EEP was modelled as a closed population in all scenarios, with no supplementation.

8.3.1.12 Genetic management

The initial population structure in the model was replaced by an analytical studbook file containing all historical data on the EEP up to the 31/12/2010. One neutral loci was modelled to obtain an estimate of the effects of population dynamics on retention of genetic diversity. The genetic management options in the model were activated as the scimitar-horned oryx EEP is actively managed by a coordinator (Gilbert 2010b).

The breeding plan was set to maintain the population at the carrying capacity as long as the population dynamics allowed it. The EEP population has a mean inbreeding coefficient of $F = 0.184$, consequently individuals were not paired if the inbreeding coefficient of the offspring exceeded $F = 0.5$. Pairs were constructed using the methods employed by EEP coordinators whereby breeding priority is assigned to individuals with the lowest mean kinships (Chapter Five). The VORTEX program (Lacy *et al.* 2009) allows the pairing of individuals based on either dynamic mean kinship lists, whereby the *MK* coefficients are adjusted after each pair is made, or static *MK* lists, whereby *MK* coefficients are not adjusted after individuals are paired. Alternatively, individuals can be paired randomly with reference to mean kinship (Lacy *et al.* 2009; Miller & Lacy 2005).

Complexity of the model increases from random mating to the static MK method to the dynamic MK method, so the genetic management strategy was sensitivity tested. The genetic management selected was based on the result of the sensitivity analysis.

The number of times a simulation attempted to identify a mate for any one individual was limited to 10 times to ensure that each iteration was manageable. The maximum number of females paired to one male was limited to the mean harem size in the EEP population.

8.3.2 Model variations

The baseline model was created in VORTEX v.9.99b using the parameters specified in table 8.1. Additional scenarios were created to test the sensitivity of the genetic management strategy (N = 3), the impact of varying female reproduction values (N = 14), varying lethal equivalents per diploid (N = 17), and differing carrying capacity (N = 16) on population viability and retention of genetic diversity. The different scenarios in the carrying capacity model (K) enabled estimation of the minimum viable population size (MVP_K) for a 99% probability of population persistence for 100-years (Reed *et al.* 2003c). All of these scenarios had the same variables as the baseline model with the exception that the parameter being sensitivity tested varied within specified limits (Tables 8.1 and 8.2).

A baseline model was created for each of four fragmentation models based on real-life fragmentation of the scimitar-horned oryx EEP (Figure 7.1, Chapter Seven). The fragmentation models separated the EEP population into a number of different sub-units depending on the model: 1) EU and non-EU, which separated the EEP population into two sub-units; 2) EU, UK and non-EU, which separated the EEP into three sub-units; 3) Bluetongue, which separated the EEP into five sub-units; and 4) Countries, which separated the EEP into 16 sun-units (Table 7.4, Chapter Seven).

Additional scenarios were created whereby dispersal between each of the sub-units was determined by the historical dispersal rates, no dispersal, and then dispersal rates between all sub-units of 1-10% in increasing increments of 1%. This resulted in 12 scenarios for each fragmentation model.

In total 98 scenarios were created across eight different population models (Table 8.2). Each scenario was run 500 times. A number of different population metrics were generated for each simulation, resulting in a number of mean and median demographic and genetic metrics for each scenario (Table 8.3). Metapopulation data were used for the fragmentation models and scenarios, but additional within-population data were extracted

for the baseline scenarios for each fragmentation model. Scenarios were grouped for post-simulation analyses (Table 8.4).

The mean time to extinction, mean population size of extant populations, mean gene diversity of extant populations, and the mean inbreeding coefficient of extant populations were analysed for each set of grouped scenarios, alongside an evaluation of the viability of the population (a 99% probability that the population survived for 100-years). Data were not normally distributed and did not respond to transformation. Kruskal-Wallis tests were applied with post-hoc Mann-Whitney tests incorporating a bonferroni correction to adjust the level of significance of multiple paired tests (Table 8.5) (Dytham 2011).

Some scenarios in the genetic management strategy, female reproduction, lethal equivalents, and carrying capacity models did not have any simulated populations go extinct. In contrast, all simulated populations went extinct for other scenarios e.g. in the countries model. In these instances Kruskal-Wallis tests were not applied to all analytical parameters.

Table 8.1 Parameters for the PVA model. Variations in scenario parameters are specified in Table 8.2

Parameter	Value
Model definitions	
Replications	500
Years	100-years
Extinction definition	Only one sex remains
Inbreeding	
Inbreeding depression (Lethal equivalents) *	6.97
% *LE* due to recessive alleles	50%
Reproduction	
Breeding system	Polyganous
Maximum age of reproduction (and lifespan)	10
Minimum female breeding age	3
Minimum male breeding age	4
Maximum number of broods per year	2
Distribution of broods per year:	-
1 brood	89.69%
2 broods	10.31%
Maximum number of progeny per brood:	2
litter size of 1	99.49%
litter size of 2	0.51%
Sex ratio at birth (% males)	50%
Density dependent reproduction	Yes
% of females breeding †	=[64–(N/(0+N))
Environmental variation in % of females breeding	10.97% (Appendix K)
% males in the breeding pool	42%
Mortality	
Female mortality	
Mean mortality 0 – 1 years	27.19
SD due to environmental variation 0 – 1 years	7.40
Mean mortality 1 – 2 years	4.64
SD due to environmental variation 1 – 2 years	2.99
Mean mortality 2 – 3 years	4.87
SD due to environmental variation 2 – 3 years	4.10
Mean mortality >3 years	7.25
SD due to environmental variation >3 years	1.77
Male mortality	
Mean mortality 0 – 1 years	35.72
SD due to environmental variation 0 – 1 years	7.07
Mean mortality 1 – 2 years	14.74
SD due to environmental variation 1 – 2 years	7.48
Mean mortality 2 – 3 years	8.85
SD due to environmental variation 2 – 3 years	7.17
Mean mortality 3 – 4 years	9.06
SD due to environmental variation 3 – 4 years	10.35
Mean mortality >4 years	14.34
SD due to environmental variation >4 years	4.86
Environmental stochasticity	
Environmental variation of survival & reproduction	Concordant
Environmental variation correlation among populations	0.50

Table 8.1 continued

Parameter	Value
Catastrophes	
Type of catastrophes	2
Catastrophe 1:	Disease/Infection (low level)
Global/local	Global
Frequency %	5% (once every 19 years)
Severity on reproduction	1.0 (no impact)
Severity on survival	0.97 (3% increase in mortality)
Catastrophe 2:	Political (low level)
Global/local	Global
Frequency %	10% (once every 3-4 years)
Severity reproduction	1.0 (no impact)
Severity survival	0.97 (3% increase in mortality)
Dispersal	
Dispersal age:	
Youngest disperser	1 year (males & females)
Oldest disperser	5 years (males & females)
% survival of dispersers	98%
Dispersal rates	See Tables 8.9 - 8.12 for matrices
Carrying capacity (*K*)	
Carrying capacity	430 [§]
SD in K due to environmental variation	0
Future change in K	No
Population harvest & supplementation	
Harvest	Yes
First year of harvest	year 5
Last year of harvest	Year 100
Interval between harvests	10 years
Number of females to be harvested	5
Age 1 year	4
Age 2 year	0
Adult	1
Number of males to be harvested	5
Age 1	4
Age 2	0
Age 3	0
Adult	1
Optional criterion for harvest (density dependent)	=(N/K)>0.80
Population supplementation	No
Genetic management	
Replace initial population with studbook file	Yes
Number of neutral loci to be modelled	1

Table 8.1 continued

Parameter	Value
Breeding plan	
Breed to maintain population at *K*	Yes
Prevent matings with *F* greater than	0.50
Pair according to mean kinships	Yes
Using a dynamic MK list	Yes
Number of times to try and find a mate	10
Maximum number of females to one male	15
Sensitivity testing	
* Lethal equivalents (*LE*)	*LE* = 0.0 – 12.0
† Female reproduction	F_R = 42 – 55%
§ Carrying capacity	*K* = 100 - 1500

Table 8.2 The eight population viability models with associated scenarios. The table details the variations in scenario input parameters

Model	Variable	Description
1. Genetic management scenarios		
EEP dynamic MK	Dynamic MK	Individuals were paired according to mean kinship coefficients. The MK list was updated each time a new pairing was made
EEP static MK	Static MK	Individuals were paired according to mean kinship coefficients. The MK list was not updated (remained static) as pairings were made
EEP random	Random	Pairs were selected at random as long as the inbreeding coefficient of offspring was predicted to be below *F*=0.50
2. Female reproduction scenarios		
EEP F 42%	42%	The reproductive rate, described by the percentage of adult females breeding in the population ± the proportion of total variation attributable to environmental variation (7.87%), was increased from 42% to 55% in increments of 1% per model
EEP F 43%	43%	
EEP F 44%	44%	
EEP F 45%	45%	
EEP F 46%	46%	
EEP F 47%	47%	
EEP F 48%	48%	
EEP F 49%	49%	
EEP F 50%	50%	
EEP F 51%	51%	
EEP F 52%	52%	
EEP F 53%	53%	
EEP F 54%	54%	
EEP F 55%	55%	

Table 8.2 continued

Model	Variable	Description
3. Lethal equivalent (inbreeding depression) scenarios		
EEP *LE* = 0.0	0.0	The mean impact of inbreeding on juvenile survival, quantified by the number of lethal equivalents per diploid, was increased from *LE*=0 to *LE*=12 in increments of 1.0 for each successive model. Four additional models were constructed for lethal equivalents of *LE*=0.929, *LE*=6.97, *LE*=3.14 and *LE*=9.26 representing the values calculated from EEP data before and after data transformation, and from Ralls *et al*. (1988) for mammals and scimitar-horned oryx, respectively
EEP *LE* = 0.929	0.929	
EEP *LE* = 1.0	1.0	
EEP *LE* = 2.0	2.0	
EEP *LE* = 3.0	3.0	
EEP *LE* = 3.14	3.14	
EEP *LE* = 4.0	4.0	
EEP *LE* = 5.0	5.0	
EEP *LE* = 6.0	6.0	
EEP *LE* = 6.97	6.97	
EEP *LE* = 7.0	7.0	
EEP *LE* = 8.0	8.0	
EEP *LE* = 9.0	9.0	
EEP *LE* = 9.26	9.26	
EEP *LE* = 10.0	10.0	
EEP *LE* = 11.0	11.0	
EEP *LE* = 12.0	12.0	
4. Carrying capacity scenarios		
EEP *K* = 100	100	The carrying capacity for the EEP was modelled as current capacity (*K* = EEP), and then from *K* = 100 to *K* = 1500 in increments of 100 for successive models
EEP *K* = 200	200	
EEP *K* =300	300	
EEP *K* = 400	400	
EEP *K* = EEP	430	
EEP *K* = 500	500	
EEP *K* = 600	600	
EEP *K* = 700	700	
EEP *K* = 800	800	
EEP *K* = 900	900	
EEP *K* = 1000	1000	
EEP *K* = 1100	1100	
EEP *K* = 1200	1200	
EEP *K* = 1300	1300	
EEP *K* = 1400	1400	
EEP *K* = 1500	1500	
5. EU and non-EU dispersal scenarios		
EU nonEU baseline	Matrix	The impact of differing dispersal rates on the viability of the EEP population was modelled using the EU & non-EU model of population fragmentation. Annual dispersal rates ranged from 0% to 10% and increased between models in increments of 1%. An additional model was constructed with historical dispersal rates (matrix) calculated from the international studbook
EU nonEU 0	0%	
EU nonEU 1	1%	
EU nonEU 2	2%	
EU nonEU 3	3%	
EU nonEU 4	4%	
EU nonEU 5	5%	
EU nonEU 6	6%	
EU nonEU 7	7%	
EU nonEU 8	8%	
EU nonEU 9	9%	
EU nonEU 10	10%	

Table 8.2 continued

Model	Variable	Description
6. EU, UK and non-EU dispersal scenarios		
EU UK nonEU baseline	Matrix	The impact of differing dispersal rates on the viability of the EEP population was modelled using the EU, UK & non-EU model of population fragmentation. Annual dispersal rates ranged from 0% to 10% and increased between models in increments of 1%. An additional model was constructed with historical dispersal rates (matrix) calculated from the international studbook
EU UK nonEU 0	0%	
EU UK nonEU 1	1%	
EU UK nonEU 2	2%	
EU UK nonEU 3	3%	
EU UK nonEU 4	4%	
EU UK nonEU 5	5%	
EU UK nonEU 6	6%	
EU UK nonEU 7	7%	
EU UK nonEU 8	8%	
EU UK nonEU 9	9%	
EU UK nonEU 10	10%	
7. Bluetongue dispersal scenarios		
BT Baseline	Matrix	The impact of differing dispersal rates on the viability of the EEP population was modelled using the bluetongue model of population fragmentation. Annual dispersal rates ranged from 0% to 10% and increased between models in increments of 1%. An additional model was constructed with historical dispersal rates (matrix) calculated from the international studbook
BT 0	0%	
BT 1	1%	
BT 2	2%	
BT 3	3%	
BT 4	4%	
BT 5	5%	
BT 6	6%	
BT 7	7%	
BT 8	8%	
BT 9	9%	
B 10	10%	
8. Countries dispersal scenarios		
Countries baseline	Matrix	The impact of differing dispersal rates on the viability of the EEP population was modelled using the countries model of population fragmentation. Annual dispersal rates ranged from 0% to 10% and increased between models in increments of 1%. An additional model was constructed with historical dispersal rates (matrix) calculated from the international studbook
Countries 0	0%	
Countries 1	1%	
Countries 2	2%	
Countries 3	3%	
Countries 4	4%	
Countries 5	5%	
Countries 6	6%	
Countries 7	7%	
Countries 8	8%	
Countries 9	9%	
Countries 10	10%	

Table 8.3 Mean and median population metrics generated by the 500 simulations run for each scenario

Demographic parameter	Abbreviation	Genetic parameter	Abbreviation
Probability of extinction	*PE*	Gene diversity	*GD*
Median time to extinction	$\tilde{x}$ *PE*	Number of alleles	-
Mean time to extinction	$\bar{x}$ *PE*	Mean inbreeding	*F*
Deterministic growth rate	Deterministic *r*	Lethal alleles	-
Stochastic growth rate	Stochastic *r*		
Population size	*N*		

Table 8.4 A summary of the grouped scenarios that were analysed. *ST: sensitivity testing

Analyses	Models/scenarios
Genetic management strategy (*ST)	Dynamic MK Static MK Random mating
Female reproduction (*ST)	42 - 55% (14 scenarios)
Lethal equivalents (*ST)	0 – 12 (17 scenarios)
Carrying capacity (*ST)	100-1500 (16 scenarios)
Impact of population fragmentation	EEP baseline EU & non-EU baseline EU, UK and non-EU baseline Bluetongue baseline Countries baseline
EU & non-EU dispersal	0 – 10% (12 scenarios)
EU, UK and non-EU dispersal	0 – 10% (12 scenarios)
Bluetongue dispersal	0 -10% (12 scenarios)
Countries dispersal	0 – 10% (12 scenarios)

Table 8.5 The bonferroni corrected levels of significance for paired Mann-Whitney U tests for each set of scenario analyses

Model	Bonferroni corrected level of significance
Genetic management strategy	0.025
Female reproduction models	0.0038 (0.0045*)
Lethal equivalent models	0.0031
Carrying capacity models	0.0033
Impact of fragmentation models	0.0125
EU and non-EU dispersal models	0.0045
EU, UK and non-EU dispersal models	0.0045
Bluetongue dispersal models	0.0045
Countries dispersal models	0.0045

* the level of significance for mean time to extinction

8.4 Results

8.4.1 Model parameters

8.4.1.1 Inbreeding

Juvenile mortality did not depend linearly on the inbreeding coefficient (r_4 = 0.749, P = 0.087). When individuals with an inbreeding coefficient of F = 0.0 were removed, so the data only reflected increasing values of inbreeding, there was a clear association (r_3 = 0.976, P = 0.005), although it should be noted that sample size was very small (Figure 8.1). Juvenile mortality was higher for individuals with F = 0.0 than those with inbreeding coefficients of F = 0.125, 0.25, and 0.375. It was possible to fit a line to the data to calculate the lethal equivalents, although a polynomial would have been a better fit. A polynomial line was not applied because using multiple parameters to describe *LE* was beyond the scope of VORTEX.

Ralls *et al.* (1988) estimated scimitar-horned oryx to have a mean of b = 4.63*2 = 9.26 *LE* per individual (based on death with 180 days of birth). The number of *LE* per diploid calculated from the SHO studbook data was b = 0.4594*2 = 0.9188. After the data were transformed the number of lethal equivalents per diploid was b=3.4869*2 = 6.97 (Figure 8.1). There was no relationship between individual inbreeding coefficient and longevity (r_{403} = -0.077, P = 0.120).

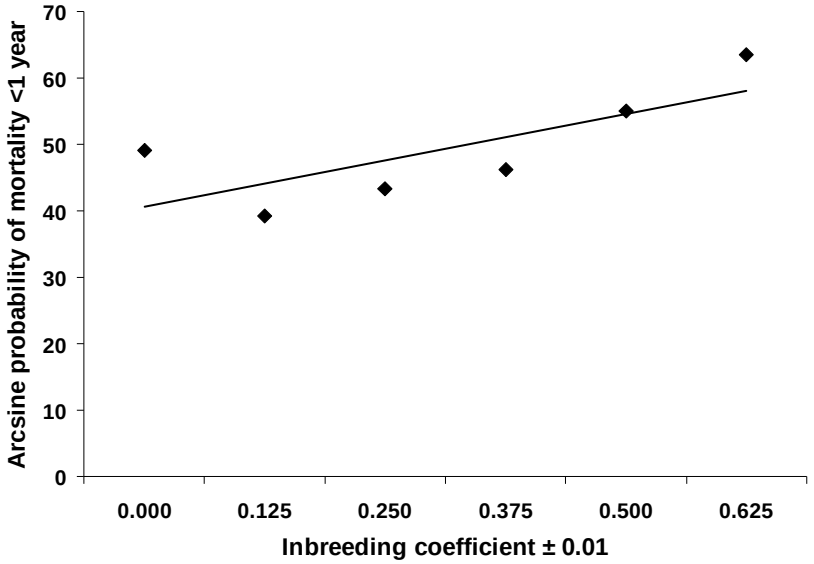


Figure 8.1 The probability of juvenile mortality in relation to the inbreeding coefficient. Samples sizes were, N = 65, 5, 17, 21, 15 and 5, for F = 0.0, 0.125, 0.250, 0.375, 0.500 and 0.625 respectively. All F values were ± 0.01. The Y intercept at X = 0 was 37.138, and the slope was b = 3.4869.

8.4.1.2 Reproduction

The age at first and last reproduction and longevity for males and females is detailed in Table 8.6. The median male and female longevity were averaged i.e. 10 years, to obtained longevity data for the PVA model. The results of the analysis on frequency of reproduction and litter size are detailed in Table 8.1

Table 8.6 Reproduction and longevity data in years for EEP male and female scimitar-horned oryx. N: sample size; *SD*: standard deviation

Parameter	*N*	Mean	*SD*	Median
Males				
Age at first reproduction (years)	129	4.3	2.2	3.6
Age at last reproduction (years)	84	8.3	4.1	7.7
Longevity (years)	586	9.0	4.4	8.0
Females				
Age at first reproduction (years)	387	3.9	2.0	3.3
Age at last reproduction (years)	210	9.2	4.3	8.9
Longevity (years)	581	11.8	5.0	11.9

The annual mean percentage of adult females (>3 years) breeding over the duration of the EEP was 42% ± σ of 7.9, $N = 3907$. The quadratic trend model that was fitted to the annual data indicates that % female reproduction declined from 1990 to 2008, and was predicted to continue to decline over the following 10 years (Figure 8.2). The proportion of the variance attributable to environmental stochasticity in the density dependent model was 7.9% (Appendix K). Female reproduction in the EEP was controlled by breeding recommendations, so scenarios were created which increased future annual female reproduction from 42% to 55%. The results of this analysis are reported under the model variation results. EEP recommendations are issued on an annual basis, so it is expected that female reproduction will vary between years and this variation is related to available space in the EEP (carrying capacity). As a result, female reproduction was modelled as being density dependent.

Studbook data were used to construct the function for density dependent reproduction which modified annual female reproduction values. When an adult female was paired with an adult male there was a 64% probability ± σ 11% that the pair would produce a live offspring (Chapter Six). Consequently, the function specified that 42% of adult females would reproduce when *N* equalled *K* and 64% would reproduce when *N* was below *K*. This resulted in density dependent reproduction being explained by = [64 – {(64-

42)*((N/K)^1)}]*(N/(0+N)). The proportion of the variance attributable to environmental stochasticity in the density dependent model was 10.97% (Appendix K).

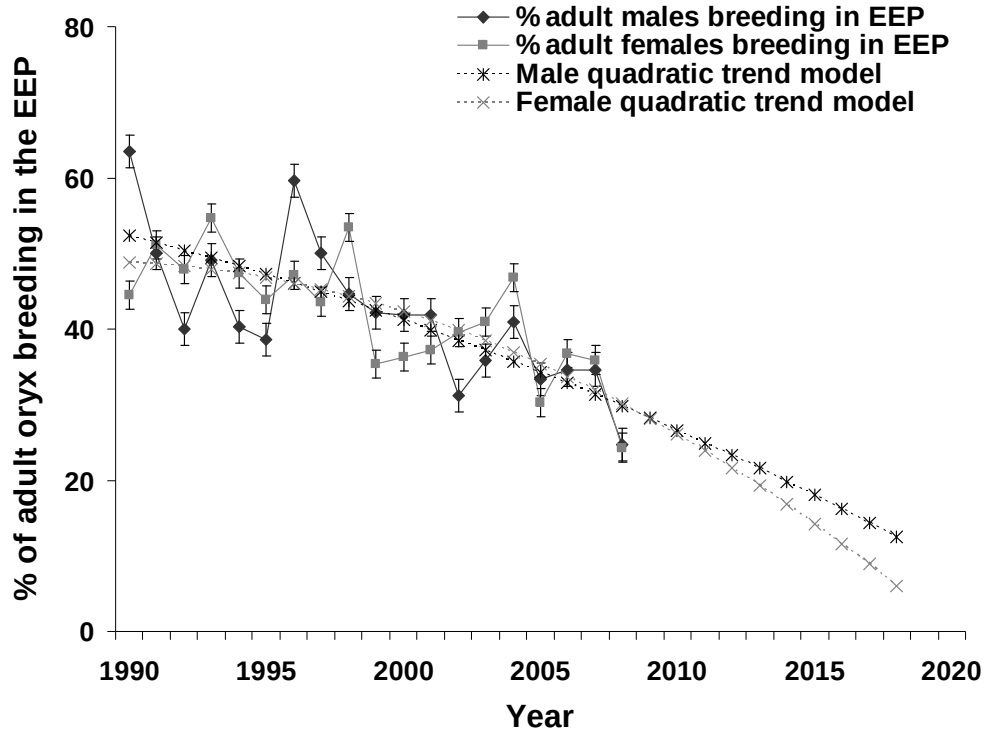


Figure 8.2 The annual percentage of males and females breeding between 1990 and 2008. Quadratic trend model was fitted to both the male and female data to predict future trends. The fitted trend equation for the male model was Yt = 53.31 – 0.92*t – 0.0169*t**2, and the equation for the female model was Yt = 48.97 – 0.070*t – 0.0486*t**2.

8.4.1.3 Mortality

Annual mortality rates varied between 1990 and 2008 with no apparent trend (Figure 8.3), and so the mean age- and sex-specific values (Table 8.1) were used to construct the PVA model. The results of the calculations for the proportion of total variance attributable to environmental variation are in Appendix K. Analysis of data on the circumstances of death of EEP oryx between 01/01/1990 and 31/12/2008 (*N* = 1367), showed that the most common circumstance of death was other (unknown), followed by euthanasia (medical), and cull. These categories, along with euthanasia and other (medical) are either ambiguous or due to decisions made by managers. Four percent of total deaths were due to environmental or behavioural conditions. When injuries from predators and deaths from infections were included, this increased to 14% of total deaths. When unknown death

circumstances and euthanasia (unknown and cull) were removed, this increased to 9% of total mortalities, and when infections and injuries from predators were included this increased to 28%. However, it is possible that environmental conditions contributed to deaths that were recorded as alternative mortalities.

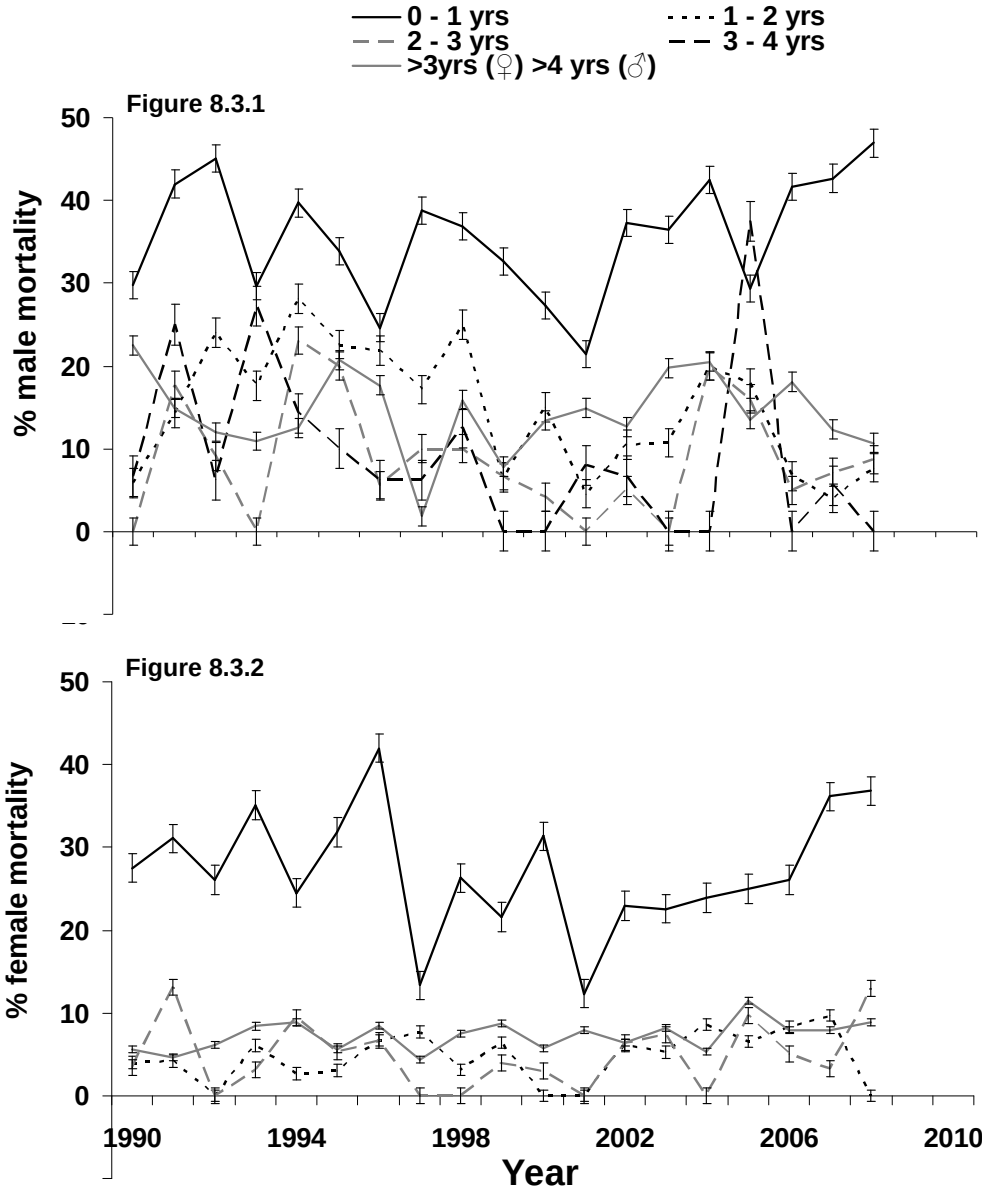


Figure 8.3 Age-specific annual mortality rates males for male (**8.1.1**) and female (**8.1.2**) scimitar-horned oryx in the EEP as a percentage of total males and female for each age class. Age classes 3-4 years and >4 years are only applicable for males, and age class >3 years is only applicable for females, due to the inter-sex differences in mean age at first reproduction (sexual maturity)

Table 8.7 Circumstances of death in the scimitar-horned oryx EEP population between 1990 and 2008. The circumstances are ranked in descending order. The five circumstances of death highlighted in light grey are those that are unidentified (other unknown), ambiguous (euthanasia medical, other medical), or are the result of decisions to euthanase individuals (cull and euthanasia). The two most common circumstances of death are highlighted in mid- and dark-grey for total deaths, male deaths and female deaths (once the unknown, ambiguous and deliberate death categories have been removed)

Circumstance of death	% Total	Rank	% ♂ deaths	Rank	% ♀ deaths	Rank
Anaesthesia / restraint	2	10	2	10	2	11
Cull	9	3	15	2	4	7
Died in transit	1	14	1	12	1	12
Environmental / behavioural	4	8	4	7	4	5
Euthanasia	3	9	4	9	3	9
Euthanasia (medical)	12	2	9	3	16	2
Infection	9	4	6	4	13	3
Injury by exhibit mate	5	6	6	5	3	8
Injury from predator	<0.5	16	<0.5	14	<0.5	15
Malicious destruction	<0.5	15	<0.5	14	<0.5	14
Old age	2	11	1	11	2	10
Other (medical)	7	5	6	6	9	4
Other (unknown)	40	1	41	1	38	1
Premature birth	1	13	1	13	1	13
Self inflicted injuries	1	12	1	12	1	12
Stillbirth	5	7	4	8	4	6

8.4.1.4 Catastrophes

Studbook analysis revealed evidence for two types of historical catastrophic population declines in the EEP population: Mortality and loss of carrying capacity. Mortality data on scimitar-horned oryx showed that infection was the most common cause of death for males and females (Table 8.7), once the unknown and euthanasia categories were removed. Catastrophic death due to infection was evident once each for males and females over the 19 year period (♂ $\bar{x}$ = 12%, σ = 6, in 2005 18% of male deaths were caused by infection; ♀ $\bar{x}$ = 12%, σ =6, in 2000 24% of female deaths were caused by infection). The impact on the population resulted in increased mortality of 2% in 2000 and 4% in 2005. Consequently, the frequency of low level disease/infection catastrophic events was modelled at a frequency of 5% (once every 19 years) and a severity of 3% impact on mortality and 0% impact on reproduction.

The second catastrophe was due to loss of 'habitat' (carrying capacity) caused by political decisions by EEP participants. In the last 10 years (since 1998) one institution has sold its entire herd of SHO to a non-EEP and non-EAZA institution, and two institutions

were excluded from the EEP and/or EAZA resulting in their oryx being removed from the EEP programme. Additionally, individual institutions have occasionally sold animals to dealers without consulting the EEP coordinator in advance. These scenarios are demographically equivalent to sudden mortality due to animals being removed from the population, and loss of habitat or carrying capacity as institutions are excluded from the EEP. These occurrences have resulted in 2-3% of the population being lost in each event, and have occurred three times every 10 years. This catastrophe was modelled with a 10% frequency (occurring once every 3-4 years, and a 3% impact on mortality).

8.4.1.5 Dispersal

The mean and median values for age at transport for male (N = 252) and female (N = 252) scimitar-horned oryx between EEP institutions are detailed in Table 8.8. Mortality during, or immediately after transport was low with 2.1% of males and 1.6% of females dying from transport related injuries or conditions e.g. capture myopathy. Consequently, a 98% transport survival rate was incorporated into the PVA model.

Dispersal rates were calculated based on historical transfers between each sub-unit of the four fragmentation models (Tables 8.9, 8.10, 8.11 and 8.12). There was no difference in dispersal rates between males and females (between EU (excluding the UK), UK and non-EU sub-units; W_3 = 3.0, P = 1.00, between the bluetongue zones; W_{12} = 49.5, P = 0.433, and between countries; W_{55} = 968, P = 0.098). As a result, the total dispersal rates, rather than sex-specific dispersal rates, were used to construct the PVA model. Total annual dispersal rates across each of the metapopulations were 0.26, 1.73, 7.52, and 8.91 for the EU non-EU model, the EU, UK, and non-EU model, the bluetongue model, and the countries model respectively.

Table 8.8 Descriptive statistics for age at transport for scimitar-horned oryx between EEP institutions. Data are in years unless otherwise specified in the table

	N	Mean	*SD*	Median
Males				
Age at 1st transport (years)	342	2	1	1
Age at last transport (years)	349	2	3	1
Minimum age 1st transport (years)	342	56 days		
Maximum age at last transport (years)	349	14		
Females				
Age at 1st transport (years)	294	3	3	1
Age at last transport (years)	312	3	4	1.4
Minimum age 1st transport (years)	294	63 days		
Maximum age at last transport (years)	312	17		

Table 8.9 Historical dispersal rates (as a percentage of the population) between the EU and non-EU sub-units in the EU non-EU fragmentation model

			To	
			1	2
			EU	non-EU
From	1	EU	99.74	0.26
	2	non-EU	0	100

Table 8.10 Historical dispersal rates (as a percentage of the population) between the three sub-units in the EU, UK & non-EU fragmentation model

			To		
			1	2	3
			EU	UK	non-EU
From	1	EU	98.90	0.84	0.26
	2	UK	0.63	99.37	0
	3	non-EU	0	0	100

Table 8.11 Historical dispersal rates (as a percentage of the population) between the five bluetongue sub-units in the bluetongue fragmentation model

			To				
			1	2	3	4	5
			BT EU 0	BT 8	BT 1 & 8	LRZ8	non-EU
From	1	BT EU 0	99.26	0.26	0.11	0.37	0.00
	2	BT 8	2.11	95.16	1.84	0.68	0.21
	3	BT 1 & 8	0.26	0.63	99.06	0.05	0.00
	4	LRZ8	0.53	0.26	0.21	99.00	0.00
	5	non-EU	0.00	0.00	0.00	0.00	100.00

Table 8.12 Historical dispersal rates (as a percentage of the population) between the 16 country sub-units in the countries fragmentation model

			To														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
			BE	CZ	DE	DK	ES	FR	GB	GR	HR	HU	IE	IL	IT	NL	PL	PT
From	1	BE	99.11	0.05	0	0	0.05	0.21	0.16	0	0	0	0	0	0	0.05	0.32	0.05
	2	CZ	0.26	98.68	0.16	0	0.16	0.05	0	0	0.11	0.11	0	0	0	0	0.47	0
	3	DE	0.26	0.11	97.41	0.16	0.26	0.79	0.16	0.05	0.16	0	0	0	0.11	0.16	0.32	0.05
	4	DK	0.11	0	0	99.84	0.00	0.05	0	0	0	0	0	0	0	0	0	0
	5	ES	0.05	0	0	0	99.90	0.05	0	0	0	0	0	0	0	0	0	0
	6	FR	0.32	0.05	0.21	0.11	0.11	98.89	0.05	0	0	0	0	0	0.05	0.16	0	0.05
	7	GB	0.11	0	0.05	0	0	0.11	99.36	0	0	0	0.32	0	0	0.05	0	0
	8	GR	0	0	0	0	0	0	0	99.89	0	0	0	0	0.11	0	0	0
	9	HR	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
	10	HU	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
	11	IE	0	0	0	0	0	0.05	0.37	0	0	0	99.58	0	0	0	0	0
	12	IL	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0
	13	IT	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
	14	NL	0.42	0	0.05	0	0	0.16	0.11	0.21	0	0	0.05	0	0	98.84	0.16	0
	15	PL	0	0	0	0.05	0	0	0	0	0	0.05	0	0	0	0.05	99.85	0
	16	PT	0	0	0	0	0.26	0		0	0	0	0	0	0	0	0	99.74

8.4.1.6 Carrying capacity

The current carrying capacity for the EEP as a contiguous population was $K = 430$. This was then separated into individual K for each population sub-unit, based on the current K, for each fragmentation model (Table 8.13)

Table 8.13 Carrying capacities for each country sub-unit grouped into EU, UK, non-EU sub-units and bluetongue sub-units

EU & non-EU model	EU, UK & non-EU model	BT model	Countries	K
EU	**EU**	**EU BT 0**	Denmark (DK)	19
			Greece (GR)	13
			Hungary (HU)	2
			Ireland (IE)	16
			Italy (IT)	9
			Poland (PL)	40
		EU 1 & 8	France (FR)	85
			Portugal (PT)	4
			Spain (ES)	30
		EU 8	Belgium (BE)	18
			Czech Rep (CZ)	11
			Germany (DE)	28
			Netherlands (NL)	20
	UK	**LRZ 8**	UK (GB)	75
Non-EU	**Non-EU**	**Non-EU**	Croatia (HR)	7
			Israel (IL)	53

8.4.1.7 Population harvest

The youngest age that an animal can be harvested from a PVA model in VORTEX (Lacy *et al.* 2009) is one year old (Miller & Lacy 2005). Adjusting for this, the studbook data shows that historically 79% of females and 81% of males harvested for reintroduction projects were from the one year age class, 4% of females and 11% of males from the two year age class, 4% of males from the three year age class, and 18% of females and 4% of males from the adult age class (Table 8.14). The mean numbers of oryx reintroduced per reintroduction event was 11, with an overall approximately equal sex ratio. Future reintroductions are likely to be for genetic augmentation using juveniles or young adults with a high probability of reproductive success (Gilbert 2010b), but including one or two adults to ensure social cohesion (Gilbert & Woodfine 2004a). Consequently, the harvesting of four males and four females from the first age class, and one male and one female from the adult age class were included in the PVA model.

Future reintroductions are likely to take place at lower frequency due to a lack of genuine opportunity for reintroductions throughout most of the species' historical range. Additionally, future reintroduction projects may use animals from the North American SSP and Middle Eastern populations as well as, or instead of, EEP animals. An interval of 10 years between harvesting (reintroduction) events was modelled to reflect this.

Table 8.14 Historical harvesting of scimitar-horned oryx from each age class in the EEP population for reintroduction projects in Northern Africa

			Female age class				Male age class				
Year	**Destination**	**Country**	**0-1**	**1-2**	**2-3**	**>3**	**0-1**	**1-2**	**2-3**	**3-4**	**>4**
1985	**Bou Hedma**	**Tunisia**	5	-	-	-	5	-	-	-	-
1995	**Souss Massa**	**Morocco**	-	1	-	-	2	3	-	-	-
1996	**Souss Massa**	**Morocco**	-	4	-	2	3	1	-	-	-
1997	**Souss Massa**	**Morocco**	2	-	1	-	4	2	-	-	-
1999	**Sidi Toui**	**Tunisia**	3	3	-	3	-	-	1	-	-
1999	**Oued Dekouk**	**Tunisia**	1	1	-	-	-	-	1	-	-
1999	**Bou Hedma**	**Tunisia**	-	-	-	-	-	-	-	1	-
2002	**Guembeul**	**Senegal**	-	2	-	-	2	-	-	-	-
2007	**Dghoumes**	**Tunisia**	-	-	-	-	-	-	1	-	1

8.4.2 Model variations

8.4.2.1 Sensitivity testing

Sensitivity testing of the genetic management models yielded differences in pre-extinction population dynamics. Consequently, the dynamic *MK* management strategy was included in the model. Similarly, the model was sensitive to annual female reproduction rates, so a density dependent function was included in the model. See Appendix L and M further the results of the sensitivity testing for these two parameters.

8.4.2.2 Lethal equivalents model

No simulated populations went extinct when the number of lethal equivalents (*LE*) per diploid was modelled at *LE* = 6.0 and lower (Figure 8.4). There was a small chance of extinction (*PE* = 0.002) at *LE* of between 6.97 and 8.0, but the probability of extinction didn't increase until *LE* increased to *LE* = 9.0 or more. Scenarios with populations that had up to 9.26 lethal equivalents per diploid met the goal of a 99% chance of population persistence over 100-years. There was no difference between the *LE* scenarios in mean time to extinction ($H_4 = 3.05$, $P = 0.550$) for those populations that went extinct, and median time to extinction was zero for all scenarios.

Mean size ($H_{16} = 5945.28$, $P < 0.001$), mean gene diversity ($H_{16} = 2776.56$, $P < 0.001$), and mean inbreeding ($H_{16} = 529.63$, $P < 0.001$) of extant populations all differed between the *LE* models with mean size and mean *GD* decreasing as lethal equivalents increased (at *LE* = 3 or more), and mean inbreeding increasing as *LE* increased (at *LE* = 5 and above except for close congeners) (Tables N.5 – N.7 Appendix N). This indicates that even when the probability of extinction was zero, the extant populations differed in size and in levels of genetic variation between the lethal equivalent scenarios. The differences in *N* for the remnant populations was observable between all *LE* levels, and a reduction in genetic diversity as *LE* increased was evident for scenarios with three or more *LE*.

The number of alleles present in the population after 100-years decreased as *LE* increased, and as expected the number of lethal alleles per diploid after 100-years increased as *LE* increased (Figure 8.5). The long-term stochastic growth rate became progressively more negative as lethal equivalents increased despite a constant positive deterministic growth rate across all scenarios (Figure 8.6). This means that the population would be expected to eventually go extinct regardless of the *LE* model unless a long-term positive growth rate could be achieved in the future.

These analyses indicated that the baseline PVA model was sensitive in terms of probability of extinction, and genetic and demographic metrics, to the number of lethal equivalents per diploid individual.

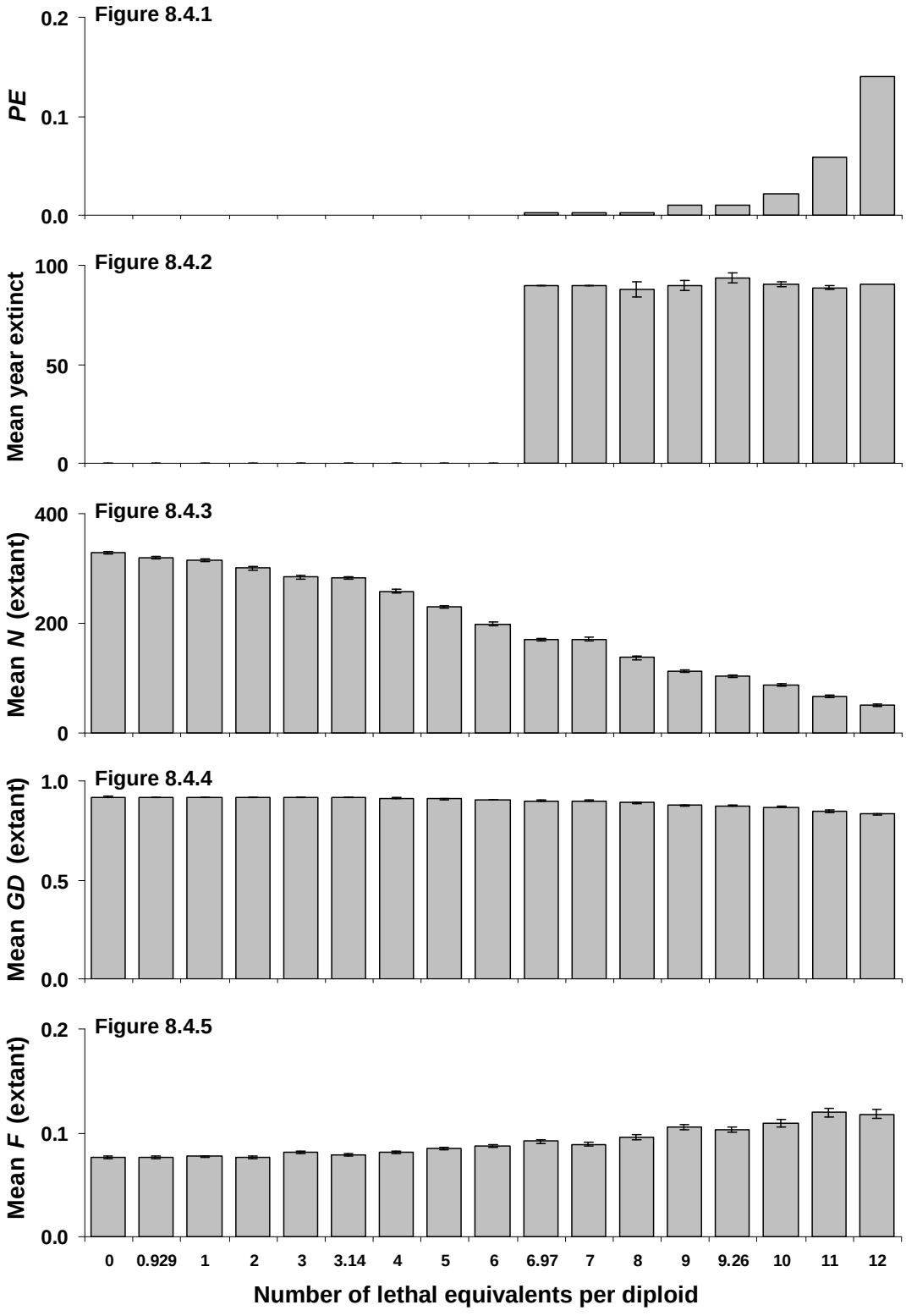


Figure 8.4 The probability of extinction (**8.4.1**), mean year of extinction (**8.4.2**), mean population size (extant populations) (**8.4.5**), mean gene diversity (extant populations) (**8.4.3**), and mean inbreeding (extant populations) (**8.4.4**) for the 16 lethal equivalent scenarios

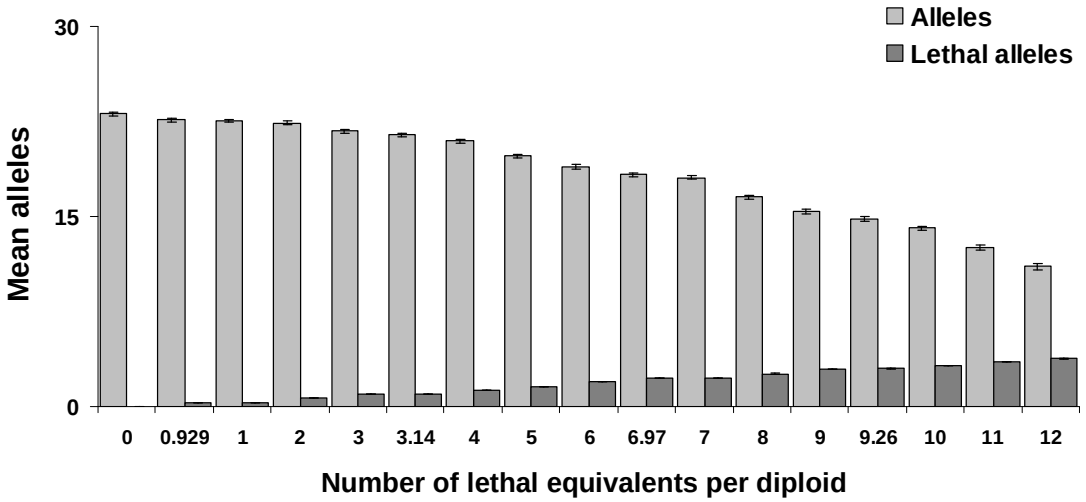


Figure 8.5 The mean number of alleles and lethal alleles per diploid present in the population after 100-years for the 16 lethal equivalent scenarios

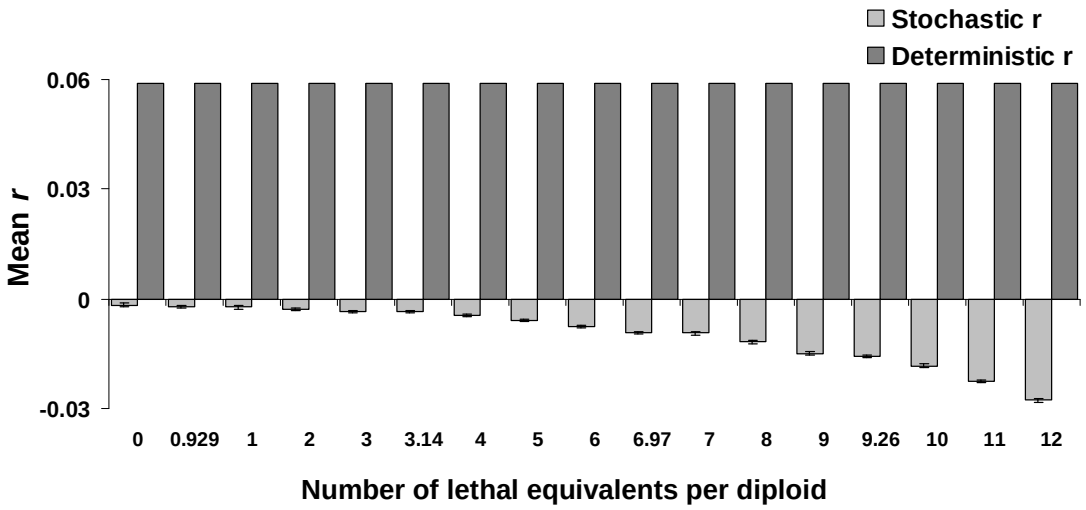


Figure 8.6 The mean stochastic and deterministic growth rates (*r*) of the population for the 16 lethal equivalent scenarios

8.4.2.3 Carrying capacity model

The probability of population extinction was highest for the K = 100 scenario (PE = 0.842), but declined sharply at carrying capacities of 200 and above (Figure 8.7). The probability of extinction reached zero at the scimitar-horned EEP capacity K = 430. Scenarios with population K = 300 or above, met the goal of a 99% chance of population persistence for 100-years. Mean time to extinction (of those scenarios where some simulated populations went extinct) increased from 74 years for K = 100 to 94 years for K = 400, and median time to extinction was zero for all scenarios except K = 100 when $\tilde{x}$ = 79 years. The K = 300 and K = 400 scenarios had only two simulated populations that went extinct, and so the analysis of mean time to extinction was limited to the K = 100 and K = 200 scenarios. The mean time to extinction did differ between these two scenarios (W = 97780, $P < 0.001$).

Mean N (H_{15} = 5868.04, $P < 0.001$), mean GD (H_{15} = 3823.09, $P < 0.001$), and mean F (H_{15} = 1854.39, $P < 0.001$) of extant populations all differed between carrying capacity scenarios. Mean N and mean GD increased as K increased and mean F decreased as K increased. Population size differed between every pairwise scenario except for K = 1400 and K = 1500 (Table N.8 Appendix N). Similarly, GD differed between every pairwise scenario at the lower carrying capacities, but once K = 900 differences between scenarios started to disappear. There was no difference between K = 1300 and above (Table N.9 Appendix N). The mean inbreeding of the extant populations revealed a similar pattern except that differences between paired scenarios started to disappear above K = 1000 (Table N.10 Appendix N). This suggests that although the probability of extinction was zero at carrying capacities of 430 and above, the N and genetic diversity of the extant populations differed between the carrying capacities until at least K = 1000.

The number of alleles retained after 100-years continued to increase as carrying capacity increased although this was very small at higher K (K = 900 and above). The number of LE per diploid plateaus once carrying capacities reached K = 1000 (Figure 8.8).

The stochastic population growth was negative until K = 900 (Figure 8.9), so at smaller carrying capacities the population would be predicted to eventually to go extinct, even though extinction did not occur within the specified time-frame of these scenarios. These analyses indicate that increasing future carrying capacity up to K = 1000 would improve genetic and demographic viability. Once carrying capacities exceed this however, there is little additional value to increasing K.

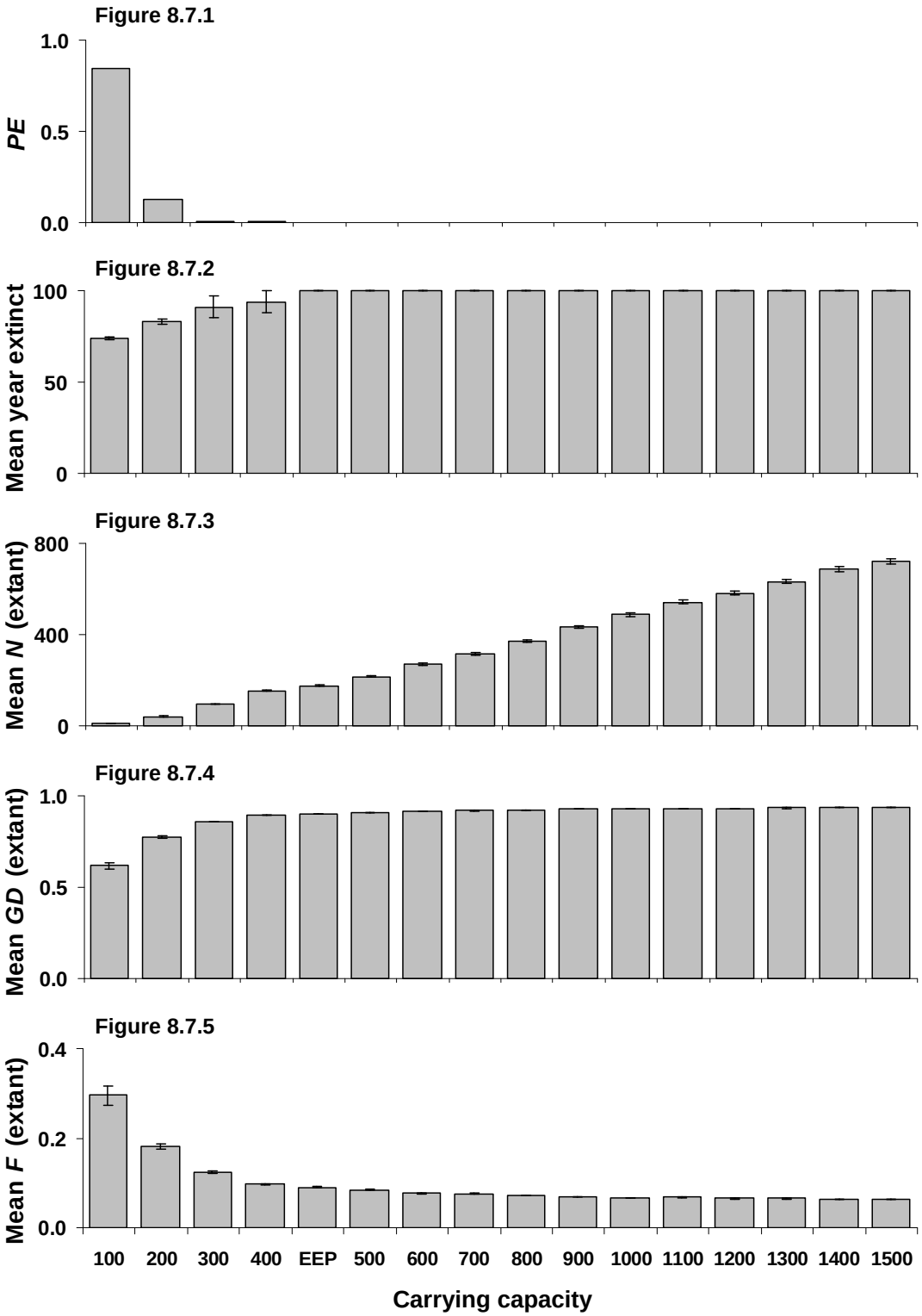


Figure 8.7 The probability of extinction (**8.7.1**), mean time to extinction (**8.7.2**), mean population size (of extant populations) (**8.7.3**), mean gene diversity of extant populations (**8.7.4**), and mean inbreeding (of extant populations) (**8.7.5**) for the 16 carrying capacity scenarios. Carrying capacity (*K*) was modelled for increasing *K* from 100 to 1500 in increments of 100

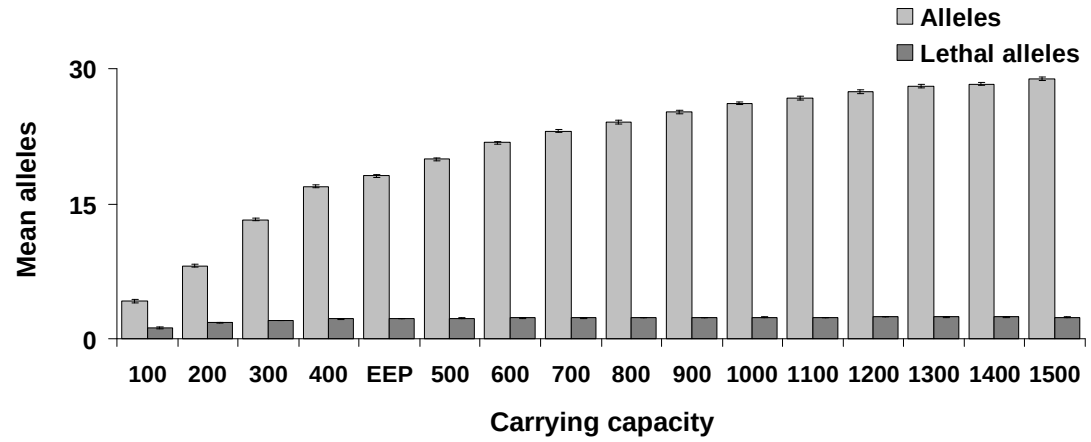


Figure 8.8 The mean number of alleles and lethal alleles per diploid present in the population after 100-years for the 16 carrying capacity scenarios

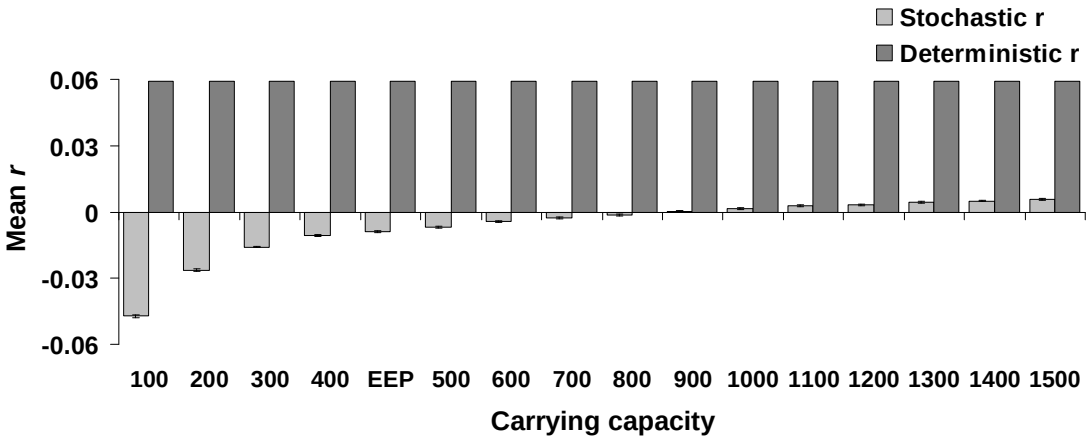


Figure 8.9 The mean stochastic and deterministic growth rates (*r*) of the population for the 16 carrying capacity scenarios

8.4.2.4 The impact of population fragmentation

The probability of population extinction increased as EEP fragmentation increased. *PE* was lowest in the contiguous EEP population (EEP baseline) (*PE* = 0.0), and increased through to the countries model, which had a probability of extinction of *PE* = 0.98 (Figure 8.10). Only the EEP baseline model met the goal of a 99% chance of population persistence for 100-years. The five fragmentation models differed in mean time to extinction (H_4 = 300.33, P < 0.001), but the difference lay between the EU, UK & non-EU model and the bluetongue model, and all fragmentation models and the countries model (Table 8.15). Median time to extinction was zero for the EEP, EU & non-EU, and EU, UK & non-EU models, and 90- and 67-years for the bluetongue and countries models, respectively.

The models also differed in the extant population size after 100-years (H_4 = 890.28, P < 0.001), and the difference lay between every pairwise comparison (Figure 8.10 and Table 8.15). The EEP simulations yielded the largest extant population size with a mean of N = 176, and the N declined as fragmentation increased until the countries model yielded a mean extant population size of N = 7.

Similarly mean *GD* (H_4 = 934.05, P < 0.001) and mean *F* (H_4 = 558.25, P < 0.001) also differed between all the models (Tables 8.16 and 8.17). *GD* decreased with increasing fragmentation and inbreeding increased with increasing fragmentation (Figure 8.10).

The increased loss of genetic diversity when the population increasingly fragmented may be attributable to an increased probability of sub-unit extinction in the fragmented populations (Figure 8.11), for example the non-EU sub-unit in the EU and non-EU model had a probability of extinction of *PE* = 0.45, and 13 out of 16 of the sub-units in the countries model had a probability of extinction of *PE* = 1.0. Correspondingly, the *N* and *GD* from those populations that did not go extinct were very small.

Retention of *GD* and alleles was higher for the metapopulation than within-population measures for all models (except the EEP as they equal each other) (Figure 8.12). This indicates that some divergence due to drift had taken place within each sub-population. This would have resulted in greater cumulative retention of genetic diversity across the whole metapopulation than would be evident from simply adding together the genetic diversity of each sub-unit.

The mean stochastic growth rate was negative for all five models (Figure 8.13), and so all populations would be predicted to decline to extinction some time after the 100 year

simulation period. The EEP model has the smallest negative growth rate, and so would be predicted to be the last simulated populations to go extinct.

These results clearly demonstrated that increasing fragmentation as per the models, increased the probability of extinction, decreased the population size and gene diversity of extant populations, and increased inbreeding in extant populations.

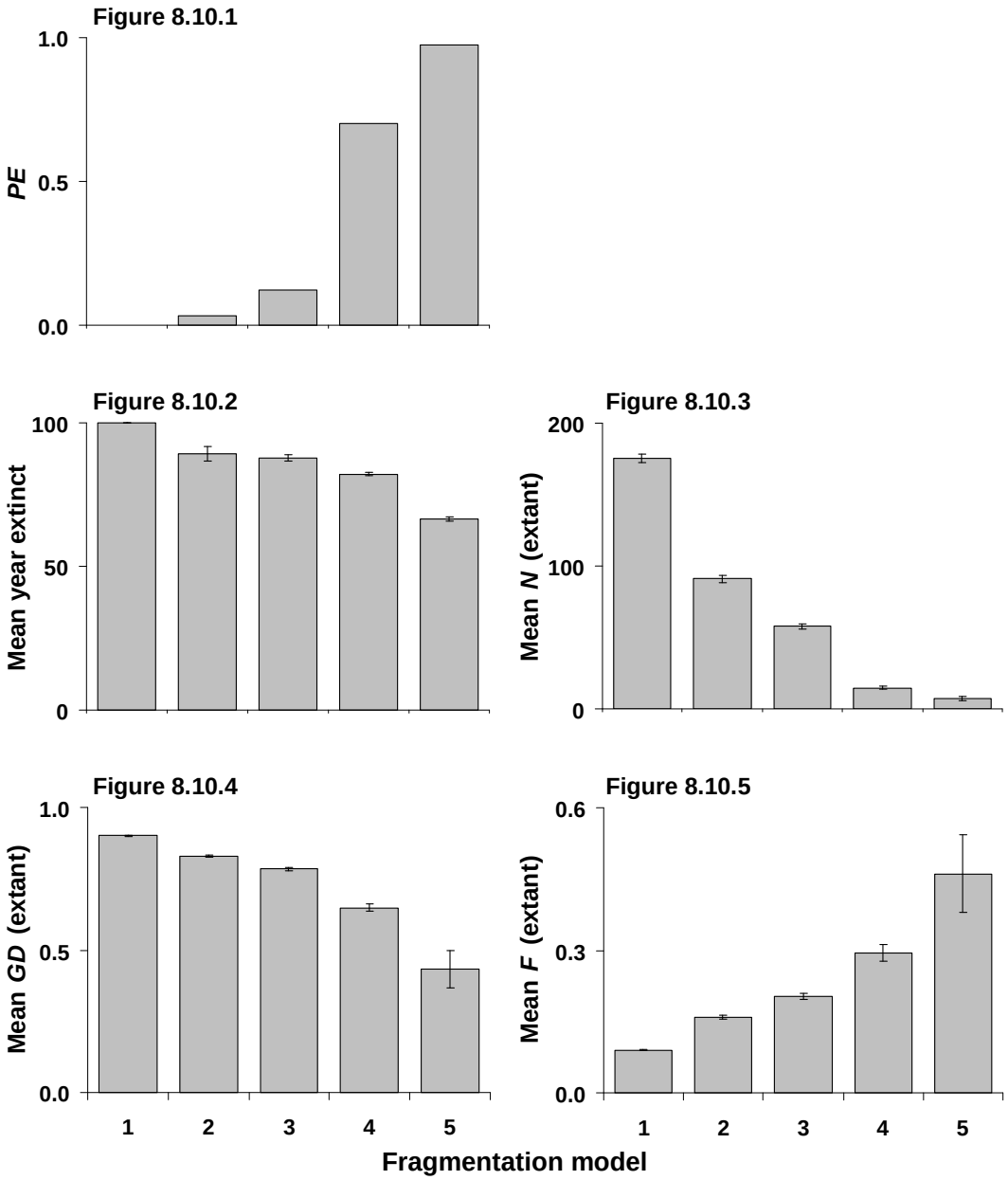


Figure 8.10 The probability of extinction (**8.10.1**), mean year of extinction (**8.10.2**), mean *N* (**8.16.3**), mean *GD* (**8.10.4**), and mean *F,* of extant populations (**8.10.5**) for the five models; EEP baseline (**1**), EU and non-EU model (**2**), EU, UK and non-EU model (**3**), Bluetongue (BT) (**4**), and countries (**5**) of fragmentation of the EEP population. Data represent the metapopulation values

Table 8.15 Results of the pairwise Mann Whitney U test with Bonferroni correction for mean time to extinction between each fragmentation model. Pairwise differences between models are highlighted in light grey

	Models			
	EU & non-EU	**EU, UK & non-EU**	**Bluetongue**	**Countries**
EEP	N/A	N/A	N/A	N/A
EU & non-EU		*W* = 684 *P* = 0.5273	*W* = 4011 *P*=0.102	*W* = 7205 *P* < 0.001
EU, UK & non-EU			*W* = 16088 *P* = 0.0002	*W* = 29056 *P* < 0.001
Bluetongue				*W* = 200314 *P* < 0.001

Table 8.16 Results of the pairwise Mann Whitney U test with Bonferroni correction for mean extant population size between each fragmentation model. Pairwise differences between models are highlighted in light grey

	Models			
	EU & non-EU	**EU, UK & non-EU**	**Bluetongue**	**Countries**
EEP	*W* = 328962 *P* < 0.001	*W* = 331141 *P* < 0.001	*W* =199699 *P* = 0.001	*W* = 78 *P* < 0.001
EU & non-EU		*W* = 261481 *P* < 0.001	*W* =184826 *P* < 0.001	*W* = 211.5 *P* < 0.001
EU, UK & non-EU			*W* =152508 *P* < 0.001	*W* = 347 *P* < 0.001
Bluetongue				*W* = 600 *P* = 0.0080

Table 8.17 Results of the pairwise Mann Whitney U test with Bonferroni correction for extant gene diversity between each fragmentation model. Pairwise differences between models are highlighted in light grey

	Models			
	EU & non-EU	**EU, UK & non-EU**	**Bluetongue**	**Countries**
EEP	*W* = 342315 *P* < 0.001	*W* = 333870 *P* < 0.001	*W* = 199338 *P* < 0.001	*W* = 78 *P* < 0.001
EU & non-EU		*W* = 259008 *P* < 0.001	*W* = 181929 *P* < 0.001	*W* = 197 *P* < 0.001
EU, UK & non-EU			*W* = 148004 *P* < 0.001	*W* = 310 *P* < 0.001
Bluetongue				*W* = 430 *P* = 0.0005

Table 8.18 Results of the pairwise Mann Whitney U test with Bonferroni correction for mean inbreeding in extant populations between each fragmentation model. Pairwise differences between models are highlighted in light grey

	Models			
	EU & non-EU	**EU, UK & non-EU**	**Bluetongue**	**Countries**
EEP	*W* = 167303 *P* < 0.001	*W* = 153538 *P* < 0.001	*W* = 134992 *P* < 0.001	*W* = 4578 *P* = 0.0031
EU & non-EU		*W* = 196655 *P* < 0.001	*W* = 136299 *P* < 0.001	*W* = 4388 *P* = 0.0042
EU, UK & non-EU			*W* =118992 *P* < 0.001	*W* = 3921 *P* = 0.0063
Bluetongue				*W* = 1267 *P* = 0.0584

Figure 8.11.1

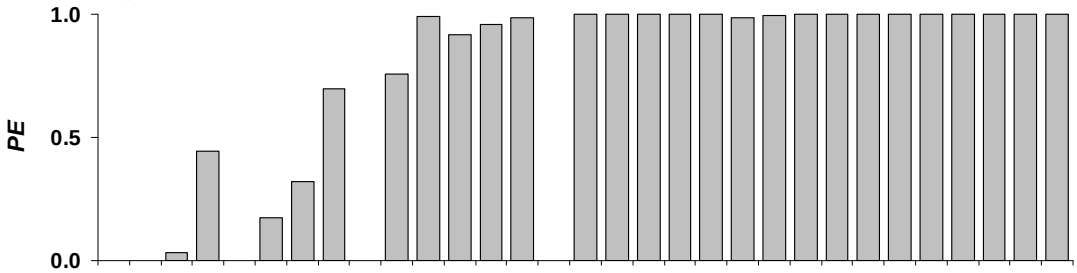


Figure 8.11.2

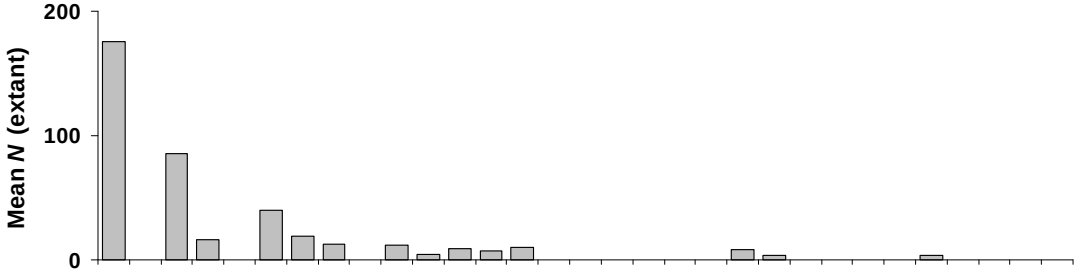


Figure 8.11.3

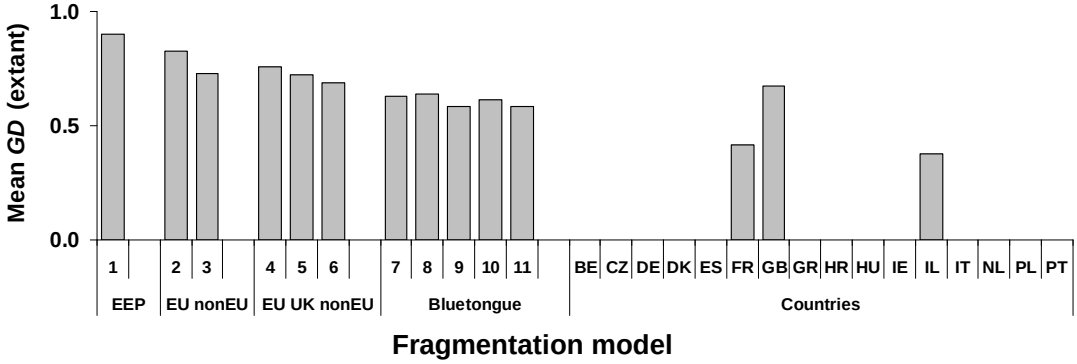


Figure 8.11 The probability of extinction (**8.11.1**), mean population size (**8.11.2**), and mean gene diversity (**8.11.3**) of extant populations, for each population subunit. Subunits are numbered 1-11 for; the complete EEP (1), EU (2) and non-EU (3), and EU (4), UK (5) and non-EU (6), BT 0 (7), BT 8 (8), BT 1 & 8 (9), LRZ 8 (10), and non-EU (11)

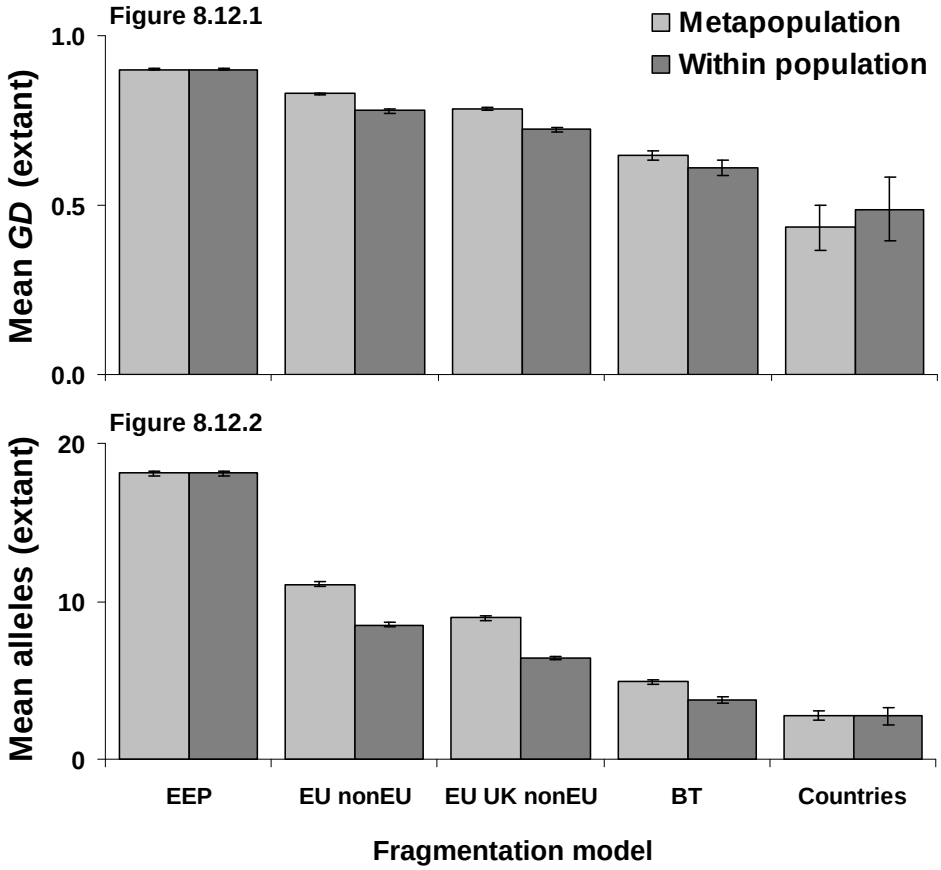


Figure 8.12 The metapopulation genetic variation and within population genetic variation as represented by mean gene diversity (figure 8.27.1) and mean alleles (figure 8.27.2) in extant populations after 100-years for the five fragmentation models

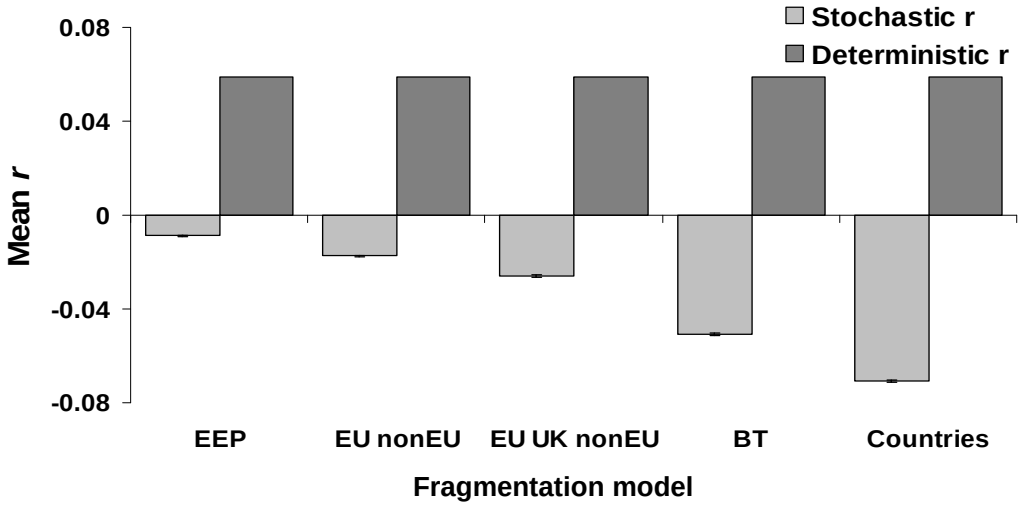


Figure 8.13 The mean stochastic and deterministic growth rates (*r*) of the population for the five fragmentation models

8.4.2.5 EU & non-EU dispersal model

The probability of extinction was smallest for the 1% dispersal scenario, but increased as dispersal increased between sub-units. The current level of dispersal (EEP) and 0% dispersal resulted in higher extinction probabilities than the 1% dispersal scenario, but lower than the remaining dispersal scenarios. Despite this, none of the scenarios could meet the goal of a 99% chance of population persistence after 100-years.

There was no difference in the mean time to extinction between different dispersal rate scenarios in the EU & non-EU model ($H_{11} = 9.42$, $P = 0.583$), but there was a difference between scenarios for the mean *N* ($H_{11} = 975.37$, $P < 0.001$), mean *GD* ($H_{11} = 535.95$, $P < 0.001$) and mean *F* ($H_{11} = 95.92$, $P < 0.001$) of extant populations after 100-years (Figure 8.14).

Most of the difference for the extant *N* lay between scenarios with more than 3% dispersal between sub-units, except for close congeners, although extant *N* did differ between the EEP and 1% dispersal scenarios (Table N.11 Appendix N). The data indicated that the 1% scenario had the largest extant *N*. Most of the difference in *GD* lay between the 1% and 2% dispersal scenarios, and then the higher levels of dispersal (5-6% upwards) (Table N.12 Appendix N). In this instance the 2% dispersal scenario had the highest retention of *GD* after 100-years, but there was no difference between the 1% and 2% dispersal scenarios. In contrast the difference in mean *F* between scenarios followed no discernable pattern (Table N.13 Appendix N).

Retention of alleles differed between the dispersal scenarios, and the 1% scenario retained the highest number of alleles in the extant population, but also retained the highest number of lethal equivalents (Figure 8.15).

All of the dispersal scenarios had a negative stochastic *r* despite having a positive deterministic *r* (Figure 8.16). This would result in the eventual extinction of all of the simulated populations in all of the scenarios for this model. However, the 1% scenario had the least negative growth rate, and so was predicted to persist for longer than the populations in the remaining scenarios.

The 1% model had the lowest *PE*, the least negative *r*, and the largest *N* of extant populations out of the 12 scenarios. It also retained the most alleles, and was predicted to retain one of the highest levels of *GD* after 100-years. Consequently, it is reasonable to conclude that a 1% dispersal of individuals between the EU and non-EU population sub-units in this fragmentation model provides the scimitar-horned oryx EEP population the best chance of population persistence and long-term viability.

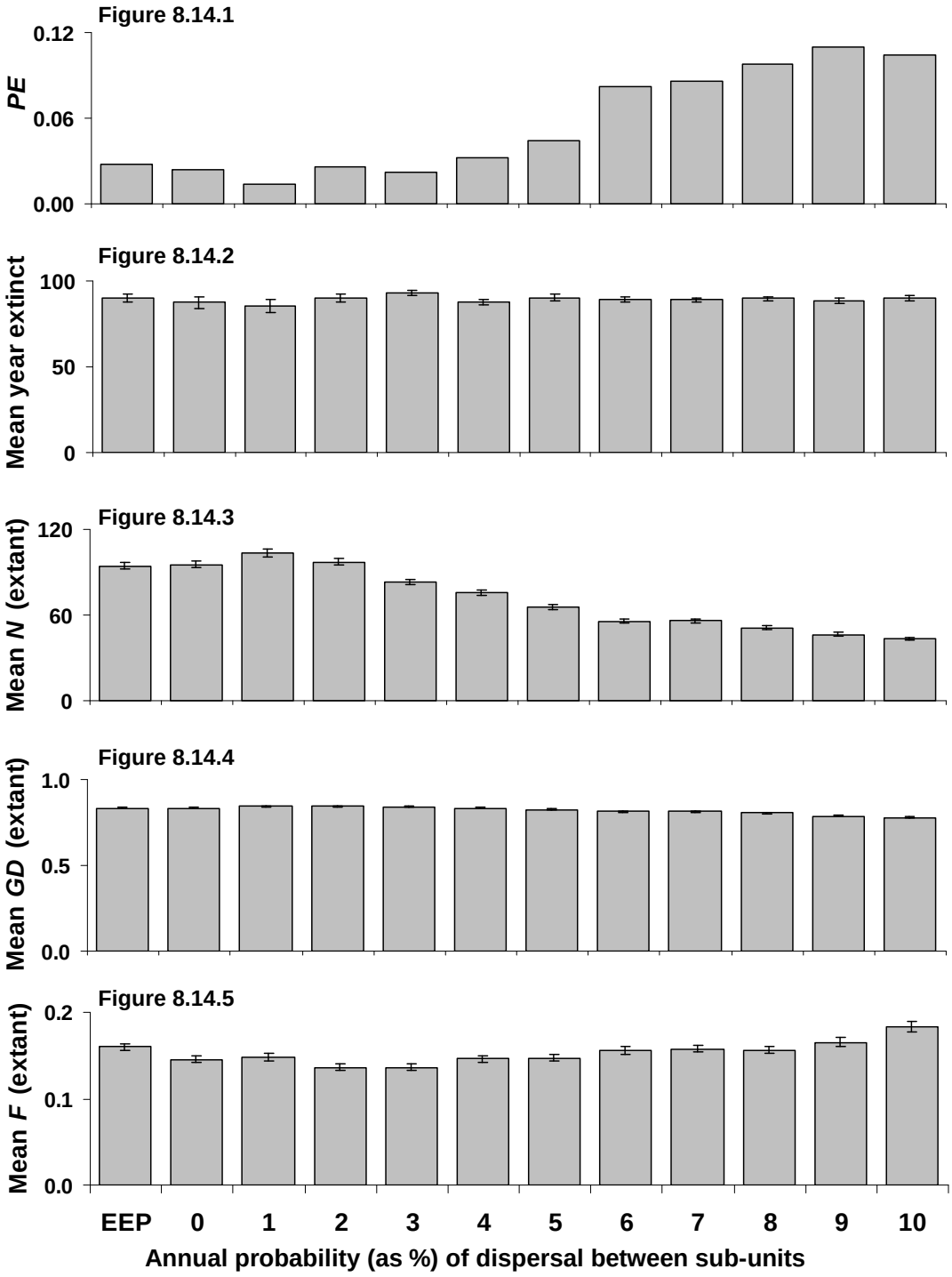


Figure 8.14 EU and non-EU dispersal model. The probability of extinction (**8.14.1**), mean year of extinction (**8.14.3**), mean *N* (of extant populations) (**8.14.5**), mean *GD* of extant populations (**8.14.2**), and mean inbreeding (of extant populations) (**8.14.4**) for the 12 dispersal scenarios

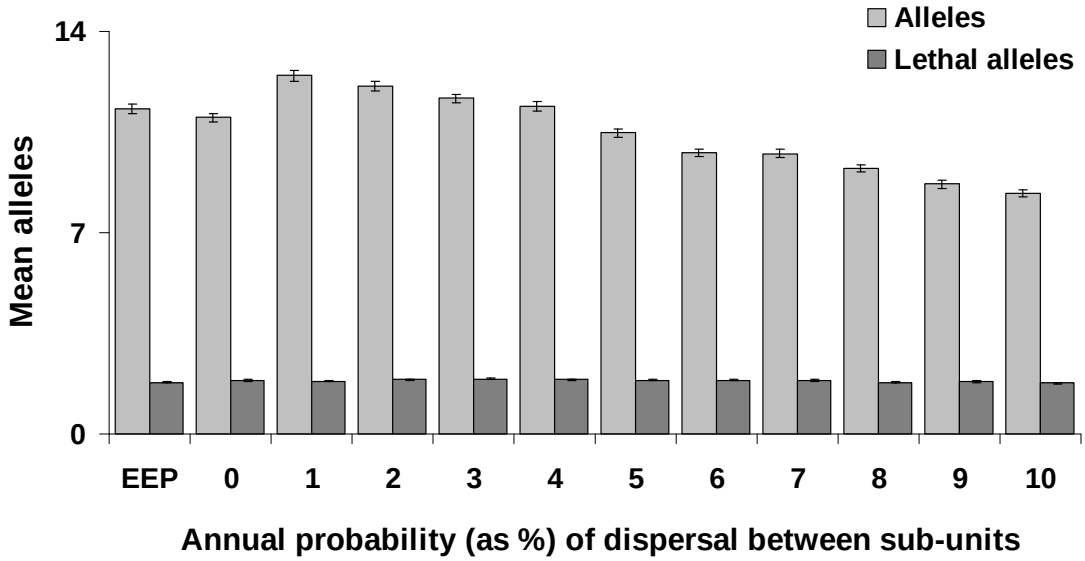


Figure 8.15 The mean number of alleles and lethal alleles per diploid present in the population after 100-years for the 12 EU and non-EU dispersal scenarios

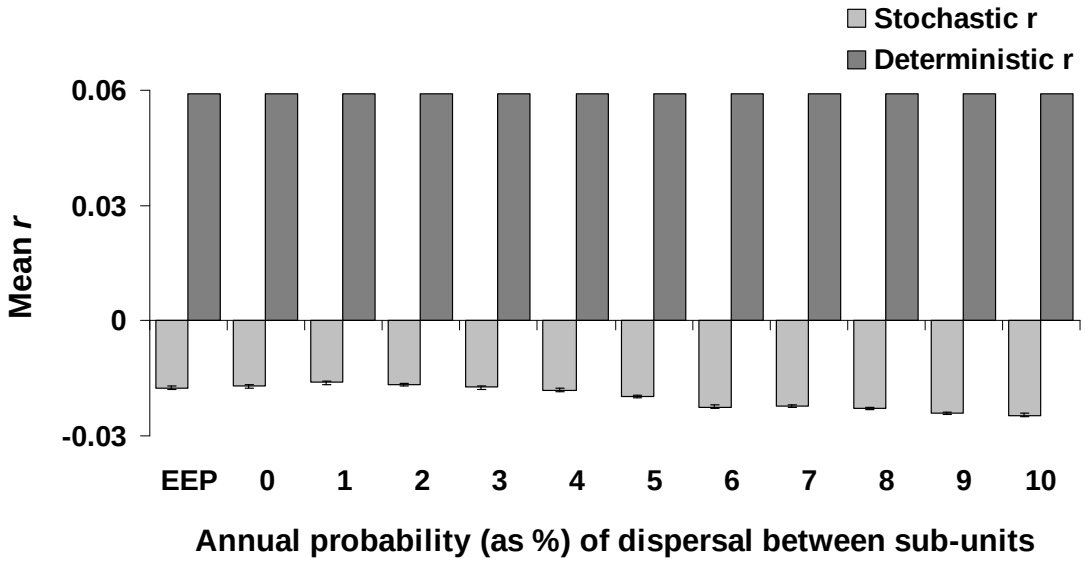


Figure 8.16 The mean stochastic and deterministic growth rates (*r*) of the population for the 12 EU and non-EU dispersal scenarios

8.4.2.6 EU, UK & non-EU dispersal model

The probability of metapopulation extinction was highest for the EEP scenario (*PE* = 0.12), and lowest for the 2% dispersal scenario (*PE* = 0.04). The remaining scenarios varied between *PE* = 0.08 – 0.05 (Figure 8.17). No scenario met the goal of a 99% probability of population persistence for 100-years.

The scenarios differed in the mean time to extinction (H_{11} = 22.34, *P* = 0.022), but this difference only lay between the 2% and 3%, 2% and 5%, and 2% and 9% scenarios (Figure 8.17, Table N.14 Appendix N).The 2% scenario resulted in the shortest mean time to extinction (*TE*) (*TE* = 85 years), so although fewer populations went extinct for this scenario, those that did decline to extinction did so more rapidly.

There was also a difference in the mean *N* of extant populations between the scenarios (H_{11} = 186.62, *P* < 0.001). The 2% scenario yielded the largest extant *N* (*N* = 82), and this differed from all other scenarios except the 1% (*N* = 81) and 3% (*N* = 76) scenarios. The EEP had the smallest extant *N* (*N* = 58), followed by the 0% dispersal scenario (*N* = 62) (Figure 8.17, Table N.15 Appendix N).

Differences in mean *GD* between scenarios were apparent (H_{11} = 169.80, *P* < 0.001), but this was mainly attributable to the 0% and 10% scenarios yielding less *GD* after 100-years than the other dispersal scenarios (Figure 8.17, Table N.16 Appendix N).

The mean *F* of extant populations varied between scenarios (H_{11} = 76.12, *P* < 0.001), but this only occurred for those scenarios with the lowest dispersal rates (0% - 1%), which had the highest levels of *F*. Inbreeding decreased and remained low for all scenarios once a 2% dispersal rate was achieved. Consequently, there was no benefit to a population, in terms of its mean *F*, in increasing dispersal between sub-units above 2%.

The 2% dispersal scenario retained the highest number of alleles, but it was the 4% dispersal scenario that retained the largest number of *LE* (Figure 8.18). All of the scenarios had a negative stochastic *r* despite having a positive deterministic *r* (Figure 8.19). All populations would be expected to eventually decline to extinction over a protracted period of time. However, the 2% scenario resulted in the smallest negative *r*, and so would be expected to persist for longer than the other populations.

The 2% scenario had the smallest *PE*, the least negative stochastic *r*, retained the largest number of alleles, yielded the largest extant *N*, and was the minimum dispersal rate needed to minimise *F*. Consequently, maintaining a 2% dispersal between sub-units would be the best strategy for maintaining population viability for this particular fragmentation model.

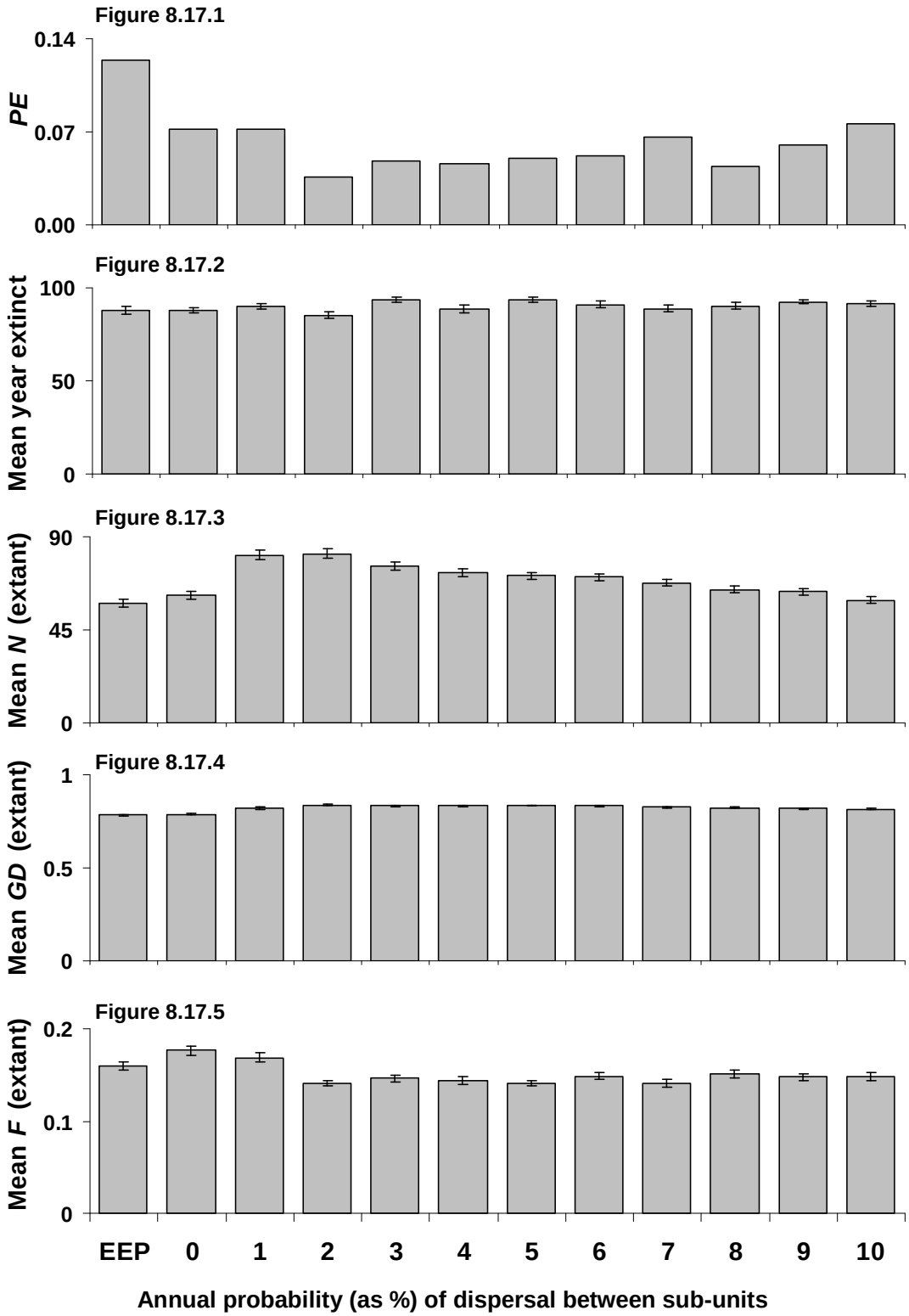


Figure 8.17 EU, UK and non-EU dispersal model. The probability of extinction (**8.17.1**), mean year of extinction (**8.17.2**), mean population size (of extant populations) (**8.17.3**), mean gene diversity of extant populations (**8.17.4**), and mean inbreeding (of extant populations) (**8.17.5**) for the 12 dispersal scenarios

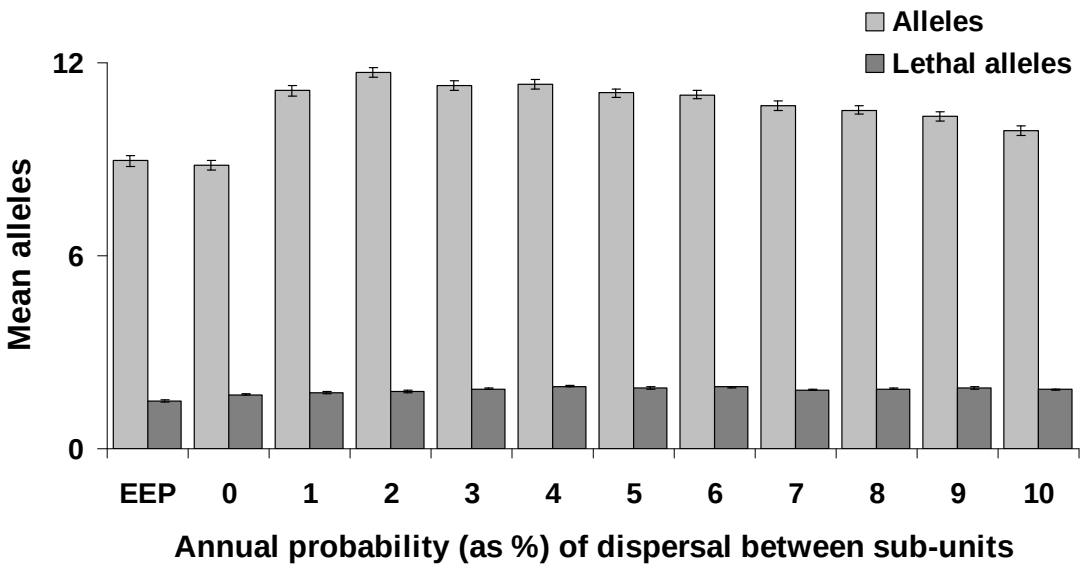


Figure 8.18 The mean number of alleles and lethal alleles per diploid present in the population after 100-years for the 12 EU, UK and non-EU dispersal scenarios

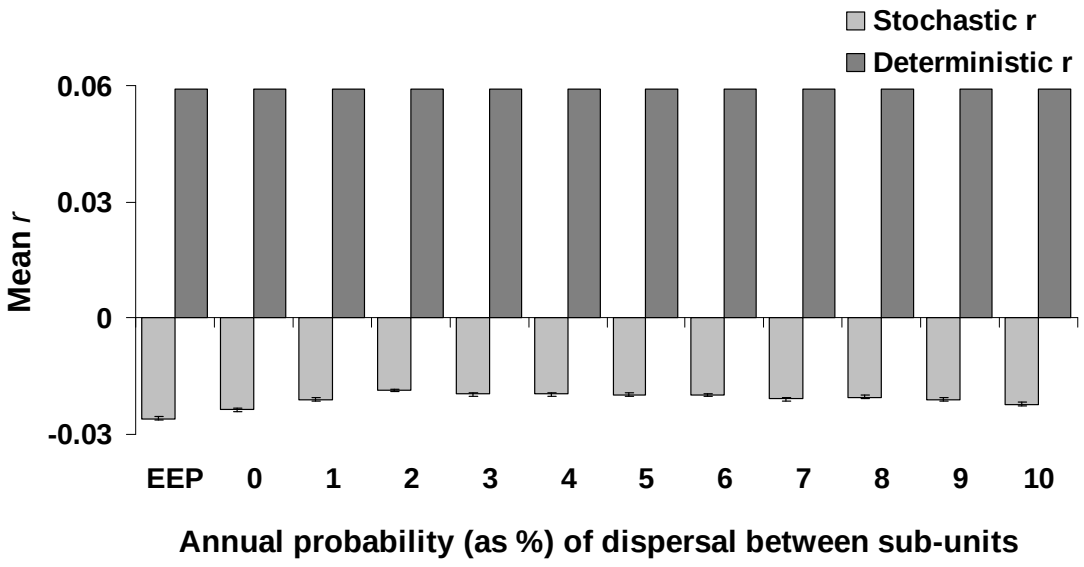


Figure 8.19 The mean stochastic and deterministic growth rates (*r*) of the population for the 12 EU, UK and non-EU dispersal scenarios

8.4.2.7 Bluetongue dispersal model

The probability of metapopulation extinction was highest for the 0% dispersal scenario (*PE* = 0.86), followed by the EEP scenario (*PE* = 0.86), and then the 1% dispersal scenario (*PE* = 0.18). All the remaining scenarios had a *PE* that varied between *PE* = 0.02 – 0.05) (Figure 8.20). None of the scenarios could meet the goal of a 99% chance of population persistence for 100-years.

The mean *TE* differed between scenarios (H_{11} = 135.26, *P* <0.001), but only for the EEP and 0% dispersal scenarios, which had a shorter time to extinction at *TE* = 82 and 77 years, respectively (Figure 8.20, Table N.18 Appendix N).

The *N* of the extant populations also differed (H_{11} = 763.23, *P* <0.001). Population size increased from 0% dispersal up to 3% dispersal, and then remained static until *N* declined from the 8% dispersal rate down to 10% (Figure 8.20). This trend was borne out in the Mann-Whitney analyses (Table N.19 Appendix N), and suggested that very low levels, and very high levels of dispersal, reduced the extant population size.

The mean *GD* of extant populations after 100-years varied between scenarios (H_{11} = 653.80, *P* <0.001), but this was only evidenced amongst the lower dispersal rates (up to 2%). Increasing dispersal above 2% neither increased nor decreased *GD* (Figure 8.20, Table N.20 Appendix N).

Inbreeding was highest at the 0% dispersal rate (mean *F* = 0.03), and then declined to *F* = 0.002 for the 4% dispersal scenario (H_{11} = 401.89, *P* <0.001). Once the dispersal rate equalled 4% and above, inbreeding neither increased nor decreased (Figure 8.20, Table N.21 Appendix N).

Allele retention after 100-years was highest for the 4% dispersal model, but the 8% dispersal scenario retained the largest number of *LE* after 100-years (*LE* = 2.0) (Figure 8.21). The mean stochastic growth rate was negative for all scenarios despite a positive deterministic *r* (Figure 8.22). This is likely to result in eventual population extinction for all scenarios unless future *r* can be increased. The population growth rate was the least negative for the 6% dispersal scenarios, and so this population would be predicted to persist for longer.

Once a dispersal rate of 4% was reached, population size, allele retention and growth rates were maximised, and inbreeding was minimised. However, dispersal rate of only 3% were required to maximise *GD*. The retention of LE in the population was highest at the 6% dispersal rate. In conclusion, a 4% dispersal rate would maximise population viability for this model of scimitar-horned oryx EEP fragmentation.

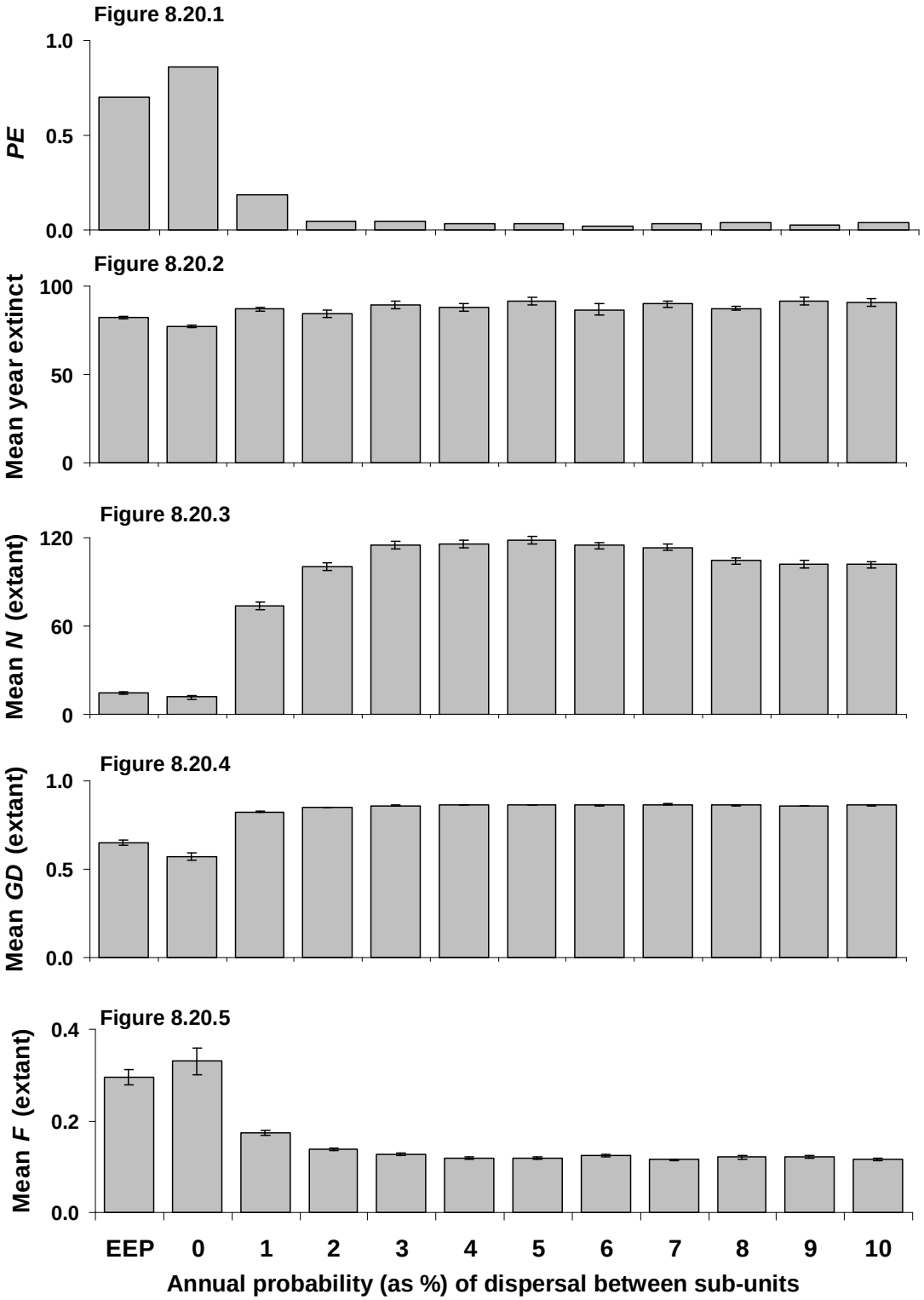


Figure 8.20 Bluetongue dispersal model. The probability of extinction (**8.20.1**), mean year of extinction (**8.20.2**), mean population size (of extant populations) (**8.20.3**), mean gene diversity of extant populations (**8.20.4**), and mean inbreeding (of extant populations) (**8.20.5**) for the 12 dispersal scenarios

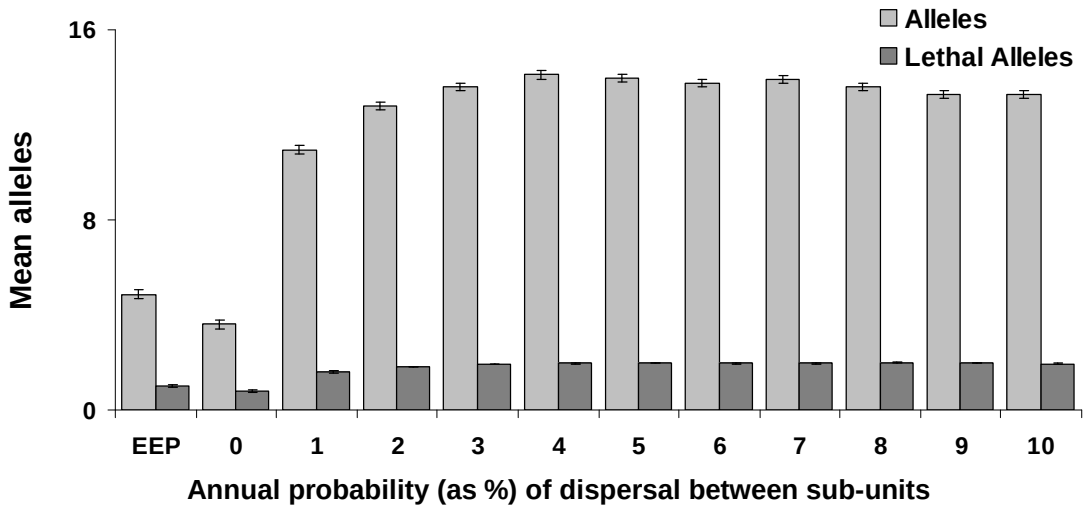


Figure 8.21 The mean stochastic and deterministic growth rates (*r*) of the population for the 11 bluetongue dispersal scenarios

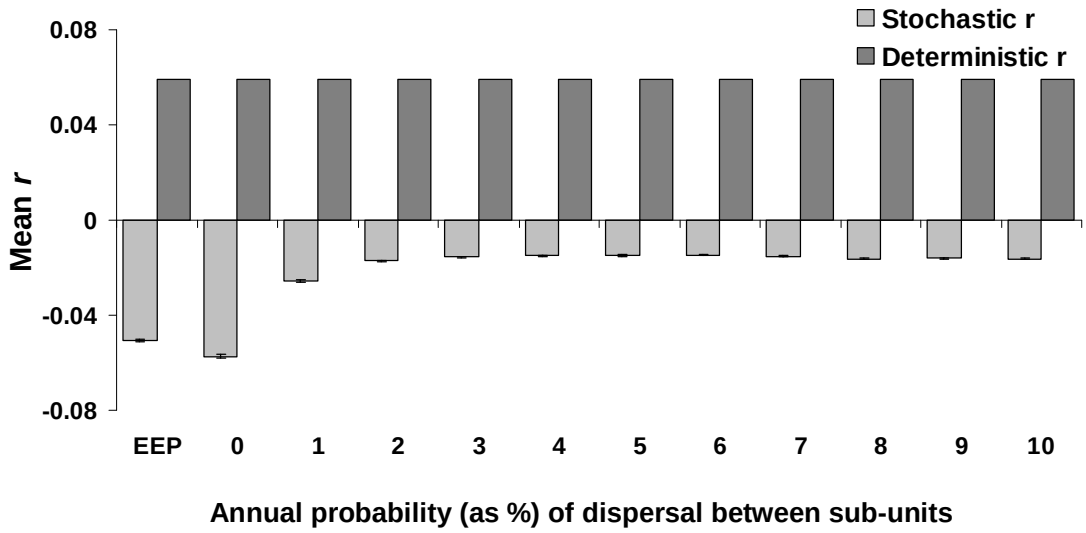


Figure 8.22 The mean number of alleles and lethal alleles per diploid present in the population after 100-years for the 11 bluetongue dispersal scenarios

8.4.2.8 Countries dispersal model

The chance of any of the simulated populations in any scenario surviving for 100-years, regardless of dispersal between the sub-units, was very small for this fragmentation model. The EEP and 0% dispersal scenarios had a probability of metapopulation extinction of *PE* = 0.98 and 0.95, respectively. All other scenarios had a probability of extinction of *PE* = 1.0. Consequently, the 99% survival goal was not met for any of the scenarios.

There was a difference between the scenarios in mean time to extinction (H_{11} = 4354.46, $P < 0.001$) with *TE* decreasing as dispersal increased (Figure 8.23, Table N.22 Appendix N).

All scenarios except the EEP and 0% dispersal scenarios had an extinction probability of *PE* = 1.0 for their simulated populations. Consequently, analysis of *N*, *GD*, and *F* in the extant populations was limited to the EEP and 0% dispersal scenarios. There was no difference between these two scenarios for population size ($W = 215$, $P = 0.4560$), gene diversity ($W = 210$, $P = 0.3691$), or the mean inbreeding coefficient ($W = 264$, $P = 0.4840$) of the extant populations (Figure 8.23). Furthermore, whilst more alleles were retained for the 0% than the EEP dispersal scenario, standard deviations indicate that this is not reflective of a real difference (Figure 8.24).

The stochastic growth rate was negative for all scenarios, but the EEP and 0% dispersal scenarios showed the least negative population growth at $r = -0.07$ (Figure 8.25). Growth rate reached a maximum of $r = -0.14$ for several scenarios, corresponding to the rapid population extinction in these cases.

This model showed very little population persistence, or population viability at any dispersal rate. This is likely to be due to the small starting *N* of the metapopulation sub-units. All but three sub-units (Israel, France and the UK) went extinct in the EEP and 0% dispersal scenarios, and this increased the probability of extinction of the metapopulation.

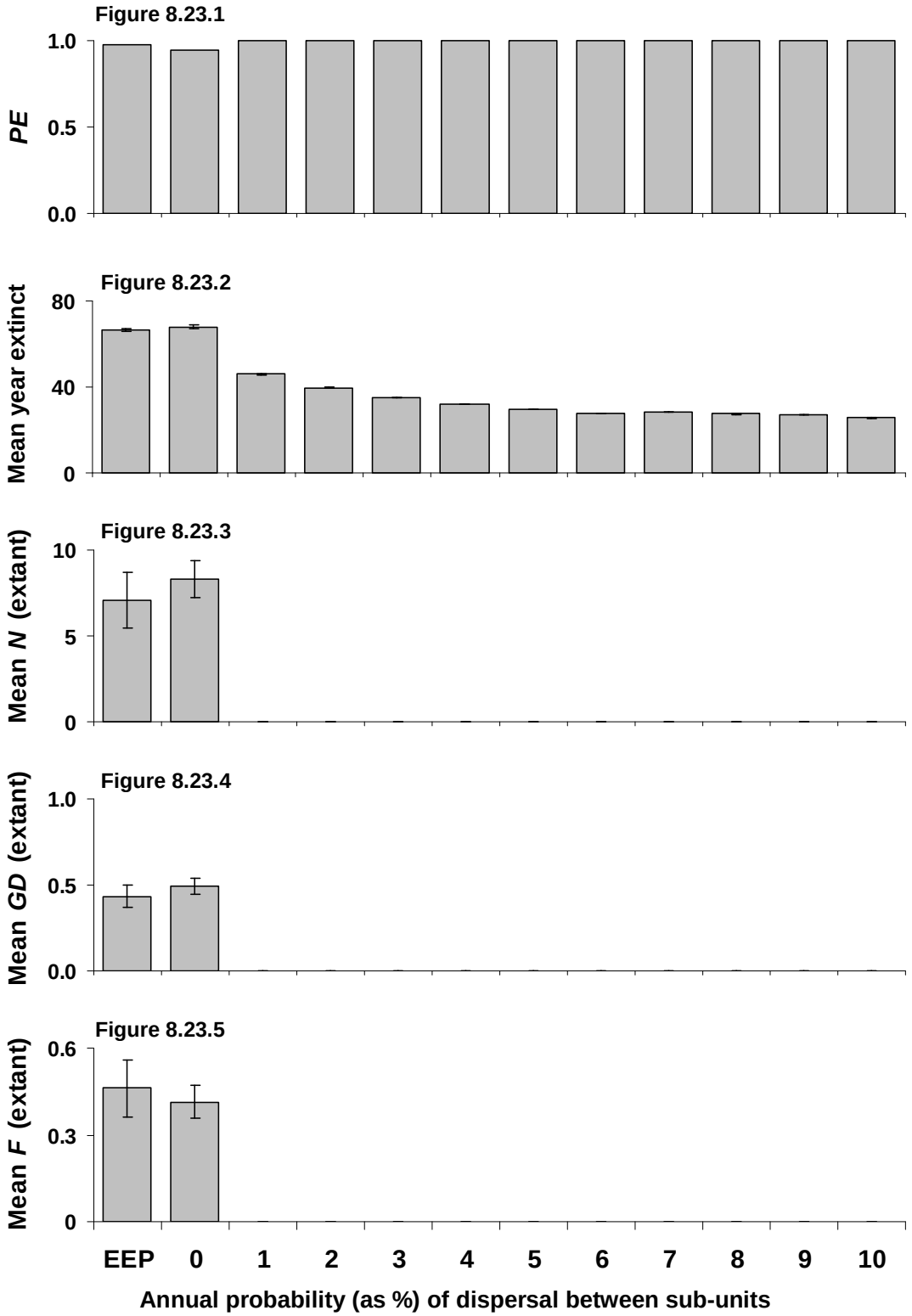


Figure 8.23 Countries dispersal model. The probability of extinction (**8.23.1**), mean year of extinction (**8.23.2**), mean population size (of extant populations) (**8.23.3**), mean gene diversity of extant populations (**8.23.4**), and mean inbreeding (of extant populations) (**8.23.5**) for the 12 dispersal scenarios.

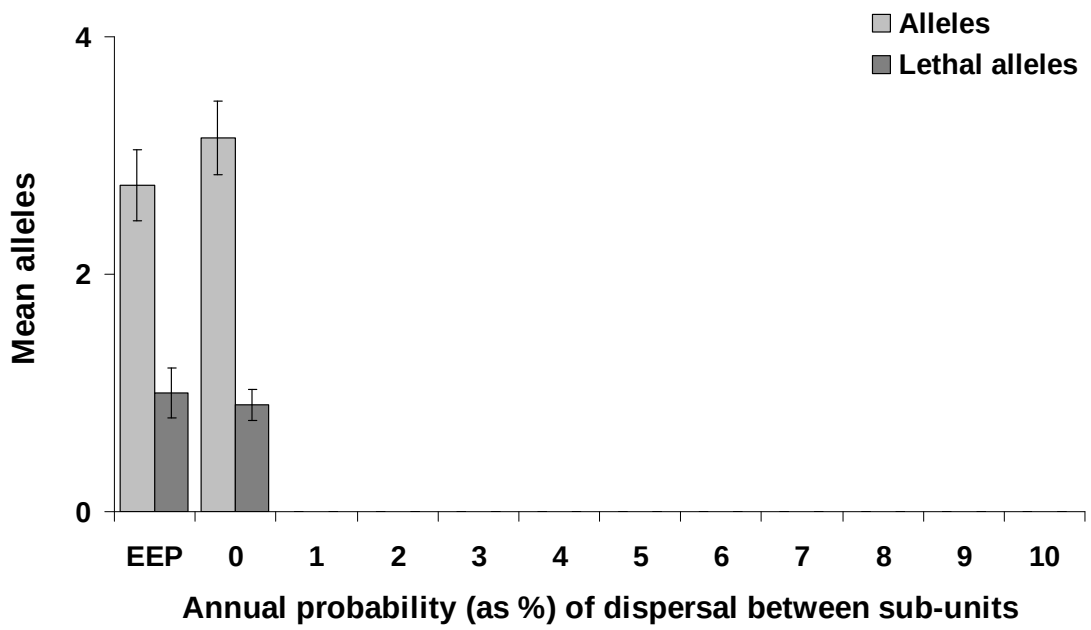


Figure 8.24 The mean number of alleles and lethal alleles per diploid present in the population after 100-years for the 12 countries dispersal scenarios

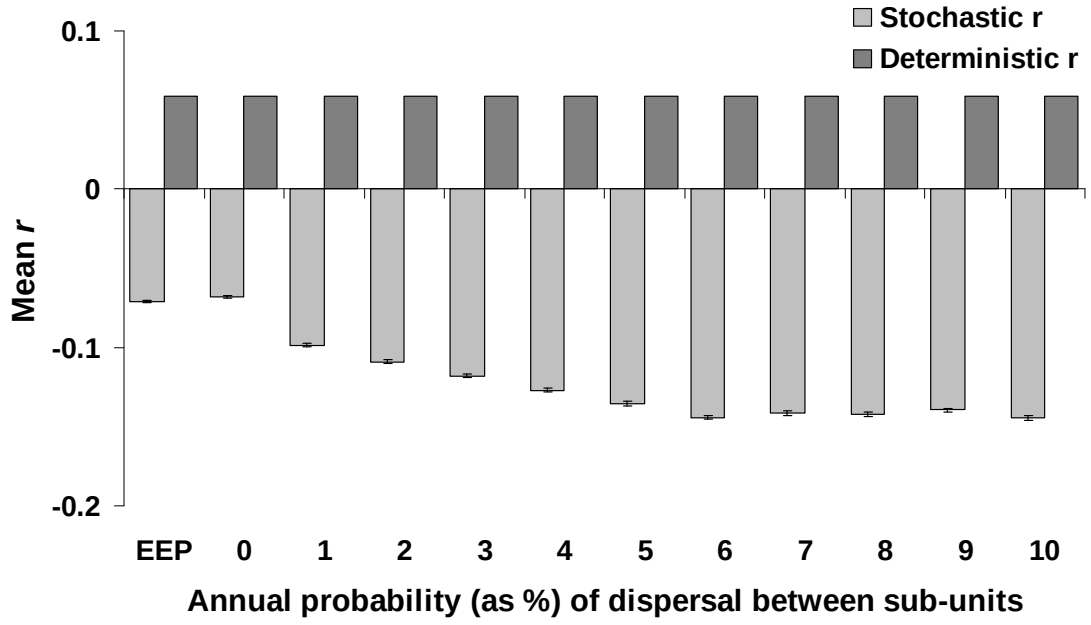


Figure 8.25 The mean stochastic and deterministic growth rates (*r*) of the population for the 12 countries dispersal scenarios

8.4.2.9 Goals summary

The ability of the simulated populations to meet the goal of ensuring a 99% probability of population persistence for 100-years was affected by the adult female reproduction rate, the number of lethal equivalents per diploid, carrying capacity and population fragmentation (Table 8.19). The simulated populations could not achieve the population viability goal until female reproduction equalled 53%, or above. However, lethal equivalents did not impact on the goal until LE = 10.0, or above. Populations with very low carrying capacities (K = 100 and 200) had less than 99% chance of survival for 100-years, but once K = 300 or more, the goal was achievable.

It should be noted that although many of the scenarios that tested the sensitivity of different population parameters were predicted to persist for 100-years, all but the populations with a carrying capacity above K = 900 would eventually become extinct, because they had a negative population growth rate. If the simulations had been run for more than 100-years, all but the populations with the highest carrying capacities would have declined to extinction. It should also be noted that lethal equivalents and female reproduction did impact on the population growth rate, but it still remained negative. This suggests that the carrying capacity is the principle determinant of growth in the simulated population, as a function of density-dependence. Consequently, if the EEP population of scimitar-horned oryx is to be maintained in captivity in perpetuity, then the size needs to increase to at least N = 900 with a corresponding increase in growth rate.

Any fragmentation of the EEP population prevented the 99% population viability goal from being met. As population sub-division is already evident in the EEP, for example there has been no exchange of animals between Israel and the EU population since the 1970s, it is unlikely that the population can achieve the viability goal.

Table 8.19 A summary of the population viability for the eight models and 96 scenarios

Model	Scenario	99% probability of survival for 100-years	*r*
Genetic management	Dynamic MK	Yes	- ve
	Static MK	Yes	- ve
	Random	Yes	- ve
Female reproduction	42%	No	- ve
	53%	No	- ve
	44%	No	- ve
	45%	No	- ve
	46%	No	- ve
	47%	No	- ve
	48%	No	- ve
	49%	No	- ve
	50%	No	- ve
	51%	No	- ve
	52%	No	- ve
	53%	Yes	- ve
	54%	Yes	- ve
	55%	Yes	- ve
Lethal equivalents	*LE* = 0.0	Yes	- ve
	LE = 1.0	Yes	- ve
	LE = 2.0	Yes	- ve
	LE = 3.0	Yes	- ve
	LE = 3.14	Yes	- ve
	LE = 4.0	Yes	- ve
	LE = 5.0	Yes	- ve
	LE = 6.0	Yes	- ve
	LE = 6.97	Yes	- ve
	LE = 7.0	Yes	- ve
	LE = 8.0	Yes	- ve
	LE = 9.0	Yes	- ve
	LE = 9.26	Yes	- ve
	LE = 10.0	No	- ve
	LE = 11.0	No	- ve
	LE = 12.0	No	- ve
Carrying capacity	*K* = 100	No	- ve
	K = 200	No	- ve
	K = 300	Yes	- ve
	K = 400	Yes	- ve
	K = 430	Yes	- ve
	K = 500	Yes	- ve
	K = 600	Yes	- ve
	K = 700	Yes	- ve
	K = 800	Yes	- ve
	K = 900	Yes	+ ve
	K = 1000	Yes	+ ve
	K = 1100	Yes	+ ve
	K = 1200	Yes	+ ve
	K = 1300	Yes	+ ve
	K = 1400	Yes	+ ve
	K = 1500	Yes	+ ve

Table 8.19 continued

Model	Scenario	99% probability of survival for 100-years	*r*
EU and non-EU	Baseline	No	-ve
	0%	No	-ve
	1%	No	-ve
	2%	No	-ve
	3%	No	-ve
	4%	No	-ve
	5%	No	-ve
	6%	No	-ve
	7%	No	-ve
	8%	No	-ve
	9%	No	-ve
	10%	No	-ve
EU, UK and non-EU	Baseline	No	-ve
	0%	No	-ve
	1%	No	-ve
	2%	No	-ve
	3%	No	-ve
	4%	No	-ve
	5%	No	-ve
	6%	No	-ve
	7%	No	-ve
	8%	No	-ve
	9%	No	-ve
	10%	No	-ve
Bluetongue	Baseline	No	-ve
	0%	No	-ve
	1%	No	-ve
	2%	No	-ve
	3%	No	-ve
	4%	No	-ve
	5%	No	-ve
	6%	No	-ve
	7%	No	-ve
	8%	No	-ve
	9%	No	-ve
	10%	No	-ve
Countries	Baseline	No	-ve
	0%	No	-ve
	1%	No	-ve
	2%	No	-ve
	3%	No	-ve
	4%	No	-ve
	5%	No	-ve
	6%	No	-ve
	7%	No	-ve
	8%	No	-ve
	9%	No	-ve
	10%	No	-ve

8.5 Discussion

The baseline model was sensitive to different genetic management strategies in terms of levels of genetic diversity and demographic stability, but not to the probability of extinction. PVAs on small captive populations should reflect the actual management strategy used in real-life population management to improve model accuracy.

The baseline model was also sensitive to annual female reproduction modelled within the limits observed from the historical scimitar-horned oryx EEP population. This highlights the need to obtain good quality data on key parameters, and to modify parameters with density-dependent functions where appropriate.

The results presented in this chapter clearly demonstrate the importance of including realistic levels of inbreeding depression in PVA models, and this is supported in the literature (Brook *et al.* 2002; Frankham 2010a; O'Grady *et al.* 2006). The baseline model was sensitive to *LE* in terms of the probability of extinction, the size of extant populations, the remnant gene diversity, and the level of inbreeding after 100-years. Even when the probability of extinction was zero, the *N*, *GD* and *F* of extant populations differed between *LE* scenarios. Furthermore, the population growth rate declined with increasing *LE*.

It is clear from the results that obtaining precise estimates for the number of lethal equivalents per diploid is extremely important, and yet many studies, and CBSG PVAs, rely on the default value of 3.14 *LE* per diploid (Frankham 2010b). This value is taken from a paper published in 1988 (Ralls *et al.* 1988) that calculated the mean number of lethal equivalents for 40 captive populations of 38 mammal species (including scimitar-horned oryx). The data set was limited to individuals held at one institution (Washington National Zoo), and calculated *LE* for juvenile mortality based only on the first 180 days post-partum. The paper itself acknowledged the limitations of the data (Ralls *et al.* 1988), but it is still often used as a default source of *LE* in PVAs. The value of 3.14 *LE* per diploid is likely to be an underestimate for many species, and *LE* for wild populations are thought to be considerably higher at approximately 12 *LE* per diploid (Frankham 2010b). Ralls *et al.* (1988) calculated 9.26 *LE* for the scimitar-horned oryx population in Washington, but a value of 6.97 *LE* was calculated from the EEP data. This difference could be caused by captive husbandry masking inbreeding depression in the EEP population, purging of deleterious alleles, a different founder base resulting in differing levels of ancestral inbreeding, or it could be an artefact of using different data sets. Whatever the cause, there was a difference in the probability of extinction, mean *N*, *GD*, and *F* of extant populations

after 100-years between the 3.14, 6.97 and 9.26 *LE* scenarios. This indicates the importance of obtaining estimates of *LE* for specific populations, not just specific species that are subject to PVAs.

The model was also sensitive to the carrying capacity. Once *K* reached 300, the goal of achieving a 99% probability of population persistence for 100-years was realised. The model exhibited a negative stochastic population growth rate until *K* = 900, indicating that this was the MVP required to maintain the scimitar-horned oryx EEP in perpetuity. Despite this, the remnant mean *N* and *GD* continued to decline until *K* = 1400 and *K* = 1200 respectively, and mean *F* continued to increase until *K* = 1000. The MVP_K for the stated viability goal was *K* = 300, but to retain a scimitar-horned oryx EEP population into the foreseeable future, a MVP_K of 900 would be needed. These two definitions are based purely on extinction probabilities and do not take into account other demographic or genetic variables. If these other factors are also considered, then the MVP_K for the scimitar-horned oryx EEP would be 1200 – 1400. A scimitar-horned oryx EEP population of this size is unlikely to be achievable, and even a population of 900 would exclude or reduce the population size of other species held in captivity (Chapter Six). However, it is important that the EEP population is maintained above 300 individuals to achieve a moderate level of viability.

The probability of extinction increased with increasing population sub-division, and the non-fragmented baseline EEP model was the only model that exhibited a 99% probability of population persistence for 100-years. Once the population was sub-divided, even when it was divided into only two sub-units (the EU and non-EU model), the probability of extinction increased, and mean and median time to extinction, the genetic diversity and demographic stability decreased. As the population was fragmented into an increasing number of sub-units (EU, UK, and non-EU, to the bluetongue, through to the countries model), the probability of extinction and mean *F* continued to increase, and the mean *GD* and extant *N* continued to decrease. Some of this trend is attributable to the sub-units being so small that they were extremely susceptible to demographic and genetic stochasticity (Leus *et al.* 2011b). However, it is important to note that 13 out of the 16 sub-units in the countries model had a *PE* = 1.0, and the remaining three sub-units had a minimum probability of extinction of *PE* = 0.98. Comparatively, four out of five sub-units in the bluetongue model had a minimum *PE* = 0.90. Even if sub-units had a low *PE* e.g. EU sub-unit in the EU and non-EU model, the remnant *N* and genetic diversity was

substantially decreased. This high risk of sub-unit extinction, and small extant sub-unit *N*, had a substantial impact on the viability of these fragmented simulated populations.

The results from the simulations that included migration between sub-units in the fragmented populations indicate that no amount of migration could completely counter the effects of sub-division. Consequently, the 99% probability of population persistence goal was not achievable for any scenario. Despite this, there was an optimal level of migration for each fragmentation model to maximise population viability. Overall, the optimal amount of migration between sub-units per annum increased from 1% to 4% as fragmentation increased, except for the countries model where no amount of migration could increase population viability.

Theoretical models and experiments with model species indicate that population sub-division should lead to increased retention of genetic diversity (Margan *et al.* 1998; Wang & Caballero 1999). However, these conclusions are based on a number of assumptions e.g. they are based on replicate sub-populations that have an equal, large and constant *N*, that cannot often be met by real populations (Lacy & Lindenmayer 1995; Lacy 2000a; Montgomery *et al.* 2010; Wang & Caballero 1999). When populations are fragmented by deterministic forces such as habitat fragmentation, or legislation preventing the dispersal of individuals (Ballou *et al.* 2010a; Boyce 1992), sub-division can lead to genetic impoverishment and an increased probability of extinction (Lacy 2000a). The fragmentation models presented here are based on the realistic sub-division of an actual population, and show that any fragmentation is detrimental to the viability of the scimitar-horned oryx EEP.

The stochastic population growth rate was negative in all scenarios for all the models with the exception of carry capacity scenarios with a $K = 900$ or more. This suggests that any simulated population with a carrying capacity below 900 would eventually decline to extinction under the modelled conditions, if the simulations extended beyond 100-years. Populations with a positive growth rate are much more resilient, although not immune from extinction (Holsinger 2000). Increasing annual female reproduction would not address this issue unless there was a corresponding increase in carrying capacity.

Detailed long-term studbook data, which included the entire period of contemporary population management in Europe, was used to parameterise the model. This provided accurate input parameters, but precluded independent model validation. Consequently, the only method of validation was through some sensitivity testing on genetic management strategies, female reproduction, and lethal equivalents. Whilst sensitivity testing has value

(Kohmann *et al.* 2005), the lack of independent verification of the model's predictive accuracy leaves it open to criticism (Coulson *et al.* 2001; Ellner *et al.* 2002; Patterson & Murray 2008).

The use of historical data to parameterise the model makes the assumption that future processes and trends will be reflective of past population dynamics. This assumption may not be met. Additionally, whilst VORTEX (Lacy *et al.* 2009) was the most appropriate existing software program, some population dynamics are not included in the programming (Lacy 1993b). These considerations mean that results need to be interpreted cautiously (Lindenmayer *et al.* 2000), and they are best thought as relative rather than absolute predictions, of fragmentation and management actions (Ball *et al.* 2003; Fieberg & Ellner 2000; Patterson & Murray 2008). Although the PVA is imperfect, it is still a useful tool for conservation planning (Lindenmayer *et al.* 2003).

All PVAs should be considered 'a work in progress' (Patterson & Murray 2008), and the model for the scimitar-horned oryx EEP is no exception. Additional research and sensitivity testing may help to refine the model further. It would also be useful to extend the model to include the global captive population in order to evaluate the wider impact of population fragmentation. A global analytical studbook will need to be created before this can happen to obtain a more realistic overview of changes in genetic diversity.

One of the most notable conclusions in this chapter is the impact of lethal equivalents on population dynamics. The widespread use of the default value of 3.14 *LE* per diploid may be under-estimating the impact of lethal equivalents on population viability for many species. It is time to re-visit this issue and a multi-taxa study using regional and global studbooks to calculate *LE* values is now overdue.

The results presented here demonstrate the risks posed to small populations by fragmentation. The causes of fragmentation may be subtle and diverse e.g. the costs of animal transport may make some zoological institutions reluctant to import animals over large distances (Rodeano, M. *pers. comm.*, 2011). This may result in less sustainable captive populations than are recognised by population managers. Every effort needs to be made to prevent the fragmentation of small populations, or alternatively, increase the size of managed populations. This may not be possible on a regional level, so population managers may need to take a global perspective to managing species in captivity.

9.0 Chapter nine: discussion and conclusion

The maintenance of endangered species in captivity is becoming an increasingly important tool in combating biodiversity loss. Captive populations are often regarded as 'assurance populations' to protect against extinction when species are extirpated in the wild (Conway 2011; Frankham 2005b). Indeed, some species are now entirely dependent on captive breeding for their continued survival (IUCN 2010). To fulfil this role, captive populations need to be genetically diverse, demographically stable, and self-sustainable, but it is becoming increasing apparent that many captive populations, including those subject to intensive management, cannot fulfil these criteria (Ballou & Traylor-Holzer 2011; Conde *et al.* 2011b; Lees & Wilcken 2009; Lees & Wilcken 2011; Leus *et al.* 2011a; Leus *et al.* 2011b; Long *et al.* 2011; Oberwemmer, Bingaman Lackey & Gusset 2011; Traylor-Holzer 2011). This thesis addressed the issue of sustainability of captive populations by examining some of the factors that influence it, particularly, the quality of data used as the basis of intensive population management, and economic fragmentation of populations.

The scimitar-horned oryx was used as a case study throughout the thesis. The species is extinct in the wild, but has a large captive population and is intensively managed through three regionally coordinated breeding programmes. The EEP is the largest intensively managed antelope population in the EAZA region (EAZA 2010b). Overall, it has a better chance of persistence than many other species. So, if this population is not sustainable, what does that mean for other species?

This thesis presents a series of original studies to inform the conservation management of the scimitar-horned oryx, and contribute to an understanding of the challenges underpinning the sustainability of all captive species.

9.1 Summary

The chapters in this thesis are sequential with the results from earlier chapters informing the methodology of later chapters. Taken together they present a cohesive overview of the challenges faced by endangered species in captivity, in particular the scimitar-horned oryx (Figure 9.1).

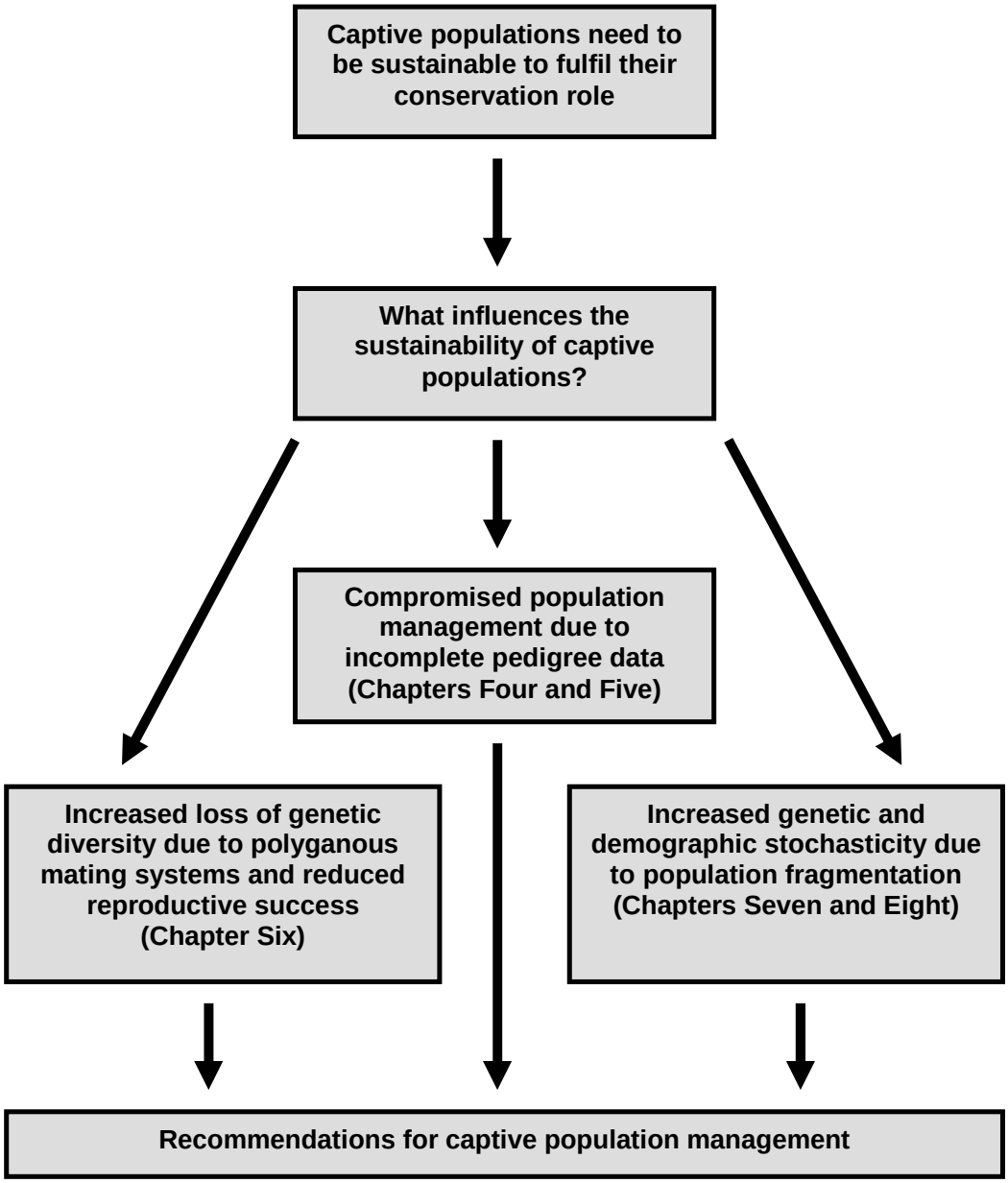


Figure 9.1 diagrammatic representation of the flow of themes through the thesis

When pedigree data are incomplete pedigree analyses over- or underestimate genetic metrics including inbreeding coefficients (*F*) (Lacy 1993a). Prior to this thesis, it was not known how much pedigree data could be missing before *F* was no longer estimatable. For the first time, Chapter Four tested the reliability of *F* derived from incomplete pedigrees, and established that a threshold of 62.5% pedigree completeness was needed for the estimation of inbreeding coefficients. These results were applied to the examination of

sustainable populations in Chapter Seven, and the evaluation of inbreeding depression in the scimitar-horned oryx in Chapter Eight. Furthermore, I recommend that captive population managers ensure pedigree data are at least 62.5% complete before making population management decisions based on inbreeding coefficients.

Populations with less than 62.5% pedigree completeness need alternative population management strategies, and AZA, EAZA, and ZAA recommend that analytical studbooks are created with 'best guesses' to complete the pedigree. The analytical studbook forms the basis of population management in place of the true studbook (Willis 1993; Willis 2001; AZA 2004). Whilst this practice is widespread (AZA 2004), the validity of this approach had not been independently evaluated. Chapter Five quantitatively tested the accuracy of the scimitar-horned oryx analytical studbook and the assumptions that underpin it. This was the first time that molecular data had been used to evaluate both a true and analytical studbook for any species.

The results revealed differences between molecular estimates of relatedness and the statistical estimates derived from the true and analytical studbooks until data completeness exceeded 87.5%. Once it did, concordance between the molecular data and the analytical studbook was evident, although tenuous. The results indicated that missing pedigree data had a substantial impact on the accuracy of relatedness values derived from studbooks, although the founder assumption and cryptic errors may have contributed as well. The conclusions supported the use of analytical studbooks, with their potentially incorrect assumptions, as a more appropriate strategy for population management than true studbooks when pedigree data were incomplete. As a consequence, the scimitar-horned oryx analytical studbook was used as the basis of population analysis in Chapters Six, Seven and Eight, and data from the Arabian oryx analytical studbook was used in place of data from the true studbook in Chapter Seven. I also recommend that population managers preferentially use analytical studbooks in population management; especially if more than 87.5% of pedigree data are complete.

The long-term retention of genetic diversity contributes towards population sustainability. Chapter Six examined the impact of different genetic and demographic variables on the retention of gene diversity in the scimitar-horned oryx EEP population. Whilst this is a well-studied discipline, deterministic predictions of variable parameter values had not previously been simulated for any captive population. The results demonstrated the importance of maximising effective population size, generation length, and reproductive success. As a consequence, I argue that captive management of the

scimitar-horned oryx EEP population should be orientated away from management that reduces these variables. This includes reducing the size of large polyganous herds, males should be rotated more frequently between groups, and established reproductive technology should be used to supplement traditional population management in order to maximise retention of genetic diversity. This may cause a conflict between the social requirements of the species and maximising the retention of gene diversity.

Chapter Six demonstrated that the genetic viability of the scimitar-horned oryx EEP population was not being maximised under existing management regimes. Chapter Seven examined this concept further by simulating the impact of fragmentation on the genetic viability and sustainability of populations under intensive management.

The impact of fragmentation has been extensively studied using experimental and wild populations, and metapopulation theory is well-developed (Frankham *et al.* 2010; Wang & Caballero 1999; Wright 1943; Wright 1969), but little attention has been paid to the fragmentation of actual populations in captivity. For the first time, in Chapter Seven I simulated the fragmentation of the scimitar-horned oryx EEP population using existing legislative and economic barriers, and then supplemented the results with further analyses on the Arabian oryx, mhorr gazelle and dorcas gazelle EEP populations. I then examined the short term viability and long-term sustainability of regional and global populations of multiple taxa under intensive management. The simulated fragmentation of EEP populations yielded small population sub-units with increased inbreeding and reduced effective population sizes. Consequently, gene diversity was predicted to decline at a faster rate in fragmented metapopulations than in EEP populations under panmixia.

Examination of regional and global populations revealed that 87% of regional, and 63% of global, populations had effective population sizes below the minimum N_e needed for short-term viability (N_e = 50), and no regional or global population had an N_e needed for self-sustainability (N_e = 500). Regional populations under intensive management had higher N_e/N ratios than global populations demonstrating that effective management can retain more gene diversity. Consequently, I recommend that population management is coordinated at a global scale in order to move towards population sustainability.

Chapter Seven examined the sustainability of captive populations using generalised models. Chapter Eight extended this by examining in detail the viability of one population, the scimitar-horned oryx EEP population, under the five fragmentation models established in Chapter Seven.

Population viability analysis (PVA) had not been applied to any scimitar-horned oryx population prior to the simulations in Chapter Eight. Furthermore, PVAs had not previously been used to simulate fragmentation in any captive population. The results demonstrated that any population sub-division reduced predicted population viability below the threshold of 99% probability of persistence for 100-years. Migration between population sub-units could not compensate for sub-division. A minimum viable population size of 300 was required for a 99% probability of population persistence for 100-years in a panmictic population, but a minimum panmictic population size of 900 was needed to ensure a self-sustaining population. The model was sensitive to lethal equivalents, and I recommend that future PVAs for any species calculate accurate lethal equivalent values rather than relying on default values obtained from the literature.

9.2 The sustainability of captive populations

Captive populations are not currently self-sustainable as evidenced by the results presented in this thesis. These findings are in agreement with other authors, although this is the first time this conclusion has been based on quantitative analysis of effective population sizes of regional and global populations for multiple-taxa (Ballou & Traylor-Holzer 2011; Conde *et al.* 2011b; Lees & Wilcken 2009; Lees & Wilcken 2011; Leus *et al.* 2011a; Leus *et al.* 2011b; Long *et al.* 2011; Oberwemmer *et al.* 2011; Traylor-Holzer 2011). Population viability can be retained in the immediate future, but the zoological community needs to reorientate existing paradigms and develop new management techniques, if captive populations are to become sustainable and fulfil their conservation objectives.

Numerous factors cause the lack of sustainability in captive populations, and these will vary between populations, species, and regions. Nevertheless, there are some issues that are widely applicable, namely population size, fragmentation, and effective management.

Chapters Six and Seven demonstrated that census sizes of thousands are necessary to attain sustainability, and Chapter Eight established that a minimum viable population size of 900 was needed for the scimitar-horned oryx EEP to ensure long-term persistence. A fundamental lack of space means that it is not possible to maintain sustainable populations for all the species currently in captivity, or for all species threatened with extinction. The

zoological community now needs to decide how and where to prioritise its limited resources.

AZA recently attempted to do this by classifying coordinated captive breeding programmes under a 'traffic light' system, whereby populations that were predicted to retain 90% of gene diversity for 100-years were designated 'green' (Ballou & Traylor-Holzer 2011). Populations unable to meet the 90/100 goal were designated as 'amber', and populations with less than 50 individuals were 'red'. The intention was to direct limited management resources towards the populations with the best chance of attaining sustainability, with 'green' populations receiving the most intensive management and 'red' populations receiving the least intensive management (Bingaman-Lackey, L. *pers. comm.* 2010). ZAA also recently classified their managed populations using a traffic light system to prioritise resources. In this case the least sustainable programmes (red) were described as needing immediate attention, and the 'green' as "performing well" (Hibbard *et al.* 2011). These schemes evaluated the status of the intensively managed populations in their care, but did not assess the need for them in the context of species vulnerability to threat or integrated conservation action. As resources are limited, I recommend that captive breeding is directed towards those species that have the greatest need of it, and for which it is a viable strategy. This will require inter-regional coordination amongst the zoological community, and between conservation practitioners operating in the species natural habitat, to ensure that resources are appropriately directed and maximised. It will also require robust evaluation criteria encompassing biological and logistical metrics in order to prioritise species breeding programmes.

9.3 Population fragmentation

Theoretically, population fragmentation can increase viability by maintaining genetic diversity across a metapopulation even though it is reduced in population sub-units (Frankham 2006; Frankham 2008; Frankham *et al.* 2010). This relies on a number of assumptions that are unlikely to be met for intensively managed populations, predominantly because population sizes are too small to withstand stochasticity. Additionally, fragmentation caused by external factors is likely to result in less viable unequal sub-units than decision-led fragmentation based on contemporary metapopulation theory. Chapters Seven and Eight demonstrated that fragmentation reduced sustainability and increased the risk of population extinction for scimitar-horned oryx. In the wild, population fragmentation is caused by habitat destruction, degradation, sub-division, and

over-exploitation of resources (Pullin 2002). In comparison the sub-division of captive populations is caused by economic concerns directly expressed as reluctance to transport animals over very large distances, and enacted through legislation designed to protect the agricultural industry. As a result the conservation of endangered species in captivity is being unnecessarily compromised.

Existing and emerging reproductive technologies may be able to provide an alternative solution to translocating individuals between captive populations, and importing new founders from the wild, although legislation still applies to the transport of gametes and embryos (DEFRA 2011).

The zoological community needs to mitigate against the effects of fragmentation by lobbying national governments and the European Union to reduce the impact of legislation on migration within, and between, intensively managed populations.

9.4 The effectiveness of population management

The contemporary population management is established on strong scientific foundations (Ballou *et al.* 2010a) but it makes a number of assumptions that often cannot be met by captive populations. One assumption is that complete pedigree data are available in studbooks for genetic and demographic analyses. This reliance on studbooks is flawed because many have incomplete pedigree data, thereby compromising the effectiveness of management and reducing population sustainability (Leus *et al.* 2011a; Leus *et al.* 2011b) as evidenced in Chapters Four to Seven. Existing strategies to compensate for this are limited, and new solutions are needed. Solutions for herd species could include a move away from studbook-based management and toward group management (Leus *et al.* 2011b) guided by the application of molecular genetic techniques, but any new technique needs to be thoroughly evaluated before application.

The establishment of specific goals such as the retention of 90% of founder gene diversity for 100-years can misrepresent a population's prospects of persistence. For example, does it mean that populations are not worth investing limited resources in if they fail to achieve the goals set for them? Or does it mean that populations are sustainable if they do achieve the goal? The answer to both questions is no. The goal represents an acceptable loss of genetic diversity and is an implicit acknowledgement that we cannot sustainably manage that population (Ballou & Traylor-Holzer 2011). The timescale involved also absolves individual responsibility for the management of the population, because no individual has control over the objective. The overall objective should be to

retain as much genetic diversity as possible. Short-term objectives would then provide a better reference to managing captive populations, and can be adjusted as circumstances change.

Whilst current management techniques are imperfect, it is important to recognise the value of managing captive populations. Chapter Six demonstrated that management can increase the retention of genetic diversity, and regionally managed populations yielded higher N_e/N ratios than largely unmanaged global populations in Chapter Seven. Current management practices should not be wholly abandoned, in fact they are well suited for some captive populations, but categorically applying them to every species is not necessarily appropriate. Management solutions need to be flexible to compensate for differing challenges and scenarios.

Global management would also benefit captive populations because management maximises the prospects of attaining sustainability. This strategy has been previously advocated by Iyengar *et al*. (2007), Lees and Wilcken (2009), WAZA (2005e), and Wood *et al*. (2008) although this is the first time it has been based on quantitative analysis of multiple taxa. Whilst global coordination of captive populations does not necessarily mean the integration of regional populations, the legislative and economic barriers that impact regional management need to be removed if effective global coordination is to take place. Global coordination for species such as the scimitar-horned oryx should include all captive and reintroduced populations as part of a comprehensive conservation plan.

9.5 Limitations

The use of studbook data in population research presents a challenge in as much as it may be poor quality and erroneous. Whilst this very issue was a focal point of this thesis, the analyses relied on the same data that was proven to be flawed. The methodology compensated for this where possible by only including studbook data with a minimum of 62.5% pedigree completeness, and using modified data to test population dynamics. Despite this, the quality of the studbook data may have been reduced due to inaccurate founder assumptions of non-relatedness and cryptic errors in identifying individuals, assigning parentage, and in collating the data from individual institutions. Historical record keeping by zoological institutions was often poor, resulting in a lack of consistency between institutions, and missing records (Bingaman Lackey 2010; Princée 1995; Willis 1993). This is a particular challenge for the scimitar-horned oryx international studbook. No records were kept for the European founders caught in Chad in 1967, or their offspring,

for their first two years in captivity. Records only began when they were transferred to other institutions, and by that time it was not possible to discern which individuals were the founders and which were the F_1 generation. Subsequently, all the receiving institutions recorded the animals as founders, resulting in an increase in the number of founders recorded in the studbook (Gilbert 2010a).

The husbandry and management of social groups also impacts on the quality of records. Individuals held in isolation, in breeding pairs or family groups are usually readily identifiable, although it does not necessarily follow that records are accurate. Species that are managed in herds or groups, and do not have individually distinguishable features such as unique stripe patterns, are more vulnerable to misidentification, particularly if identifiers like ear tags are lost (Gilbert & Woodfine 2004a; Princée 1995).

The scimitar-horned oryx population has been particularly affected by this issue (Gilbert 2010a; Gilbert & Woodfine 2004a), and cryptic errors in the studbook are a notable concern. Records for the scimitar-horned oryx go back to 1875, and it is not possible to resolve this issue using molecular genetic analyses because 79% of animals listed in the studbook are dead (Gilbert 2010a). Consequently, the extent of cryptic errors in the studbook is, and will remain, unknown. Erroneous studbook data reduces the quality of the pedigrees derived from them, which then impacts on the accuracy of analyses.

The results and conclusions obtained from this thesis are also limited by the underlying assumptions of the software programs used in the analyses. Deterministic and stochastic simulations generate predictions based on the parameters that are entered in the programs, and it is not possible to model all population dynamics (Lacy 1993b; Lacy 2000a; Lacy & Ballou 2002; Miller & Lacy 2005). The predictions are therefore subject to user-error and software limitations.

Despite all of these limitations, the results obtained in this thesis are valid because they relied on comparative analyses of different models, rather than absolute predictions of future population dynamics. Any limitations that existed in the analyses were applied equally to all models and scenarios, therefore allowing comparisons of varying parameters and different fragmentation models.

9.6 Recommendations for captive management

A number of recommendations are presented based on the results in this thesis. The recommendations are separated into those specifically for the management of the scimitar-

horned oryx EEP population, and those that apply to the management of all captive populations.

9.6.1 Recommendations for the management of scimitar-horned oryx

1. Gene diversity has been, and is predicted to be, lost from the scimitar-horned oryx EEP population at an accelerated rate because the N_e/N, and consequently the N_e, is small. The N_e/N can be maximised by reducing the size, and increasing the number, of polyganous groups along with an increased rotation of males between herds. This would result in a more equal sex ratio and family sizes thereby increasing N_e/N and the retention of gene diversity. Applying this will require a change in the management of scimitar-horned oryx which may be in conflict with its social behaviour, and institutional requirements. These principles, and therefore the recommendation, apply equally to all species with polyganous and polyandrous group management structures.
2. Assisted reproductive techniques have been developed for the scimitar-horned oryx (Kouba *et al.* 2001; Morrow & Monfort 1998; Morrow *et al.* 2000; O'Brien & Roth 2000; Roth *et al.* 1998; Roth *et al.* 1999), but have not been applied to its management. The fragmentation of the EEP population, and the need for global coordination, means that this technology can play a vital role in conserving genetic diversity in both captive and reintroduced populations, and should now be utilised.
3. At least 900 individuals are required to ensure the sustainability of the scimitar-horned oryx EEP population (Chapter Eight), but an estimated 2655 individuals are needed to ensure an N_e of 500, and prevent the net loss of genetic diversity (Chapter Six) from the population. This is more than twice the number of oryx currently in the EEP population, and over 1000 individuals more than the global population, respectively (Gilbert 2010a). These population sizes are unachievable for the coordinated captive breeding programme without displacing other endangered taxa. Consequently, I recommend that the species is coordinated at a global scale combining captive and reintroduced populations in fenced protected areas. However, as most of the estimated captive population is not subject to intensive population management (Chapter Two), I recommend that large free-ranging populations encompassing individuals from all the founder lineages are established back in their historic range as soon as possible. Reintroduction projects that aim to do this should be supported by the captive breeding programmes.

9.6.2 Recommendations for the management of captive populations

1. The sustainability of captive populations is reduced by economic fragmentation. I recommend that zoo and aquaria associations lobby administrative authorities to reduce the barriers to migration within and between coordinated captive breeding programmes.
2. Wild populations are increasingly threatened with extinction, and captive populations are typically small and unsustainable. Many species are likely to need an integrated management approach that encompasses captive, semi-captive and free-ranging populations. Consequently, species in need of intensive management should be coordinated at a global level to maximise resources and increase effective and census metapopulation size, thereby giving endangered species the best chance of survival.
3. The zoological community needs to develop robust criteria to prioritise limited resources to those species that will benefit from captive breeding. This will require a re-evaluation of species diversity in captivity, and a shift in existing paradigms.
4. Alternative management solutions need to be developed for populations with incomplete pedigree data.

9.7 Recommendations for further research

Detailed recommendations for research are discussed in each chapter. Here, I provide general research recommendations

9.7.1 Recommendations for further research for scimitar-horned oryx

The scimitar-horned oryx international studbook is flawed, and the analytical studbook provides only a limited solution for population management. Additionally, the large numbers of scimitar-horned oryx in the Middle East and on Texan ranches have not been evaluated in relation to the known population. In order to maximise captive genetic diversity and re-establish genetically diverse populations in the oryx's former range, I recommend a global molecular genetics study is initiated to evaluate the relationship within and between the different populations. The results will inform the global management of the species.

9.7.2 Recommendations for further research for captive populations

This thesis does not include an evaluation of the impact of adaptation to captivity on the sustainability of captive populations. Adaptation to captivity can pose a serious threat to species integrity and the fitness of reintroduced populations (Frankham 2003; Frankham *et al.* 2010), and therefore impact sustainability. This issue is often overlooked because of the difficulty in evaluating it for endangered species due to long generation lengths and confounding factors (Frankham 2010b). I recommend that despite the difficulties, this research undertaken as it will make a notable contribution to the management of endangered species in captivity.

I have previously recommended that new management techniques are developed to address the limitations of current management strategies. The development of any new approach to managing captive populations needs to be accompanied by rigorous research to evaluate the impact on population viability and sustainability, and species integrity.

9.8 Conclusion

The presumption behind the establishment of specific goals such the retention of 90% of founder gene diversity for 100-years, is that populations will be maintained in captivity for a limited period of time before being re-established in the wild. However, some populations have already been in captivity for 100-years, and there is no indication that the rate of biodiversity loss is decreasing, in fact the opposite is true. If captive populations are to make a genuine contribution to conservation, they need to be self-sustainable. Currently, they are not.

If the zoological community wants to make a genuine contribution to biodiversity conservation, it needs to sustainably manage its populations or re-establish them as free-ranging populations in the wild as soon as possible. To achieve sustainability, population management needs to shift from regional to global populations; the zoological community needs to invest in existing technology such as assisted reproductive and molecular genetic techniques, and develop new tools, methods and strategies to ensure the populations in their care are sustainable. If a population cannot achieve sustainability, then attention should be directed to other populations and programmes to maximise limited resources.

The methods and techniques developed for the preservation of captive populations are increasingly applicable to populations in their natural habitat (Gusset & Dick 2010). Habitat destruction and fragmentation are continuing apace, and parks and protected areas

are being fenced to protect the remaining biodiversity (Robert 2006). The lines between the wild and captivity are becoming increasingly blurred. The zoological community now needs to decide if it will rise to the challenge of combating biodiversity loss and maintain sustainable populations of endangered species in captivity, semi-captivity, and the wild.

This thesis argues that captive populations like the scimitar-horned oryx EEP are not currently sustainable. It challenges some of the existing methods of population management, and makes a contribution to the understanding of intensive population management in general, and the management of the scimitar-horned oryx EEP, in particular. In argues for a fundamental shift in population management paradigms, and presents a challenge to the international zoo community to fulfil its potential for conserving biodiversity.

Appendix A: animal and plant reintroductions

The table was generated from an ISI Web of Knowledge search (http://wok.mimas.ac.uk). The search generated a total of 956 results which was reduced to 598 published papers once duplications and non-relevant subject material were removed. The definition of a 'captive' source included individuals that were captive-born, captive-reared, or plants and animals that spent a part of their lifecycle or development in captivity. The definition of a 'wild' source was wild-caught individuals that were translocated between one *in-situ* location and another and only held in captivity for disease or quarantine reasons. The IUCN categories were based on the 2004 listings and not the status at the time of release, or the local status of the species

Species	Common name	IUCN status	Source	Reference
ANIMALIA				
ACTINOPTERYGII				
Acipenser fulvescens	Lake sturgeon	LC	Captive/Wild	1, 2, 3
Catostomus latipinnis	Flannelmouth sucker	-	Unknown	1, 4
Barbus treurensis	Treur River barb	EN	Unknown	1, 5
Erimonax monachus	Spotfin chub	-	Captive	1, 6
Micropterus salmoides	Largemouth bass	-	Captive	1, 7
Morone saxatilis	Striped bass	-	Captive	1, 8
Etheostoma percnurum	Duskytail darter	-	Captive	1, 6
Oncorhynchus kisutch	Coho salmon	-	Unknown	1, 9
Oncorhynchus nerka	Sockeye salmon	LC	Captive	1, 10
Oncorhynchus tshawytscha	Chinook salmon	-	Captive	1, 9, 11
Salmo salar	Atlantic salmon	LC	Unknown	1, 12, 13, 14
Salmo trutta	Sea trout	LC	Unknown	1, 13
Salvelinus namaycush	Lake trout	-	Unknown	1, 15
Noturus baileyi	Smoky madtom	CR	Captive	1, 6
Noturus flavipinnis	Yellowfin madtom	VU	Captive	1, 6
AMPHIBIA				
Hyla arborea	European tree frog	LC	Unknown	1, 16
Litoria aurea	Green & golden bell frog	VU	Captive	1, 17, 18
AVES				
Anser erythropus	Lesser white-fronted goose	VU	Unknown	1, 19, 20
Branta sandvicensis	Hawaiian goose	VU	Captive	1, 21, 22
Cygnus buccinator	Trumpeter swan	LC	Captive	1, 23
Himantopus novaezelandiae	Black stilt	CR	Captive	1, 24
Ciconia ciconia	White stork	LC	Unknown	1, 25
Mycteria cinerea	Milky stork	VU	Captive	1, 26
Gypaetus barbatus	Bearded vulture	LC	Captive	1, 27
Gyps fulvus	Griffon vulture	LC	Captive	1, 28
Haliaeetus albicilla	White-tailed sea eagle	LC	Unknown	1, 29
Milvus milvus	Red kite	NT	Wild	1, 30, 31
Neophron percnopterus	Egyptian vulture	EN	Unknown	1, 32
Pandion haliaetus	Osprey	LC	Wild	1, 33, 34
Gymnogyps californianus	Californian condor	CR	Captive	1, 35
Vultur gryphus	Andean condor	NT	Unknown	1, 36
Falco femoralis	Aplomado falcon	LC	Unknown	1, 37
Falcon peregrinus	Peregrine falcon	LC	Captive	1, 38, 39, 40
Crax rubra	Great currasow	NT	Captive	1, 41

Appendix A continued

Species	Common name	IUCN list	Source	Reference
AVES continued				
Penelope obscura bronzina	Dusky-legged guan	LC	Captive	1, 42
Penelope superciliaris jacupemba	Rusty-margined guan	LC	Captive	1, 42
Colinus virginianus ridgwayi	Masked bobwhite	NT	Captive	1, 43
Alectoris rufa	Red-legged partridge	LC	Captive	1, 44
Crossoptilon mantchuricum	Brown-eared pheasant	VU	Captive/wild	1, 45
Catreus wallichii	Cheer pheasant	VU	Unknown	1, 46
Perdix perdix	Grey partridge	LC	Captive	1, 44, 47
Tetrao tetrix	Black grouse	LC	Unknown	1, 48
Tetrao urogallus	Capercaillie	LC	Captive	1, 49
Tympanuchus cupido	Greater prairie chicken	VU	Wild	1, 50, 51
Grus americana	Whopping crane	EN	Captive	1, 52, 53, 54
Chlamydotis undulata	Houbara bustard	VU	Captive	1, 55
Crex crex	Corncrakes	NT	Captive	1, 56, 30
Dryolimnas aldabranus	Aldabra rail	-	Wild	1, 57
Gallirallus australis greyi	North Island weka	VU	Captive	1, 58
Acanthisitta chloris	Rifleman	LC	Wild	1, 59
Philesturnus carunculatus carunculatus	South Island saddleback	NT	Wild	1, 60
Corvus corax	Raven	LC	Unknown	1, 61
Corvus hawaiiensis	Hawaiian crow	EW	Captive	1, 62, 63
Lichenostomus melanops cassidix	Helmeted honeyeater	LC	Unknown	1, 64, 65
Notiomystis cincta	Hihi	VU	Wild	1, 66
Petroica australis	North Island robin	LC	Unknown	1, 67
Foudia rubra	Mauritius fody	EN	Captive	1, 68
Leucopsar rothschildi	Bali mynah	CR	Captive	1, 69
Myadestes obscurus	‘Oma’o	VU	Captive/wild	1, 70
Picoides borealis	Red-cockaded woodpecker	VU	Wild	1, 71, 72
Ramphastos vitellinus ariel	Channel-billed toucan	LC	Captive	1, 73
Puffinus gavia	Fluttering shearwaters	LC	Wild	1, 74
Amazona barbadensis	Yellow-shouldered Amazon parrot	VU	Captive	1, 75
Ara macao	Scarlet macaw	LC	Captive	1, 76, 77
Ara ararauna	Blue-gold macaw	LC	Wild	1, 78
Neophema chrysogaster	Orange-bellied parrot	CR	Captive	1, 79
Rhynchopsitta pachyrhyncha	Thick-billed parrot	EN	Unknown	1, 80
Athene cunicularia	Burrowing owl	LC	Captive	1, 81, 82
Bubo bubo	Eagle owl	LC	Captive	1, 83
Strix uralensis	Ural owl	LC	Unknown	1, 84
Tyto capensis	African grass owl	LC	Captive	1, 85
BIVALVIA				
Argopecten irradians	Bay scallops	-	Unknown	1, 86
BRANCHIOPODA				
Daphnia longispinia	Zooplankton	-	Wild	1, 87
GASTROPODA				
Patella ferruginea	Limpit	-	Wild	1, 88
Cittarium pica	West Indian topshell	-	Unknown	1, 89
Io fluvialis	Spiny riversnail	EN	Wild	1, 90
Placostylus spp.	Placostylus land snails	VU	Wild	1, 91

Appendix A continued

Species	Common name	IUCN list	Source	Reference
INSECTA				
Maculinea nausithous	Dusky large blue butterfly	NT	Wild	1, 92
Maculinea teleius	Large blue butterfly	NT	Wild	1, 92
Parnassius apollo	Swallowtail butterfly	VU	Captive	1, 93
Pseudophilotes baton schiffermuelleri	Baton blue butterfly	-	Wild	1, 94
Decticus verrucivorus	Warbiter cricket	-	Captive	1, 95
Deinacrida mahoenui	Mahoenui giant weta	-	Wild	1, 91
Ischnura gemina	San Francisco Forktail	VU	Wild	1, 96
Cicindela formosa generosa	Tiger beetle	-	Unknown	1, 97
Nicrophorus americanus	American burying beetle	CR	Captive/wild	1, 98, 99
Agasicles hygrophila	Alligatorweed flea beetle	-	Unknown	1, 100
MALACOSTRACA				
Astacus astacus	Noble Crayfish	VU	Wild	1, 101, 102
Austropotamobius pallipes	White-clawed crayfish	VU	Captive	1, 103
MAMMALIA				
Bison bison athabascae	Wood bison	NT	Unknown	1, 104
Bison bonasus	European bison	VU	Captive	1, 105, 106
Capra ibex	Alpine ibex	LC	Unknown	1, 107, 108, 109
Gazella dama	Dama gazelle	CR	Captive	1, 110
Gazella dama mhorr	Mhorr gazelle	CR	Captive	1, 111
Gazella gazella	Mountain gazelle	VU	Captive	1, 112, 113
Gazella subgutturosa marica	Arabian gazelle	VU	Captive	1, 114
Oryx dammah	Scimitar-horned oryx	EW	Captive	1, 115
Oryx leucoryx	Arabian oryx	EN	Captive	1, 116, 117, 118
Ovis Canadensis	Penninsula bighorn sheep	LC	Captive	1, 119
Rupicapra rupicapra	Chamois	LC	Wild	1, 120
Blastocerus dichotomus	Marsh deer	VU	Unknown	1, 121
Capreolus capreolus	Roe deer	LC	Wild	1, 122
Cervus elaphus	Elk	LC	Wild	1, 123, 124
Cervus elaphus corsicanus	Corsican red deer	LC	Captive	1, 125
Cervus elaphus nannods	Tale elk	LC	Unknown	1, 126
Elaphurus davidianus	Pere David's deer	EW	Captive	1, 127
Rangifer tarandus	Caribou	LC	Unknown	1, 128
Sus scrofa	Wild boar	LC	Wild	1, 129
Canis lupus baileyi	Mexican wolf	LC	Captive	1, 130
Canis lupus	Grey wolf	LC	Wild	1, 131, 132
Canis rufus	Red wolf	CR	Captive	1, 130, 133, 134
Lycaon pictus	African wild dog	EN	Captive	1, 135, 136
Vulpes velox	Swift fox	LC	Captive/wild	1, 137, 138, 139
Acinonyx jubatus	Cheetah	VU	Unknown	1, 135
Felis silvestris silvestris	European wildcat	LC	Unknown	1, 140
Lynx canadensis	Lynx	LC	Wild	1, 141
Lynx lynx	Eurasian Lynx	LC	Captive	1, 142, 143, 144
Lynx rufus	Bobcat	LC	Unknown	1, 145
Panthera leo	Lion	VU	Wild	1, 135
Panthera pardus	Leopard	NT	Wild	1, 135, 146
Crocuta crocuta	Spotted hyaena	LC	Wild	1, 135
Lontra canadensis	River otter	LC	Wild	1, 147, 148, 149
Lutra lutra	Eurasian otter	NT	Wild	1, 150, 151, 152
Martes americana	American marten	LC	Wild	1, 153, 154
Martes pennanti	Fisher	LC	Wild	1, 155
Meles meles	Eurasian badger	LC	Wild	1, 156

Appendix A continued

Species	Common name	IUCN list	Source	Reference
MAMMALIA continued				
Mustela nigripes	Black-footed ferret	EN	Captive	1, 157, 158, 159
Mustela putorius	Scottish polecat	LC	Captive	160
Monachus monachus	Monk seal	CR	Unknown	161
Ursus americanus	Black bear	LC	Unknown	1, 162, 163
Ursus arctos	Brown bear	LC	Wild	1, 164, 165
Phascogale tapoatafa	Brush-tailed phascogale	NT	Unknown	1, 166
Myrmecobius fasciatus	Numbat	EN	Unknown	1, 167
Lagostrophus fasciatus	Hare-wallaby	EN	Captive	1, 168, 169
Lagorchestes hirsutus	Rufous hare-wallaby	VU	Unknown	1, 170, 171
Onychogalea fraenata	Bridled nailtail wallaby	EN	Unknown	1, 172
Petrogale xanthopus	Yellow-footed rock wallaby	NT	Captive	1, 173
Trichosurus vulpecula	Brushtail possum	LC	Wild	1, 174
Phascolarctos cinereus	Koala	LC	Captive	1, 175, 176
Bettongia lesueur	Burrowing bettong	NT	Wild	1, 177
Bettongia penicillata	Brush-tailed bettong	CR	Unknown	1, 178
Oryctolagus cuniculus	Wild rabbit	NT	Unknown	1,179
Sylvilagus palustris hefneri	Lower keys marsh rabbit	LC	Wild	1, 180
Ornithorhynchus anatinus	Platypus	LC	Unknown	1, 181, 182
Tachyglossus aculeatus multiaculeatus	Short-beaked echidna	LC	Wild	1, 183
Perameles bougainville	Western barred bandicoot	EN	Wild	1, 184
Perameles gunnii	Eastern barred bandicoot	NT	Unknown	1, 185, 186
Macrotis lagotis	Greater bilby	VU	Unknown	1, 187, 188
Equus ferus przewalskii	Takhi	CR	Captive	1, 189, 190
Equus hemionus	Asiatic wild ass	EN	Unknown	1, 191
Ceratotherium simum simum	White rhino	NT	Captive	1, 192, 193
Diceros bicornis	Black rhino	CR	Unknown	1, 194, 195
Alouatta caraya	Black howler monkey	LC	Wild	1, 196
Ateles geoffroyi	Spider monkey	EN	Captive	1, 197
Leontopithecus chrysopygus	Black lion tamarins	EN	Captive/wild	1, 198
Leontopithecus rosalia	Golden lion tamarins	EN	Captive	1, 199, 200, 201
Pan troglodytes	Chimpanzees	EN	Captive	1, 202, 203
Pongo pygmaeus	Bornean orangutan	EN	Unknown	1, 203
Hylobates agilis albibarbis	Agile gibbons	EN	Captive	1, 204
Varecia variegata variegata	Black & white ruffed lemur	CR	Captive	1, 205
Castor fiber	European beaver	LC	Wild	1, 206, 207, 208
Arvicola terrestris	Water vole	LC	Captive	1, 209
Microtus rossiaemeridionalis	Russian common vole	LC	Captive	1, 210
Neotoma magister	Allegheny woodrats	NT	Unknown	1, 211, 212
Glis glis	Edible dormouse	LC	Wild	1, 213
Cynomys gunnisoni	Gunnison's prairie dog	LC	Unknown	1, 214
Sciurus vulgaris	Red squirrel	LC	Wild	1, 215
MAXILLOPODA				
Cyclops abyssorum	Zooplankton	-	Wild	1, 87
REPTILIA				
Melanosuchus niger	Black caiman	NT	Unknown	1, 216
Crocodylus palustris	Mugger crocodile	VU	Unknown	1, 217
Leiolopisma acrinasum	Fiordland skink	-	Wild	1, 218
Geochelone nigra hoodensis	Giant tortoises	VU	Captive	1, 219
Testudo hermanni	Hermann's tortoise	NT	Unknown	1, 220, 221
Testudo hermanni hermanni	Mediterranean tortoises	EN	Unknown	1, 222
Emys orbicularis	European pond turtle	NT	Wild	1, 223

Appendix A continued

Species	Common name	IUCN list	Source	Reference
PLANTAE				
BRYOPHYTA				
Scorpidium scorpioides	Scorpidium moss	-	Wild	1, 224
Sphagnum angustifolium	Sphagnum	-	Unknown	1, 225
Sphagnum capillifolium	Sphagnum	-	Unknown	1, 225
Sphagnum fuscum	Sphagnum	-	Unknown	1, 225
Sphagnum magellanicum	Magellan's sphagnum	-	Unknown	1, 225
Sphagnum papillosum	Papillose sphagnum	-	Unknown	1, 225
FILICOPSIDA				
Woodsia ilvensis	Wood fern	-	Unknown	1, 226
Osmunda regalis	Fern	-	Captive	1, 227
LILIOPSIDA				
Gladiolus imbricatus	Gladiolus	-	Unknown	1, 228
Bulbophyllum membranaceum	Orchid	-	Captive	1, 229
Bulbophyllum vaginatum	Orchid	-	Captive	1, 229
Grammatophyllum speciosum	Giant orchid	-	Captive	1, 229
Habenaria radiata	White egret orchid	-	Captive	1, 230
Ipsea malabarica	Orchid	-	Captive	1, 231
Spiranthes brevilabris	Short-lipped ladies'-tresses	-	Captive	1, 232
Scirpus spp.	Tule	-	Unknown	1, 233
Helonias bullata	Swamp pink	-	Unknown	1, 234
Aristida beyrichlana	Wiregrass	-	Unknown	1, 235
Hubbardia heptaneuron	Hubbardia bor	-	Unknown	1, 236
Nassella pulchra	Bunchgrass	-	Unknown	1, 237
Zea mays	'Chococito' maize race	-	Unknown	1, 238
Zizania texana	Texas wildrice	-	Wild	1, 239
MAGNOLIOPSIDA				
Argyroxiphium sandwicense	Mauna kea silversword	VU	Unknown	1, 240
Cirsium dissectum	Meadow thistle	-	Wild	1, 241
Cirsium pitcheri	Pitcher's thistle	-	Captive	1, 242
Echinacea laevigata	Echinacea	-	Unknown	1, 243
Pseudophoenix sargentii	Buccaneer palm	-	Unknown	1, 244
Senecio hadrosomus	Senecio	-	Captive	1, 245
Tetraneuris herbacea	Lakeside daisy	-	Unknown	1, 246
Warea amplexifolia	Florida sandhill	-	Unknown	1, 247
Pediocactus knowltonii	Knowlton's cactus	-	Unknown	1, 248
Silene douglasii var. oraria	Silene	-	Wild	1, 249
Abronia umbellate spp.	Pink sand verbena	-	Unknown	1, 250
Succisa pratensis	Devil's-bit scabious	-	Wild	1, 241
Castanea dentate	American chestnut	-	Unknown	1, 251
Nepeta rtanjensis	Nepeta	-	Captive	1, 252
Hibiscus dasycalyx	Neche River rose mallow	-	Unknown	1, 253
Purshia subintegra	Purshia	-	Captive	1, 254
Syzygium travancoricum	Syzygium	CR	Captive	1, 255
Agalinis acuta	Sandplain false foxglove	-	Unknown	1, 256
Castilleja fasciatus	Golden paintbrush	-	Unknown	1, 257
Cordylanthus maritimus	Salt marsh bird's beak	-	Wild	1, 258
Schwalbea americana	American chaffseed	-	Captive	1, 259

1: IUCN, 2009(Nielsen *et al.* 2007); **2:** Bezold & Peterson, 2008; **3:** Drauch & Rhodes, 2007; **4:** Mueller & Wydoski, 2004; **5:** Engelbrecht & Roux, 1998; **6:** Shute *et al.*, 2005; **7:** Mittelbach *et al*., 1995; **8:** Bouchard, 2003; **9:** Pearsons & Temple, 2007; **10:** Hebdon *et al*., 2004; **11**: Narum *et al*., 2007; **12:** Moravec, 2003; **13:** Saura *et al*., 1990; **14:** Bagliniere *et al*., 1990; **15:** Keller *et al*., 1990; **16:** Zvirgzds

et al., 1995; **17:** Daly *et al*., 2008; **18:** Stockwell *et al*., 2008; **19:** von Essen, 1996; **20:** von Essen, 1991; **21:** Black, 1998; **22:** Black *et al*., 1997; **23:** Lumsden & Drever, 2002; **24:** van Heezik *et al*., 2005; **25:** Olsson & Rogers, 2009; **26:** Yaacob, 1994; **27:** Schaub *et al*., 2009; **28:** Mihoub *et al*., 2009; **29:** Love & Ball, 1979; **30:** Carter & Newbery, 2004; **31:** Carter *et al*., 1999; **32:** Terrasse, 1990; **33:** Dennis & Dixon, 2001; **34:** Martell, 1995; **35:** Meretsky *et al*., 2001; **36:** Lieberman *et al*., 1993; **37:** Anon, 2006; **38:** Jacobsen *et al*., 2008; **39:** Kirmse, 2001; **40:** Holroyd & Banasch, 1990; **41:** Fournier & Janik, 2008; **42:** Pereira & Wajntal, 1999; **43:** Carpenter *et al*., 1991; **44:** Meriggi *et al*., 2007; **45:** Zhang *et al*., 2004; **46:** Garson *et al*., 1992; **47:** Parish & Sotherton, 2007; **48:** Dobler & Siedle, 1993; **49:** Spittler, 1994; **50:** Hoffman & Beauprez, 1997; **51:** Hoffman *et al*., 1992; **52:** Hartup *et al*., 2005; **53:** Kreger *et al*., 2003; **54:** Ellis *et al*., 1992; **55:** Judas, 2000; **56:** Mudenda *et al*. 2008; **57:** Wanless *et al*., 2002; **58:** Graeme & Graeme, 1995; **59:** Leech *et al*., 2007; **60:** Pierre, 1999; **61:** Koch *et al*., 1986; **62:** Valutis & Marzluff, 1999; **63:** Kuehler *at al*., 1995; **64:** Pearce & Lindenmayer, 1998**; 65:** McCarthy, 1995; **66:** Castro *et al*., 1995; **67:** Lewis *et al*., 2009; **68:** Cristinacce *et al*., 2008; **69:** Collins *et al*., 1998; **70:** Fancy *et al*., 2001; **71:** Carrie *et al*., 1999; **72:** Rudolph *et al*., 1992; **73:** Coimbra Filho, 2000; **74:** Bell, 1995; **75:** Sanz & Grajal, 1998; **76:** Brightsmith *et al*., 2005; **77:** Nader *et al*., 1999; **78:** Plair *et al*., 2001; **79:** Brown *et al*., 1995; **80:** Koschmann, 1995; **81:** Leupin & Low, 2001; **82:** Martell *et al*., 2001; **83:** Foerstel, 1990; **84:** Stuerzer, 1999; **85:** Brown *et al.,* 2007; **86:** Tarnowski & Homer, 2008; **87:** Kohout & Fott, 2006; **88:** Espinosa *et al*., 2008; **89:** Coates *et al*., 2003; **90:** Ahlstedt, 1991; **91:** Sherley, 1995; **92:** Wynhoff, 1998; **93:** Witkowski *et al*., 1997; **94:** Marttila *et al*., 1997; **95:** Cunningham *et al*., 1997; **96:** Hannon & Hafernik, 2007; **97:** Brust, 2002; **98:** Kozol *et al.*, 1996; **99:** Wetzel, 1996; **100:** Buckingham *et al*., 1983; **101:** Sint & Fureder, 2004; **102:** Taugbol, 2004; **103:** Rogers & Watson, 2007; **104:** Larter *et al*., 2000; **105:** Belousova *et al*., 2005; **106:** Olech & Perzanowski, 2002; **107:** Gauthier & Villaret, 1990; **108:** Wiersema, 1990; **109:** Grodinsky & Stuwe, 1987; **110:** Cano *et al*., 1993; **111:** Wiesner & Muller, 1998; **112:** Dunham, 2001; **113:** Dunham *et al*., 1993; **114:** Haque & Smith, 1996; **115:** Gordon & Gill, 1993; **116:** Harding *et al.,* 2007; **117:** Spalton *et al*., 1999; **118:** Spalton, 1993; **119:** Ostermann *et al*., 2001; **120:** Frkovic, 2008; **121:** Figueira *et al*., 2005; **122:** Calenge *et al*., 2005; **123:** Hicks *et al*., 2007; **124:** Witmer, 1990; **125:** Kidjo *et al*., 2007; **126:** Johnson & Cushman, 2007; **127:** Jiang *et al*., 2000; **128:** Collins *et al*., 2003; **129:** Vernesi *et al*., 2003; **130:** Hedrick & Fredrickson, 2008; **131:** Carroll *et al*., 2003; **132:** Fritts *et al*., 1997; **133:** Phillips *et al*., 1995; **134:** Moore, 1990; **135:** Hayward *et al*., 2007b; **136:** Woodroffe & Ginsberg, 1999; **137:** Bremner-Harrison *et al*., 2004; **138:** Smeeton & Weagle, 2000; **139:** Carbyn *et al*., 1994; **140:** Buettner & Worel, 1990; **141:** Steury & Murray, 2004; **142:** Vandel *et al*., 2006; **143:** Boer *et al*., 2000; **144:** Scott *et al*., 1999; **145:** Warren *et al*., 1990; **146:** Hayward *et al*., 2007; **147:** Raesly, 2001; **148:** Serfass *et al*., 1999; **149:** Serfass *et al*., 1993; **150:** van't Hof & van Langevelde, 2004; **151:** Fernandez-Moran *et al*., 2002; **152:** Weber *et al*., 1991; **153:** Swanson & Kyle, 2007; **154:** Swanson *et al*., 2006; **155:** Aubry & Lewis, 2003; **156:** Balestrieri *et al*., 2006; **157:** Wisely *et al*., 2008; **158:** Miller *et al*., 1998; **159:** Russell *et al*., 1994; **160:** Solow *et al*., 2006; **161:** Marchessaux, 1990; **162:** Smith *et al*., 1991; **163:** Alt & Beecham, 1984; **164:** Dupre *et al*., 2000; **165:** Arquilliere, 1998; **166:** Soderquist, 1995; **167:** Friend & Thomas, 1995; **168:** Hardman & Moro, 2006a; **169:** Hardman & Moro, 2006; **170:** Gibson *et al*., 1995; **171:** McLean *et al*., 1995; **172:** McCallum, 1995; **173:** Lapidge, 2005; **174:** Pietsch, 1995; **175:** Norton, 1995; **176:** Ellis *et al*., 1990; **177:** Short & Turner, 2000; **178:** Pizzuto *et al*., 2007; **179:** Letty *et al*., 1998; **180:** Faulhaber *et al*., 2006; **181:** Souter & Williams, 2001; **182:** Carey & Smallridge, 1998; **183:** Rismiller & McKelvey, 1995; **184:** Richards & Short, 2003; **185:** Backhouse *et al*., 1995; **186:** Dufty *et al*., 1995; **187:** Moseby & O'Donnell, 2003; **188:** Southgate & Possingham,1995; **189:** Stauffer, 2005; **190:** Boyd, 1998; **191:** Rowen & Saltz, 1996; **192:** Boer *et al*., 1999a; **193:** Boer *et al*., 1999b; **194:** van der Westhuizen, 2003; **195:** Vande Weghe, 1998; **196:** Lindbergh, 1987; **197:** McKinney & Schutt, 2005; **198:** Valladares-Padua *et al*., 2000; **199:** Beck, 1998; **200:** Castro *et al*., 1998; **201:** Bush *et al*., 1996; **202:** Ancrenaz *et al*., 2001; **203:** Grundmann & Didier, 2000; **204:** Cheyne, 2006; **205:** Wyner *et al*., 1999; **206:** Jacob, 2003; **207:** Zurowski & Kasperczyk, 1990; **208:** Zurowski, 1979; **209:** Moorhouse *et al*., 2009; **210:** Banks *et al*., 2002; **211:** Serfass, 2008; **212:** Schlie & Bookhout, 1985; **213:** Jurczyszyn, 2006; **214:** Davidson *et al*., 1999; **215:** Fornasari *et al*, 1997; **216:** Pacheco *et al.,* 1991; **217:** Jayson *et al.,* 2006; **218:** Thomas & Whitaker, 1995; **219:** Gibbs *et al*., 2008; **220:** Bertolero *et al*., 2007; **221:** Servan & Dupre, 2003; **222:** Devaux, 1990; **223:** Miquet & Cadi, 2002; **224:** Kooijman *et al*., 1994; **225:** Rochefort & Bastien, 1998; **226:** McHaffie, 2006; **227:** Zenkteler, 2002; **228:** Jogar & Moora, 2008; **229:** Yam & Thame, 2005; **230:** Takahashi *et al*., 2008; **231:** Martin, 2003; **232:** Stewart *et al*. 2003; **233:** Johnson & Cushman, 2004; **234:** Dodds & Hartman, 1995; **235:** Coffey *et al*., 2002; **236:** Yadav *et al*., 2009; **237:** Buisson *et al*., 2008; **238:** Reyes *et al*., 2000; **239:** Power, 1996; **240:** Friar *et al*., 2000; **241:** Smulders *et al*., 2000; **242:** Bowles *et al*., 1993; **243:** Alley & Affolter, 2004; **244:** Lippincott, 1995; **245:** Ortega & Gonzalez, 1990; **246:** McClain & Ebinger, 2008; **247:** Black *et al*., 2001; **248:** Cully, 1996; **249:** Kephart, 2004; **250:** Kaye, 1995; **251:** Pierson *et al*., 2007; **252:** Misic *et al*., 2005; **253:** Smith & Creech, 1995; **254:** Maschinski *et al*., 2004; **255:** Anand *et al*., 2004; **256:** Dunwiddie *et al*., 1996; **257:** Lawrence & Kaye, 2006; **258:** Helenurm & Parsons, 1997; **259:** Obee, 1997.

Appendix B: institution list for the scimitar-horned oryx studbook

The table lists institutions that supplied data to the scimitar-horned oryx international studbook. The chapter numbers indicate where that data was used. The mnemonics are used as abbreviations for institutions in data chapters

Mnemonic	Institution	Country	Chapter
AALBORG	Aalborg Zoo	Denmark	4, 5, 6, 7, 8
AFRICAN	African region	African region	6
AL AIN	Al Ain Zoo	UAE	6
ALGIERS	Jardin D'Essai du Hamma	Algeria	6
AMERSFOOR	Dierenpark Amersfoort	Netherlands	5, 6, 7, 8
AMOUGIES	Amo Safari Park	Belgium	4, 6
AMSTERDAM	Artis Zoo	Netherlands	4, 5, 6, 7, 8
ANIMALES	Vivo Animales	USA	4
ASKANIYA	Zoologicheskii Park Askaniya Nova	Ukraine	4, 6
ATTICAZOO	Attica Zoological Park S.A.	Greece	6, 7, 8
AYWAILLE	Monde Sauvage Safari	Belgium	6, 7, 8
A-Z RANCH	Wildlife A-Z Ranch	USA	4
BADOCA PK	Badoca Park	Portugal	6
BAMBERGER	Bamberger Ranch	USA	4
BANGKOK	Dusit Zoological Park	Thailand	6
BANKS J	John Banks	USA	4
BARCELONA	Parc Zoologic de Barcelona	Spain	4, 6, 7, 8
BELPASSO	Parco Zoo di Sicilia	Italy	4, 6
BERLIN TP	Tierpark Berlin-Friedrichsfelde	Germany	4, 6, 7
BERLINZOO	Zoologischer Garten Berlin	Germany	4, 5, 6, 7, 8
BLANCKEND	Van Blanckendaell Park Zoo	Netherlands	6
BODE W	Werner Bode	Germany	4, 6
BOGOR	Taman Safari Indonesia	Indonesia	4, 6
BOISSIERE	Espace Zoologique la Boissiere du Dore	France	6, 7, 8
BONIZOO	Bonizoo	France	4, 6
BORDI G	Zoo Farm Roma	Italy	4, 6
BOU HEDMA	Bou Hedma National Park	Tunisia	6
BOUILLON	Parc Animalier	Luxembourg	6
BRACKETT	Little Ponderosa Animal Farm	USA	4
BRATISLAV	Zoologicka Zahrada Bratislava	Slovakia	4, 6
BRAVA	Sociedade Agricola da Brava	Portugal	6
BRIJUNI	Brijuni National Park	Croatia	6
BURFORD	Cotswold Wildlife Park and Gardens	UK	4, 5, 6, 7, 8
BUSCH TAM	Busch Gardens Tampa Bay	USA	6
BUSSOLENG	Parco Natura Viva	Italy	6, 7, 8
CABARCENO	Parque de la Naturaleza de Cabarceno	Spain	6, 7, 8
CABOSSE	Zoo de Jurques	France	6, 7, 8
CAIRO ZOO	Giza Zoological Gardens	Egypt	6
CASA JE	Jardin Exotique de Casablanca	Morocco	6
CATSKILL	Catskill Game Farm	USA	4
CHAD	Chad	Chad	4, 6
CHARD	Wildlife Park at Cricket St Thomas	UK	5, 6
CHESINGTN	Chessington World of Adventures	UK	6, 7, 8
CHESTER	North of England Zoological Society	UK	5, 6, 7, 8
CINCO CAN	Cinco Canyon Ranch (Jeff Soele	USA	4
CLEARWATR	Clearwater Ranch	USA	4
CLIFTON	U.S. Department of Agriculture	USA	4, 6
CZECH REP	Czech Republic	Czech Republic	6
DDCR	Dubai Desert Conservation Reserve	UAE	5
DEALER	Unknown dealer	Unknown	6

Appendix B continued

Mnemonic	Institution	Country	Chapter
DEBRECEN	Nagyerdei Kult-rpark KHT	Hungary	6, 7, 8
DELFTS	Wolfgang Delfts	Namibia	4, 6
DGHOUMES	Dghoumes National Park	Tunisia	6
DIAMOND K	Diamond K Ranch	USA	4
DICKERSON	Dickerson Park Zoo	USA	4
DOSWELL	Paramount's Kings Dominion	USA	4
DUBBO	Western Plains Zoo	Australia	5
DUBLIN	Zoological Society of Ireland-Dublin	Ireland	6, 7, 8
DVURKRALV	Zoo Dvur Kralove	Czech Republic	4, 6
EDINBURGH	Edinburgh Zoo	UK	5, 6
ELCHE SAF	Rio Safari Elche	Spain	6
ENGLAND	England	UK	6
ESTEPONA	Parque de la Naturaleza Selwo	Spain	4, 5, 6, 7, 8
FASANO	Zoosafari	Italy	6
FERNDALE	International Animal Exchange Inc	USA	6
FOTA	Fota Wildlife Park	Ireland	6, 7, 8
FREJUS	Parc Zoologique de Frejus	France	4, 6
FRIGUIA	STB Kanta – Friguia Zoo	Tunisia	6
FUENGIROL	Zoo de Fuengirola	Spain	6
GDANSK	Miejski Ogrod Zoologiczny Wybrzeza	Poland	6, 7, 8
GELSNKRKN	Zoom Erlebniswelt Gelsenkirchen	Germany	6
GENK	Limburgse Zoo	Belgium	6
GETTORF	Tierpark Gettorf	Germany	4, 6
GHAMADAN	Ghamadan Zoo	Jordan	6
GRAMMONT	Tierhandlung Peeters	Belgium	6
GUERNO	Parc Zoologique de Chateau de Branfere	France	4, 6, 7, 8
HAI BAR	Hai Bar Yotvata Nature reserve	Israel	6
HAI KEF Z	Hai Kef Zoo	Israel	6
HANNOVER	Zoo Hannover	Germany	6
HILVARENB	Safaripark Beekse Bergen	Netherlands	6
HITACHI	Hitachi City Kamine Zoological Park	Japan	4
HODENHAGN	Serengeti Safaripark Hodenhagen	Germany	4, 6
HODONIN Z	Zoologicka Zahrada Hodonin	Czech Republic	6
HOHENSTAD	Walter Sensen	Germany	4
HOLIDAY	Earl Tatum	USA	6
ISRAEL	Israel	Israel	6
JACKSONVL	Jacksonville Zoo and Gardens	USA	4
JERUSALEM	The Tisch Family Zoological Gardens	Israel	5, 6, 7, 8
KARLSRUHE	Zoologischer Garten Karlsruhe	Germany	4, 5, 6, 7, 8
KATOWICE	Silesian Zoological Garden	Poland	4, 6, 7, 8
KNOWSLEY	Knowsley Safari Park	UK	6, 7, 8
KREFELD	Zoo Krefeld	Germany	6, 7, 8
KRECHTING	Tierpark Krechting	Germany	4, 6
KVIV ZOO	Kyiv Zoological Park	Ukraine	4, 6
L RUHE	Louis Ruhe	Germany	4, 6
LA LAJITA	La Lajita Oasis Park	Spain	4, 6
LA PALMYR	Zoo de la Palmyre	France	4, 5, 6, 7
LABENNE	Oceafaunia Parc de Labenne	France	6
LCS DOS	Lion Country Safari	USA	4
LE PAL	Le Pal, Parc Animalier	France	4, 5, 6, 7, 8
LE VIGEN	Parc Paysager et Animalier du Reynou	France	6
LEIPZIG	Zoologischer Garten Leipzig	Germany	4, 5, 6, 7, 8
LENAERTS	Anvoy Belgie	Belgium	6
LISBON	Jardim Zoologico Lisboa	Portugal	4, 5, 6, 7, 8
LISIEUX Z	Centre d'Etude Rech Zool Augeron	France	4, 6, 7, 8
LITTLEROC	Little Rock Zoological Gardens	USA	4

Appendix B continued

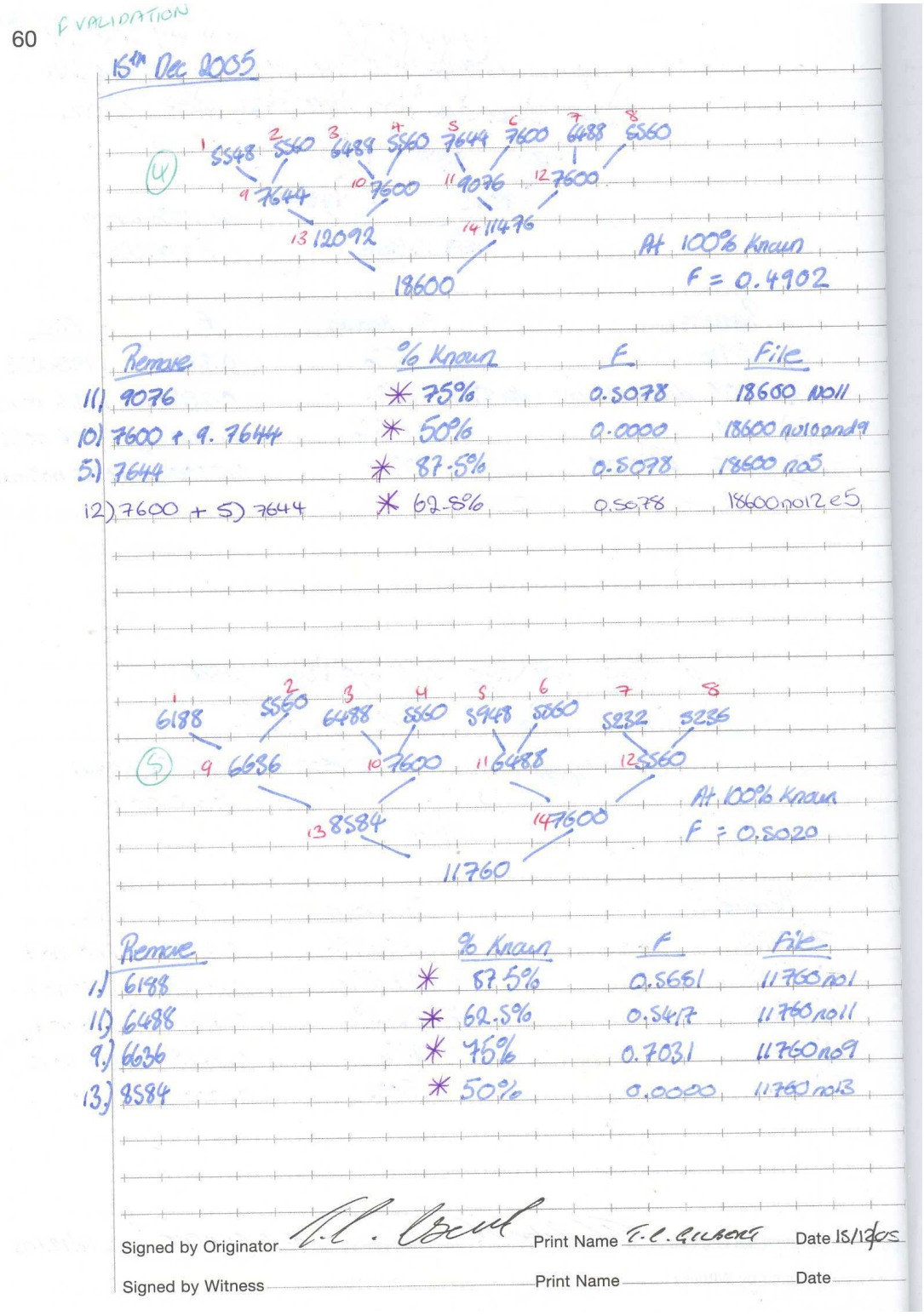
Mnemonic	Institution	Country	Chapter
LODZ	Miejski Ogrod Zoologiczny w Lodz	Poland	5, 6, 7, 8
LONDON RP	Zoological Society of London	UK	6
LONGLEAT	Longleat Safari Park	UK	4, 5, 6, 7, 8
LOSANGELE	Los Angeles Zoo and Botanical Gardens	USA	6
MADRID Z	Zoo Aquarium de Madrid	Spain	5, 6
MALLORCA	Auto Safari Reserva Africana	Spain	6
MALTON	Flamingo Land Ltd	UK	5, 6, 7, 8
MANCHESTR	Belle Vue Zoopark	UK	6
MANOR HS	Manor House Wildlife Park	UK	5, 6, 7, 8
MARWELL	Marwell Wildlife	UK	4, 5, 6, 7, 8
MCALPINE	The Hon. Sir William McAlpine	UK	6
MCCOMBS	Red McCombs Ranch	USA	4
MCLEAN	McLean Ranches	USA	4
MEMPHIS	Memphis Zoological Garden & Aquarium	USA	4
MENACHEM	Kfar Menachem Zoo	Israel	6
MONROE	Louisiana Purchase Gardens & Zoo	USA	4
MONTPELLI	Parc de Lunaret	France	5, 6, 7, 8
MOROCCO	Morocco	Morocco	6
MOULIN	Parc Zool de Moulin de Richard	France	6
MT CARMEL	Hai Bar Carmel	Israel	6
MT ULLA	Lazy 5 Ranch	USA	6
MUNSTER	Westfalischer Zoologischer Gtn Munster	Germany	6
NAT BRIDG	Natural Bridge Zoological Park	USA	4
NELSON S	Circle Bar Ranch	USA	4
NISHIMURO	Nanki Shirahama Adventure World	Japan	4
NZP-CRC	NZP-Conservation & Research Center	USA	4
NZP-WASH	Smithsonian National Zoological Park	USA	4
OBTERRE	Parc de la Haute Touche	France	4, 6, 7, 8
ODAN	Odejewski ‘Odan’	Poland	6
OMAHA	Omaha's Henry Doorly Zoo	USA	4
OPOLE	Ogrod Zoologiczny w Opolu	Poland	4, 6, 7, 8
OSIJEK	Osijek Zoo	Croatia	6
OUED DEK	Oued Dekouk Nature Reserve	Tunisia	5, 6
PABICH D	Dariusz Pabich	Poland	6
PAPHOS BP	Pafos Bird Park	Cyprus	6
PARIS ZOO	Parc Zoologique de Paris MNHN	France	4, 6
PASQUALE	Martino Pasquale	Italy	4, 6
PEGASO R	Rancho Pegaso	USA	4
PELISSANE	Parc Zoologique de la Barben	France	6, 7, 8
PENROSE	Animal World	USA	4
PESSAC	Parco Zool. De Bordeaux Pessac	France	4, 6
PLAISANCE	African Safari	France	4, 6, 7, 8
PLANCKNDL	Wild Animal Park Mechelen Planckendael	Belgium	6, 7, 8
PLOCK	Miejski Ogrod Zoologiczny Plock	Poland	6, 7, 8
PLZEN	Zoological and Botanical Garden Plzen	Czech Republic	6, 7
POMBIA	Pombia Safari Park	Italy	4, 6
POOLE Q	Livestock Quarantine Services	UK	6
PRAHA	Zoological Garden Prague	Czech Republic	4, 5, 6, 7, 8
PRETORIA	National Zoological Gardens of South Africa	South Africa	5
PRIVATE	Private collection	Unknown	6
PT ST PER	Planete Sauvage	France	4, 6, 7, 8
PUBLIC	General public	Unknown	4, 6
PUNTAVERD	Parco Zoo Punta Verde	Italy	6, 7
QALQILYAH	Qalqilyah Zoo	Israel	6
QUADROS	Quadros Photographic Safari Park	Portugal	6
RABAT	Parc Zoologique Natl. de Rabat	Morocco	4, 6

Appendix B continued

Mnemonic	Institution	Country	Chapter
RAMAT GAN	Zoological Center Tel Aviv	Israel	6, 7, 8
SAN ANTON	San Antonio Zoo & Aquarium	USA	6
SANDIEGOZ	San Diego Zoo	USA	6
SCOTTSBLU	Riverside Zoo	USA	4
SD-WAP	San Diego Wild Animal Park	USA	6
SELLES	Chateau de Selles	France	4, 6
SENEGAL	Senegal	Senegal	6
SEVILL RN	La Reserva Natural Castillo de las Guarda	Spain	6
SEVILLE	Seville	Spain	6
SIDI TOUI	Sidi Toui National Park	Tunisia	5, 6
SINAI	Sinai	Egypt	6
SLAUGHTER	Southern Exposure Wildlife Park	USA	6
SOEST	G Frans Van den Brink	Netherlands	4, 6
SOFIAZOO	Sofia Zoological Gardens	Bulgaria	6
SOUS MASS	Souss Massa National Park	Morocco	4, 6
TABERNAS	Oasys Parque del Desiertode Tabernas	Spain	6
TIPP STAT	Tipperary Sanctuary for Endangered Wildlife	Australia	5
TOKYOTAMA	Tama Zoological Park	Japan	4
TURIN	Giardino Zoologico Della Citta Di Tori	Italy	6
UNKNOWN	Unknown location	Unknown	6
VALBREMBO	Parco Faunistico Le Cornelle	Italy	4, 5, 6, 7, 8
VALCORBA	Parco Faunistico Valcorba	Italy	5, 6
VESZPREM	Kittenberger Zoo	Hungary	5, 6, 7, 8
WALVISBAY	Walvis Bay Quarantine Station	Namibia	6
WARSAW	Miejski Ogrod Zoologiczny Warsaw	Poland	4, 5, 6, 7, 8
WEAVER O	Owen Weaver	USA	4
WHIPSNADE	Whipsnade Wild Animal Park	UK	5, 6, 7, 8
WILD WRLD	Wildlife World Zoo	USA	6
WILDS	The Wilds	USA	4
WOBURNLTD	Woburn Safari Park	UK	5, 6, 7, 8
WROCLAW	Miejski Ogrod Zoologiczny we Wroclawiu	Poland	4, 6, 7, 8
ZAGREB	Zooloski vrt Zagreb	Croatia	5, 6, 7, 8
ZEEHANDLR	Eric Zeehandelaar	USA	4, 6
ZOO KOKI	Zoo Koki	Spain	6
ZOOANIMAL	John Rens Zoo Animal Brokers	Netherlands	6
ZOOKOSICE	Zoologicka Zahrada Kosice	Slovakia	6
ZOOSAFARI	Zoo Safari Swierkocin	Poland	6

Appendix C: a logbook page illustrating hypothetical pedigrees

A scanned logbook page illustrating the pedigrees of two individuals (studbook numbers 18600 and 11760) with the random removal of individuals, the resulting pedigree completeness, derived *F,* and the name of the archived PM2000 file



Appendix D: the use of microsatellites in genetic research

Results from an ISI Web of Knowledge search using filter criteria 'microsatellites* captive breeding'. 70 results were returned relating to 61 studies on 65 species. Nine of the results were for the genetic analysis of populations using other molecular methods such as allozyme anlaysis

Common name	Species	Reference
ACTINOPTERYGII		
Atlantic sturgeon	*Acipenser oxyrinchus*	1, 2
Cape Fear shiner	*Notropis mekistocholas*	1, 3
Asian arowana	*Scleropages formosus*	1, 4
Lake Victoria cichlid	*Paralabidochromis chilotes*	1, 5
Brown-marbled grouper	*Epinephelus fuscoguttatus*	1, 6
Malabar Grouper	*Epinephelus malabaricus*	1, 6
Japanese flounder	*Paralichthys olivaceus*	1, 7
Common sole	*Solea solea*	1, 8
Rainbow trout	*Oncorhynchus mykiss*	1, 9
Atlantic Salmon	*Salmo salar*	1, 10, 11
Brown trout	*Salmo trutta*	1, 12
Pipefish & seahorses	Syngnathidae	1, 13
AMPHIBIA		
Eastern tiger salamander	*Ambystoma tigrinum tigrinum*	1, 14
AVES		
White-headed duck	*Oxyura leucocephala*	1, 15
Bearded vulture	*Gypaetus barbatus*	1, 16
Lesser Kestrel	*Falco naumanni*	1, 17
Peregrine falcon	*Falco peregrinus*	1, 18
Red-legged partridge	*Alectoris rufa*	1, 19
Common quail	*Coturnix coturnix coturnix*	1, 20
Quail	*Coturnix coturnix japonica*	1, 20
Peafowl	*Pavo cristatus*	1, 21
Gran Canarian blue chaffinch	*Fringilla teydea polatzeki*	1, 22
White-breased thrasher	*Ramphocinclus brachyurus*	1, 23
Eurasian Eagle-owl	*Bubo bubo*	1, 24
CRUSTACEA		
Kuruma shrimp	*Penaeus japonicus*	1, 25
Marine shrimp	*Litopenaeus vannamei*	1, 26
MAMMALIA		
Cuvier's gazelle	*Gazella cuvieri*	1, 27
Mhorr gazelle	*Gazella dama mhorr*	1, 27
Dorcas gazelle	*Gazella dorcas*	1, 27
Vietnamese sika deer	*Cervus Nippon pseudaxis*	1, 28
Black muntjac	*Muntiacus crinifrons*	1, 29
White-tailed Deer	*Odocoileus virginianus*	1, 30
Iberian wolf	*Canis lupus sigantus*	1, 31
Fossa	*Cryptoprocta ferox*	1, 32
Cheetah	*Acinonyx jubatus*	1, 33
South China Tiger	*Panthera tigris amoyensis*	1, 34
European Mink	*Mustela lutreola*	1, 35
Black-footed Ferret	*Mustela nigripes*	1, 36
American Mink	*Mustela vison*	1, 37

Appendix D continued

Common name	Species	Reference
MAMMALIA continued		
Flying fox	*Pteropus ssp.*	1, 38
Rodrigues fruit bat	*Pteropus rodricensis*	1, 39
Parma wallaby	*Macropus parma*	1, 40
Tammar Wallaby,	*Macropus eugenii*	1, 41
European wild rabbit	*Oryctolagus cuniculus*	1, 42
Persian wild ass	*Equus hemionus onager*	1, 43
Baird's Tapir	*Tapirus bairdii*	1, 44
Western Barred Bandicoot	*Perameles bougainville*	1, 45
Greater Bilby	*Macrotis lagotis*	1, 46
Goeldi's monkey	*Callimico goeldii*	1, 47
Common marmoset	*Callithrix jacchus*	1, 48
Bolivian squirrel monkey	*Saimiri boliviensis*	1, 49
Common squirrel monkey	*Saimiri sciureus collinsi*	1, 49
Guianan Squirrel Monkey	*Saimiri sciureus sciureus*	1, 49
Rhesus macaques	*Macaca mulatta*	1, 50, 51
Mongoose Lemur	*Eulemur mongoz*	1, 52
Common Hamster	*Cricetus cricetus*	1, 53
Golden Hamster	*Mesocricetus auratus*	1, 54
Idaho Ground Squirrel	*Spermophilus brunneus*	1, 55
REPTILIA		
Galapagos land Iguana	*Conolophus subcristatus*	1, 56
San Esteban chuckwalla	*Sauromalus varius*	1, 57
European pond turtle	*Emys orbicularis*	1, 58
Galapagos giant tortoise	*Chelonoidis nigra*	1, 59, 60
Seychelles giant tortoise		1, 61

1: IUCN 2009; **2:** Henderson *et al*. 2004; **3:** Saillant *et al*. 2005; **4:** Yue *et al*. 2004; **5:** Fiumera *et al*. 1999; **6:** Zhu *et al*. 2005; **7:** Sekino *et al*. 2004; **8:** Blonk *et al*. 2009; **9:** Silverstein *et al*. 2004; **10:** Herbinger *et al*. 2006 ; **11:** Karlsson *et al*. 2010; **12:** Campos *et al*. 2006; **13:** Jones & Avise 2001; **14:** Bulut *et al*. 2009; **15:** Munoz-Fuentes *et al*. 2008; **16:** Gautschi *et al*. 2003a; **17:** Alcaide *et al*. 2010; **18:** Jacobsen *et al*. 2008; **19:** Baratti *et al*. 2005; **20:** Barilani *et al*. 2005; **21:** Hale *et al*. 2004; **22:** Suarez *et al*. 2009; **23:** Temple *et al*. 2009; **24:** Isaksson & Tegelstrom 2002; **25:** Jerry *et al*. 2004; **26:** Luvesuto *et al*. 2007; **27:** Ruiz-Lopez *et al*. 2009; **28:** Thevenon *et al*. 2003; **29:** Ni *et al*. 2009; **30:** Anderson *et al*. 2002; **31:** Ramirez *et al*. 2006; **32:** Vogler *et al*. 2009; **33:** Harley *et al*. 2000; **34:** Xu *et al*. 2007; **35:** Michaux *et al*. 2005; **36:** Wisely *et al*. 2003; **37:** Belliveau *et al*., 1999; **38:** Comeaux & McCracken 1996; **39:** O'Brien *et al*. 2007; **40:** Ivy *et al*. 2009; **41:** Hynes *et al*. 2005; **42:** Surridge *et al*. 1999; **43:** Nielsen *et al*. 2007; **44:** Norton & Ashley 2004; **45:** Smith & Hughes 2008; **46:** Smith *et al*. 2009; **47:** Vasarhelyi 2002; **48:** Nievergelt *et al*., 2000; **49:** Lavergne *et al*. 2003; **50:** Morin *et al.* 1997; **51:** Satkoski *et al*. 2008; **52:** Pastorini *et al*., 2004; **53:** Neumann & Jansman, 2004; **54:** Fritzsche *et al*. 2006; **55:** Garner *et al*. 2005; **56:** Tzika *et al*. 2008; **57:** Mcaliley *et al*. 2006; **58:** Velo-Anton *et al*. 2008; **59:** Burns *et al*. 2003; **60:** Milinkovitch *et al*. 2004; **61:** Palkovacs *et al*. 2003

Appendix E: Iyengar *et al.* 2006

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Short communication

Structure and evolution of the mitochondrial control region in oryx

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1. Introduction

The main non-coding region within mitochondrial DNA (mtDNA), the control region (CR), is situated between the transfer RNA genes $tRNA^{PRO}$ on the 5′ side of the light (L)-strand and $tRNA^{PHE}$ on the 3′ side of the heavy (H)-strand in mammals. Comparative studies have shown that the CR is highly structured, with sequences conserved across divergent taxa, suggesting strong selective constraints for these regions (e.g., Larizza et al., 2002; Saccone et al., 1991; Sbisá et al., 1997; in mammals; Randi and Lucchini, 1998; Ruokenen and Kvist, 2002 in birds). It has been found to be organised into three domains: the 'ETAS domain' adjacent to the $tRNA^{PRO}$ gene, the 'central domain,' and the 'CSB domain' adjacent to the $tRNA^{PHE}$ gene (Fig. 1). The ETAS domain contains a number of 15 bp conserved sequences called termination associated sequences (TAS), first identified by Doda et al. (1981) as being associated with the termination of the nascent H strand during replication. Sbisá et al. (1997) subsequently identified two conserved blocks of approximately 60 bp within ten mammalian orders that they called ETAS1 and ETAS2 (extended TAS since they contained TAS sequences), which have been suggested to have roles in the regulation of replication and transcription. The CSB (conserved sequence block) domain contains the main regulatory elements of the mitochondrial genome: the origin of replication of the H strand (O_H), promoters for the transcription of both the heavy strand (HSP), and the light strand (LSP), along with conserved sequence blocks (CSBs 1–3) that are thought to be involved in the processing of the RNA primers for H strand replication (Walberg and Clayton, 1981). The CSB1 element is present in all animals studied to date, it always contains or is adjacent to the O_H, and is considered to be critical for the replication process (Sbisà et al., 1997). The CSB2 and CSB3 elements, found in most mammals, were thought to be absent in artiodactyls from early studies carried out on the cow (Anderson et al., 1982). However, a subsequent study by Ghivizzani et al. (1993) showed that partial sequences similar to both CSB2 and CSB3 were in fact present in the cow in the form of a functionally analogous fused CSB2 + 3 element. This feature has been reported in most artiodactyls (Sbisà et al., 1997) but Ghivizzani et al. (1993) found that a more distant artiodactyl, the pig (Suidae), possessed complete CSB2 and CSB3 elements. These authors, therefore, proposed that the loss/fusion of these elements among other artiodactyls occurred at a point after the divergence of Suidae, about 40–50 million years ago (MYA).

The ETAS and CSB domains have been found to frequently contain variable length tandem repeats (VNTRs) that are responsible for inter-specific length variation and intra-specific heteroplasmy. Repetitive sequences (RS) have been described at several positions in the CR of vertebrates (RS1–5), with most of them occurring near the TAS motifs (the RS2 location), or between CSB1 and CSB2 (the RS3 location) (Hoelzel et al., 1994).

The CR is also known as the D-loop because of the three-stranded displacement (D) loop structure created by the nascent short H strand that displaces the parental H strand (Saccone et al., 1991). Characterisation and analysis of this region has helped to obtain information on the organisation and evolution of the sequence at different levels of

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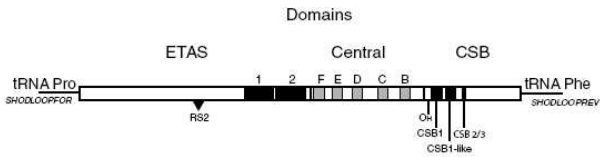

Fig. 1. Schematic diagram of the mtDNA control region of oryx. Locations of RS2, ETAS1, and ETAS2 elements within the ETAS domain, conserved blocks F, E, D, C, B within the central conserved domain, and the O_H, CSB1, CSB1-like, and CSB2 + 3 elements within the CSB domain are shown.

divergence, to identify conserved sequences of putative functional importance, and proved useful in phylogenetic, phylogeographic, and population genetic studies of diverse species (e.g., Douzery and Randi, 1997 in Cervidae; Randi and Lucchini, 1998 in Galliformes; and Larizza et al., 2002 in Rodentia). The CR is the most rapidly evolving part of the mitochondrial genome, with each of the three domains presenting a distinct pattern of variation. The ETAS and CSB domains evolve rapidly, while the central domain maintains a high degree of conservation across taxa (Pesole et al., 1999; Sbisà et al., 1997). Within Bovidae, CR 5′ variable sequences have been used to study the phylogeography of many species (e.g., Grant's gazelles, Arctander et al., 1996; impala and greater kudu, Nersting and Arctander, 2001; African buffalo, Simonsen et al., 1998, kob, Birungi and Arctander, 2000; hartebeest, topi, and wildebeest, Arctander et al., 1999; roan antelope, Matthee and Robinson, 1999; Alpers et al., 2004; and sable antelope, Pitra et al., 2002).

The scimitar horned oryx (SHO) (*Oryx dammah*) belongs to the Hippotragini tribe within the Antilopinae subfamily of the Bovidae family, along with addax (*Addax nasomaculatus*), roan (*Hippotragus equinus*), sable (*Hippotragus niger*), and two other oryx species, the Arabian oryx (*Oryx leucoryx*), and the gemsbok or Plains oryx (*Oryx gazella*). Simultaneous morphological and molecular phylogenetic studies have revealed that the Alcelaphini tribe consisting of bonteboks, topis, hartebeests, and gnus is a closely related sister group to Hippotragini, with the Caprini tribe consisting of sheep, goats, chamois, and musk ox, positioned basally within the same monophyletic clade of Caprini + Alcelaphini + Hippotragini (Hassanin and Douzery, 2003; Ropiquet and Hassanin, 2004). Using large DNA datasets and a Bayesian relaxed molecular clock approach, these authors also obtained estimates for the Caprini + Alcelaphini + Hippotragini group splitting from the rest of the Antilopinae subfamily, and the Alcelaphini + Hippotragini group splitting from Caprini, at around 15.1 million years ago (MYA) and 13.1 MYA, respectively (Hassanin and Douzery, 2003), and around 13.4 and 12 MYA, respectively (Ropiquet and Hassanin, 2004), during the middle Miocene. The Hippotragines are all currently listed on the IUCN Red list of threatened species, with the SHO considered extinct in the wild (IUCN, 2004, http://www.redlist.org/). SHO, however, exists in large numbers in captivity, and as part of a larger genetic study on the SHO, we obtained complete CR sequences from several captive animals and compared them to sequences available in GenBank for the two other oryx species, the Arabian oryx and the gemsbok. We report here on the structure and evolution of the CR in this genus and also explore the phylogenetic content of this sequence by comparison to sequences from other Hippotragines and a few non-Hippotragines such as the bontebok/blesbok (*Damaliscus pygargus*), hartebeest (*Alcelaphus buselaphus*), and sheep (*Ovis aries*).

2. Materials and methods

2.1. Samples

Mostly faecal samples, but also a number of blood samples, were obtained from captive animals held in various zoos. Between three and six fresh (<12 h) faecal pellets were placed into 50 ml tubes containing ~30 g silica gel (Type III indicating, Sigma) with a small piece of filter paper separating the faecal material from the silica gel (Wasser et al., 1997). The tubes were then held at ambient temperature for several weeks prior to being stored long term at 4 °C. Blood samples were collected by zoo veterinarians and shipped within 24 h for processing or held at −20 °C and shipped frozen.

2.2. DNA extraction

DNA was extracted from faecal samples in a dedicated area using the QIAamp® DNA stool minikit (Qiagen) according to manufacturer's instructions but with the following modifications: ~100 mg (approximately half a pellet) dried faecal material was separated on a sterile Petri dish, placed in an Eppendorf tube with 1.8 ml ASL buffer and allowed to incubate at 37 °C for 12–24 h, and the final post-extraction elution step was carried out for 30 min. A maximum of 15 samples were processed at one time with 1–2 negative controls. DNA was extracted from blood using the protocol described in Bruford et al. (1998) and diluted to approximately 10 ng/μl for use.

2.3. Primer design and PCR amplification

Species specific primers were designed to the CR of SHO from a sequence in GenBank (Accession No. AJ235324).

Primers (*SHODLOOPFOR* 5′-TCAAGGAAGAAGCTA TAGCC and *SHODLOOPREV* 5′-CATCTAGGCATTT TCAGTGA) bind within $tRNA^{PRO}$ and $tRNA^{PHE}$ gene sequences and amplify the entire CR sequence. PCR amplification was carried out within dedicated areas in a 30 µl volume containing 2 µl template DNA, 1×PCR buffer (ABgene), 2.5 mM $MgCl_2$, 24 µg BSA, 200 µM each dNTP, 200 nM each primer, and 0.5 U Taq DNA polymerase (ABgene). Amplification conditions were as follows: initial denaturation for 4 min, followed by 35–40 cycles of 94 °C for 30 s, 55 °C for 60 s, and 72 °C for 60 s followed by a final extension at 72 °C for 10 min. In a number of cases ($n = 4$), amplification was carried out using both blood and faecal DNA in order to confirm identical sequences from both sources. Sequencing of PCR products was carried out using an ABI377 sequencer (Applied Biosystems).

2.4. Sequence and phylogenetic analyses

All SHO sequences obtained in this study, were checked by eye, edited, and aligned using ClustalW implemented in BioEdit 5.0.9 (Hall, 1999) along with sequences for Arabian oryx and gemsbok from GenBank. Secondary structures and thermal stabilities of the ETAS domain were calculated in the program MFOLD 3.1 (Zuker, 2003). Information on nucleotide composition was obtained using MEGA 2.1 (Kumar et al., 2001). The model of DNA substitution that best fitted the data was selected using MODELTEST, version 3.06 (Posada and Crandall, 1998). Phylogenetic relationships within the genus *Oryx* were analysed using maximum likelihood (ML) and maximum parsimony (MP) approaches in PAUP, version 4.0b10 (Swofford, 2002), using a heuristic search with the tree bisection reconnection (TBR) swapping algorithm. An addax sequence from GenBank was used as the outgroup in the analyses since previous studies have found that whilst being closely related to oryx species, the addax represents a separate sister taxon (Hassanin and Douzery, 1999). Robustness of phylogenies was assessed by bootstrapping with 1000 replicates. Mean uncorrected p and HKY + I + Γ distances between haplotypes were measured in MEGA and PAUP, respectively.

Additional phylogenetic analyses were carried out using sequences for addax (AJ235310), roan (AJ235321), sable (AF181115), bontebok (AJ235319), hartebeest (AJ235312), and sheep (AF089809) from GenBank. In this case, only ~750 bases were successfully used, since stretches of sequences that could not be satisfactorily aligned had to be deleted. Once again, both ML and MP approaches were used, with the sheep sequence placed as the outgroup, and bootstrapping carried out with 1000 replicates. Substitution saturation in the CR sequence was tested using the saturation index described by Xia et al. (2003) and implemented in the program DAMBE (Xia and Xie, 2001). The observed saturation index (I_{SS}) was compared with the critical index value ($I_{SS.C}$) at which sequences will be saturated and fail to recover the true tree. If I_{SS} is not smaller than $I_{SS.C}$, severe substitution saturation is suggested (Xia et al., 2003).

3. Results

3.1. Structure and composition of the control region

Nine unique SHO CR sequences are reported here, one of which (SHO Leipzig) was found to be identical to the SHO sequence used for initial primer design (GenBank Accession No. AJ235324). CR sequences were either 1246 or 1247 bp long for SHO, and 1240 and 1223 bp for Arabian oryx and gemsbok, respectively. The gemsbok sequence had deletions of 15, 3, and 10 bp at SHO sequence positions 392, 1110, and 1117, respectively, while the Arabian oryx sequence had deletions of 13 and 2 bp at SHO sequence positions 146 and 1110, respectively, in addition to an insertion of 6 bp at SHO sequence position 459 (complete sequence alignment shown in Fig. 2).

Three domains within the CR of oryx were evident from the presence of conserved sequences, as well as by observing the distribution of variability. These were the highly variable ETAS domain with 28% variable sites, the conserved central domain with 5% variable sites, and the highly variable CSB domain with 19% variable sites. The CR length heterogeneity could be ascribed to the two peripheral ETAS and CSB domains, with the former showing higher heterogeneity than the latter (Fig. 2). A bias in the number of transitions over transversions was apparent across all domains, with Ti:Tv ratios varying between approximately 10:1 (CSB domain), 14:1 (central domain), and 15:1 (ETAS domain). The RS2 region in the ETAS domain contained a number of repeats of a short core sequence of alternating purines and pyrimidines and constituting a GYRCAT (Y = C/T, R = A/G) motif that are characteristically seen within TAS sequences in vertebrates (Sbisà et al., 1997). Five such motifs were present across all three species of oryx, with three situated within ETAS2 (Fig. 2). Analysis of potential secondary structure formation within this region revealed stable clover leaf-like structures in all three species of oryx (Figs. 3A–C). The ETAS1 and ETAS2 elements were found to be practically contiguous, with only one nucleotide separating the two elements. Variability between ETAS1 and ETAS2 was different, with 10/60 nucleotides variable within ETAS1 (16.7%), as opposed to 17/72 (23.6%) within ETAS2. The putative point of arrest of D-loop synthesis, proposed to be a TCCCC element in pigs and a GCCCC element in cattle and cervids (Douzery and Randi, 1997), is proposed to be a TCCCC element within ETAS1 in oryx (Fig. 2).

The central domain was the most conserved part of the CR, with only 5% variable sites. Several conserved blocks (F, E, D, C, and B boxes) previously reported within bovids and cervids (Douzery and Randi, 1997) could be clearly identified (Fig. 2).

Within the CSB domain of oryx, a putative initiation site for H strand replication (O_H), based on previous reports on cow (Saccone et al., 1991) and various cervids (Douzery and Randi, 1997) was identified, along with putative LSP and HSP sequences (Fig. 2). Situated 19 nucleotides down-

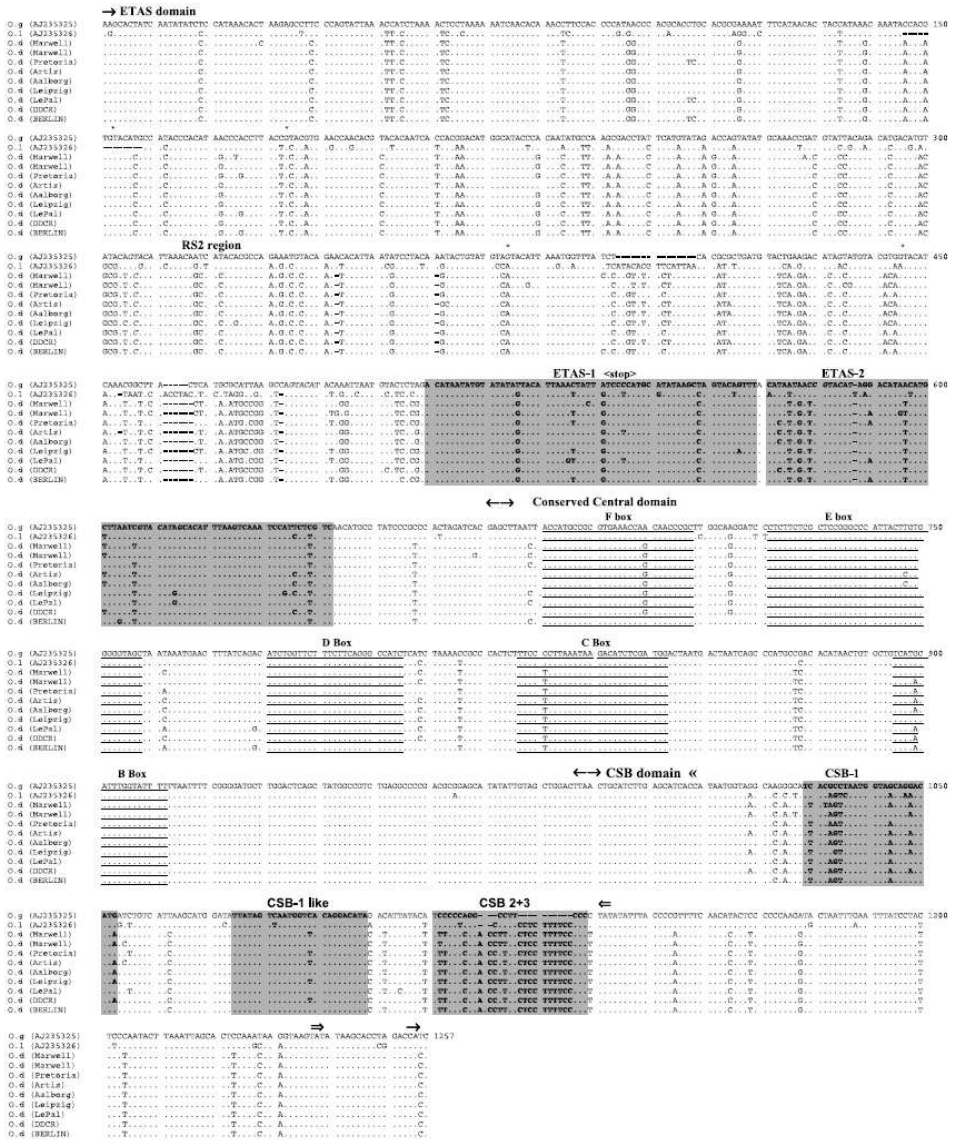

Fig. 2. Alignment of the entire D-loop sequence of oryx. O.g = *O. gazella* or gemsbok, O.l = *O. leucoryx* or Arabian oryx, O.d = *O. dammah* or SHO. The three domains (ETAS, conserved central, and CSB) are shown. ETAS1 and 2 elements within the ETAS domain, conserved boxes F-B within the central domain, CSB1, CSB1-like, and CSB2 + 3 elements within the CSB domain are highlighted. GYRCAT motifs within the ETAS domain are marked with asterisks and motifs conserved across all species are italicised. Putative point of arrest of replication (<stop>), putative origin of replication O_H (∝), putative LSP (⇐), and putative HSP (⇒) are indicated.

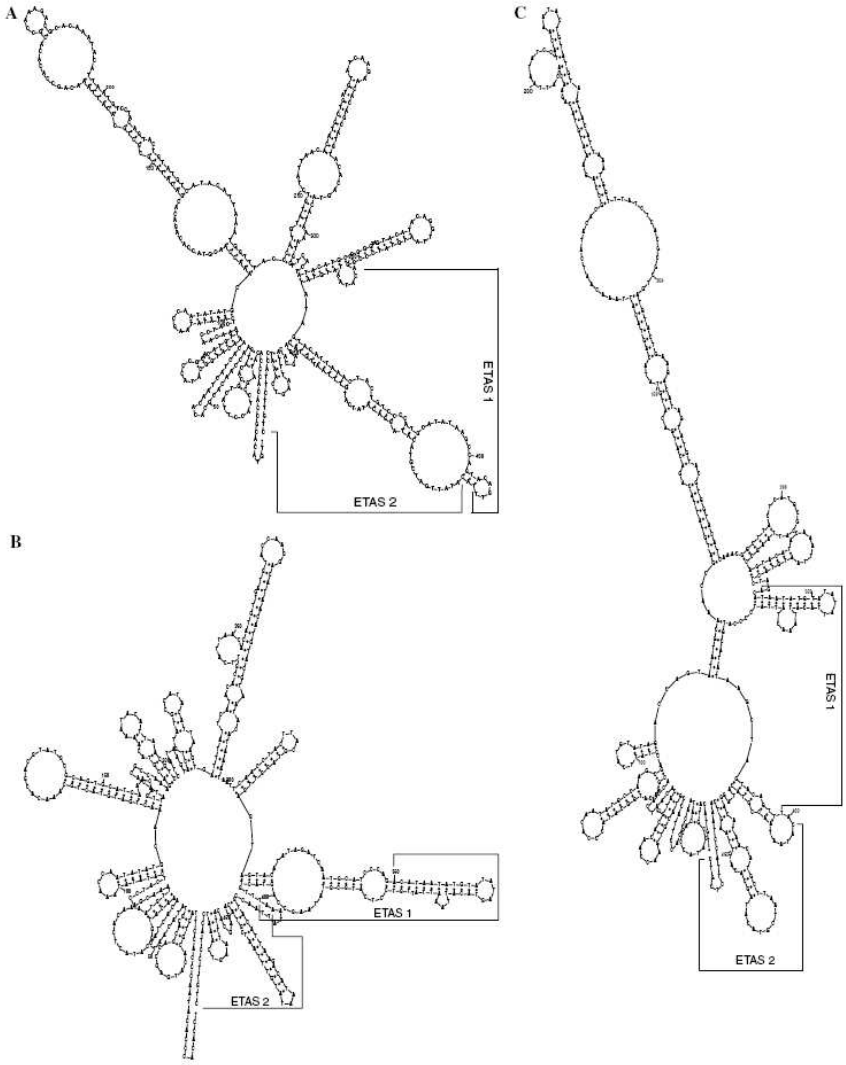

Fig. 3. Potential secondary structures obtained with ~470 nucleotides of the ETAS domain. (A) *O. dammah* (SHO), $\Delta G = -60.0$ kcal/mole, position 1 corresponds to position 151 in sequence alignment. (B) *O. leucoryx* (Arabian oryx), $\Delta G = -62.3$ kcal/mole, position 1 corresponds to position 159 in sequence alignment. (C) *O. gazella* (gemsbok), $\Delta G = -60.2$ kcal/mole, position 1 corresponds to position 151 in sequence alignment.

stream of the putative O_H, we identified a CSB that we have described as the CSB1 element since the O_H has always been found to be located immediately next to or within the CSB1 element in mammals (Sbisà et al., 1997). However, 21 nucleotides further downstream of this CSB element, we found another CSB that we have described as a CSB1-like element (as described in cervids by Douzery and Randi, 1997). This CSB1-like element was found to be more conserved across oryx species than the upstream CSB1 element (2/25 variable sites as opposed to 9/25 variable sites). To investigate this further, we aligned oryx sequences with sequences from GenBank for addax (AJ235310), roan (AJ235321), sable (AF181115, AF181106), bontebok (AJ235319), hartebeest (AJ235312), sheep (AF089809,

AY829430), and cow (*Bos taurus*, NC001567, AF034438). The duplicated CSB1 element was found in all Hippotragine species (oryx, addax, roan, and sable), but not in any of the non-Hippotragine species studied, such as the closely related bontebok and hartebeest, the more distant sheep, and the even more distant cow. Upon alignment of the single CSB1 element present in non-Hippotragines with the CSB1 element situated next to the O_H in the Hippotragines, a low level of overall sequence conservation was observed, although high levels of sequence conservation within the non-Hippotragine genera was seen (Fig. 4A). However, upon alignment of the single CSB1 element of the non-Hippotragines with the further downstream CSB1-like element identified in this study, a much higher level of conservation was observed across all taxa (Fig. 4B). A fused CSB2 + 3 element, similar to that observed in other artiodactyls (Anderson et al., 1982; Sbisà et al., 1997), was also identified in oryx (Fig. 2). No repetitive sequences were found in the RS3 region between CSB1 and CSB2, as previously reported in a number of vertebrates (Hoelzel et al., 1994).

The length and base composition of the three domains, shown in Table 1, reveal that A + T was greater than G + C in all domains. The ETAS domain showed a base content where A > C > T > G, whereas the CSB domain showed a base content where A > T > C > G. The central domain, in contrast, had a different composition (T > C > A > G), with an increase of G and a decrease of A. No significant difference in nucleotide frequencies was detected between the three different oryx species.

3.2. Phylogenetic relationships, sequence divergence, and saturation analysis

In the case of sequences from genus *Oryx*, the HKY + I + Γ model (freqA = 0.3223, freqC = 0.2676, freqG = 0.1447, freqT = 0.2654; Ti/Tv ratio = 31.488; proportion of invariable sites (I) = 0.5171; α = 0.559) was selected by the Akaike Information criterion (AIC) using MODELTEST. Model selection by the AIC has been reported to offer several advantages over hierarchical

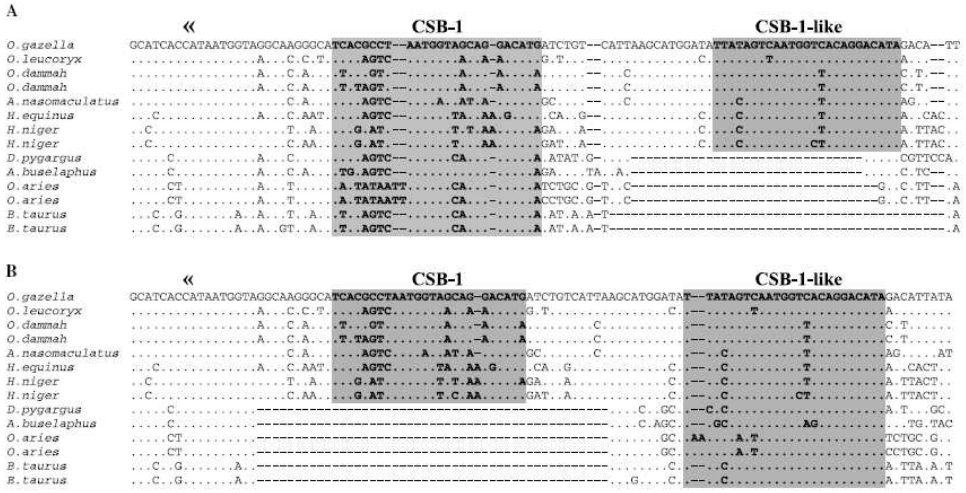

Fig. 4. Two possible alignments of putative O_H («), CSB1, and CSB1-like sequences of Hippotragines (*O. gazella* = gemsbok, AJ235325, *O. leucoryx* = Arabian oryx, AJ235326, *O. dammah* = SHO, this study: Marwell, Leipzig, *A. nasomaculatus* = addax, AJ235310, *H. equinus* = roan, AJ235321, *H. niger* = sable, AF181115, AF181106) and non-Hippotragines (*D. pygargus* = bontebok, AJ235319, *A. buselaphus* = hartebeest, AJ235312, *O. aries* = sheep, AF089809, AY829430, *B. taurus* = cow, NC001567, AF034438).

Table 1
Sequence lengths and nucleotide frequencies of the three domains within the control region in three species of oryx

	ETAS domain					Central domain					CSB domain				
	bp	% A	% C	% G	% T	bp	% A	% C	% G	% T	bp	% A	% C	% G	% T
O. gazella	652	37.4	26.1	12.1	24.4	316	23.7	26.9	20.6	28.8	255	32.0	25.8	15.2	27.0
O. leucoryx	658	37.8	24.8	11.9	25.5	316	23.7	26.9	20.3	29.1	265	31.6	26.3	12.8	29.3
O. dammah	663	36.6	26.8	12.7	23.9	316	23.3	27.0	20.7	28.9	267	32.0	25.0	12.0	30.9

Figures are based on 1 sequence each for *O. gazella* (gemsbok) and *O. leucoryx* (Arabian oryx), and on 10 sequences for *O. dammah* (SHO).

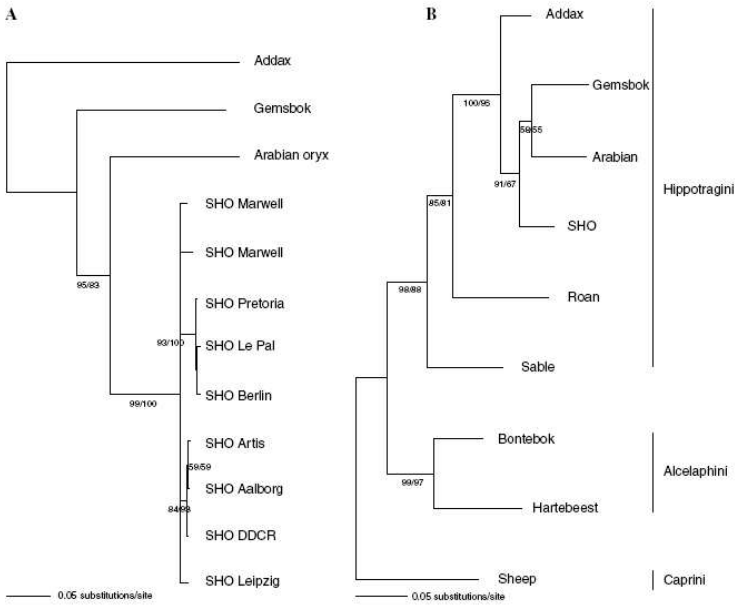

Fig. 5. Maximum likelihood trees of (A) entire CR sequence using addax as the outgroup and (B) ~750 nucleotide CR sequence using sheep as the outgroup. Maximum parsimony trees showed identical topologies. Numbers next to nodes are bootstrap support in 1000 replicates under likelihood on the left and parsimony on the right. Only values >50% are shown.

likelihood ratio tests (Posada and Buckley, 2004). One most parsimonious tree (MPT) was obtained (number of parsimony informative characters = 132, tree length = 428, consistency index CI = 0.77, Homoplasy index HI = 0.23, retention index RI = 0.59, Rescaled consistency index RC = 0.46). Both MP and ML methods produced identical trees using addax as the outgroup. Arabian oryx and SHO grouped together in a monophyletic group with high bootstrap values, with gemsbok positioned basal to this group (Fig. 5A). Genetic distance estimates from the sequences also provided support for a slightly closer relationship between SHO and Arabian oryx, with mean uncorrected p and HKY + I + Γ distances between SHO and Arabian oryx of 10.4% and 24.2%, respectively, between SHO and gemsbok of 11.9% and 30.7% respectively, and between Arabian oryx and gemsbok of 12.4% and 33.5%, respectively.

Additional phylogenetic analyses using a shorter CR sequence and all taxa within Hippotragini (oryx, addax, roan, and sable), two representative taxa from the closely related Alcelaphini tribe (bontebok and hartebeest) and one from the more distant Caprini tribe (sheep, used as the outgroup sequence) were also carried out. In this case, the HKY + Γ (freqA = 0.3222, freqC = 0.2539, freqG = 0.1474, freqT = 0.2765; Ti/Tv ratio = 4.8257, α = 0.2463) model was selected by the AIC. One MPT was obtained (number of parsimony informative characters = 143, tree length = 437, CI = 0.70, HI = 0.30, RI = 0.48, RC = 0.33). Once again, both MP and ML methods produced identical topologies (Fig. 5B). The bontebok and hartebeest, placed together with very high bootstrap, were basal to the monophyletic group of all Hippotragini taxa again, with high bootstrap support. Within Hippotragini, sable was placed basal to roan, with a terminal group consisting of the closely related oryx species and addax. In this case, the Arabian oryx failed to group with SHO, and instead, grouped with gemsbok with low bootstrap support.

The index of substitution saturation, I_{SS}, was much smaller (0.213) than the critical I_{SS} value, $I_{SS.C}$ (0.773), indicating that the sequences were not significantly saturated at this level of phylogenetic analysis (Xia et al., 2003). Numbers of transitions and transversions when plotted against uncorrected p distance also suggest a lack of extensive saturation, with 64% of the variation in transitions and 75% of the variation in transversions being within a linear regression (Fig. 6). A mean divergence of 15.1% was calculated from the basal node of sheep to the terminal taxa of oryx and addax. Based on the previous estimates of 15.1 or 13.4 MY for the shared common ancestor of Caprini and the Hippotragini/Alcelaphini tribes (Hassanin and Douzery, 2003; and Ropiquet and Hassanin, 2004, respectively), a mean evolutionary rate of between 1% and 1.13%/MY was calculated for the CR.

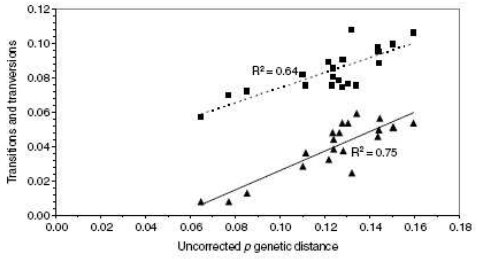

Fig. 6. Saturation plot for the CR sequence. Observed transitions (■) and transversions (▲) were plotted against uncorrected *p* distance values.

4. Discussion

4.1. Organisation of the control region

The presence of conserved regions, nucleotide composition data, and frequency of variable nucleotides, all supported the separation of the CR sequence into three characteristic domains in oryx. Consistent with previous studies on mammals (Pesole et al., 1999), the ETAS and CSB domains were found to be highly variable, with the central domain being highly conserved. Small size differences were noted between the three oryx species due to the presence of indels. CR size differences within the same genus and also within the same subspecies have been previously reported (e.g., in rodents, Larizza et al., 2002). In the RS2 region of the ETAS domain, five GYRCAT motifs were conserved across all oryx species but additional motifs were also seen. We detected stable clover leaf-like secondary structures within this region in all three species of oryx. These secondary structures, which include the TAS sequences and have been observed in a number of vertebrates (e.g., Freeman et al., 2001; Randi and Lucchini, 1998; Sbisà et al., 1997), are thought to play an important role in the termination of replication. Oryx ETAS1 and ETAS2 elements were found to be separated by just 1 nucleotide. Practically contiguous ETAS1 and ETAS2 elements have been previously observed in a number of mammals, including artiodactyls (Sbisà et al., 1997). The ETAS1 element is thought to contain recognition signals for the termination of nascent DNA or RNA, while the ETAS2 is proposed to bind termination factors (Sbisà et al., 1997), suggesting that high levels of conservation would be expected in both elements. We observed a higher degree of conservation within ETAS1 when compared to ETAS2 in oryx. A higher degree of conservation of ETAS1, as opposed to ETAS2 sequences, has also been reported in red backed voles by Matson and Baker (2001).

We have identified putative O_H, HSP, and LSP sequences within the CSB domain, and discovered a duplicated CSB element in oryx. The CSB element situated adjacent to the putative O_H, designated the CSB1 element, showed low sequence conservation across oryx species but the CSB1 element further downstream to this CSB element and designated the CSB1-like element, showed a high degree of sequence conservation across oryx species and addax, and in fact, across all the diverse species studied, suggesting that this is likely to be the functional CSB1 element in oryx and other Hippotragines. The duplicated CSB1 element found in oryx was also present in other Hippotragines such as addax, roan, and sable, but not in the non-Hippotragine species analysed (bontebok, hartebeest, sheep, cow). The bontebok and hartebeest belong to the Alcelaphini tribe, a sister group to the Hippotragini tribe, both within a monophyletic clade also containing the more distant and basal Caprini tribe to which the sheep belongs (Hassanin and Douzery, 2003). The cow, on the other hand, belongs to the Bovinae sub-family, which is a totally separate and distinct sister lineage to the Antilopinae sub-family (Hassanin and Douzery, 2003). Thus, our results suggest that a duplication event of the CSB1 element occurred within the Hippotragini tribe after its separation from the closely related Alcelaphini tribe. This separation has been estimated to have occurred approximately 13.1 MYA by Hassanin and Douzery (2003), and approximately 12MYA by Ropiquet and Hassanin (2004). Duplication of CSB1 elements within the CSB domain has been previously observed in many other species (e.g., pygmy sperm whale, hedgehog, opossum, some rodents, and some cervids, Sbisà et al., 1997; Douzery and Randi, 1997; Larizza et al., 2002), and it is thought that the DNA/RNA transition in this region facilitates slippage events (Larizza et al., 2002). CSB1-like elements have also been reported within other regions in the CR. Both red backed voles (Matson and Baker, 2001) and subterranean mole rats (Reyes et al., 2003) have been found to possess CSB1-like elements, but within the ETAS domain and not the CSB domain.

4.2. Sequence divergence and phylogenetic content of the control region

A bias towards transitions over transversions was highly apparent across all domains of the CR in oryx. This has frequently been reported in previous studies (e.g., cervids, Douzery and Randi, 1997; avian species, Randi and Lucchini, 1998; and rodents, Matson and Baker, 2001; Reyes et al., 2003) and can result in a saturation of transitions when phylogenetic reconstructions including very divergent taxa are made. However, CR sequences continue to be widely used to infer phylogenetic relationships among individuals, populations, and species (e.g., Douzery and Randi, 1997; Matson and Baker, 2001; Randi and Lucchini, 1998; Reyes et al., 2003). The phylogenetic utility of the entire 1.2 kb CR sequence (excluding indels) was clearly evident in analyses of all three oryx species using addax as the outgroup. Further investigations of the phylogenetic utility of a shorter version of the CR sequence across more distantly related taxa (Hippotragini, Alcelaphini, and Caprini) also revealed a highly congruent phylogeny. The Hippotragini tribe was monophyletic, with sable being placed most basal,

followed by roan, and a terminal group consisting of the closely related oryx and addax. A close relationship of oryx and addax compared to roan or sable has been observed in previous molecular phylogenetic studies of Hippotragini (Hassanin and Douzery, 1999). Also consistent with earlier reports (Hassanin and Douzery, 2003; Ropiquet and Hassanin, 2004), bontebok and hartebeest were placed together as a sister clade to the monophyletic Hippotragini clade. In this case, a close association of Arabian oryx and SHO was not seen, with the Arabian oryx grouping instead with gemsbok but with low bootstrap support. A close relationship between SHO and Arabian oryx was however, evident from the genetic distance estimates and the results shown in Fig. 5A.

Saturation analyses revealed that transitions were saturated sooner than transversions, a common observation with CR sequences (e.g., Douzery and Randi, 1997). However, saturation levels were not extreme, and despite deletion of a considerable length of unalignable sequence, we were able to resolve phylogenies at this level of genetic divergence. Using molecular estimates of divergence times obtained by Hassanin and Douzery (2003) and Ropiquet and Hassanin (2004) for the separation of the Caprini and the Alcelaphini/Hippotragini tribes, a mean evolutionary rate of 1–1.13%/MY was estimated for the CR sequence. This estimate, which represents an average evolutionary rate and does not depict the heterogeneity in substitution rates between the peripheral domains and the conserved central domain, is likely to be an underestimate, because a considerable amount of variable sequence was deleted from the analyses, and because a degree of saturation was evident. This figure is however, concordant with results from a comprehensive study of mtDNA nucleotide substitution rates in mammals where the CR was observed to evolve at an average rate of 1.26%/MY in diverse groups such as primates, carnivores, and whales, with horses and donkeys (order Perissodactyla) demonstrating rates of evolution of 1.42%/MY (Pesole et al., 1999).

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Appendix F: Iyengar *et al.* 2007

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Remnants of ancient genetic diversity preserved within captive groups of scimitar-horned oryx (*Oryx dammah*)

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Abstract

Scimitar-horned oryx, now considered extinct in the wild, persists in large numbers in captivity. In this first molecular genetic study on this species, we explore the patterns of genetic diversity across European, North American, and a few other captive groups using microsatellite markers and mitochondrial control region sequencing. Strong population structure was not evident from microsatellite data but we discovered deep divergence within the mitochondrial DNA haplotypes from a network analysis where three disconnected networks were obtained, with estimated divergence times of *c.* 2.1–2.7 million years. Mismatch distribution analyses suggest population expansions *c.* 1.2 and 0.5 million years ago. We discuss our findings in the context of historical climatic changes in North Africa and use information obtained on current patterns of genetic diversity within captive groups to make recommendations for future captive management and reintroduction strategies.

Keywords: antelope, captive breeding, conservation genetics, Hippotragini, North Africa, oryx

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Introduction

Captive breeding is seen to play an increasingly important role in the conservation of threatened species. Several species such as the scimitar-horned oryx (*Oryx dammah*), the Przewalski's horse (*Equus ferus przewalskii*), and the black-footed ferret (*Mustela nigripes*) have been successfully retained in captivity after extinction in the wild. This trend is likely to continue in the future since thousands of threatened species are thought to require captive breeding over the next few hundred years in order to prevent them from going extinct (Tudge 1995). Regional and international programs now exist for many endangered species in captivity where coordinated breeding and management is practised. Efforts are made to preserve the genetic variation of the wild population from which founders were drawn, to minimize loss of this initial diversity as a consequence of inbreeding, and to produce appropriate animals for reintroduction to the species' former range (Russello & Amato 2004).

The scimitar-horned oryx (SHO) belongs to the Hippotragini tribe within the Antilopinae subfamily of Bovidae, along with addax (*Addax nasomaculatus*), roan (*Hippotragus equinus*), sable (*Hippotragus niger*), and two other oryx species, the Arabian oryx (*Oryx leucoryx*) and the Plains oryx or Gemsbok (*Oryx gazella*). During the middle ages, SHO is known to have spanned right across North Africa, from Mauritania on the Atlantic coast to Sudan on the Red Sea, along the interface between true desert and the less arid 'North Saharan/Mediterranean' habitat and the 'Sahelian' habitat (region bordering the Sahara to the south and varying in width from several hundred kilometres to over 1000 km) (Newby 1978, 1980). Populations on the northern fringe of the Sahara are thought to have disappeared by the beginning of the 20th century, with the southern Sahelian range remaining almost continuous until the 1960s (Fig. 1). Continued fragmentation eventually led to the extermination of the species from across this region, with the last confirmed sightings made in Chad in the mid-1980s (Newby 1988). Reasons for the decline include drought, loss of habitat, over-hunting, and competition with domestic

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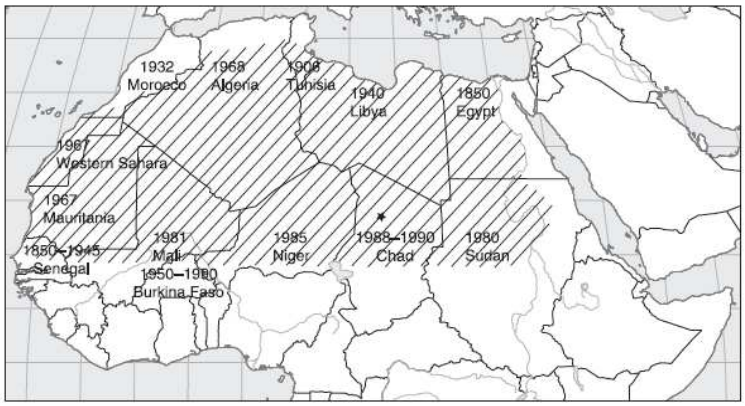

Fig. 1 Historical distribution of SHO across North Africa with approximate extinction times within regions. The star in Chad indicates the approximate location of the base camp in 1967 from which SHO were captured within a 200-km radius. Outline map obtained from BYU Geography Department (www.geog.byu.edu/outlinemaps.dhtml). Information obtained from Wakefield *et al.* (2004).

livestock (Jackson 1978; Newby 1988; Dixon *et al.* 1991). SHO is now officially classified as extinct in the wild (IUCN 2006) but exists in large numbers in captivity, and there may be as many as 6000 animals held in zoos, private collections, and ranches worldwide (Gilbert 2005). Although there are records from the 1930s of a small number of individuals that may have contributed to the modern captive groups, the vast majority of founders were captured in Chad in the 1960s (Wakefield *et al.* 2004). This consisted of three animals caught in 1963 and taken to the USA, and a greater number (*c.* 44) captured in 1967 (Fig. 1), of which *c.* 26 were taken to USA, *c.* 18 were brought to Europe, and a handful of individuals were sent to zoos in South Africa and Japan (A. Rost, personal communication). Descendants of the North American and European animals are now managed within the Species Survival Plan (SSP) of the American Association of Zoos & Aquaria (AZA), and the European Endangered Species Programme (EEP) of the European Association of Zoos & Aquaria (EAZA), respectively. Other coordinated captive breeding programmes exist in Australasia and Japan, and there are also SHO in parts of the world that are not covered by any such programme.

There is considerable interest in the re-introduction of SHO to parts of its former range and as many as 13 North African countries have become signatories to the '*Action plan for the conservation and restoration of Sahelo-Saharan antelope and gazelle*' (Beudels-Jamar *et al.* 1998). Captive-bred SHO have already been successfully released into protected areas in Tunisia, Morocco and Senegal (Gordon & Gill 1993; Wakefield *et al.* 2004). In this first genetic study on the SHO, we explored the extent and patterns of genetic variation within the EEP and SSP groups which represent the most significant captive management programmes in terms of size (*c.* 460 and 230 individuals, respectively) and numbers of founders (Gilbert 2005). Small numbers of additional samples from other captive groups and from museums were also used for comparison. We report on the current mitochondrial and microsatellite diversity in these captive groups, and based on our findings, make inferences on historical patterns of demography and suggest future management strategies for this species.

Materials and methods

Samples

We obtained faecal, blood, and ear/muscle biopsy samples. Three to six fresh faecal pellets were placed in 50 mL tubes containing *c.* 30 g silica gel (Type III indicating, Sigma) with a small piece of filter paper separating the faecal material from the silica gel (Wasser *et al.* 1997). The tubes were held at ambient temperature for several weeks prior to being stored long term at 4 °C. Blood was collected by zoo veterinarians and shipped within 24 h for processing, or held at –20 °C and shipped frozen. All tissue samples were placed in 100% alcohol and shipped at ambient temperature. A total of 122 faecal and 35 blood/skin samples were obtained from EEP participating zoos (UK, Spain, France, Greece, Holland, Germany, Portugal, Denmark, Poland, Croatia, Czech Republic, and Israel). Sixty-nine faecal samples could be allocated to specific

individuals but the remaining 53 were unidentifiable. An additional 6 and 19 faecal samples were also obtained from Pretoria zoo, South Africa, and Dubai Desert Conservation Reserve (DDCR), United Arab Emirates, respectively (total faecal sample $n = 147$). The 35 blood/skin samples included two samples from Marwell zoo, which were from animals that were translocated to Australia in 1987. Forty-eight tissue samples were obtained from various zoos/ranches within the USA, which consisted largely of muscle biopsies taken using remote injection darts by trained professionals, and a smaller number of blood/necropsy samples taken during veterinary procedures/autopsies. A small number of museum samples were also obtained, which consisted of pieces of pelt from animals collected in Sudan (1824, $n = 1$, 1911, $n = 3$) and Chad (1925, $n = 1$), and a tooth from an animal collected in Chad (1960s).

DNA extraction

DNA was extracted from faecal samples in a dedicated area using the QIAamp DNA stool mini kit (QIAGEN) according to manufacturer's instructions but with the following modifications: *c.* 100 mg dried faecal material was placed in an Eppendorf tube with 1.8 mL ASL buffer, mixed thoroughly, and allowed to incubate at 37 °C for 12–24 h, and the final postextraction elution step was carried out for 30 min. A maximum of 15 samples were processed at one time with 1–2 negative controls. DNA was extracted from blood using the protocol described in Bruford *et al.* (1998), and from skin biopsies using a standard phenol–chloroform protocol (Milligan 1998). In the case of museum samples, pieces of pelt were cryopulversied in liquid nitrogen using an MM300 mixer mill (Retsch) and *c.* 200 mg of powder placed in 5 mL of extraction buffer (0.45 M EDTA pH 8, 1% sarcosyl, 0.4 mg/mL proteinase K), followed by the procedure described in Vigilant *et al.* (2001). For DNA extraction from teeth, a hand-held drill was used to make a hole in the root and a small amount of material collected and used as described above. All these procedures were carried out in contamination-free areas with appropriate negative controls.

Microsatellite analyses

A set of six microsatellite loci previously described in sheep (MAF46, MAF50, OarFCB304, OarAE119, OarCP26) or cattle (RBP3) and found to amplify polymorphic alleles in Arabian oryx (Marshall *et al.* 1999) was used. Polymerase chain reactions (PCR) were carried out in a 15-μL volume containing 2 μL template, 1× PCR buffer (ABgene, 75 mM Tris-HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20), 1.0–3.0 mM $MgCl_2$, 12 μg BSA (Roche), 200 μM each dNTP, 200 nM each primer and 0.4 U DNA polymerase (ABgene). Amplification conditions consisted of initial denaturation for 4 min, followed by 30–45 cycles (blood and faecal DNA, respectively) at 94 °C for 30 s, 54–62 °C annealing temperature for 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. The 5′ end of the forward primer was fluorescently labelled and the products were separated using gel electrophoresis on an ABI PRISM 377. Alleles were sized relative to an internal standard (HD400 with ROX label) and scored using GENESCAN 3.0 and GENOTYPER software (Applied Biosystems). In the case of faecal samples, heterozygous genotypes were accepted once confirmed in two separate amplifications, but homozygous genotypes were repeated 4–6 times to ensure high levels of accuracy.

Mitochondrial DNA sequencing

PCR amplification of either the complete control region (1.24 kb) (primers *SHODLOOPFOR* 5′-TCAAGGAAGA-AGCTATAGCC and *SHODLOOPREV* 5′-CATCTAGGC-ATTTTCAGTGA described in Iyengar *et al.* 2006) or a shorter 353-bp product (primer *SHODLOOPFOR* and primer *SHODLOOP350REV* 5′-TGTGGTACGTCGGTTTGC) was carried out in a 30-μL volume containing 2 μL template, 1× PCR buffer (ABgene), 2.5 mM $MgCl_2$, 24 μg BSA, 200 μM each dNTP, 200 nM each primer and 0.5 U *Taq* DNA polymerase (ABgene). The large product was amplified from all tissue samples but with faecal samples, upon failure to amplify the large product, attempts were made to amplify the smaller 353-bp product. Amplification conditions were as follows: initial denaturation for 4 min, followed by 30–40 cycles (blood and faecal DNA, respectively) of 94 °C for 30 s, 55 °C for 30–60 s (0.35-kb and 1.2-kb products, respectively) and 72 °C for 30–60 s (0.35- and 1.2-kb products, respectively), followed by a final extension at 72 °C for 10 min. In a few cases ($n = 4$), amplification of the larger product was carried out using both blood and faecal DNA in order to confirm identical sequences from both sources. In the case of museum samples, two separate PCRs were carried out for each sample amplifying overlapping products of 129 bp and 125 bp using primers *SHOMUSFOR1* (5′-GAAGCACTATCAATATATCCC) and *SHOMUSREV1* (5′-GTTATGAAATTTCCGCGTGC); and *SHOMUSFOR2* (5′-TCAACACAAACTTTCCACCC) and *SHOMUSREV2* (5′-GTTGGTTCATGTGCAGTAAG), respectively. Amplification conditions were as described above for the 0.35-kb product and sequencing was carried out on an ABI PRISM 377.

Statistical analyses

Microsatellites. Polymorphism within management groups (EEP, SSP, SA — South Africa, UAE) and overall in the entire captive population, measured as the total number of alleles, mean number of alleles per locus, and mean

observed heterozygosity was calculated using GENEPOP (Raymond & Rousset 1995). Allelic richness estimates were made using FSTAT (Goudet 2001). Tests for deviation from Hardy–Weinberg expectations and genotypic linkage disequilibrium were performed in GENEPOP followed by sequential Bonferroni correction (Rice 1989). Estimates of within-group F_{IS} were obtained using GENETIX version 4.04 (Belkhir *et al*. 2003). STRUCTURE 2.0 (Pritchard *et al*. 2000; Falush *et al*. 2003) was used to look for the presence of genetic structure among the samples. This software uses a Bayesian clustering approach to infer the number of populations (K) in a data set without a priori assignment of samples to populations. We used the population admixture model (where each individual is assumed to have inherited a proportion of its ancestry from each population) with correlated allele frequencies among populations. Ten replicates (to check for consistency) were run at each estimated group size (from $K = 1$ to $K = 5$) using a burn-in of 50 000 iterations and collection of data over 500 000 iterations. Values for the log likelihood of data across runs and values for individual membership within groups were then evaluated. Posterior probability values were calculated for the maximum log-likelihood value obtained using the formula given in Pritchard & Wen (2003). Pairwise estimates of the coefficient of relatedness (r) for all individuals were calculated using the Lynch & Ritland (1999) measure within the program IDENTIX (Belkhir *et al*. 2002). A recent study found that the Lynch and Ritland estimate (r_{xyLR}) more accurately depicted true relatedness between individuals compared to the Queller & Goodnight (1989) estimate (r_{xyQG}) (Russello & Amato 2004). Pairwise kinship coefficients were calculated as half the pairwise relatedness coefficients (Hardy 2003), and mean kinship (mk) values were then obtained for every individual as the average of pairwise kinship coefficient values to all other individuals including itself (Russello & Amato 2004).

Mitochondrial DNA. Sequences were checked by eye, edited, and aligned using BIOEDIT 5.0.9 (Hall 1999). Numbers of haplotypes, private haplotypes, polymorphic sites, and haplotype and nucleotide diversity were determined using DNASP 4.0 (Rozas *et al*. 2003). The model of DNA substitution that best fitted the data was selected using MODELTEST, version 3.06 (Posada & Crandall 1998). The Tamura-Nei + I + γ model [proportion of invariable sites (I) = 0.7786; $\alpha = 0.7361$] was selected by the Akaike information criterion (AIC). Model selection by the AIC has been reported to offer several advantages over hierarchical likelihood-ratio tests (Posada & Buckley 2004). Sequences were analysed using maximum-parsimony (MP) and maximum-likelihood (ML) approaches in PAUP, version 4.0b10 (Swofford 2002). A heuristic search with the tree-bisection–reconnection (TBR) branch swapping algorithm with 100 random taxon addition replicates was used in both cases. Node support was assessed using 1000 bootstrap replicates with 10 random taxon addition replicates in the case of MP, and 100 bootstrap replicates with one random taxon addition replicate in the case of ML (due to insufficient computer power). We also used a Bayesian likelihood approach in MRBAYES, version 3.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) using a general GTR + I + G model, allowing MRBAYES to estimate the various model parameters. The analysis was carried out using the default setting of four Markov chains (three heated and one cold) for 3 000 000 generations, sampling once every 100 generations. Four separate analyses were carried out simultaneously starting with different random trees. Post-burn-in trees (22 500) from all four analyses were used to estimate posterior probabilities. A homologous sequence from the Arabian oryx (GenBank: AJ235326) was used as the outgroup in all analyses.

Mean uncorrected p distances between groups of haplotypes were measured in MEGA version 2.1 (Kumar *et al*. 2001). Genealogical relationships among sequences were determined by a minimum spanning network using the statistical parsimony method (Templeton *et al*. 1992) implemented in TCS 1.18 (Clement *et al*. 2000). The algorithm within this program estimates the 95% statistical confidence limit for the maximum number of nucleotide substitutions between two haplotypes (the parsimony limit) and sequentially connects taxa into networks within this limit. A mismatch distribution of pairwise substitutional differences among haplotypes and a range of neutrality statistics capable of detecting the genetic traces of population growth, decline, or stability, were examined using DNASP version 4.0 (Rozas *et al*. 2003). Values for Fu's F-statistic (F_S, which specifically tests for population growth and detects excesses of low-frequency alleles) and Fu and Li's F^* and D^* statistics were obtained (Fu 1997). Observed values of F_S were compared with values obtained upon 1000 simulations in order to determine 99% confidence intervals. To estimate time since expansion (t), we used the formula $\tau = 2ut$ where $u = 2\mu k$, where μ is the mutation rate per site per million years and k is the length of the sequence. For estimation of recent population size, we used estimates of theta (θ) and the formula $\theta = 2N_{ef}\mu$, where N_{ef} is the female effective population size and μ is the mutation rate in substitutions/site/generation. This estimate is representative of recent rather than historical population size since genealogical information is not used (Crandall *et al*. 1999).

Results

Historical demography

Forty haplotypes (two indels considered) were identified among 141 SHO samples upon sequencing the entire

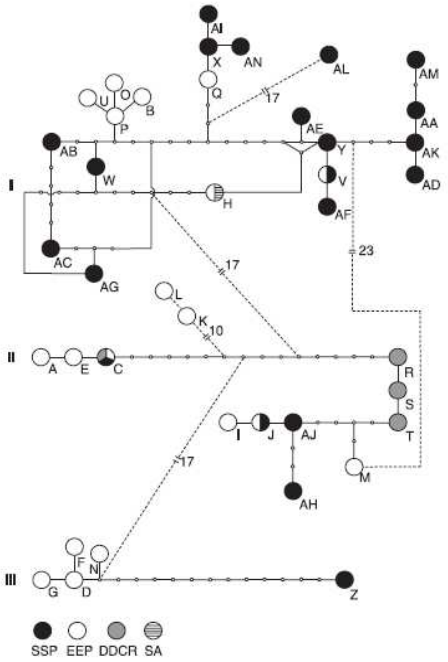

Fig. 2 Statistical parsimony network for haplotypes A–AN. Each single line represents a mutation step and small circles represent missing haplotypes. Numbers of mutation steps required for connecting the three networks and two sets of unconnected haplotypes are also shown.

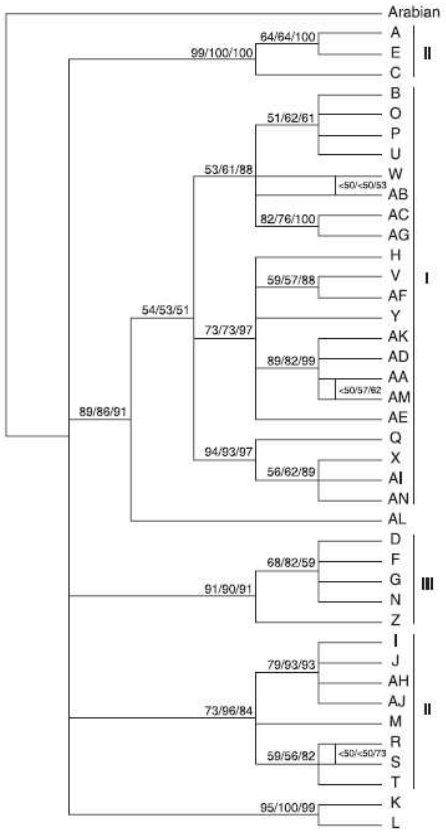

Fig. 3 Consensus tree depicting haplotype relationships. Numbers above nodes are bootstrap support for the node using ML and MP, and posterior probabilities using Bayesian analysis (ML/MP/Bayesian). Weak associations found in one/two analyses are shown in a smaller font between haplotypes W & AB, AA & AM, and R & S). Network groupings of the haplotypes are also shown.

control region (Table 1). Sequencing of the smaller 353-bp product from a further 45 samples where amplification of the 1.2-kb product was unsuccessful, did not provide additional information. The sequences reported are likely to represent cytoplasmic mtDNA (*cymt*) and not nuclear copies of mtDNA (*numt*) because we designed species-specific primers for this study and obtained identical sequences from both blood and tissue samples in a few individuals. A network analysis produced three disconnected networks with 95% confidence (connection limit = 15) indicating deep divergence between haplotypes (Fig. 2). Three unconnected haplotypes were also observed (K-L and AL). Numbers of mutation steps required for connecting haplotypes between the three separate networks and for connecting the unconnected haplotypes are shown in Fig. 2. A minimum of 24 mutation steps were necessary to connect haplotypes between networks. Four most parsimonious trees were produced using maximum parsimony (MP), with topologies very similar to those obtained with maximum likelihood (ML) and Bayesian analyses. A consensus tree is shown in Fig. 3 with MP and ML bootstrap support values and Bayesian posterior probabilities. Haplotypes within networks I and III were found to group together with fairly high bootstrap and posterior probability values, but network II haplotypes grouped into two separate clades (A, E, C) and (I, J, AH, AJ, M, R, S, T). Mean uncorrected p distances between haplotypes in networks I and II, I and III, and II and III were 2.62%, 2.35%, and 3.01%, respectively. From a previous study on the

Table 1 Variable sites within the mtDNA control region haplotypes

	1111222223 3007723570 0785650085	3333333344 2367889900 5616494717	4444444444 1334455666 2240178019	4444444444 7778888999 0353457358	5555555555 2233555668 6706679240	5555555555 8899900122 5614704246	5555566666 4556735556 8199872399	6666677777 7888990000 9179369701	1111 1111111111 0000000000 2344834561	1111111111 0000000011 4390245733	SAMPLES Captive group (location, number)
A	CCTTATGCAT	AGTAGGTTC	GGAACTCCTC	CGACAGTTTC	ACCCAGTCTG	ATTATAATAT	TAACCTCAAA	CTTGGTACTA	TAGAGAATCA	TTTATTTTTT	T EP (Mar,1)
B	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	GTTT.....	....A ..C...G...	.....	.....A..G	G...	C....G..T.	A.C...C..C . EEP (Mar,2; Sel,2; LeP,3; Leip,2)
C	....	....	....	....	....	....	....	....	....	....A.....	A..... . EEP (Mar,8; Ches,2; Karls,1; War,2; Wob,3; Edin,3; DDCR,2; Mad,1); SSP (Fos Rim,1; Bam, 1)
D	T..C....G.	...G.TA...	....	...TGA....	.TT.....	....A G...C.....	..G..T....	...A...TC.	C..GA..C..	A.C.....	C. . EEP (Mar,1; Whip,1; Wob,1)
E	....	....	....	....	....	....	....	....	....	....A.....	..... . EEP (Mar,1)
F	T..C....G.	...G.TA...	....	...TGA....	.TT.....	....A G...C.....	..G.T.T...	...A...TC.	C..GA..C..	A.C.....	C. . EEP (Mar,1)
G	T..C....G.	...G.TA...	..G.....	...TGA....	.TT.....	....A G...C.....	..G..T....	...A...TC.	C..GA..C..	A.C.....	C. . EEP (Mar,1)
H	TTCCG...G.	.....	....T.CC.	....T.TTCT	..T.....	TT.....CA	..C.....	.....A..	T..A..G..	C.A..G..T.	A..... C . SA (Pret,2) EEP (Cots,2; Manor,2; Sel,4)
I	T..C..A.G.	..C..T....	A.....T..	.....T..	.....T	..TT....C.	.....	C	...T.C....	...A..C..	A..... C . EEP (Artis,1)
J	T..C..A.G.	....T....	A.....T..	.....T..	.....T	.TT....C.	.....	C	...T.C....	...A..C..	A..... C . EEP (Artis,4); SSP (Bam,2; Wilds,1)
K	T..C..A.G.	....T....	.....GC G	....T....	.....	.TT.A....	..C...G.GC	.....	...T.C....	...A..C..	A..... . EEP (LaPalm,1; Aal 3; Lun,1; Karls,3; Ber, 3; Leip,3; Zag, 3)
L	T..C...AGC	G....T....	.....	.....	T.T.....	.TT.A....	..C...G.GC	.....	.....	CG..A.....	A..... . EEP (LaPalm, 1)
M	T..C....G.	....T....	A.....	.....T..	.....	.T.....C.	.....	C	...T.C....	...A..G...	A..C.....C . EEP (Aal,2)
N	T..C....G.	...G.TA...	.....	...TGA.C.	.TT.G...A	G...C..C..	.G...T....	...A.C.TC	C..GA..C.	A..G...C.	EEP (Cots,1)
O	TTCCG...G.	....T.CC.	....T.TTCT	..T.....	.GTT.....A	..C...G...	.....	.....A..G	...A..G...	C....G..T.	A.C...C.C . EEP (Whip,2)
P	TTCCG...G.	....T.CC.	....T.TTCT	..T.....	TTT.....A	..C...G...	.....	.....A..G	...A..G...	C....G..T.	A.C...C.C . EEP (War,1; Pra,5; Lis,1; Zag,1)
Q	TTCCG...G.	.....CC.	....T.TTCT	..T....C.	.TT.....	....A ..CG.....	.....	.....A..G	T..A..G...	C....G....	A.C..... . EEP (Sel,1; Ber,1; Pra,1)
R	T..C....G.	....T....	A.....T..	.....	.....C.T	.T.....C.	.....	C	...T.....	...A.....	A...C....C . UAE (DDCR,1)
S	T..C....G.	....T....	A.....	.....T..	.....	.T.....C.	.....	C	...T.....	...A.....	A...C....C . UAE (DDCR,1)
T	T..C....G.	....T....	A.....	.....T..	.....	.T.....C.	.....	C	...T.C....	...A.....	A...C....C . UAE (DDCR,1)
U	TTCCG...G.	....T.CC.	....T.TTCT	..T.....	TTT.....	.C...G...	.....	.....A..G	...A..G...	C....G..T.	A.C...C..C . EEP (Ber,1)
V	TTCCG...G.	....T.CC.	....T.TTCT	..T.....	TT.....CA	.C.....	G...A...	T..A.....	C.A..G..T.	A.C...C..C	EEP (Amers,1), SSP (SDiego,3; Mem,1)
W	TTCCG...G.	....T.CC.	....T.TTCT	..T.....	TTT.....A	.C...G...	.....	.....A..	T..A..G...	C....G..T.	A.C...C..C . SSP (Bam,2)
X	TTCCGC..G.	.....	.....CC.	....T.TTCT	..T....C.	TT.....A	.CG.....	.....	.....A..G	T..A..G...	C....G.... A.C..... . SSP (Bam,4; HDZ,12)
Y	TTCCG...G.	.....	....T.CC.	....T.TTCT	...T.....	TT.....CA	..C.....	.G.....A..	T..A..G...	C.A..G..T.	A.C..... C . SSP (Bam,1)
Z	T..C....G.	...G.TA...	.....	....C....	AGT.AC...	TT.....A	GC..C.G...	.G...G..	..CA.....C	C..GA.GC.G	AC.....-. . SSP (Bam,1; Wilds,2)
AA	TTCCG...G.	....T.CC.	....T.CC.	....GT.TTCT	..T.....	TT..C.CA	..C.....	.G....A..	T.....G...	C.A..G....	A.C..C...C . SSP (Bam,1)
AB	TTCCG...G.	....T.CC.	....T.TTCT	..T.....	TTT.....A	.C...G...	.....	.....A..	T..A..G...	C....G....	A.C...CCCC . SSP (Fos Rim,1)
AC	TTCCG...G.	....T.CC.	....T.TTCT	..T....C.	TT.....A	.C...G...	.....	.....A..G	...A..G..G	C....G....	A.C...CCCC G SSP (Fos Rim,1)
AD	TTCCG...G.	....T.CC.	....GT.TTCT	..T.....	TT...C.CA	.C.....	.....	.....A..	T.....G...	C.A..G....	A.C.....C . SSP (HDZ,1)
AE	TTCCG...G.	....T.CC.	....T.TTCT	..T.....	TT.....A	.C.....	.....	.G....A..	T..A..G.C.	C.A..G..T.	A.C.....C . SSP (Bam,1)
AF	TTCCG...G.	....T.CC.	....T.TTCT	..T.....	TT.....CA	.C.....	.....	.G....A..	T..A.....	C.A..G..T.	A.C..C...C . SSP (SDiego,1)
AG	TTCCG...G.	....T.CC.	....T.TTCT	..T....C.	TT.....A	.C...G...	.....	.....A..G	...A..G..G	C....G....	A.....C..C C SSP (Nat,3)
AH	T..C..A.G.	....T....	A.....T..	.....	.....T	.TT....C.	.....	.....G..C	...T.C..G.	...A..C..	A....C...C . SSP (Fos Rim,1)
AI	TTCCGC..G.	.....	.....CC.	....T.TTCT	..T....C.	.T.....A	.CG.....	.....	.....A..G	T..A..G...	C....G.... A.C..C.... . SSP (HDZ,2)
AJ	T..C..A.G.	....T....	A.....T..	.....	.....T	.TT....C.	.....	C	...T.C....	...A..C..	A.....C . SSP (Bam,1)
AK	TTCCG...G.	.....	.....T.CC.	...GT.TTCT	..T.....	TT...C.CA	.C.....	.....	.G....A..	T.....G...	C.A..G.... A.C..... . SSP (HDZ,1)
AL	TTCCG...G.	.A..AT..CT	.A..T.T.C.	.....	..T...C.	.TT.....T.A	.C..C..C..	C.....A..	TC.AA.G...	CG...G....	A..... . SSP (HDZ,1)
AM	TTCCG...G.	....T.CC.	..GT.TTCT	..T.....	..T...C.CA	.C.....	.....	.G....A..	T.....G...	C.A..G....	A.C..C.CCC . SSP (HDZ,1)
AN	TTCCGC..G.	.....	.....CC.	....T.TTCT	..T.....C.	.TT.....A	.CG.....	.....	.....A..G	T..A..G...	C.....G.... A.C.....C.. . SSP (HDZ,1)

Mar = Marwell, Sel = Selwo, LeP = Le Pal, Leip = Leipzig, Ches = Chester, Karls = Karlsruhe, War = Warsaw, Wob = Woburn, Edin = Edinburgh, DDCR = Dubai Desert Conservation Reserve, Mad = Madrid, Fos Rim = Fossil Rim, Bam = Bamberger, Whip = Whipsnade, Pret = Pretoria, Cots = Cotswold, Wilds = The Wilds, La Palm = La Palmyre, Aal = Aalborg, Lun = Lunaret, Ber = Berlin, Zag = Zagreb, Pra = Prague, Lis = Lisbon, Amers = Amersfoort, SDiego = San Diego, Mem = Memphis, HDZ = Henry Doorly zoo, Nat = National zoo. GenBank Accession nos are DQ159406–DQ159445 for haplotypes A-AN.

Table 2 Genetic diversity within captive SHO

	EEP	SSP	SA	UAE	TOTAL
Mt Dloop haplotypes (1188nt)					
Sample size	86	48	2	5	141
No. of haplotypes	19	21	1	4	40
No. of private haplotypes	15	18	0	3	—
No. of polymorphic sites (% variation)	72 (6.1)	82 (6.9)	0	17 (1.4)	99 (8.3)
Haplotype diversity (SD)	0.880 (0.018)	0.875 (0.040)	0	0.900 (0.161)	0.926 (0.01)
Nucleotide diversity (SD)	0.017 (0.0004)	0.015 (0.0019)	0	0.008 (0.002)	0.018 (0.0005)
Microsatellites					
Sample size	120	48	4	6	178
Total no. of alleles	27	35	11	9	82
No. of private alleles	0	7	0	0	—
Allelic richness	2.92	3.34	2.20	1.71	—
Average no. of alleles/locus	5.4	7.0	2.2	1.8	16.4
Observed heterozygosity (%)	54	57	32	27	42
Average range	6.2	8.0	3.4	4.0	8.0
F_{IS} (95% CI)	0.128 (0.065–0.185)	0.020 (−0.082–0.101)	0.348 (−0.500–0.600)	0.222 (−0.250–0.390)	0.134 (0.083–0.179)

SD, standard deviation; average range, average range of allele size expansion in repeat motif number.

and Z (3.63%, 3.71%, and 3.79%, respectively), all found within the SSP group. A number of haplotypes representing all three divergent networks (X, W, AE, Y, and AA from network I, C, J, and AJ from network II, and Z from network III) were detected within Bamberger Ranch in Texas, USA. Both haplotypes AA and Z showing one of the maximal *p* distance values were found within Bamberger. Another large ranch in Texas (Fossil Rim) was found to contain haplotypes spanning two networks (AB and AC from network I, and C and AH from network II). Within HDZ, although only network I haplotypes were detected (X, AI, AN, AL, AM, AK, AD), the majority (all except X) were found to be unique to individuals from this zoo. Haplotypes from across all three networks were also found in the EEP group but network I haplotypes were underrepresented. Haplotypes E and D were found in the two individuals translocated to Australia from Marwell zoo in 1987. Haplotypes K and L, which were disconnected to the rest of the networks, were found in several individuals, including two individuals sent in 1999/2000 to Sidi Toui National Park, Tunisia, from La Palmyre zoo, France, as part of a reintroduction programme (Table 1). UAE samples consisted of three closely related haplotypes R, S, and T, and one slightly more distant haplotype, C, which was shared with both EEP and SSP groups. Only one haplotype (H) was found between two individuals from Pretoria zoo and was shared with individuals from the EEP group.

Current microsatellite diversity in captive groups

We successfully amplified six microsatellite loci in 106 out of 147 faecal samples (72%). Thirteen repeat samples were identified from the multilocus genotypes obtained and were deleted, leaving 93 samples for all analyses. One locus each within the EEP and SSP group (MAF46 and FCB304, respectively) was found to deviate significantly from Hardy–Weinberg equilibrium after Bonferroni correction. Both loci demonstrated heterozygote deficits but since the same locus was not found to consistently deviate across both groups, this is not thought to be a consequence of null alleles. Tests for linkage disequilibrium after Bonferroni correction revealed one association that remained significant (OarCP26 and MAF50). Both loci, mapped to sheep chromosome 4, are separated by 30 cM (www.thearkdb.org/, Roslin Institute), a distance considered adequate to ensure linkage equilibrium by some authors (e.g. Luo *et al*. 2004). However, given the significant result, we eliminated locus MAF50 from all analyses.

Total number of alleles, number of private alleles, allelic richness, average allelic range in repeat unit length and average heterozygosity were all once again, higher in the SSP than in the EEP group (Table 2). To check that this was not an effect of sampling large numbers of related individuals within the EEP group and not in the SSP group, we deleted one individual out of every known full-sib and parent–offspring relationship from both groups and re-analysed the remaining data (EEP $n = 102$; SSP $n = 43$). All values remained identical in both data sets (data not shown). The very small sample sets of SA and UAE showed much lower values for all these estimates but clearly require additional sampling. Within-group estimates for the inbreeding coefficient, F_{IS}, revealed the lowest value in the SSP group (0.020), with moderate levels in the EEP group (0.128).

Table 3 Results from STRUCTURE 2.0 for *K* values 1–5. The highest Ln *P*(*X*/*K*) obtained in 10 independent runs, mean inferred assignment to clusters, and numbers of individuals with > 75% assignments within each cluster are shown

K	Ln *P*(*X*/K)	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
1	−2099.8					
2	−2035.8	0.454 (10EEP,36SSP,3UAE)	0.546 (73EEP,1SSP,1UAE,2SA)			
3	−1982.8	0.321 (2EEP,29SSP,1UAE)	0.338 (30EEP,1SSP,2UAE)	0.343 (37EEP,1SSP,4UAE)		
4	−1990.2	0.266 (20EEP,1UAE)	0.291 (25EEP,1SA)	0.205 (3EEP,5SSP)	0.238 (15SSP,1UAE)	
5	−2038.0	0.208 —	0.192 —	0.209 —	0.208 —	0.184 —

The results obtained using STRUCTURE 2.0 are summarized in Table 3. Results were highly consistent across the 10 independent repeat runs suggesting adequate numbers of iterations. The highest estimated log-likelihood value (in 10 runs) and the highest posterior probability (0.9991) was seen in the case of $K = 3$ and there was evidence of differential clustering of EEP and SSP samples, with the majority of SSP samples clustering separately to a large proportion of the EEP samples with > 75% assignment. However, overall proportions assigned to groups were fairly symmetric ($-1/K$ in each group) suggesting that there was no support for strong genetic structure across the sample set (Pritchard & Wen 2003). The F_{ST} value between EEP and SSP groups was also low, at 0.047 ($P < 0.001$).

Individuals important to global captive breeding based on mk *values*

Values obtained for *mk* in every individual were ranked from lowest to highest in order to prioritize the genetically most important animals, that is, those with the lowest *mk* values (Fig. 5). Almost all (44/48, 92%) SSP individuals had *mk* values lower than the median, while the majority (81/120, 68%) of the EEP individuals had *mk* values higher than the median. The UAE and SA samples fell on both sides of the median, with 4/6 lower and 2/6 higher than the median in the case of UAE and 2/4 lower and 2/4 higher than the median in the case of SA, respectively. The two genetically most important living animals were from Bamberger and possessed four of the eight SSP private alleles. Three SSP private alleles were found in six individuals from HDZ (ranked 2nd, 13th, 25th, 28th, 31st, and 38th). Fifteen of the HDZ individuals sampled were dead (marked with asterisks in Fig. 5), a number of which have produced no offspring (8th, 13th, 22nd, 28th, 45th, 55th). In Fossil Rim, three of the SSP private alleles were found in two individuals (ranked 14th and 44th). One individual from Fresno (ranked 7th) and two individuals each from San Diego and The Wilds (ranked 5th and 20th, respectively) had one SSP private allele each The highest ranked EEP individuals were from Selwo zoo, Spain (4th), and Artis zoo, Holland (11th), followed by the two animals sent to Sidi Toui from La Palmyre (18th and 23rd, respectively).

Discussion

Inferences on historical patterns of demography

Deep sequence divergence between the SHO mtDNA haplotypes was evident by the three disconnected networks, suggesting historical population isolation. Estimated divergence times between the networks ranged from *c.* 2.1–2.7 million years. These levels of divergence are likely to represent remnants of ancient divergence within SHO, since the genus *Oryx* along with a number of other arid-adapted bovid species first appear in the fossil record *c.* 2.7–2.5 Ma (Vrba 1995; Bobe & Eck 2001; deMenocal 2004), and molecular phylogenetic studies support a recent divergence of this genus within the Hippotragini tribe, with all three species consistently found as terminal taxa with very short branch lengths (Gatesy *et al.* 1997; Hassanin & Douzery 1999; Iyengar *et al.* 2006).

Significantly large negative values for Fu's F_S were obtained both when all haplotypes were analysed simultaneously, and when network I haplotypes were analysed separately. In addition, nonsignificant values for Fu and Li's F* and D* were seen in both these cases, a pattern that is highly suggestive of an ancient population expansion. A unimodal pattern of mismatch distribution characteristic of a population explosion was however, not observed when all haplotypes were considered. Results from a mismatch distribution are sometimes considered tentative since a number of factors such as the time of population expansion, population size before expansion, and subdivision of populations, have been found to affect the results (Marjoram & Donnelly 1994). It is possible that both population subdivision (clearly apparent from the three disconnected networks), and population size before

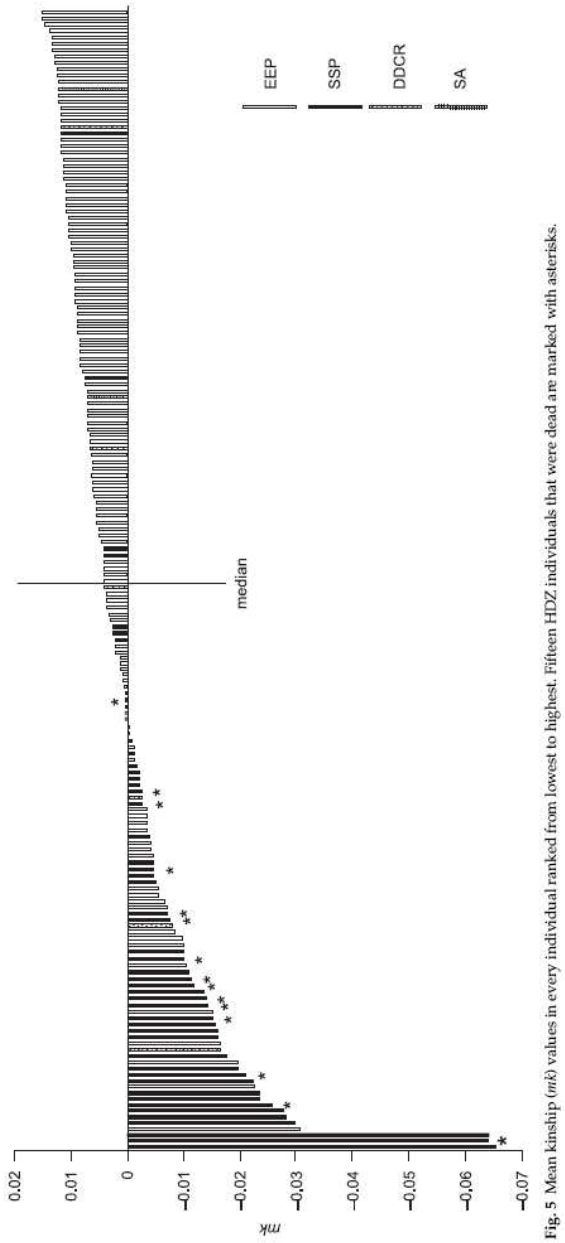

Fig. 5 Mean kinship (*mk*) values in every individual ranked from lowest to highest. Fifteen HDZ individuals that were dead are marked with asterisks.

expansion, are responsible for this observation. In the case of network I haplotypes however, a mismatch distribution showing a unimodal pattern was supported by a significantly low raggedness statistic (Harpending 1994). Times of population expansion were estimated to be *c.* 0.5 Ma and *c.* 1.2 Ma with network I or all haplotypes, respectively.

There is considerable evidence to suggest that glaciation events caused severe climatic changes within Africa. Throughout the Quaternary period which was dominated by ice ages that occurred every 41 000 years until 0.9 Ma, and every 100 000 years thereafter, it has been found that during glacial cycles, the climate was colder and drier in Africa with an increase in savannah and desert regions and a reduction in rainforests (e.g. deMenocal 2004; Hewitt 2004a). Studies have shown that at the last glacial maximum (LGM) *c.* 23 000–14 500 years ago, increased aridity in Africa resulted in the expansion of the Sahara desert zone hundreds of kilometre further south than at present, compressing the Sahelian zone equator wards (Thomas & Thorp 1995). However, other studies have found that at this time, areas in the northwest of the Sahara retained greater winter rainfall and consequently formed a belt of semidesert to the south of the present day desert margin (Hooghiemstra *et al.* 1992). Even in the central part of the Sahara, areas above 1500 m are thought to have resembled semidesert at the LGM since evidence suggests that winter rainfall occurred in these regions and maintained scattered vegetation (Maley 2000). Although many phylogeographical studies of large mammals across Africa have found evidence for the existence of glacial refugial areas within the west, east, and south of the continent (reviewed in Hewitt 2004b), there are very few studies that have investigated the evolutionary history of the fauna and flora of North Africa. Unsurprisingly, however, these studies have also found genetic evidence for conspicuous palaeoclimatic effects in this region (Brown *et al.* 2002; Cosson *et al.* 2005).

We propose therefore, that the population expansion signals detected at 1.2 Ma and 0.5 Ma in SHO are the result of the restriction of populations within suitable refugial areas during glacial cycles followed by expansion during favourable interglacial conditions. Since evidence already suggests that suitable semidesert habitat was available to the north and the south of the Sahara, and possibly even within the Sahara itself at higher altitudes, SHO populations may have become restricted into three or more such refugial areas during an ancient glaciation, resulting in the three divergent networks that are seen. A star-shaped topology characteristically seen when populations have undergone rapid range expansion following restriction into small refugia as seen in many species from temperate regions (e.g. Hull & Girman 2005) was, however, not seen in the networks. Perhaps refugial areas in this region were large, retaining sizeable numbers of diverse ancestral haplotypes. In this case, small numbers of ancestral haplotypes expanding into new haplotypes would then reveal small groups of star-shaped topologies as seen in network I (e.g. P, B, O, and U). Since all the samples used in this study are most likely to have originated from just one location (Chad), we are unfortunately unable to obtain a more complete picture of the various phylogeographical groups that may have existed, and an extensive survey of museum samples will prove useful in this context.

Following the ice ages, in the early part of the Holocene (9500–4500 years ago), there is extensive evidence to suggest that conditions were much more humid in the Sahara than it is at present ('the early Holocene pluvial episode'), with savannah extending right into the desert. Relict savannah plant species have been found in the Sahara and rock art left by early humans in the area suggest the existence of savannah species such as elephants and hippos during this time (e.g. Lézine 1989; deVivo & Carmignotto 2004). Thus, having undergone repeated population restriction and expansion during glacial cycles, the existence and maintenance of enormous numbers of SHO across North Africa in the past few thousand years is highly possible, providing support for the census estimate obtained in this study of *c.* 1 million individuals in the recent past. SHO was considered the most numerous large mammal of the Sahel during the middle ages, and as recently as 1936, herds of up to 10 000 animals were sighted in Chad (Bassett 1975; Newby 1988). High levels of genetic diversity must have been maintained within SHO populations since they were migratory, travelling large distances (over 600 km annual round trip recorded in Chad) in search of grazing (Newby 1988).

Genetic diversity preserved within captive groups

The SSP group was found to retain higher levels of genetic diversity with both mtDNA and microsatellites, reflecting the greater number of founder individuals taken to the USA from the initial captures made in Chad. Overall mean uncorrected *p* distance across all SHO haplotypes was 2.0%, a value that is comparable to those reported within other Hippotragines (1.9% in roan, Alpers *et al.* 2004) and the closely related Alcelaphines [1.7% in topi (*Damaliscus lunatus*), 2.4% in wildebeest (*Connochaetes taurinus*), Arctander *et al.* 1999]. However, the maximal levels of divergence between haplotypes reported in other Hippotragines are far greater than those seen in SHO. For example, in sable, Pitra *et al.* (2002) observed a mean sequence divergence of 14.6% between three clades representing regions in eastern and southern Africa, and in roan, Alpers *et al.* (2004) observed a maximal divergence of 27.5% between two haplotypes from Senegal and Botswana. In this study, a mean divergence of only 2.7% was observed between the three SHO networks with a maximum value of 3.8% between haplotypes AM and Z. Therefore, given

that SHO spanned across vast areas of North Africa, and that populations may have become isolated into ice age refugia resulting in highly divergent groups of haplotypes (networks), it appears that some of the range of diversity may have become lost. The detection of a novel transition within the museum sample from Sudan (1911) provides some evidence for the existence of greater diversity in the past, but more extensive sampling is required in order to elucidate historical patterns.

The lack of strong evidence for population genetic structure using microsatellites could be a result of the small number of loci used in this study since Evanno *et al.* (2005) have reported a drop in detection of signal of population genetic structure with five loci in comparison to 10 loci. However, other studies have successfully detected evidence for population structure using just five microsatellite loci (Pritchard *et al.* 2000; Hufbauer *et al.* 2004). Consequently, we interpret our finding of a lack of population structure as being a result of very large numbers of SHO existing largely in panmixia within the Sahelian region after the early Holocene pluvial episode. SHO are thought to have been highly nomadic, travelling vast distances on a regular basis (Newby 1988; Wacher 1988). Mean observed microsatellite heterozygosity in SHO across all groups was identical to that seen in wild populations of roan (42%, Alpers *et al.* 2004), and values seen within the SSP and EEP groups (57% and 54%) were very similar to that seen in captive populations of Arabian oryx (54% across six loci, four of which were the same as those used in this study) (Marshall *et al.* 1999).

Future captive breeding and reintroductions

Information from the SHO stud book database containing multigenerational captive breeding records suggests that the sample set used in this study includes, albeit to varying degrees, 80% and > 85% of the original founder lineages from the SSP and EEP groups, respectively (data not shown). Future captive breeding must maintain and actively manage the high levels of genetic diversity seen within the SSP group. Results suggest that Bamberger, HDZ, and Fossil Rim hold some of the most valuable global SHO genetic diversity. Demographic studies on SHO within the SSP has revealed an ageing population where far greater numbers of older rather than younger individuals are being held, and breeding is inadequate (also indicated by the very low F_{IS} value seen within SSP in this study), and it has been recognized that managed captive breeding is urgently required (Spevak 2004). Results from this study further highlight this need. Loss of some genetic diversity is already apparent in HDZ where a number of dead individuals of genetic importance have left no known progeny. Within the EEP group, although levels of overall genetic diversity are lower, mtDNA haplotypes from all networks are represented (except for a degree of under-representation of network I haplotypes), and levels of microsatellite allelic richness are high. Also, large numbers of younger individuals are held as a result of sustained managed captive breeding, rendering the population more 'stable' in the long term (Gilbert 2005). Although small sample sizes in the UAE and SA groups preclude conclusions, it is clear that management programmes running within individual countries need to keep in mind the requirement for animal import from other regions in order to prevent extensive inbreeding. Since all Australasian SHO are likely to have descended from a few individuals from Marwell, for example the two animals sampled in this study which had common EEP haplotypes and high *mk* values (ranked 147th and 149th), there is a need for future animal import from both within and outside the EEP into these regions. Two SHO sent to Sidi Toui National Park as part of 18 individuals sent from the EEP to various parks in Tunisia, possessed distinct haplotypes and low *mk* (ranked 18th and 24th), making them important in a global context. Studbook information indicates that the remaining animals sent to Tunisia are related at varying degrees to other animals held within the EEP. It is of interest to carry out further genetic analyses on these reintroduced animals and on animals to be re-introduced in the future in order to establish how best to maintain and supplement genetic diversity in these groups.

In conclusion, based on our findings, we recommend that a 'global' perspective for the captive genetic management of SHO is maintained, and that individuals across networks continue to be intermixed as currently practised. Individuals from various management programmes and regions need to be effectively utilized for sustained future captive breeding in order to ensure that the vital remnants of genetic diversity are retained and represented in future reintroduction programmes.

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Appendix G: assumptions in the scimitar-horned oryx analytical studbook

Table G.1 Parentage assumptions and hypothetical individuals

Females					Males				
SB ID	Studbook		Assumption		SB ID	Studbook		Assumption	
	Sire	Dam	Sire	Dam		Sire	Dam	Sire	Dam
5012	UNK	UNK	5004	5020	5004	UNK	UNK	WILD	WILD
5064	UNK	UNK	WILD	WILD	5024	UNK	UNK	5004	5020
5076	5028	UNK	-	5020	5060	UNK	UNK	WILD	WILD
5132	UNK	UNK	5084	5064	5084	UNK	UNK	5060	5064
5148	UNK	UNK	5060	5064	5128	UNK	UNK	5084	5064
5264	UNK	UNK	5128	5148	5212	5140	UNK	-	5020
5308	5140	UNK	-	5020	5276	5140	UNK	-	5020
5408	5140	UNK	-	5168	5828	UNK	UNK	5536	5464
5534	UNK	UNK	WILD	WILD	5840	UNK	UNK	5680	5704
5576	UNK	UNK	5428	5436	5860	UNK	UNK	5560	5548
5848	UNK	UNK	5592	5704	5876	UNK	UNK	5140	5308
5852	UNK	UNK	5592	5704	5896	UNK	UNK	5236	5688
5868	UNK	UNK	5560	5548	5900	UNK	UNK	5356	5360
5872	UNK	UNK	7120	7192	6208	UNK	UNK	5592	5596
6072	UNK	5532	5276	-	6212	UNK	UNK	5520	5524
6120	UNK	UNK	5680	5704	6460	UNK	5868	5860	-
6220	UNK	UNK	5592	5596	6616	5760	UNK	-	5664
6420	UNK	UNK	5744	5808	7008	UNK	UNK	5400	5534
6448	UNK	6052	5684	-	7016	UNK	UNK	7600	6636
6540	5736	UNK	-	6068	7348	5444	UNK	-	5448
6584	5956	5548	8900	-	7600	5560	6488	-	9644
6628	5760	UNK	-	5664	7896	UNK	UNK	5296	5424
6636	5956	6188	8900	-	8000	UNK	UNK	7016	6636
6700	UNK	6308	5900	-	8532	6616	UNK	-	6628
6864	UNK	UNK	5744	6420	8644	UNK	UNK	5296	5424
6900	UNK	UNK	5736	6236	8848	UNK	UNK	5296	7896
7048	UNK	6052	5900	-	8900	UNK	UNK	5560	5552
7116	UNK	5868	5684	-	8984	UNK	UNK	5544	HYP001
7676	UNK	6636	7016	-	9272	UNK	7116	6460	-
7928	UNK	UNK	7008	5534	9296	UNK	UNK	5412	5264
7932	UNK	UNK	7008	5534	9324	UNK	6480	6476	-
8652	UNK	UNK	5236	6788	9652	UNK	UNK	6460	5576
8712	UNK	5868	6460	-	10346	6616	UNK	-	9424
8760	UNK	7048	6460	-	11456	6616	UNK	-	5664
9060	6164	UNK	-	5440	11700	9476	UNK	-	6288
9276	UNK	6700	6460	-	11888	UNK	UNK	8984	9640
9320	UNK	6448	6460	-	11988	UNK	UNK	9272	8760
9424	6616	UNK	-	6628	12760	UNK	UNK	5592	5596
9640	UNK	UNK	5896	5300	13648	UNK	UNK	9652	9320
9644	UNK	UNK	5560	5948	13664	UNK	UNK	9652	9320
9728	UNK	UNK	6460	8712	13672	UNK	UNK	9272	9276
11508	5840	UNK	-	5848	13676	UNK	UNK	9652	9320
11600	UNK	UNK	8308	7980	13812	11476	12092	-	19756
12056	UNK	UNK	8180	6804	14836	8900	13140	-	19760
12684	9476	UNK	-	6288	15348	11476	12092	-	19756
13532	UNK	8528	9932	-	15356	UNK	UNK	7008	7928
13628	UNK	UNK	11888	9640	15560	UNK	UNK	9304	8320
13656	UNK	UNK	9652	9728	15768	UNK	UNK	12352	13136
13660	UNK	UNK	9652	7048	15800	8900	13140	-	19760

Appendix G: Table G.1 continued

Females					Males				
SB ID	Studbook		Assumption		SB ID	Studbook		Assumption	
	Sire	Dam	Sire	Dam		Sire	Dam	Sire	Dam
13668	UNK	UNK	9652	9320	16444	UNK	UNK	8900	9644
15616	13580	UNK	-	6072	16764	UNK	14556	14584	-
16420	UNK	UNK	9652	13660	17128	UNK	10420	14380	-
16448	UNK	UNK	11888	13628	17372	UNK	UNK	9652	13660
16864	8900	13140	-	19760	17584	UNK	14556	14584	-
17232	UNK	UNK	7008	7928	18168	UNK	15052	14380	-
17852	UNK	10420	8308	-	18300	UNK	UNK	15356	7928
17940	10696	UNK	-	8652	18510	UNK	UNK	14612	22404
18464	UNK	UNK	6460	13660	18511	UNK	UNK	16872	16924
18468	UNK	UNK	5876	5872	18600	11476	12092	-	19756
18984	7012	UNK	-	6072	18980	7012	UNK	-	6072
19256	UNK	UNK	16444	16448	19476	10696	UNK	-	8652
19756	UNK	UNK	7600	7644	19752	UNK	UNK	8180	8660
19760	UNK	UNK	8900	9644	20584	UNK	UNK	8900	19760
19764	UNK	UNK	11476	11600	23096	UNK	20180	19752	-
19768	UNK	UNK	11476	11600	23784	UNK	19760	19752	-
19860	11476	12092	-	19756	24104	20168	UNK	-	17852
19908	11476	12092	-	19756	26200	UNK	21720	22708	-
20448	UNK	13660	13648	-	26886	UNK	UNK	17856	21504
20772	UNK	UNK	17372	19544	27504	17044	UNK	-	10596
20781	UNK	UNK	16968	10596	27728	15768	UNK	-	20660
21228	UNK	UNK	18168	7980	28484	9324	UNK	-	25252
21720	UNK	13656	13676	-	28800	UNK	26116	23624	-
21852	UNK	UNK	14584	20696	29020	UNK	UNK	9088	10960
22184	UNK	19760	19752	-	29268	UNK	25708	23624	-
22240	UNK	19764	19752	-	29844	UNK	26116	26200	-
22428	UNK	UNK	20584	19792	29900	UNK	UNK	26200	21116
22540	UNK	UNK	19744	19792	30212	UNK	23012	26200	-
22584	UNK	7932	19212	-	30288	UNK	26116	26200	-
23720	20296	UNK	-	21228	30424	UNK	UNK	22036	19756
23792	19156	UNK	-	8652	30520	24104	UNK	-	24128
23964	UNK	19764	22516	-	30612	UNK	29172	24796	-
24604	UNK	21380	23096	-	30772	UNK	29172	24796	-
24809	UNK	UNK	14612	22404	30988	UNK	29676	19476	-
24810	UNK	UNK	14612	22404	31000	22036	UNK	-	19756
24848	UNK	UNK	23096	19756	31060	UNK	27644	28800	-
24852	UNK	UNK	23096	19756	31136	UNK	28264	29136	-
24904	UNK	21116	13676	-	31216	UNK	UNK	28832	20660
25076	15768	UNK	-	20660	31276	28324	UNK	-	17996
25176	UNK	23012	13676	-	31324	28324	UNK	-	17996
25252	17044	UNK	-	6552	31328	28324	UNK	-	26344
25356	17044	UNK	-	6552	31376	UNK	27820	24796	-
25708	UNK	13660	13676	-	31610	UNK	26464	26886	-
25820	23164	UNK	-	8652	31820	UNK	UNK	28800	29192
26116	UNK	23152	22468	-	31904	UNK	UNK	22036	19756
26344	17044	UNK	-	17996	32100	UNK	UNK	28800	23012
26372	17044	UNK	-	6552	32248	UNK	26892	29460	-
26604	UNK	13660	22468	-	32320	UNK	18468	26992	-
26740	UNK	UNK	23096	19756	32512	UNK	28024	30396	-
26884	UNK	UNK	14380	23132	32516	UNK	21820	30396	-
26888	UNK	UNK	14380	24112	32532	UNK	30272	29844	-
26892	UNK	UNK	23144	14556	32540	UNK	26604	29844	-
27032	22036	UNK	-	19756	32560	UNK	29200	29844	-
27080	22036	UNK	-	19756	32587	UNK	UNK	29844	25708

Appendix G: Table G.1 continued

Females					Males				
SB ID	Studbook		Assumption		SB ID	Studbook		Assumption	
	Sire	Dam	Sire	Dam		Sire	Dam	Sire	Dam
27520	17044	UNK	-	10596	32640	UNK	UNK	22036	19756
27644	UNK	23012	23388	-	32656	UNK	UNK	22036	19756
27820	UNK	24848	24852	-	32716	UNK	27964	29844	-
27964	UNK	23152	23624	-	32736	UNK	27644	29844	-
28048	UNK	13660	23624	-	32832	UNK	23152	29844	-
28232	UNK	26116	23624	-	32848	UNK	18468	26992	-
28308	UNK	25708	23624	-	32905	UNK	25176	29900	-
28396	9324	UNK	-	17996	33150	UNK	UNK	30212	21116
28564	22464	UNK	-	20772	33212	UNK	25708	30288	-
28600	UNK	25176	23624	-	33604	UNK	UNK	30656	21820
28892	UNK	27820	24852	-	33976	UNK	UNK	31120	21820
28968	UNK	26604	23624	-	34136	UNK	UNK	30772	24848
29172	UNK	24848	24796	-	34244	18980	UNK	-	17428
29192	UNK	23152	23624	-	34270	UNK	23012	31060	-
29200	UNK	23012	23624	-	34449	UNK	31756	31060	-
29260	UNK	26116	23624	-	34464	UNK	UNK	30772	24848
29536	24104	UNK	-	17852	34516	UNK	UNK	30772	24848
29576	24104	UNK	-	17852	34568	UNK	UNK	31120	21820
29688	23144	27264	-	26892	35272	29460	UNK	-	32504
29808	UNK	25176	26200	-	35278	UNK	UNK	27596	28940
29964	UNK	26604	26200	-	35612	UNK	32984	30904	-
30016	UNK	24848	24796	-	35674	UNK	UNK	31204	29388
30056	UNK	UNK	22036	19756	35710	UNK	UNK	34092	32240
30156	UNK	UNK	22036	19756					
30252	UNK	UNK	26200	27964					
30556	24104	UNK	-	24128					
30928	UNK	27416	29136	-					
30958	UNK	26116	28800	-					
31140	UNK	29432	18316	-					
31184	UNK	28308	28800	-					
31212	UNK	UNK	28800	28048					
31264	28324	UNK	-	17996					
31622	UNK	24809	26886	-					
31756	UNK	28600	29268	-					
31828	UNK	UNK	28800	29192					
31832	UNK	28048	28800	-					
31908	UNK	29808	28800	-					
31924	UNK	UNK	22036	19756					
31944	UNK	UNK	28832	20660					
32108	28324	UNK	-	17996					
32192	UNK	26892	29460	-					
32536	UNK	24904	29844	-					
32592	UNK	28048	29844	-					
32624	UNK	29964	29844	-					
32684	UNK	26116	29844	-					
32700	UNK	23012	29844	-					
32772	28832	UNK	-	20660					
32808	28832	UNK	-	20660					
32836	UNK	28308	29844	-					
33154	UNK	UNK	30212	21116					
33158	UNK	27964	30212	-					
33248	UNK	UNK	30656	21820					
33332	UNK	UNK	30288	29808					

Appendix G: Table G.1 continued

Females					Males				
SB ID	Studbook		Assumption		SB ID	Studbook		Assumption	
	Sire	Dam	Sire	Dam		Sire	Dam	Sire	Dam
33344	UNK	26116	30288	-					
33360	UNK	UNK	30656	21820					
33440	UNK	28600	30288	-					
33460	UNK	UNK	27596	17852					
33636	UNK	29964	30288	-					
34248	18980	UNK	-	17428					
34734	UNK	UNK	32028	29172					
34750	UNK	UNK	32028	29172					
34928	UNK	UNK	32028	29172					
35308	UNK	UNK	32028	29172					
35714	UNK	UNK	31748	29388					
35718	UNK	UNK	34092	32240					
35954	28792	UNK	-	32420					
HYP001	5896	5300	-	-					

Table G.2 Birth date, gender, location and birth type assumptions. **CB**: captive born, **UNK**: unknown birth type

SB ID	Studbook				Assumption			
	Sex	Birth date	1st location	Birth	Sex	Birth date	1st location	Birth
5828	M	~1970	UNK	CB	-	10/04/1970	MEMPHIS	-
5872	M	-	-	CB	F	-	-	-
6208	M	~1972	UNK	CB	-	23/05/1972	BERLIN TP	-
6212	M	~1972	UNK	CB	-	25/09/1972	SANDIEGOZ	-
6220	F	~1972	UNK	CB	-	24/02/1974	BERLIN TP	-
8900	M	~1979	UNK	CB	-	11/05/1970	PRAHA	-
9644	F	UNK	UNK	CB	-	01/02/1973	PRAHA	-
11600	F	-	UNK	UNK	-	-	BERLIN TP	CB
12056	F	UNK	UNK	UNK	-	10/06/1982	PRAHA	CB
15768	M	07/05/1987	-	CB	-	30/07/1987	-	-
18464	F	-	UNK	CB	-	-	HAI BAR	-
18511	M	~1990	ENGLAND	CB	-	29/09/1990	CHESTER	-
19752	M	UNK	UNK	CB	-	27/03/1983	PRAHA	-
19756	F	UNK	UNK	CB	-	10/04/1983	DVURKRALV	-
19760	F	UNK	UNK	CB	-	06/05/1984	MUNSTER	-
19764	F	~1991	UNK	CB	-	01/07/1988	BRATISLAV	-
19768	F	UNK	UNK	CB	-	17/07/1990	EDINBURGH	-
20781	F	~1992	ENGLAND	CB	-	09/06/1993	WHIPSNADE	-
26884	F	~1998	UNK	CB	-	16/07/2000	BERLIN TP	-
26886	M	~1998	ENGLAND	CB	-	08/07/1997	EDINBURGH	-
26888	F	~1998	UNK	CB	-	09/04/2001	BERLIN TP	-
26892	F	~1998	UNK	CB	-	26/04/1998	BERLINZOO	-
HYP001	-	-	-	-	F	~1972	UNK	CB

Appendix H: Lynch-Ritland relatedness values and mean kinship coefficients with associated sample type

Sample type with the *LR* values derived from molecular analysis and *MK* coefficients derived from the analytical studbook (ASB) and true studbook (TSB) for scimitar-horned oryx. Key to sample type: **F**: faecal samples; **Bl**: blood samples; **Sk**: skin samples; **Ts**

Females					Males				
SB ID	**Sample**	**LR**	**ASB**	**TSB**	**SB ID**	**Sample**	**LR**	**ASB**	**TSB**
13836	F	-0.0127	0.0395	0.0739	17044	F	-0.0023	0.0545	0.0703
14752	Bl & Ts	-0.0057	0.0804	0.0965	17128	Bl	-0.0317	0.0323	0.0015
14764	Bl & Ts	-0.0088	0.0804	0.0859	21336	Bl & F	-0.0047	0.1059	0.0688
15680	Bl	-0.0140	0.0631	0.0882	23264	Bl & F	-0.0139	0.1568	0.1665
16552	F	-0.0107	0.0424	0.0799	24796	Bl	-0.0226	0.1213	0.1309
18984	F	-0.0190	0.0313	0.0607	24852	Bl & Sk	-0.0359	0.1389	0.5000
19952	F	-0.0296	0.0715	0.0974	25236	Bl	-0.0183	0.1117	0.1226
20248	F	-0.0140	0.0624	0.0729	25632	Bl & Sk	-0.0228	0.1182	0.1289
20460	F	-0.0163	0.0519	0.0637	26052	Bl	-0.0247	0.1117	0.1226
20768	F	-0.0174	0.0447	0.0841	26944	Bl	-0.0163	0.1393	0.1480
21744	F	-0.0118	0.0464	0.0861	27504	F	-0.0093	0.0633	0.0743
21820	Bl	-0.0123	0.1309	0.1891	28324	Bl & Ts	0.0149	0.1132	0.0806
22348	F	-0.0331	0.0675	0.0723	28380	F	-0.0331	0.1264	0.1485
22420	F	-0.0142	0.0527	0.0565	28412	Bl & Ts	-0.0244	0.0592	0.0575
22460	Bl	-0.0134	0.1293	0.1674	28484	Bl & Ts	-0.0256	0.0655	0.5000
23268	F	-0.0176	0.0610	0.0710	28988	F	-0.0228	0.0583	0.0562
23348	F	-0.0083	0.0683	0.0848	29036	F	0.0004	0.1154	0.1279
23544	F	-0.0079	0.0924	0.0410	29620	F	-0.0165	0.1117	0.1226
24388	F	-0.0084	0.0626	0.0763	30124	Bl & F	-0.0288	0.1023	0.1689
24848	Bl & Sk	-0.0012	0.1411	0.5000	30600	F	-0.0365	0.0333	0.5000
25312	F	-0.0062	0.0454	0.0422	30768	F	-0.0190	0.0325	0.5000
26140	F	-0.0139	0.0719	0.0891	30776	Bl & F	-0.0102	0.1179	0.1263
26576	F	-0.0433	0.0311	0.5000	30868	F	0.0000	0.0831	0.0877
27144	Bl	-0.0121	0.1247	0.1421	31100	Sk	-0.0153	0.0588	0.0745
27516	F	-0.0076	0.0500	0.0682	31204	Bl	-0.0095	0.1627	0.1801
27556	Bl	-0.0173	0.1264	0.1485	31276	Sk	0.0071	0.0907	0.0817
27820	Bl & Sk	-0.0100	0.1422	0.5000	31300	Sk	0.0095	0.0911	0.0782
28024	Bl	-0.0071	0.1538	0.1830	31312	Sk	0.0101	0.0899	0.0773
28032	F	-0.0516	0.0290	0.5000	31320	Sk	0.0085	0.0905	0.0782
28184	F	-0.0245	0.0719	0.0891	31324	Sk	-0.0044	0.0907	0.0817
28500	Bl & Ts	-0.0130	0.0648	0.0743	31328	Sk	0.0058	0.0894	0.0817
29044	F	-0.0078	0.1161	0.0876	31332	Sk	0.0035	0.0849	0.0662
29172	Bl & Sk	-0.0101	0.1336	0.5000	31340	Bl & Ts	0.0014	0.0877	0.0612
29388	Bl	-0.0014	0.1533	0.1814	31748	Bl	-0.0105	0.1492	0.1831
29664	F	-0.0025	0.1247	0.1421	32512	Bl	-0.0096	0.1603	0.1846
29692	F	-0.0195	0.1045	0.1292	32516	Bl	-0.0057	0.1492	0.1891
30016	Bl / Sk	-0.0140	0.1336	0.5000	33604	Bl	-0.0112	0.1442	0.5000
30252	F	-0.0275	0.0777	0.0836					
30284	Bl & F	-0.0089	0.1179	0.1263					
30752	Bl & F	-0.0042	0.1184	0.1287					
30876	F	-0.0136	0.0817	0.0871					
31268	Sk	0.0075	0.0908	0.0817					
31288	Sk	0.0031	0.0906	0.0828					
31316	Sk	0.0060	0.0927	0.0779					
32056	Bl & Ts	-0.0251	0.0911	0.0782					
32196	Bl	-0.0330	0.1357	0.1435					
33248	Bl	-0.0057	0.1442	0.5000					
33360	Bl	-0.0247	0.1442	0.5000					

Appendix I: OIE listed diseases for scimitar-horned oryx

Countries highlighted in bold are scimitar-horned oryx EEP countries. Title legend: 1) disease never occurred; 2) disease absent during the report period; 3) current unresolved disease events; 4) disease suspected; 5) infection present (with no clinical disease); 6) demonstrated clinical disease; 7) disease restricted to certain zones of the country

Disease	1	2	3	4	5	6	7
Anthrax	AE; CU; SG	CY; **CZ; DE; DK**; DZ; EG; **ES; FR; HR; HU; IE; IL**; JP; KR; LK; LY; MA; **NL; PL; PT**; TN; UA	**HR**	CA; US	MX	BG; SD; SN; TD; ZA	AU; CA; CN; **GR**; NE; US
Bluetongue	**IE; PL;** KR; LK; NZ; TH; UA	BG; CA; CY; **CZ**; **DK**; EG; **HR**; **HU**; JP; NE; **NL**; SG	CY; **ES**; **GB**; **GR**; MA	**BE**	AU; **FR; IT**; MX; SA; ZA	DZ; **ES; GR; IL**; LY; **PT**; TN ; US ; ZA	CU; **DE; FR; GB; IT**
Bovine anaplasmosis	AE; **CZ; DK; GB; IE**; NZ; SG	CY; **FR; GR; HR; HU; IT**; JP; KR; LY; **NL**; NE; TN	CA	MX	CA; EG; MX	CU; **IL**; TH; US; ZA	AU; SD
Bovine babesiosis	AE; CA; CY; NZ; SG	**BE;** BG; **CZ; DK; HR; HU**; JP; KR; **NL**; TN; UA; US		IT, MX	EG; MX	CU; **GB; IE; IL**; LK; LY; ZA	AU; **ES; GR**; NE; SD
Bovine brucellosis	SG	AE; AU; **BE;** BG; CA; **CZ; DE; DK; FR; GB; HR; IL;** JP; MA; **NL;** NZ; SD; UA			CY ; EG; **PL**; MX	BF; CU; DZ; **ES; GR; IE; IT;** LK; LY; ML; **PT;** TH; TN; SA; YE; ZA	CN; US
Bovid genital campylobacteriosis	AE; EG; KR; SG	CU; CY; **CZ; DE; DK; GR; HR; HU; IL; IT**; LY; **PL**; **PT**; TN; UA		CA	NL	AU; **FR**; **GB**; **IE**; JP; NZ; US	
Bovine spongiform encephalopathy	AU; BF; CN; CU; CY; DZ; EG; **HR**; **HU**; KR; LK; LY; MA; ML; MX; NZ; SD; SG; TN; UA; ZA	**CZ**; **DE**; **DK**; **GR**; **IL**; NE; **NL**			**IE; PL**	**GB; PT**	CA; **ES; FR**

Appendix I continued

Disease	1	2	3	4	5	6	7
Bovine tuberculosis	AE; LK	AU; **BE**; BG; CU; CY; **CZ**; **DK**; **IL**; JP; NE; SG; SD		CA	**DE; EG; HR; IE ;** UA	BF; DZ; **GB**; **GR**; **IE**; KR; LY; **MA**; **NL**; NZ; **PL**; **PT**; SA; TD; TH; TN; ZA	CN; **ES; FR; HU; IT**; MX; US
Bovine viral diarrhoea	**HR**; LK; SD; SG; UA	**GR**; **IT**; LY; NE; TN		US	BG; **CZ;** MX	AU; CA; CU; CY; **DE**; **DK**; **FR**; **GB**; ID; **IE**; **IL**; JP; KR; **NL**; NZ; US	CN; **ES; HU**
Brucellosis *Brucella abortus*	SG	AU; BG; CY; **CZ**; **DE**; **DK**; **FR**; **HR**; **HU**; **IE**; **IL**; JP; MA; **NL**; NZ; SA; UA	**BE**	CA; MX	**IT**; MX	CU; DZ; **GR**; KR; LK; **PT**; TH; TN; YE; ZA	CN; ES; GB; ZA
Contagious bovine pleuropenumonia	BG; CU; CY; DZ; **GR**; **HR**; ID; KR; LK; LY; MA; MX; SG; TH; TN; UA	AU; CA; **CZ; DE;** **DK**; EG; **ES; FR; GB; HU; IE; IL; IT**; JP; **NL;** NZ; **PL; PT**; SN; US; ZA		MR	BF; SD; TD	ML; NE	
Crimean congo haemorrhagic fever	AE; AU; **BE**; CA; CU; CY; **CZ**; **DE**; **DK**; DZ; **ES**; **FR**; **GB**; **GR**; **HR**; **HU**; **IE**; **IL**; **IT**; KR; LK; LY; MA; **NL**; NZ; **PT**; SD; SG; TN; UA; US: YE	BG; JP; NE; MX				ZA	
Echinoccosis / hydatidosis	LK; **IE; SG**	CU; CY; **DK; HR; IL; IT;** KR; **NL;** NZ; TN; UA		CA; US	BG; **CZ**; EG; ZA	AU; **DE;** DZ; **GB;** JP; LY; **PL**	CN; **ES; FR; GR; HU**
Enzootic bovine rhinotracheitis	LK; LY; ML; SG	CU; **CZ; DK; IL**; KR; TN; SA; UA		**IT**	**HR; NL**	AU; **BE**; CA; CY; **DE**; **FR**; **GB**; **IE**; JP; MX; NZ; **PL**; SA; US	**GR; HU; ES**

Appendix I continued

Disease	1	2	3	4	5	6	7
Epizootic haemorrhagic disease	AE; BF; CU; **CZ; DK; DE;** DZ; **ES; GB**; **HR;HU; IE; IT;** KR; ML; **NL;** NZ; **PL; PT**; SA; SD; SG; TN; UA	CY; **FR; GR; IL**; JP; LK; MA; NE		CA			US
Foot and mouth disease	CU; NZ	AE; AU; BG; CA; CY; **CZ**; **DE**; **DK**; DZ; EG; **ES; FR** ; **GR; HR ; HU**; **IE; IL; IT**; JP; MA; **NL; PL; PT**; SG; TN; UA; US	BG; **IL;** KR; **LY**; ZA		SA	BF; LK ; SA; SD; SN; TD; TH; YE	CN; ML; NE; ZA
Heartwater	AE; AU; BG; CA; CU; CY; **CZ; DK**; DZ; **DE;** EG; **ES; GB**; **GR**; **HR; HU; IE; IL;** KR; LK; LY; **NL;** NZ; **PL**; SG; TN; UA; US	**FR**; JP; **PT**		BF	ZA	ZA	SD
Leptospirosis	EG	BG; **CZ; GR**; KR; LY; SG; TN		MX	**HR;** MX; **NL; IT**; UA; US	AU ; CA; CU; **DE; DK; ES; GB; IE; IL; IT;** JP; LK; NZ; US	**FR; HU**
Lumpy skin disease	AU; **BE**; CA; **CZ**; CU; CY; **DE**; **DK**; DZ; **ES**; **FR**; **GB**; **GR**; **HR**; **HU**; **IE**; **IT**; JP; KR; LK; LY; MA; MX; **NL**; NZ; **PL**; **PT**; SA; SG; TN; US	EG; **IL**			SD	BF; SN; ZA	MA; NE

Appendix I continued

Disease	1	2	3	4	5	6	7
New world screwworm *Cochliomyia hominivorax*	AE; AU; BG; **BE**; CA; CN; CY; **CZ; DE; DK**; DZ; EG; **ES** ; **FR; GB; GR; HR; HU; IE; IL; IT**; KR; LK; MA; ML; NE; **NL;** NZ; **PL; PT;** SA; SD; SG; SN; TH; TN; YE; ZA	JP; LY; MX; US				CU	
Paratuberculosis	AE; EG	**BE**; **PL**; TN		US	**CZ; HR; NL; IT**	CA; **DE; DK; GB; GR; IE; IL; IT**; JP; LY; US	**ES; FR; HU**
Peste des petit ruminants	AU; **BE**; BG; CA; CU; CY; **CZ**; **DE; DK; ES; FR; GB; GR; HR; HU; IE; IT;** JP; KR; LK; MX; **NL;** NZ; **PL; PT;** SG; TH; UA; US; ZA	EG; **IL**; LY; MA; TN	CN ; DZ			AE; BF; TD; SN; SA; SD; YE	CN; NE
Q fever	AE; CU; EG; LK; MA; MX; NZ; SD; SG; UA	**BE**; **CZ**; **GR**; JP; KR; LY; **PT**; TN	**NL**		AU; **HR; NL;** SA; ZA	CA; BG; CY; **DE**; **DK**; **GB**; **IE**; **IL**; **PL**; US	**ES; FR; HU; IT**
Rabies	CY; NZ	AU; **BE**; **CZ; DE**; **DK**; EG; **FR**; **GR; IE**; JP; **PT**; SG	ID		BG; **NL**; SA	CA; CU; DZ; **HR; IL**; LK; LY; MA; **PT;** SA; SN. TN; TD; TH; UA; US; YE; ZA	CN; **ES; HU; IT**; MX; NE; SD

Appendix I continued

Disease	1	2	3	4	5	6	7
Rift valley fever	AU; AE; **BE**; CA; **CZ**; CU; CY; **DE**; **DK**; DZ; **ES**; **GB**; **GR**; **HR**; **HU**; ID; **IE**; **IL**; **IT**; JP; KR; MA; MX; **NL**; NZ; **PL**; **PT**; SG; TN; UA; US	EG; **FR**; SD; SN; YE	ZA		SA	ZA	
Rinderpest	CA; CU; CY; DZ; **ES**; MA; MX; NZ; **PT**; TN; UA; US	AE; AU; **BE**; BF; BG; **CZ; DE; DK**; EG; **FR; GB; GR; HR; IE; IL; IT**; JP; KR; LK; LY; ML; MR; NE**; NL; PL**; SA; SD; SG; SN; TD; TH; YE					
Surra *Trypanosoma evansi*	AU; BG; CA; CU; CY; **CZ; DK; DE; GR; GB; HR; HU; IE;** KR; MX; **NL;** NZ; **PL**; SG; UA; US; ZA	AE; **BE**; JP; LK; LY; **PT**; SA; SD; TN				BF; **IL**	**ES**
Theileriosis	AU; BG; CA; CY; **CZ**; **DE**; **DK**; **FR**; **HR**; **IE;** MX; **NL;** NZ; **PL**; SG; UA; US	AE; CU; **GB**; **GR**; **HU**; JP; KR; LY; TN; ZA		**IT**	EG	**IL**	SA; SD
Trichinellosis	AE; CU; CY; LY; SG	AU; **BE; DE; DK**; JP; KR; LK; MX; **NL;** NZ; **PT**; TN		CA; US	BG; **CZ;** EG; **HR; IE; IT;** UA; ZA	**IL; PL**	CN; **ES; FR; GR; HU**
Trichomonosis	LK; SG; TN; UA	AE; CU; CY; **CZ; DE; DK; GR; HR; IL**; JP; KR; LY; MX; NZ; **PL; PT**		AU; CA	**IT**	**GB**; US; ZA	**ES; FR; HU**; US

Appendix I continued

Disease	1	2	3	4	5	6	7
Trypanosomosis	AE; AU; **BE**; BG; CA; **CZ**; **DE**; **DK**; **FR**; **GB**; **HR**; **HU**; **IE**; **IT**; KR; LK; MA; MX; **NL**; **PL**; **PT**; SG; UA; US	**ES**; **GR**; **IL**; **LY**; TN		MR	EG	BF; JP; TH; ZA	CN; SD; ZA
Vesicular stomatitis	AE; **BE;** BG; CU; CY; **CZ; DE; DK**; DZ; EG; **ES; GB; GR; HR; HU; IE; IL; IT**; JP; KR; LY; MA; ML; **NL;** NZ; **PL; PT;** SA; SD; SG; TN; UA; ZA	CA; **FR**					MX; US

Countries underlined are scimitar-horned oryx range states. AE: United Arab Emirates; AU: Australia; **BE: Belgium;** BF: Burkina Faso; BG: Bulgaria; CA: Canada; CN: China; CU: Cuba; CY: Cyprus; **CZ: Czech Republic; DE: Germany;** DZ: Algeria; EG: Egypt; **ES: Spain; FR: France; GB: Great Britain; GR: Greece; HR: Croatia; HU: Hungary;** ID: Indonesia; **IE: Ireland; IL: Israel; IT: Italy;** JP: Japan; KR: South Korea; LK: Sri Lanka; LY: Libya; MA: Morocco; ML: Mali; MR: Mauritania; MX: Mexico; NE: Niger; **NL: Netherlands;** NZ: New Zealand; **PL: Poland; PT: Portugal;** SA: Saudi Arabia; SD: Sudan; SG: Singapore; SN: Senegal; TD: Chad; TH: Thailand; TN: Tunisia; TW: Taiwan; UA: Ukraine; US: United States of America; YE: Yemen; ZA: South Africa (OIE 2011)

Appendix J: Species listing for the global population analyses

Species 1-111 are included in Figures 7.10 and 7.12. Species highlighted in light grey correspond to the species included in Figures 7.11 and 7.13.

#	Species	Scientific	Population	TAG
1	Puerto Rican crested toad	*Peltophryne lemur*	SSP & ISB	Amphibian
2	Aruba Island rattlesnake	*Crotalus unicolor*	SSP & ISB	Reptile
3	Chinese alligator	*Alligator sinensis*	EEP, SSP & ISB	Reptile
4	Arabian oryx	*Oryx leucoryx*	SSP	Antelope & giraffe
5	Black-faced impala	*Aepyceros melampus petersi*	ISB	Antelope & giraffe
6	Bongo	*Tragelaphus eurycerus isaaci*	EEP, SSP & ISB	Antelope & giraffe
7	Cuvier's gazelle	*Gazella cuvieri*	EEP & ISB	Antelope & giraffe
8	Dorcas gazelle	*Gazella dorcas*	EEP & ISB	Antelope & giraffe
9	Giant eland	*Taurotragus derbianus gigas*	ISB	Antelope & giraffe
10	Mhorr gazelle	*Gazella dama mhorr*	EEP & ISB	Antelope & giraffe
11	Okapi	*Okapia johnstoni*	EEP, SSP & ISB	Antelope & giraffe
12	Yellow-backed duiker	*Cephalophus silvicultor*	SSP & ISB	Antelope & giraffe
13	Lowland anoa	*Bubalus depressicornis*	EEP, SSP & ISB	Cattle
14	Muskox	*Ovibos moschatus*	ISB	Cattle
15	Grevy's zebra	*Equus grevyi*	EEP, SSP & ISB	Equid
16	Hartmann's zebra	*Equus zebra hartmannae*	EEP & ISB	Equid
17	Somali wild ass	*Equus asinus somalicus*	EEP, PMP & ISB	Equid
18	Pygmy hippopotamus	*Hexaprotodon liberiensis*	EEP, SSP & ISB	Hippopotamus
19	Black rhinoceros	*Diceros bicornis*	EEP, SSP & ISB	Rhinoceros
20	One-horned rhinoceros	*Rhinoceros unicornis*	EEP & SSP	Rhinoceros
21	Sumatran rhinoceros	*Dicerorhinus sumatrensis*	ISB	Rhinoceros
22	White rhinoceros	*Ceratotherium simum simum*	EEP, SSP & ISB	Rhinoceros
23	Vicugna	*Vicugna vicugna*	EEP & ISB	Camelid
24	Baird's tapir	*Tapirus bairdii*	SSP & ISB	Tapir
25	Malayan tapir	*Tapirus indicus*	EEP, SSP & ISB	Tapir
26	Babirusa	*Babyrousa babyrussa*	EEP & SSP	Pig
27	Bonobo	*Pan paniscus*	EEP, SSP & ISB	Ape
28	Gorilla	*Gorilla gorilla*	EEP, SSP & ISB	Ape
29	Moloch gibbon	*Hylobates moloch*	EEP & ISB	Ape
30	Orang-utan	*Pongo pygmaeus*	EEP, SSP & ISB	Ape
31	Pileated gibbon	*Hylobates pileatus*	EEP & ISB	Ape
32	Black lion tamarin	*Leontopithecus chrysopygus*	EEP & ISB	Callitrichid
33	Cotton-top tamarin	*Saguinus oedipus*	SSP	Callitrichid
34	Goeldi's monkey	*Callimico goeldii*	EEP & ISB	Callitrichid
35	Golden lion tamarin	*Leontopithecus rosalia*	EEP, SSP & ISB	Callitrichid
36	Golden-headed lion tamarin	*Leontopithecus chrysomelas*	EEP, SSP & ISB	Callitrichid
37	Pied tamarin	*Saguinus bicolor*	EEP, SSP & ISB	Callitrichid
38	Black howler monkey	*Alouatta caraya*	EEP, SSP & ISB	Cebid
39	Diana monkey	*Cercopithecus diana*	EEP	Old world monkey
40	Drill	*Mandrillus leucophaeus*	ISB	Old world monkey
41	Douc langur	*Pygathrix nemaeus*	ISB	Old world monkey
42	Golden monkey	*Rhinopithecus roxellana*	ISB	Old world monkey
43	Lion-tailed macaque	*Macaca silenus*	SSP & ISB	Old world monkey
44	Alotran gentle lemur	*Hapalemur alaotrensis*	EEP & ISB	Prosimian
45	Aye-aye	*Daubentonia madagascariensis*	EEP & ISB	Prosimian
46	Black and white ruffed lemur	*Varecia variegata variegata*	EEP, SSP & ISB	Prosimian

Appendix J continued

#	Species	Scientific	Population	TAG
47	Black lemur	*Eulemur macaco macaco*	EEP, SSP & ISB	Prosimian
48	Black lemur	*Eulemur macaco flavifrons*	EEP, SSP & ISB	Prosimian
49	Crowned sifaka	*Propithecus verreauxi coronatus*	EEP & ISB	Prosimian
50	Grey gentle lemur	*Hapalemur griseus*	EEP & ISB	Prosimian
51	Red ruffed lemur	*Varecia rubra*	EEP, SSP & ISB	Prosimian
52	Western grey lemur	*Hapalemur occidentalis*	ISB	Prosimian
53	Goodfellow's tree kangaroo	*Dendrolagus goodfellowi*	EEP & ISB	Marsupial
54	Grizzled grey tree kangaroo	*Dendrolagus inustus*	ISB	Marsupial
55	Matschie's tree kangaroo	*Dendrolagus matschiei*	EEP, SSP & ISB	Marsupial
56	Southern koala	*Phascolarctos cinereus victor*	ISB	Marsupial
57	Giant anteater	*Myrmecophaga tridactyla*	EEP, SSP & ISB	Xenarthra
58	Asian small-clawed otter	*Aonyx cinereus*	SSP	Small carnivore
59	Fossa	*Cryptoprocta ferox*	EEP, SSP & ISB	Small carnivore
60	Giant otter	*Pteronura brasiliensis*	EEP, SSP & ISB	Small carnivore
61	Red panda	*Ailurus fulgens refulgens*	EEP, SSP & ISB	Small carnivore
62	Madagascar giant jumping rat	*Hypogeomys antimena*	ISB	Rodent
63	Giant panda	*Ailuropoda melanoleuca*	SSP & ISB	Bear
64	Polar bear	*Ursus maritimus*	EEP, SSP & ISB	Bear
65	Sloth bear	*Melursus ursinus*	EEP, SSP & ISB	Bear
66	Spectacled bear	*Tremarctos ornatus*	EEP, SSP & ISB	Bear
67	African wild dog	*Lycaon pictus*	SSP & ISB	Canid & hyaenid
68	Bush dog	*Speothos venaticus*	EEP & ISB	Canid & hyaenid
69	Maned wolf	*Chrysocyon brachyurus*	EEP, SSP & ISB	Canid & hyaenid
70	Mexican grey wolf	*Canis lupus baileyi*	SSP & ISB	Canid & hyaenid
71	Red wolf	*Canis rufus gregoryi*	SSP & ISB	Canid & hyaenid
72	Amur leopard	*Panthera pardus orientalis*	EEP, SSP & ISB	Felid
73	Amur tiger	*Panthera tigris altaica*	EEP, SSP & ISB	Felid
74	Arabian leopard	*Panthera pardus nimr*	ISB	Felid
75	Bengal tiger	*Panthera tigris tigris*	ISB	Felid
76	Black-footed cat	*Felis nigripes*	EEP & ISB	Felid
77	Caracal	*Caracal caracal*	SSP	Felid
78	Cheetah	*Acinonyx jubatus*	EEP, SSP & ISB	Felid
79	Chinese leopard	*Panthera pardus japonensis*	EEP & ISB	Felid
80	Fishing cat	*Prionailurus viverrinus*	EEP & ISB	Felid
81	Gordon's wild cat	*Felis silvestris gordoni*	ISB	Felid
82	Indochinese tiger	*Panthera tigris corbetti*	ISB	Felid
83	Pallas' cat	*Felis manul*	EEP & ISB	Felid
84	Sand cat	*Felis margarita*	EEP & ISB	Felid
85	Snow leopard	*Uncia uncia*	EEP, SSP & ISB	Felid
86	South China tiger	*Panthera tigris amoyensis*	ISB	Felid
87	Sri Lankan leopard	*Panthera pardus kotiya*	EEP & ISB	Felid
88	Sri Lankan rusty-spotted cat	*Prionailurus rubiginosus phillipsi*	ESB & ISB	Felid
89	Sumatran tiger	*Panthera tigris sumatrae*	EEP, SSP & ISB	Felid
90	Oriental white stork	*Ciconia boyciana*	EEP & ISB	Ciconiiformes
91	Great hornbill	*Buceros bicornis*	EEP & ISB	Coraciiformes
92	Blue-billed currasow	*Crax alberti*	PMP & ISB	Cracid & cuckoo
93	Red-billed currasow	*Crax blumenbachii*	ISB	Cracid & cuckoo
94	Blyth's trapoan	*Tragopan blythii*	ESB & ISB	Galliformes
95	Congo peafowl	*Afropavo congensis*	EEP, SSP & ISB	Galliformes

Appendix J continued

#	Species	Scientific	Population	TAG
96	Horned Guan	*Oreophasis derbianus*	ISB	Galliformes
97	Vietnamese pheasant	*Lophura hatinhensis*	ISB	Galliformes
98	Black-necked crane	*Grus nigricollis*	ISB	Gruiformes
99	Buff crested bustard	*Lophotis ruficrista*	PMP & ISB	Gruiformes
100	Hooded crane	*Grus monacha*	ESB, PMP & ISB	Gruiformes
101	Kori bustard	*Ardeotis kori*	SSP & ISB	Gruiformes
102	Red-crowned crane	*Grus japonensis*	EEP, SSP & ISB	Gruiformes
103	Siberian white crane	*Grus leucogeranus*	EEP & ISB	Gruiformes
104	Wattled crane	*Bugeranus carunculatus*	ESB, SSP & ISB	Gruiformes
105	White-naped crane	*Grus vipio*	EEP, SSP & ISB	Gruiformes
106	Maroon-fronted parrot	*Rhynchopsitta pachyrhyncha terrisi*	SSP & ISB	Parrot
107	Spix's macaw	*Cyanopsitta spixii*	ISB	Parrot
108	St Vincent parrot	*Amazona guildingii*	ISB	Parrot
109	Lesser bird-of-paradise	*Paradisaea minor*	PMP & ISB	Passeriformes
110	Red bird-of-paradise	*Paradisaea rubra*	PMP & ISB	Passeriformes
111	Mauritius pink pigeon	*Columba mayeri*	EEP, SSP & ISB	Pigeon

Appendix K: Calculations for demographic and environmental stochasticity as a proportion of total variance

General equation

The variance attributable to demographic stochasticity (σ_{DS}) is explained by:

$$\sigma_{DS} = \sqrt{\frac{\hat{p}\hat{q}}{n-1}}$$

Where $\hat{p}$ is the proportion of observations in a category, and $\hat{q}$ is $1-\hat{p}$ (Miller & Lacy 2005)

The variance attributable to environmental stochasticity is explained by:

$$\sigma_{EV} = \sqrt{\sigma^2_{EV}} = \sqrt{\sigma^2_{TOT} - \overline{\sigma^2_{DS}}}$$

Where σ^2_{TOT} is the total variance across the data, and $\overline{\sigma^2_{DS}}$ is the mean binomial variance across individual mortality rates or female breeding rates (Miller & Lacy 2005).

Females breeding (variable female breeding model)

$$\sigma_{DS} = \sqrt{\frac{0.0788 * 0.9212}{3907-1}} = 0.004311$$

$$\sigma_{EV} = \sqrt{\sigma^2_{EV}} = \sqrt{0.0788^2 - 0.0043^2} = 0.078683 = 7.87\%$$

Females breeding (density dependent female breeding model)

$$\sigma_{DS} = \sqrt{\frac{0.42 * 0.58}{3907-1}} = 0.0079$$

$$\sigma_{EV} = \sqrt{\sigma^2_{EV}} = \sqrt{0.11^2 - 0.0079^2} = 0.1097 = 10.97\%$$

Mortality

Females: Age class 0 – 1 years

$$\sigma_{DS} = \sqrt{\frac{0.2719 * 0.7281}{1008 - 1}} = 0.014021$$

$$\sigma_{EV} = \sqrt{\sigma^2{}_{EV}} = \sqrt{0.0753^2 - 0.0140^2} = 0.073983 = 7.3983\%$$

Females: Age class 1 – 2 years

$$\sigma_{DS} = \sqrt{\frac{0.0464 * 0.9536}{629 - 1}} = 0.008394$$

$$\sigma_{EV} = \sqrt{\sigma^2{}_{EV}} = \sqrt{0.0311^2 - 0.0084^2} = 0.029946 = 2.9946\%$$

Females: Age class 2 – 3 years

$$\sigma_{DS} = \sqrt{\frac{0.0487 * 0.9513}{551 - 1}} = 0.009175$$

$$\sigma_{EV} = \sqrt{\sigma^2{}_{EV}} = \sqrt{0.0420^2 - 0.0092^2} = 0.040986 = 4.0986\%$$

Females: Age class >3 years

$$\sigma_{DS} = \sqrt{\frac{0.0725 * 0.9275}{3929 - 1}} = 0.004138$$

$$\sigma_{EV} = \sqrt{\sigma^2{}_{EV}} = \sqrt{0.0182^2 - 0.0041^2} = 0.017723 = 1.7723\%$$

Males: Age class 0 – 1 years

$$\sigma_{DS} = \sqrt{\frac{0.3572 * 0.6428}{1113 - 1}} = 0.014369$$

$$\sigma_{EV} = \sqrt{\sigma^2{}_{EV}} = \sqrt{0.0721^2 - 0.0144^2} = 0.070654 = 7.0654\%$$

Males: Age class 1 – 2 years

$$\sigma_{DS} = \sqrt{\frac{0.1474 * 0.8526}{529 - 1}} = 0.015428$$

$$\sigma_{EV} = \sqrt{\sigma^2{}_{EV}} = \sqrt{0.0764^2 - 0.0154^2} = 0.074826 = 7.4826\%$$

Males: Age class 2 – 3 years

$$\sigma_{DS} = \sqrt{\frac{0.0885 * 0.9115}{361 - 1}} = 0.014969$$

$$\sigma_{EV} = \sqrt{\sigma^2{}_{EV}} = \sqrt{0.0732^2 - 0.0150^2} = 0.071653 = 7.1653\%$$

Males: Age class 3 – 4 years

$$\sigma_{DS} = \sqrt{\frac{0.0906 * 0.9094}{303 - 1}} = 0.016517$$

$$\sigma_{EV} = \sqrt{\sigma^2{}_{EV}} = \sqrt{0.1048^2 - 0.0165^2} = 0.103490 = 10.3490\%$$

Males: Age class >4 years

$$\sigma_{DS} = \sqrt{\frac{0.1434 * 0.8566}{1209 - 1}} = 0.010084$$

$$\sigma_{EV} = \sqrt{\sigma^2{}_{EV}} = \sqrt{0.0496^2 - 0.0101^2} = 0.048564 = 4.8564\%$$

Appendix L: sensitivity testing of the genetic management strategies

None of the populations went extinct in the mean kinship models and only three populations went extinct in the random mating model, resulting in a zero probability of extinction under genetic management, and a 0.6% probability of extinction under the random mating model (Figure L.1). All three scenarios met the goal of 99% probability of population persistence for 100-years. The two mean kinship models were predicted to persist beyond the 100-year time-frame, and the mean time to extinction for the random mating model 97.7-years. Median time to extinction was infinite for all three models. The stochastic mean growth rates (Figure L.2) were negative across all years for the three models, indicating that extinction would be the eventual fate of the population if it had been modelled over an extended period of time.

There was a difference between the three genetic management models in the population size ($H_2 = 219.82$, $P < 0.001$), the retention of gene diversity ($H_2 = 550.54$, $P < 0.001$), and mean inbreeding ($H_2 = 322.24$, $P < 0.001$) of extant populations after 100-years, and this difference was evident between all the pairwise models (Table L.1). Furthermore, the mean number of alleles per diploid after 100-years differed between each model with the dynamic mean kinship model retaining the most alleles (and lethal alleles) and the random model retaining the least alleles (and lethal alleles) (Figure L.3). These results indicate that the model was sensitive to the genetic management strategy.

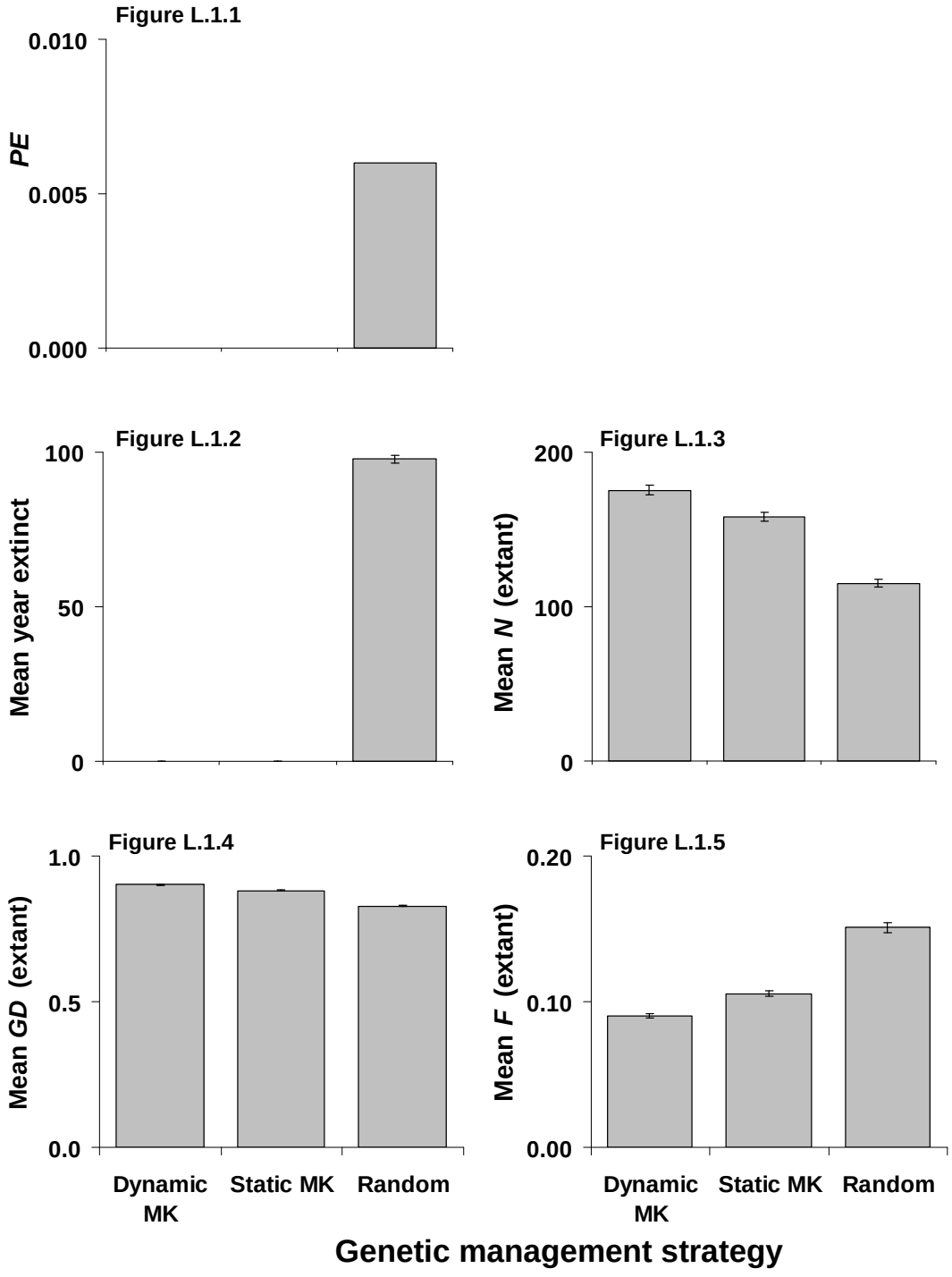

Figure L.1 The probability of extinction (**L.1.1**), mean year of extinction (**L.1.2**), mean population size (of extant populations) (**L.1.3**), mean gene diversity of extant populations (**L.1.2**), and mean inbreeding (of extant populations) (**L.1.4**) for the three genetic management scenarios (dynamic MK, static MK, and random mating).

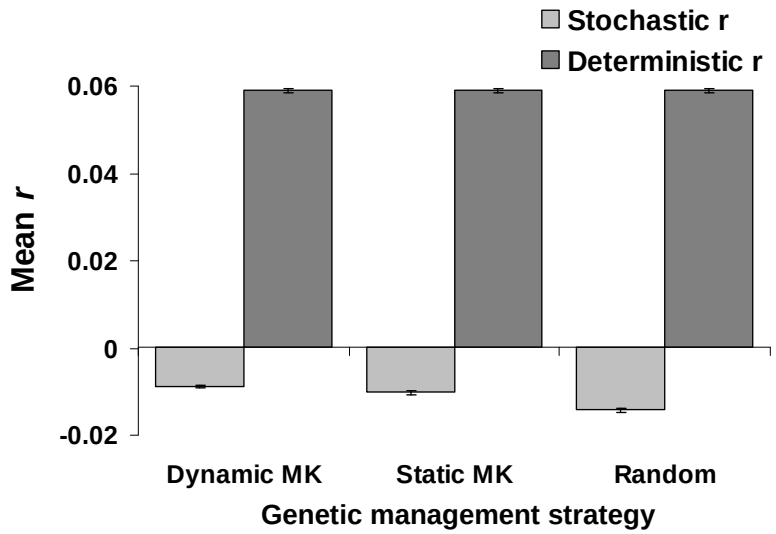

Figure L.2 The mean stochastic and deterministic growth rates (*r*) of the population for the three genetic management scenarios

Table L.1 Results from the Mann-Whitney pairwise tests for population size, gene diversity and mean inbreeding for the three genetic management scenarios

	Variable		
Model	**Population size**	**Gene diversity**	**Mean inbreeding**
Dynamic MK v static MK	W=267096.5 P=0.0002	W=296996.5 P<0.001	W=223625.5 P<0.001
Dynamic MK v random	W=313612.5 P<0.001	W=349351 P<0.001	W=171712 P<0.001
Static v random	W=297568.5 P<0.001	W=320590.5 P<0.001	W=192477.5 P<0.001

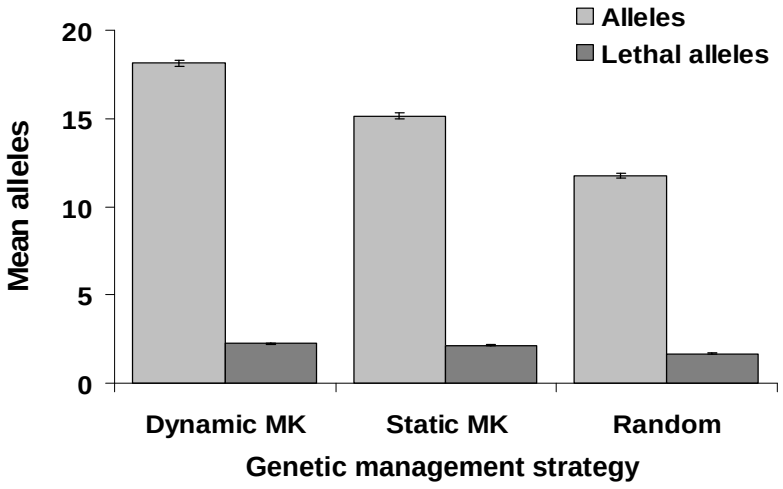

Figure L.3 The mean number of alleles and lethal alleles per diploid present in the population after 100-years for the three genetic management scenarios

Appendix M: sensitivity testing of the genetic management strategies

The goal of a 99% chance of population persistence was not met until the annual female reproductive rate reached 53%. The probability of extinction (*PE*) decreased as female reproduction increased until it reached zero for the 55% female reproduction scenario (Figure M.1). There was a corresponding increase in mean time to extinction (H_{11} = 147.85, $P < 0.001$). Median time to extinction was 83, 89, and 92 years for the 42%, 43% and 44% scenarios respectively, but zero for all of the remaining scenarios. Most of the difference in time to extinction lay between the scenarios with lower female reproduction. Once adult female reproduction reached an annual rate of 46%, time to extinction did not vary between the scenarios (Table N.1 Appendix N), indicating that increases in reproduction at low reproductive outputs increase persistence time of populations.

The mean size (H_{13} = 2911.78, $P < 0.001$) and mean gene diversity (H_{13} = 2136.51, $P < 0.001$) of extant populations increased as female reproduction increased, and mean inbreeding decreased as female reproduction increased (H_{13} = 337.22, $P < 0.001$). The mean size of extant populations differed between all of the scenarios, with the exception of 42-43% and 44-45% comparisons (Table N.2 Appendix N). This indicated that even when mean time to extinction did not differ between models (when female reproduction was high), the size of the remnant population did. Similarly, the mean gene diversity retained after 100-years increased once female reproduction increased above 45% (with the occasional exception between close congeners (Table N.3 Appendix N)). Mean inbreeding after 100-years was lower when female reproduction was high (51% and above), but did not differ between models when reproduction was low (with the exception of close congeners) (Table N.4 Appendix N). Furthermore, both the mean number of alleles and the mean number of lethal alleles per diploid after 100-years increased as female reproduction increased (Figure M.2).

The stochastic mean growth rates (Figure M.3) were negative for the 14 models even though deterministic growth rates were positive once adult female reproduction reached 46%. This would eventually result in the extinction of the population over a longer time scale than was modelled here if long-term growth rates were not improved in the future. These results indicated that the model was sensitive to both genetic and demographic metrics.

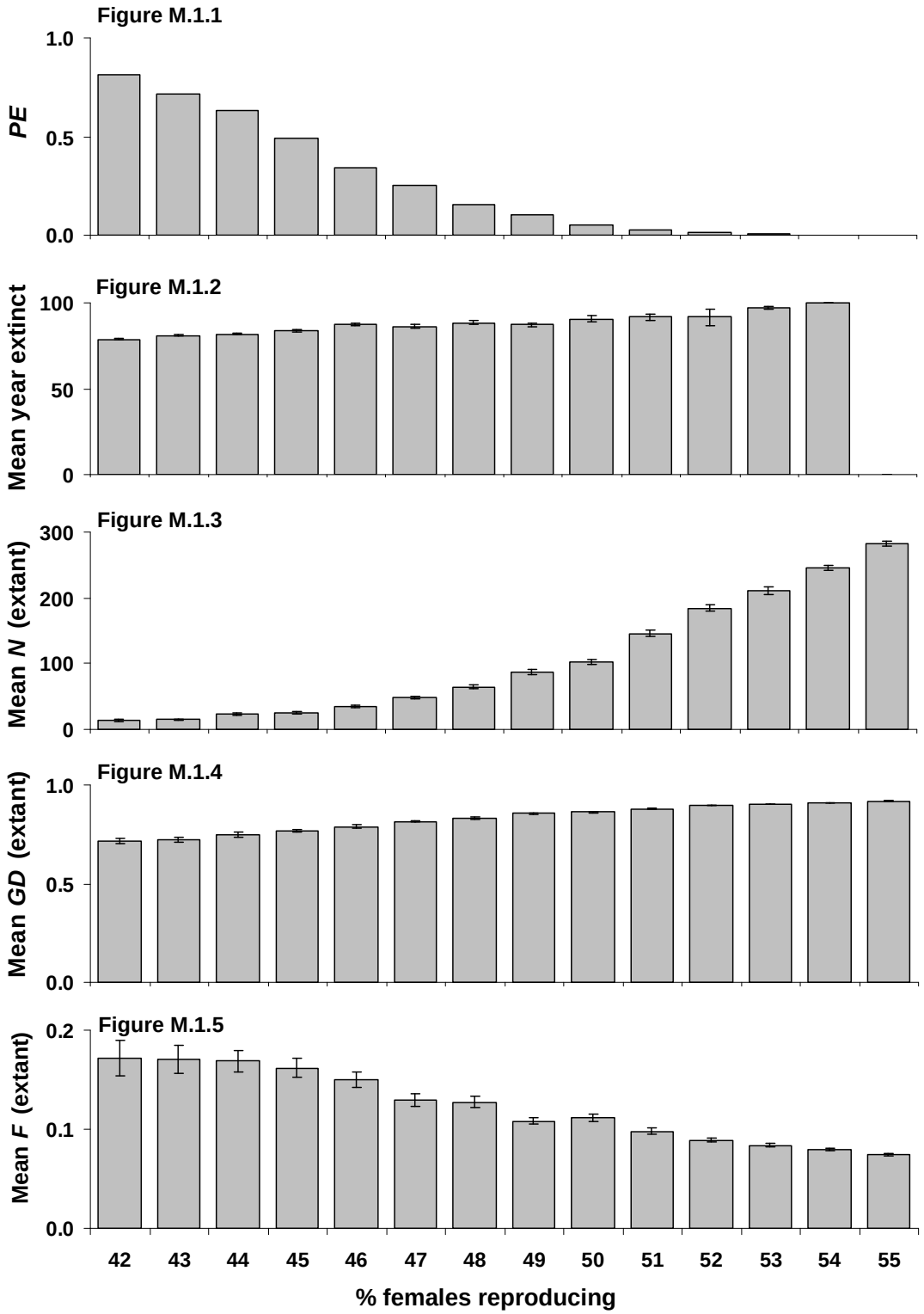

Figure M.1 The probability of extinction (**M.1.1**), mean year of extinction (**M.1.2**), mean population size (extant populations) (**M.1.3**), mean gene diversity (extant populations) (**M.1.4**), and mean inbreeding (extant populations) (**M.1.5**) for the 14 female reproduction scenarios (EEP at 42%, and then increasing female reproduction between 43 and 55% in increments of 1%)

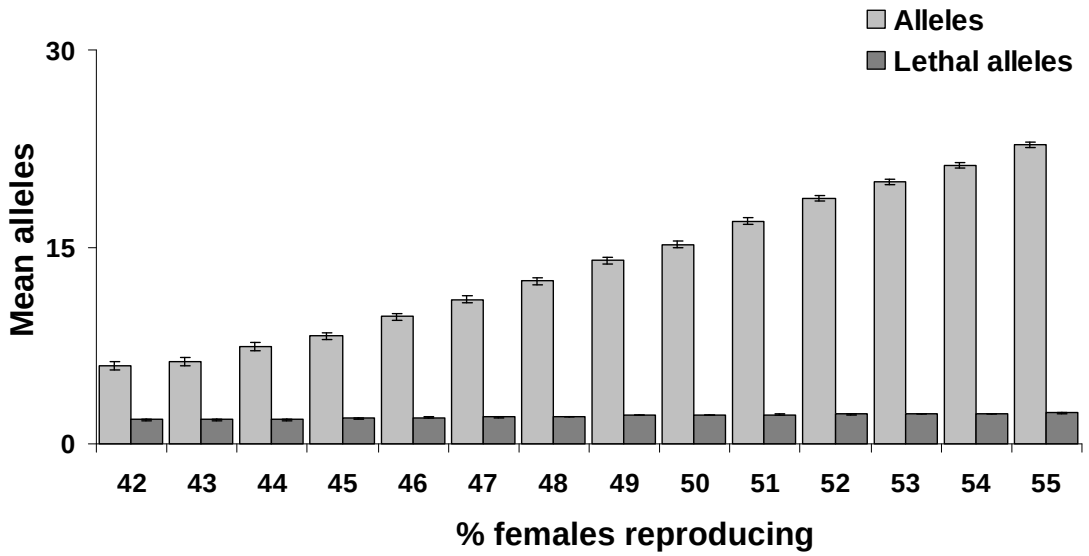

Figure M.2 The mean number of alleles and lethal alleles per diploid present in the population after 100-years for the 14 female reproduction scenarios

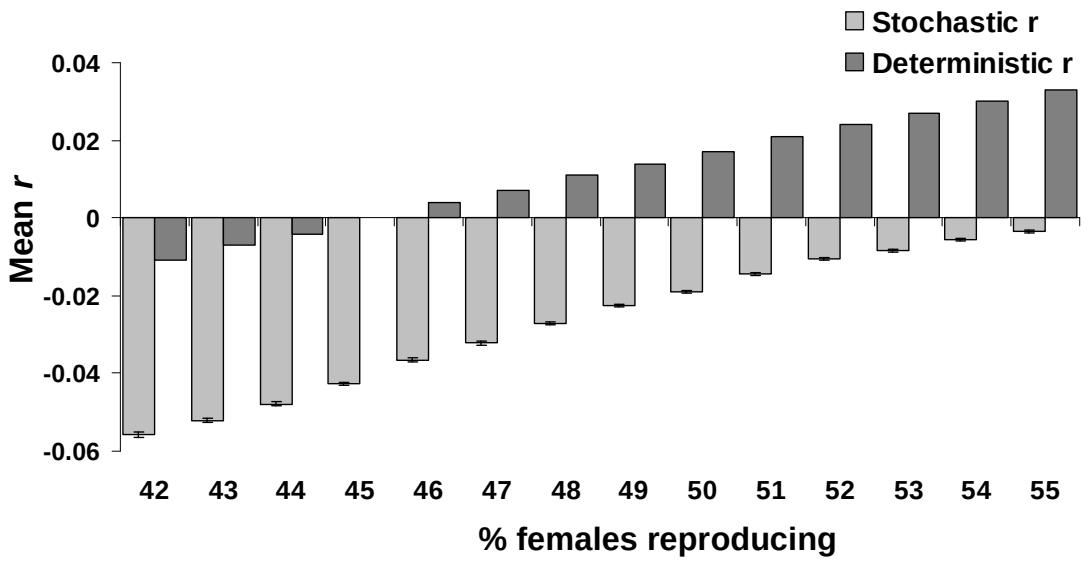

Figure M.3 The mean stochastic and deterministic growth rates (*r*) of the population for the 14 female reproduction scenarios

Appendix N: results of the Mann-Whitney U test with Bonferroni correction

N.1 Female reproduction model (Tables N.1 – N.4)

Table N.1 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean time to extinction for the 14 scenarios of the female reproduction model. The 54% and 55% scenarios are not included as none of their simulated populations went extinct. Cells highlight in light grey indicate a difference between scenarios

Percentage of females breeding						
	42%	**43%**	**44%**	**45%**	**46%**	**47%**
42%		W=149387 P=0.0173	W=138490 P=0.0009	W=121899 P<0.001	W=104295 P<0.001	W=100164 P<0.001
43%			W=118639 P=0.3169	W=102668 P=0.0062	W=85637 P<0.001	W=81378 P<0.001
44%				W=84871 P=0.787	W=69261 P<0.001	W=65486 P=0.0004
45%					W=46785 P=0.0013	W=43439 P=0.0471
46%						W=26016 P=0.3492
47%						
48%						

	48%	**49%**	**50%**	**51%**	**52%**	**53%**
42%	W=91916 P<0.001	W=89715 P<0.001	W=85741 P<0.001	W=84359 P=0.0002	W=83946 P=0.0141	W=83542 P=0.0027
43%	W=73183 P<0.001	W=70957 P=0.0004	W=66915 P<0.001	W=65583 P=0.0011	W=65117 P=0.0238	W=64740 P=0.0042
44%	W=57742 P<0.001	W=55741 P=0.0033	W=51900 P<0.001	W=50677 P=0.0022	W=50244 P=0.0366	W=49885 P=0.005
45%	W=36824 P=0.0009	W=35056 P=0.0566	W=31730 P=0.0004	W=30710 P=0.0101	W=30290 P=0.0577	W=30002 P=0.0083
46%	W=20451 P=0.2915	W=19011 P=0.7255	W=16114 P=0.0191	W=15260 P=0.0941	W=14880 P=0.1795	W=14642 P=0.0192
47%	W=12247 P=0.0809	W=11239 P=0.6826	W=9201 P=0.0049	W=8634 P=0.0546	W=8377 P=0.1521	W=8192 P=0.0112
48%		W=5086 P=0.2765	W=3690 P=0.0847	W=3307 P=0.3641	W=3078 P=0.1752	W=2985 P=0.0406
49%			W=1773 P=0.0198	W=1537 P=0.0978	W=1427 P=0.1801	W=1351 P=0.0131
50%				W=524 P=0.6030	W=415 P=0.5128	W=387 P=0.3415
51%					W=107 P=0.5105	W=88 P=0.1001
52%						W=34 P=1.000
53%						

Table N.2 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean population size of extant populations for the 14 scenarios of the female reproduction model. Pairwise differences between scenarios are highlighted in light grey

	Percentage of females breeding						
	42%	**43%**	**44%**	**45%**	**46%**	**47%**	**48%**
42%		W=10496 P=0.5936	W=10692 P=0.0008	W=11943 P<0.001	W=12253 P<0.001	W=10954 P<0.001	W=10107 P<0.001
43%			W=20387 P=0.0015	W=22606 P<0.001	W=23240 P<0.001	W=21247 P<0.001	W=19837 P<0.001
44%				W=38409 P=0.0607	W=39785 P<0.001	W=37192 P<0.001	W=35324 P<0.001
45%					W=68161 P=0.0005	W=64639 P<0.001	W=61865 P<0.001
46%						W=105714 P<0.001	W=102857 P<0.001
47%							W=137289 P=0.0004
48%							

	Percentage of females breeding						
	49%	**50%**	**51%**	**52%**	**53%**	**54%**	**55%**
42%	W=8513 P<0.001	W=7680 P<0.001	W=6623 P<0.001	W=5313 P<0.001	W=5079 P<0.001	W=4580 P<0.001	W=4396 P<0.001
43%	W=17275 P<0.001	W=15865 P<0.001	W=14048 P<0.001	W=11856 P<0.001	W=11358 P<0.001	W=10544 P<0.001	W=10209 P<0.001
44%	W=31047 P<0.001	W=28818 P<0.001	W=25457 P<0.001	W=21539 P<0.001	W=20518 P<0.001	W=18730 P<0.001	W=17930 P<0.001
45%	W=55308 P<0.001	W=51619 P<0.001	W=46002 P<0.001	W=39872 P<0.001	W=38048 P<0.001	W=35118 P<0.001	W=33886 P<0.001
46%	W=93391 P<0.001	W=87781 P<0.001	W=78349 P<0.001	W=68228 P<0.001	W=64696 P<0.001	W=59511 P<0.001	W=57195 P<0.001
47%	W=125971 P<0.001	W=118831 P<0.001	W=105484 P<0.001	W=91738 P<0.001	W=86054 P<0.001	W=78088 P<0.001	W=74256 P<0.001
48%	W=167336 P<0.001	W=159430 P<0.001	W=142136 P<0.001	W=124494 P<0.001	W=116532 P<0.001	W=105394 P<0.001	W=99173 P<0.001
49%		W=193893 P=0.0008	W=172972 P<0.001	W=151975 P<0.001	W=141344 P<0.001	W=126572 P<0.001	W=117451 P<0.001
50%			W=200934 P<0.001	W=177711 P<0.001	W=164578 P<0.001	W=146466 P<0.001	W=134501 P<0.001
51%				W=214580 P<0.001	W=199520 P<0.001	W=179077 P<0.001	W=162171 P<0.001
52%					W=227611 P=0.0001	W=205650 P<0.001	W=184949 P<0.001
53%						W=224298 P<0.001	W=201696 P<0.001
54%							W=223449 P<0.001
55%							

Table N.3 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean gene diversity of extant populations for the 14 scenarios of the female reproduction model. Pairwise differences between scenarios are highlighted in light grey

Percentage of females breeding							
	42%	**43%**	**44%**	**45%**	**46%**	**47%**	**48%**
42%		W=10518 P=0.6254	W=11178 P=0.103	W=12822 P=0.0001	W=12978 P<0.001	W=12230 P<0.001	W=11609 P<0.001
43%			W=21091 P=0.0200	W=23735 P=0.0001	W=24093 P<0.001	W=22893 P<0.001	W=21991 P<0.001
44%				W=38713 P=0.1001	W=40189 P<0.001	W=38954 P<0.001	W=38172 P<0.001
45%					W=68326 P=0.0008	W=66637 P<0.001	W=65517 P<0.001
46%						W=107431 P=0.0012	W=106791 P<0.001
47%							W=139655 P=0.0047
48%							

Percentage of females breeding							
	49%	**50%**	**51%**	**52%**	**53%**	**54%**	**55%**
42%	W=9928 P<0.001	W=9429 P<0.001	W=71660 P<0.001	W=6067 P<0.001	W=5705 P<0.001	W=5124 P<0.001	W=4621 P<0.001
43%	W=19432 P<0.001	W=18617 P<0.001	W=15761 P<0.001	W=13187 P<0.001	W=12540 P<0.001	W=11604 P<0.001	W=10713 P<0.001
44%	W=34710 P<0.001	W=33454 P<0.001	W=29242 P<0.001	W=25227 P<0.001	W=24030 P<0.001	W=22189 P<0.001	W=20524 P<0.001
45%	W=60396 P<0.001	W=58587 P<0.001	W=51594 P<0.001	W=45193 P<0.001	W=43089 P<0.001	W=40018 P<0.001	W=37185 P<0.001
46%	W=99698 P<0.001	W=96842 P<0.001	W=86401 P<0.001	W=76635 P<0.001	W=73051 P<0.001	W=67880 P<0.001	W=63149 P<0.001
47%	W=132408 P<0.001	W=129138 P<0.001	W=116607 P<0.001	W=104485 P<0.001	W=99511 P<0.001	W=92557 P<0.001	W=85740 P<0.001
48%	W=172492 P=0.0006	W=168474 P<0.001	W=154508 P<0.001	W=140020 P<0.001	W=133477 P<0.001	W=124718 P<0.001	W=115548 P<0.001
49%		W=197856 P=0.0179	W=182373 P<0.001	W=157885 P<0.001	W=147182 P<0.001	W=135946 P<0.001	W=209965 P<0.001
50%			W=193515 P<0.001	W=184614 P<0.001	W=184614 P<0.001	W=173321 P<0.001	W=160978 P<0.001
51%				W=220210 P<0.001	W=209471 P<0.001	W=196084 P<0.001	W=180730 P<0.001
52%					W=232257 P=0.0054	W=218159 P<0.001	W=200655 P<0.001
53%						W=232287 P=0.0012	W=214698 P<0.001
54%							W=230604 P<0.001
55%							

Table N.4 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean inbreeding of extant populations for the 14 scenarios of the female reproduction model. Pairwise differences between scenarios are highlighted in light grey

	Percentage of females breeding						
	42%	**43%**	**44%**	**45%**	**46%**	**47%**	**48%**
42%		W=10767 P=0.9960	W=12602.5 P=0.7675	W=4585 P<0.001	W=19879 P=0.6843	W=23136 P=0.1407	W=25902 P=0.1019
43%			W=22729 P=0.6997	W=28050 P=0.9934	W=33769 P=0.7156	W=38758 P=0.1025	W=42970 P=0.0676
44%				W=41474 P=0.6554	W=49507 P=0.2725	W=56714 P=0.0052	W=62599 P=0.0020
45%					W=76371 P=0.5434	W=86571 P=0.0080	W=94830 P=0.0020
46%						W=122811 P=0.0133	W=133682 P=0.0021
47%							W=150245 P=0.6620

	Percentage of females breeding						
	49%	**50%**	**51%**	**52%**	**53%**	**54%**	**55%**
42%	W=28151 P=0.0184	W=29227 P=0.0285	W=31217 P=0.0023	W=32351 P=0.0003	W=33013 P=0.0001	W=33742 P<0.001	W=34454 P<0.001
43%	W=46436 P=0.0069	W=48131 P=0.111	W=51204 P=0.0004	W=53027 P<0.001	W=54072 P<0.001	W=55119 P<0.001	W=56193 P<0.001
44%	W=67447 P<0.001	W=69697 P=0.0001	W=74257 P<0.001	W=76859 P<0.001	W=78423 P<0.001	W=80079 P<0.001	W=81771 P<0.001
45%	W=101711 P<0.001	W=104903 P<0.001	W=111795 P<0.001	W=115667 P<0.001	W=118276 P<0.001	W=120927 P<0.001	W=123738 P<0.001
46%	W=142617 P<0.001	W=146744 P<0.001	W=156703 P<0.001	W=162024 P<0.001	W=165796 P<0.001	W=169643 P<0.001	W=173838 P<0.001
47%	W=159056 P=0.1005	W=163187 P=0.1544	W=173856 P=0.0003	W=179912 P<0.001	W=184132 P<0.001	W=188964 P<0.001	W=193830 P<0.001
48%	W=189150 P=0.2997	W=193836 P=0.4026	W=0.205657 P=0.0023	W=211926 P<0.001	W=217260 P<0.001	W=223242 P<0.001	W=229196 P<0.001
49%		W=206522 P=0.8211	W=220193 P=0.0202	W=226947 P=0.0003	W=233688 P<0.001	W=241222 P<0.001	W=248900 P<0.001
50%			W=239127 P=0.0114	W=246183 P=0.0001	W=253399 P<0.001	W=261479 P<0.001	W=269546 P<0.001
51%				W=244894 P=0.2566	W=252971 P=0.0046	W=262662 P<0.001	W=0.27203 8 P<0.001
52%					W=253189 P=0.0615	W=263976 P<0.001	W=274348 P<0.001
53%						W=257073 P=0.0264	W=267572 P<0.001
54%							W=259645 P=0.0261
55%							

N.2 Lethal equivalent model (Tables N.5 – N.7)

Table N.5 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean population size of extant populations for the 14 scenarios of the lethal equivalents model. Pairwise differences between scenarios are highlighted in light grey

Lethal equivalent models								
	0	**0.929**	**1**	**2**	**3**	**3.14**	**4**	**5**
0		W=263429 P=0.0039	W=267552 P=0.0002	W=285311 P<0.001	W=299680 P<0.001	W=301811 P<0.001	W=320121 P<0.001	W=343051 P<0.001
0.929			W=254842 P=0.3147	W=273342 P<0.001	W=289352 P<0.001	W=290912 P<0.001	W=312223 P<0.001	W=336972 P<0.001
1				W=268433 P=0.0001	W=284494 P<0.001	W=285905 P<0.001	W=307633 P<0.001	W=332584 P<0.001
2					W=268155 P=0.0001	W=268806 P<0.001	W=294265 P<0.001	W=321704 P<0.001
3						W=250734 P=0.9154	W=277204 P<0.001	W=305075 P<0.001
3.14							W=277170 P<0.001	W=305535 P<0.001
4								W=277569 P<0.001
5								

Lethal equivalent models									
	6	**6.97**	**7**	**8**	**9**	**9.26**	**10**	**11**	**12**
0	W=355182 P<0.001	W=364781 P<0.001	W=364064 P<0.001	W=370519 P<0.001	W=370587 P<0.001	W=373653 P<0.001	W=368874 P<0.001	W=360493 P<0.001	W=339770 P<0.001
0.929	W=351595 P<0.001	W=362687 P<0.001	W=362084 P<0.001	W=369642 P<0.001	W=370074 P<0.001	W=373290 P<0.001	W=368628 P<0.001	W=360750 P<0.001	W=340250 P<0.001
1	W=348378 P<0.001	W=360163 P<0.001	W=359502 P<0.001	W=368218 P<0.001	W=369194 P<0.001	W=372611 P<0.001	W=368259 P<0.001	W=360282 P<0.001	W=339701 P<0.001
2	W=341887 P<0.001	W=356296 P<0.001	W=355430 P<0.001	W=366694 P<0.001	W=368384 P<0.001	W=372248 P<0.001	W=368032 P<0.001	W=360202 P<0.001	W=339711 P<0.001
3	W=330324 P<0.001	W=347430 P<0.001	W=346593 P<0.001	W=361930 P<0.001	W=365043 P<0.001	W=369876 P<0.001	W=366556 P<0.001	W=359574 P<0.001	W=339501 P<0.001
3.14	W=329965 P<0.001	W=347318 P<0.001	W=346210 P<0.001	W=361646 P<0.001	W=365021 P<0.001	W=369679 P<0.001	W=366649 P<0.001	W=359714 P<0.001	W=339574 P<0.001
4	W=308098 P<0.001	W=329328 P<0.001	W=328121 P<0.001	W=350286 P<0.001	W=357025 P<0.001	W=363200 P<0.001	W=362622 P<0.001	W=357892 P<0.001	W=338873 P<0.001
5	W=285608 P<0.001	W=311144 P<0.001	W=309843 P<0.001	W=338157 P<0.001	W=348670 P<0.001	W=356083 P<0.001	W=358259 P<0.001	W=355892 P<0.001	W=337975 P<0.001
6		W=278404 P<0.001	W=276832 P<0.001	W=312578 P<0.001	W=329310 P<0.001	W=338783 P<0.001	W=346087 P<0.001	W=349117 P<0.001	W=334634 P<0.001
6.97			W=248104 P=0.8013	W=286671 P<0.001	W=308606 P<0.001	W=319420 P<0.001	W=331056 P<0.001	W=339561 P<0.001	W=329161 P<0.001
7				W=286832 P<0.001	W=308079 P<0.001	W=318800 P<0.001	W=329750 P<0.001	W=337762 P<0.001	W=327709 P<0.001
8					W=276582 P<0.001	W=288834 P<0.001	W=304815 P<0.001	W=321147 P<0.001	W=318024 P<0.001
9						W=256944 P=0.0183	W=273086 P<0.001	W=292838 P<0.001	W=295936 P<0.001
9.26							W=265850 P<0.001	W=286921 P<0.001	W=291911 P<0.001
10								W=262648 P<0.001	W=271799 P<0.001
11									W=235738 P<0.001
12									

Table N.6 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean gene diversity of extant populations for the 14 scenarios of the lethal equivalents model. Pairwise differences between scenarios are highlighted in light grey

	Lethal equivalent models							
	0	**0.929**	**1**	**2**	**3**	**3.14**	**4**	**5**
0		W=252195 P=0.6703	W=253313 P=0.5025	W=253541 P<0.4712	W=268213 P=0.0001	W=268189 P<0.0001	W=273945 P<0.001	W=289602 P<0.001
0.929			W=251447 P=0.7934	W=251658 P=0.7579	W=266525 P=0.0004	W=266352 P=0.0004	W=272234 P<0.001	W=288064 P<0.001
1				W=250160 P=0.9844	W=265344 P=0.0009	W=265057 P=0.0012	W=271140 P<0.001	W=286953 P<0.001
2					W=265305 P=0.001	W=265157 P=0.0011	W=271009 P<0.001	W=286936 P<0.001
3						W=249866 P=0.933	W=255889 P=0.2170	W=272438 P<0.001
3.14							W=256427 P=0.1762	W=273027 P<0.001
4								W=266819 P=0.0003
5								

	Lethal equivalent models								
	6	**6.97**	**7**	**8**	**9**	**9.26**	**10**	**11**	**12**
0	W=298685 P<0.001	W=302223 P<0.001	W=307952 P<0.001	W=327191 P<0.001	W=335444 P<0.001	W=336694 P<0.001	W=340822 P<0.001	W=339223 P<0.001	W=329209 P<0.001
0.929	W=297171 P<0.001	W=125250 P<0.001	W=306685 P<0.001	W=326267 P<0.001	W=334746 P<0.001	W=336031 P<0.001	W=340423 P<0.001	W=338805 P<0.001	W=329087 P<0.001
1	W=296458 P<0.001	W=300639 P<0.001	W=305895 P<0.001	W=326164 P<0.001	W=334880 P<0.001	W=336021 P<0.001	W=340592 P<0.001	W=338913 P<0.001	W=329352 P<0.001
2	W=296361 P<0.001	W=300503 P<0.001	W=305838 P<0.001	W=325689 P<0.001	W=334272 P<0.001	W=335500 P<0.001	W=339779 P<0.001	W=338249 P<0.001	W=328536 P<0.001
3	W=282291 P<0.001	W=287376 P<0.001	W=292230 P<0.001	W=314079 P<0.001	W=325060 P<0.001	W=326637 P<0.001	W=332222 P<0.001	W=332382 P<0.001	W=325245 P<0.001
3.14	W=283042 P<0.001	W=288146 P<0.001	W=293172 P<0.001	W=315154 P<0.001	W=325846 P<0.001	W=327342 P<0.001	W=332950 P<0.001	W=332859 P<0.001	W=325594 P<0.001
4	W=276883 P<0.001	W=282447 P<0.001	W=287300 P<0.001	W=309715 P<0.001	W=321438 P<0.001	W=322886 P<0.001	W=329095 P<0.001	W=330025 P<0.001	W=323807 P<0.001
5	W=260478 P=0.0251	W=267298 P=0.0001	W=271427 P<0.001	W=295192 P<0.001	W=309021 P<0.001	W=310906 P<0.001	W=318040 P<0.001	W=321038 P<0.001	W=317702 P<0.001
6		W=257563 P=0.0972	W=261024 P=0.0156	W=285383 P<0.001	W=300567 P<0.001	W=302781 P<0.001	W=310398 P<0.001	W=314826 P<0.001	W=313228 P<0.001
6.97			W=252221 P=0.5143	W=275614 P<0.001	W=291142 P<0.001	W=293278 P<0.001	W=300884 P<0.001	W=306651 P<0.001	W=306238 P<0.001
7				W=273958 P<0.001	W=290597 P<0.001	W=292817 P<0.001	W=301254 P<0.001	W=307198 P<0.001	W=307519 P<0.001
8					W=268253 P<0.001	W=271039 P<0.001	W=280207 P<0.001	W=290072 P<0.001	W=294380 P<0.001
9						W=248648 P=0.4532	W=257159 P=0.0027	W=269236 P<0.001	W=275871 P<0.001
9.26							W=253626 P=0.0273	W=265824 P<0.001	W=272497 P<0.001
10								W=253198 P<0.001	W=261584 P<0.001
11									W=230328 P<0.001
12									

Table N.7 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean inbreeding of extant populations for the 14 scenarios of the lethal equivalents model. Pairwise differences between scenarios are highlighted in light grey

	Lethal equivalent models							
	0	**0.929**	**1**	**2**	**3**	**3.14**	**4**	**5**
0		W=251108 P=0.8511	W=248368 P=0.6803	W=252025 P=0.6977	W=239635 P=0.0201	W=246942 P=0.4689	W=239327 P=0.0168	W=231183 P<0.001
0.929			W=247737 P=0.5822	W=251178 P=0.8391	W=238820 P=0.0123	W=246145 P=0.3687	W=238601 P=0.0107	W=230853 P<0.001
1				W=253642 P=0.4577	W=241051 P=0.0440	W=248532 P=0.7068	W=241134 P=0.0459	W=232934 P=0.0001
2					W=237690 P=0.0060	W=245158 P=0.2649	W=237665 P=0.0059	W=229840 P<0.001
3						W=257934 P=0.0925	W=250106 P=0.9749	W=241671 P=0.0603
3.14							W=242474 P=0.0886	W=234461 P=0.0005
4								W=241975 P=0.0700
5								

	Lethal equivalent models								
	6	**6.97**	**7**	**8**	**9**	**9.26**	**10**	**11**	**12**
0	W=227819 P<0.001	W=222662 P<0.001	W=226725 P<0.001	W=213821 P<0.001	W=204988 P<0.001	W=209774 P<0.001	W=203796 P<0.001	W=199392 P<0.001	W=193279 P<0.001
0.929	W=227131 P<0.001	W=222525 P<0.001	W=226412 P<0.001	W=213822 P<0.001	W=204895 P<0.001	W= 097910 P<0.001	W=203910 P<0.001	W=199591 P<0.001	W=193387 P<0.001
1	W=229139 P<0.001	W=224195 P<0.001	W=228134 P<0.001	W=215214 P<0.001	W=205871 P<0.001	W=210773 P<0.001	W=204757 P<0.001	W=200332 P<0.001	W=193938 P<0.001
2	W=226246 P<0.001	W=221807 P<0.001	W=225694 P<0.001	W=213114 P<0.001	W=204281 P<0.001	W=209304 P<0.001	W=203439 P<0.001	W=199152 P<0.001	W=193051 P<0.001
3	W=238002 P=0.0073	W=232087 P=0.0001	W=236728 P=0.0036	W=223487 P<0.001	W=213520 P<0.001	W=218189 P<0.001	W=211854 P<0.001	W=206214 P<0.001	W=198769 P<0.001
3.14	W=230626 P<0.001	W=225696 P<0.001	W=229895 P<0.001	W=217002 P<0.001	W=207664 P<0.001	W=212509 P<0.001	W=206540 P<0.001	W=201667 P<0.001	W=195055 P<0.001
4	W=238288 P=0.0088	W=232524 P=0.0001	W=236856 P=0.0039	W=223771 P<0.001	W=213854 P<0.001	W=218290 P<0.001	W=212148 P<0.001	W=206682 P<0.001	W=199062 P<0.001
5	W=246233 P=0.3791	W=240019 P=0.0286	W=244729 P=0.2477	W=231638 P=0.0001	W=220987 P<0.001	W=225223 P<0.001	W=218721 P<0.001	W=212278 P<0.001	W=203733 P<0.001
6		W=243990 P=0.1875	W=248778 P=0.7887	W=235990 P=0.0021	W=225253 P<0.001	W=229076 P<0.001	W=222968 P<0.001	W=215719 P<0.001	W=206680 P<0.001
6.97			W=254069 P=0.2900	W=241840 P=0.1036	W=230833 P=0.0001	W=234286 P=0.0020	W=228139 P<0.001	W=220634 P<0.001	W=211005 P<0.001
7				W=236823 P=0.0063	W=225804 P<0.001	W=229605 P<0.001	W=223459 P<0.001	W=216222 P<0.001	W=207232 P<0.001
8					W=237411 P<0.0166	W=240662 P=0.0935	W=234160 P=0.005	W=225692 P=0.0001	W=215172 P<0.001
9						W=248157 P=0.5214	W=241833 P=0.6611	W=232183 P=0.0991	W=220778 P=0.038
9.26							W=239318 P=0.3160	W=229829 P=0.0283	W=218966 P=0.0117
10								W=229846 P=0.2333	W=218273 P=0.0968
11									W=210768 P=0.6719
12									

N.3 Carrying capacity model (Tables N.8 – N.10)

Table N.8 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean population size of extant populations for the 16 scenarios of the carrying capacity model. Pairwise differences between scenarios are highlighted in light grey

	Carrying capacity scenarios						
	200	**300**	**400**	**EEP**	**500**	**600**	**700**
100	W=8706 P<0.001	W=4103 P<0.001	W=3235 P<0.001	W=3173 P<0.001	W=3250 P<0.001	W=3162 P<0.001	W=3160 P<0.001
200		W=130457 P<0.001	W=104868 P<0.001	W=99513 P<0.001	W=98121 P<0.001	W=96385 P<0.001	W=96142 P<0.001
300			W=181141 P<0.001	W=163902 P<0.001	W=147270 P<0.001	W=133257 P<0.001	W=129572 P<0.001
400				W=226234 P<0.001	W=192596 P<0.001	W=159937 P<0.001	W=145614 P<0.001
EEP					W=212802 P<0.001	W=174842 P<0.001	W=155896 P<0.001
500						W=207654 P<0.001	W=180331 P<0.001
600							W=217315 P<0.001

	Carrying capacity scenarios							
	800	**900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**
100	W=3160 P<0.001	W=3160 P<0.001	W=3160 P<0.001	W=3160 P<0.001	W=3163 P<0.001	W=3160 P<0.001	W=3161 P<0.001	W=3160 P<0.001
200	W=95821 P<0.001	W=95716 P<0.001	W=95718 P<0.001	W=95703 P<0.001	W=95953 P<0.001	W=95757 P<0.001	W=95862 P<0.001	W=95706 P<0.001
300	W=127011 P<0.001	W=125053 P<0.001	W=125094 P<0.001	W=124370 P<0.001	W=124795 P<0.001	W=124606 P<0.001	W=124892 P<0.001	W=124383 P<0.001
400	W=137678 P<0.001	W=129845 P<0.001	W=128873 P<0.001	W=125833 P<0.001	W=125819 P<0.001	W=125406 P<0.001	W=125722 P<0.001	W=125071 P<0.001
EEP	W=144730 P<0.001	W=133858 P<0.001	W=131913 P<0.001	W=127905 P<0.001	W=127503 P<0.001	W=127007 P<0.001	W=127154 P<0.001	W=126494 P<0.001
500	W=161903 P<0.001	W=144015 P<0.001	W=138370 P<0.001	W=132041 P<0.001	W=130594 P<0.001	W=129421 P<0.001	W=128572 P<0.001	W=127966 P<0.001
600	W=190765 P<0.001	W=164163 P<0.001	W=152449 P<0.001	W=142313 P<0.001	W=138728 P<0.001	W=135560 P<0.001	W=132809 P<0.001	W=131535 P<0.001
700	W=218291 P<0.001	W=185713 P<0.001	W=168100 P<0.001	W=154652 P<0.001	W=148773 P<0.001	W=143180 P<0.001	W=138205 P<0.001	W=136262 P<0.001
800		W=216015 P<0.001	W=193755 P<0.001	W=175837 P<0.001	W=167011 P<0.001	W=157136 P<0.001	W=149264 P<0.001	W=145466 P<0.001
900			W=224612 P<0.001	W=202208 P<0.001	W=190191 P<0.001	W=175556 P<0.001	W=164106 P<0.001	W=158407 P<0.001
1000				W=226297 P<0.001	W=212238 P<0.001	W=194752 P<0.001	W=180958 P<0.001	W=173333 P<0.001
1100					W=235123 P=0.0009	W=215539 P<0.001	W=199167 P<0.001	W=190395 P<0.001
1200						W=230667 P<0.001	W=213620 P<0.001	W=204047 P<0.001
1300							W=232018 P=0.0001	W=221597 P<0.001
1400								W=239633 P=0.0201

Table N.9 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean gene diversity of extant populations for the 16 scenarios of the carrying capacity model. Pairwise differences between scenarios are highlighted in light grey

	Carrying capacity scenarios							
	200	**300**	**400**	**EEP**	**500**	**600**	**700**	
100	W=8672 P<0.001	W=4263 P<0.001	W=3321 P<0.001	W=3206 P<0.001	W=3178 P<0.001	W=3160 P<0.001	W=3166 P<0.001	
200		W=135677 P<0.001	W=109353 P<0.001	W=103890 P<0.001	W=100698 P<0.001	W=98751 P<0.001	W=98170 P<0.001	
300			W=192909 P<0.001	W=177411 P<0.001	W=162748 P<0.001	W=150435 P<0.001	W=146242 P<0.001	
400				W=230917 P=0.0001	W=206580 P<0.001	W=184510 P<0.001	W=176523 P<0.001	
EEP					W=223106 P<0.001	W=197650 P<0.001	W=188598 P<0.001	
500						W=224151 P<0.001	W=214491 P<0.001	
600							W=240314 P=0.0296	

	Carrying capacity scenarios							
	800	**900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**
100	W=3160 P<0.001	W=3161 P<0.001	W=3162 P<0.001	W=3160 P<0.001	W=3160 P<0.001	W=3160 P<0.001	W=3160 P<0.001	W=3160 P<0.001
200	W=97331 P<0.001	W=96564 P<0.001	W=96447 P<0.001	W=96441 P<0.001	W=96357 P<0.001	W=95898 P<0.001	W=96118 P<0.001	W=95963 P<0.001
300	W=141337 P<0.001	W=135843 P<0.001	W=133416 P<0.001	W=134459 P<0.001	W=131329 P<0.001	W=130146 P<0.001	W=130337 P<0.001	W=129207 P<0.001
400	W=167375 P<0.001	W=156432 P<0.001	W=150445 P<0.001	W=153333 P<0.001	W=144585 P<0.001	W=143511 P<0.001	W=142399 P<0.001	W=140054 P<0.001
EEP	W=178099 P<0.001	W=165314 P<0.001	W=158037 P<0.001	W=161422 P<0.001	W=151029 P<0.001	W=149745 P<0.001	W=148235 P<0.001	W=145382 P<0.001
500	W=202609 P<0.001	W=188495 P<0.001	W=178235 P<0.001	W=182328 P<0.001	W=169458 P<0.001	W=167851 P<0.001	W=164989 P<0.001	W=161358 P<0.001
600	W=227286 P<0.001	W=212412 P<0.001	W=199945 P<0.001	W=204292 P<0.001	W=189390 P<0.001	W=187684 P<0.001	W=183734 P<0.001	W=179078 P<0.001
700	W=237170 P=0.0042	W=221954 P<0.001	W=208805 P<0.001	W=213551 P<0.001	W=197676 P<0.001	W=196001 P<0.001	W=191658 P<0.001	W=186785 P<0.001
800		W=235491 P=0.0012	W=221252 P<0.001	W=225770 P<0.001	W=209920 P<0.001	W=207992 P<0.001	W=203091 P<0.001	W=197976 P<0.001
900			W=235308 P=0.0011	W=239721 P=0.0211	W=223364 P<0.001	W=221014 P<0.001	W=215712 P<0.001	W=210150 P<0.001
1000				W=254125 P=0.3963	W=238691 P=0.0114	W=235999 P=0.0018	W=230521 P<0.001	W=224244 P<0.001
1100					W=234872 P=0.0008	W=232548 P=0.0001	W=227143 P<0.001	W=221331 P<0.001
1200						W=247475 P=0.5434	W=241484 P=0.0549	W=235600 P=0.0013
1300							W=244524 P=0.2099	W=238315 P=0.0090
1400								W=244115 P=0.1792

Table N.10 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean inbreeding of extant populations for the 16 scenarios of the carrying capacity model. Pairwise differences between scenarios are highlighted in light grey

	Carrying capacity scenarios						
	200	**300**	**400**	**EEP**	**500**	**600**	**700**
100	W=27148 P<0.001	W=34729 P<0.001	W=36277 P<0.001	W=36746 P<0.001	W=36972 P<0.001	W=37239 P<0.001	W=37282 P<0.001
200		W=243702 P<0.001	W=264341 P<0.001	W=269915 P<0.001	W=275289 P<0.001	W=280247 P<0.001	W=281234 P<0.001
300			W=281592 P<0.001	W=291014 P<0.001	W=303246 P<0.001	W=314061 P<0.001	W=316312 P<0.001
400				W=257964 P=0.0430	W=273252 P<0.001	W=287742 P<0.001	W=291116 P<0.001
EEP					W=266194 P=0.0005	W=281845 P<0.001	W=285124 P<0.001
500						W=207654 P<0.001	W=375250 P<0.001
600							W=253751 P=0.4434

	Carrying capacity scenarios							
	800	**900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**
100	W=37421 P<0.001	W=37518 P<0.001	W=37584 P<0.001	W=37540 P<0.001	W=37602 P<0.001	W=37642 P<0.001	W=37672 P<0.001	W=37690 P<0.001
200	W=284227 P<0.001	W=286168 P<0.001	W=287895 P<0.001	W=286989 P<0.001	W=288863 P<0.001	W=289204 P<0.001	W=289969 P<0.001	W=290454 P<0.001
300	W=323332 P<0.001	W=328277 P<0.001	W=332827 P<0.001	W=330034 P<0.001	W=335097 P<0.001	W=335673 P<0.001	W=337402 P<0.001	W=338894 P<0.001
400	W=301096 P<0.001	W=307723 P<0.001	W=314990 P<0.001	W=310929 P<0.001	W=318855 P<0.001	W=319294 P<0.001	W=322484 P<0.001	W=324755 P<0.001
EEP	W=295722 P<0.001	W=302790 P<0.001	W=310659 P<0.001	W=306320 P<0.001	W=314784 P<0.001	W=315244 P<0.001	W=318811 P<0.001	W=321135 P<0.001
500	W=375250 P<0.001	W=375250 P<0.001	W=375250 P<0.001	W=375250 P<0.001	W=375250 P<0.001	W=375250 P<0.001	W=375250 P<0.001	W=375250 P<0.001
600	W=264952 P=0.0013	W=271821 P<0.001	W=281496 P<0.001	W=276880 P<0.001	W=286747 P<0.001	W=286806 P<0.001	W=291640 P<0.001	W=294122 P<0.001
700	W=261512 P=0.0137	W=267948 P=0.0001	W=278066 P<0.001	W=273300 P<0.001	W=283584 P<0.001	W=283214 P<0.001	W=288435 P<0.001	W=290907 P<0.001
800		W=256577 P=0.1660	W=267268 P=0.0002	W=262635 P=0.0067	W=272934 P<0.001	W=272747 P<0.001	W=277722 P<0.001	W=280506 P<0.001
900			W=261482 P=0.0139	W=256601 P=0.1643	W=267683 P=0.0001	W=267145 P=0.0002	W=272955 P<0.001	W=275535 P<0.001
1000				W=245476 P=0.2958	W=256088 P=0.2011	W=255553 P=0.2456	W=261155 P=0.0169	W=263749 P=0.0031
1100					W=260581 P=0.0237	W=260283 P=0.0280	W=265570 P=0.0008	W=268390 P=0.0001
1200						W=249715 P=0.9067	W=255238 P=0.2748	W=257974 P=0.0908
1300							W=256069 P=0.2027	W=258840 P=0.0600
1400								W=252779 P=0.5798

N. 4 EU and non-EU dispersal model (Tables N.11 – N.13)

Table N.11 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean population size of extant populations for the 12 scenarios of the EU & non-EU dispersal model. Pairwise differences between scenarios are highlighted in light grey

	EU & non-EU dispersal models					
	0%	**1%**	**2%**	**3%**	**4%**	
EEP	W=229199 P=0.1521	W=222284 P=0.0011	W=225431 P=0.0250	W=242358 P=0.1293	W=250002 P=0.0004	
0%		W=230852 P=0.0485	W=234229 P=0.3732	W=252367 P=0.0018	W=260505 P<0.001	
1%			W=247088 P=0.2342	W=264803 P<0.001	W=272521 P<0.001	
2%				W=255934 P<0.001	W=264053 P<0.001	
3%					W=248096 P=0.0232	

	EU & non-EU dispersal models					
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=262191 P<0.001	W=270118 P<0.001	W=268822 P<0.001	W=273703 P<0.001	W=278939 P<0.001	W=282521 P<0.001
0%	W=273215 P<0.001	W=281418 P<0.001	W=279945 P<0.001	W=285313 P<0.001	W=290561 P<0.001	W=293858 P<0.001
1%	W=284268 P<0.001	W=290528 P<0.001	W=289317 P<0.001	W=293712 P<0.001	W=297992 P<0.001	W=300808 P<0.001
2%	W=276594 P<0.001	W=283640 P<0.001	W=282362 P<0.001	W=287224 P<0.001	W=291766 P<0.001	W=294589 P<0.001
3%	W=262153 P<0.001	W=272462 P<0.001	W=270856 P<0.001	W=277155 P<0.001	W=283423 P<0.001	W=287635 P<0.001
4%	W=248967 P=0.0002	W=261211 P<0.001	W=259232 P<0.001	W=266455 P<0.001	W=273497 P<0.001	W=278125 P<0.001
5%		W=242499 P<0.001	W=240282 P=0.0001	W=248234 P<0.001	W=255984 P<0.001	W=261144 P<0.001
6%			W=208788 P=0.6778	W=215734 P=0.0930	W=224593 P<0.001	W=231340 P<0.001
7%				W=216487 P=0.0263	W=224987 P<0.001	W=231031 P<0.001
8%					W=212867 P=0.0091	W=219721 P<0.001
9%						W=207184 P=0.0640

Table N.12 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean gene diversity of extant populations for the 12 scenarios of the EU & non-EU dispersal model. Pairwise differences between scenarios are highlighted in light grey

EU & non-EU dispersal models					
	0%	**1%**	**2%**	**3%**	**4%**
EEP	W=229012 P=0.1403	W=215546 P<0.001	W=216706 P<0.001	W=223956 P=0.0073	W=231584 P=0.5029
0%		W=223744 P=0.0003	W=225144 P=0.0031	W=232786 P=0.1850	W=240474 P=0.4842
1%			W=244825 P=0.4972	W=252112 P=0.0274	W=258723 P=0.0001
2%				W=244703 P=0.1223	W=251586 P=0.0006
3%					W=246823 P=0.0477

EU & non-EU dispersal models						
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=238767 P=0.1843	W=247157 P<0.001	W=250781 P<0.001	W=253210 P<0.001	W=264569 P<0.001	W=266079 P<0.001
0%	W=248185 P=0.0048	W=256380 P<0.001	W=260184 P<0.001	W=262467 P<0.001	W=273630 P<0.001	W=275055 P<0.001
1%	W=265409 P<0.001	W=271666 P<0.001	W=274967 P<0.001	W=276496 P<0.001	W=286184 P<0.0010	W=287233 P<0.001
2%	W=258850 P<0.001	W=265372 P<0.001	W=268697 P<0.001	W=270419 P<0.001	W=280436 P<0.001	W=281614 P<0.001
3%	W=253910 P=0.0001	W=261531 P<0.001	W=265103 P<0.001	W=267111 P<0.001	W=277903 P<0.001	W=279223 P<0.001
4%	W=241588 P=0.0474	W=249276 P<0.001	W=252624 P<0.001	W=255102 P<0.001	W=265947 P<0.001	W=267628 P<0.001
5%		W=236983 P=0.0020	W=240560 P<0.001	W=243325 P<0.001	W=254200 P<0.001	W=255976 P<0.001
6%			W=214089 P=0.3637	W=217493 P=0.0337	W=228234 P<0.001	W=230438 P<0.001
7%				W=212619 P=0.2138	W=223693 P<0.001	W=226022 P<0.001
8%					W=214530 P=0.0016	W=217203 P=0.0002
9%						W=201217 P=0.5119

Table N.13 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean inbreeding of extant populations for the 12 scenarios of the EU & non-EU dispersal model. Pairwise differences between scenarios are highlighted in light grey

EU & non-EU dispersal models					
	0%	**1%**	**2%**	**3%**	**4%**
EEP	W=252589 P=0.0001	W=252578 P=0.0003	W=258201 P<0.001	W=256298 P<0.001	W=248332 P=0.0015
0%		W=238226 P=0.7554	W=244716 P=0.1350	W=243295 P=0.2905	W=234813 P=0.5526
1%			W=249662 P=0.0766	W=248283 P=0.1790	W=239642 P=0.7449
2%				W=236409 P=0.7350	W=227951 P=0.0457
3%					W=231155 P=0.1109

EU & non-EU dispersal models						
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=245625 P=0.0035	W=235419 P=0.0955	W=228146 P=0.9654	W=228808 P=0.5780	W=224299 P=0.8522	W=214999 P=0.0086
0%	W=233079 P=0.5082	W=224557 P=0.1083	W=216657 P=0.0007	W=218037 P=0.0064	W=213996 P=0.0007	W=205727 P<0.001
1%	W=238019 P=0.7179	W=229450 P=0.1974	W=221501 P=0.0022	W=222931 P=0.0167	W=218817 P=0.0023	W=210378 P<0.001
2%	W=226578 P=0.0459	W=218417 P=0.0037	W=210847 P<0.001	W=212322 P=0.0001	W=208449 P<0.001	W=200566 P<0.001
3%	W=229578 P=0.1021	W=221182 P=0.0100	W=213950 P<0.001	W=215206 P=0.0003	W=211285 P<0.001	W=203356 P<0.001
4%	W=232861 P=0.9658	W=223844 P=0.2708	W=216769 P=0.0072	W=217872 P=0.0363	W=213687 P=0.0054	W=205384 P<0.001
5%		W=219992 P=0.3116	W=213503 P=0.0135	W=214417 P=0.0547	W=210322 P=0.0094	W=202180 P<0.001
6%			W=205216 P=0.1910	W=206017 P=0.4406	W=201748 P=0.1296	W=194237 P=0.0003
7%				W=209675 P=0.6184	W=205164 P=0.7647	W=196846 P=0.0097
8%					W=199307 P=0.4437	W=191561 P=0.0034
9%						W=190581 P=0.0306

N.5 EU, UK and non-EU dispersal model (Tables N.14 – N.17)

Table N.14 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean year extinct for the 12 scenarios of the EU, UK & non-EU dispersal model. Pairwise differences between scenarios are highlighted in light grey

EU, UK & non-EU dispersal models					
	0%	**1%**	**2%**	**3%**	**4%**
EEP	W=456 P=0.5388	W=411 P=0.8043	W=323 P=0.1473	W=277 P=0.1591	W=329 P=0.8082
0%		W=1223 P=0.3054	W=1062 P=0.1926	W=931 P=0.0117	W=1073 P=0.9134
1%			W=1107 P=0.0333	W=999 P=0.1372	W=1124 P=0.4990
2%				W=266 P=0.0021	W=322 P=0.1448
3%					W=674 P=0.0390

EU, UK & non-EU dispersal models						
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=285 P=0.1729	W=319 P=0.5257	W=406 P=0.9151	W=297 P=0.6681	W=324 P=0.2304	W=393 P=0.3733
0%	W=949 P=0.0146	W=1044 P=0.1992	W=1232 P=0.7411	W=988.0 P=0.2389	W=1031 P=0.0246	W=1162 P=0.0420
1%	W=1013 P=0.1328	W=1099 P=0.6226	W=1303 P=0.6097	W=1045 P=0.7853	W=1111 P=0.2212	W=1266 P=0.3665
2%	W=263.0 P=0.0011	W=313 P=0.0281	W=383 P=0.0939	W=294 P=0.0428	W=301 P=0.0030	W=364 P=0.0089
3%	W=608 P=0.8887	W=655 P=0.4147	W=819 P=0.0486	W=614 P=0.2813	W=689 P=0.6198	W=816 P=0.3938
4%	W=458 P=0.0302	W=526 P=0.3263	W=651 P=0.9403	W=492 P=0.4008	W=515 P=0.0583	W=604 P=0.1048
5%		W=700 P=0.3510	W=865 P=0.0470	W=644 P=0.3593	W=725 P=0.6788	W=841 P=0.5694
6%			W=838 P=0.3842	W=645 P=0.8848	W=707 P=0.5764	W=818 P=0.7172
7%				W=883 P=0.4866	W=934 P=0.0945	W=1065 P=0.1562
8%					W=550 P=0.5472	W=652 P=0.7766
9%						W=1053 P=0.8289

Table N.15 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean population size of extant populations for the 12 scenarios of the EU, UK & non-EU dispersal model. Pairwise differences between scenarios are highlighted in light grey

	EU, UK & non-EU dispersal models				
	0%	**1%**	**2%**	**3%**	**4%**
EEP	W=264893 P<0.001	W=241236 P=0.0060	W=241562 P=0.0817	W=247693 P=0.0004	W=251901 P<0.001
0%		W=193145 P<0.001	W=187638 P<0.001	W=194794 P<0.001	W=196715 P<0.001
1%			W=214182 P=0.1887	W=220227 P=0.6455	W=223759 P=0.2110
2%				W=239769 P=0.0434	W=244475 P=0.0022
3%					W=230997 P=0.3532

	EU, UK & non-EU dispersal models					
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=253477 P<0.001	W=254086 P<0.001	W=255845 P<0.001	W=263571 P<0.001	W=262461 P<0.001	W=265792 P<0.001
0%	W=197369 P<0.001	W=198536 P<0.001	W=201204 P=0.0003	W=207638 P=0.0076	W=207627 P=0.0242	W=111813 P<0.001
1%	W=224918 P=0.0999	W=225763 P=0.0564	W=227610 P=0.0055	W=234888 P=0.0001	W=234061 P<0.001	W=237988 P<0.001
2%	W=246239 P=0.0003	W=247241 P=0.0001	W=249074 P<0.001	W=257853 P<0.001	W=256933 P<0.001	W=261008 P<0.001
3%	W=232384 P=0.1703	W=233327 P=0.0984	W=235731 P=0.0082	W=243716 P=0.0001	W=243141 P<0.001	W=247516 P<0.001
4%	W=229350 P=0.6274	W=230515 P=0.4136	W=232515 P=0.0886	W=241352 P=0.0017	W=241070 P=0.0004	W=245931 P<0.001
5%		W=227286 P=0.6941	W=229337 P=0.1980	W=238349 P=0.0056	W=237902 P=0.0016	W=242813 P<0.001
6%			W=226991 P=0.3701	W=235801 P=0.0191	W=235480 P=0.0060	W=240292 P<0.001
7%				W=226988 P=0.1461	W=226525 P=0.0702	W=231726 P=0.0004
8%					W=228635 P=0.6654	W=234223 P=0.0251
9%						W=226470 P=0.0791

Table N.16 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean gene diversity of extant populations for the 12 scenarios of the EU, UK & non-EU dispersal model. Pairwise differences between scenarios are highlighted in light grey

	EU, UK & non-EU dispersal models				
	0%	**1%**	**2%**	**3%**	**4%**
EEP	W=262962 P<0.001	W=230315 P=0.8763	W=221665 P=0.0044	W=228501 P=0.3445	W=229146 P=0.3952
0%		W=186137 P<0.001	W=177742 P<0.001	W=182485 P<0.001	W=182578 P<0.001
1%			W=208090 P=0.0057	W=213941 P=0.2936	W=214493 P=0.3311
2%				W=238955 P=0.0673	W=239807 P=0.0489
3%					W=227600 P=0.8975

	EU, UK & non-EU dispersal models					
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=229815 P=0.5592	W=231562 P=0.9042	W=117370 P<0.001	W=242234 P=0.0330	W=242537 P=0.0072	W=246036 P=0.0001
0%	W=182412 P<0.001	W=184194 P<0.001	W=187546 P<0.001	W=193819 P<0.001	W=194344 P<0.001	W=197947 P<0.001
1%	W=215187 P=0.4863	W=216663 P=0.7751	W=219080 P=0.4864	W=226316 P=0.0709	W=226524 P=0.0198	W=229617 P=0.0003
2%	W=240810 P=0.0202	W=242119 P=0.0071	W=244247 P=0.0003	W=252151 P<0.001	W=252237 P<0.001	W=254969 P<0.001
3%	W=228212 P=0.6994	W=229820 P=0.4103	W=232519 P=0.0606	W=240235 P=0.0024	W=240497 P=0.0003	W=243520 P<0.001
4%	W=228409 P=0.7921	W=230038 P=0.4809	W=232733 P=0.0793	W=240548 P=0.0033	W=240733 P=0.0005	W=243990 P<0.001
5%		W=227604 P=0.6394	W=230493 P=0.1178	W=238392 P=0.0054	W=238554 P=0.0009	W=242082 P<0.001
6%			W=227842 P=0.2711	W=235562 P=0.0222	W=235800 P=0.0047	W=239230 P<0.001
7%				W=225847 P=0.2375	W=226125 P=0.0864	W=229514 P=0.0025
8%					W=229178 P=0.5745	W=232913 P=0.0542
9%						W=224712 P=0.1842

Table N.17 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean inbreeding of extant populations for the 12 scenarios of the EU, UK & non-EU dispersal model. Pairwise differences between scenarios are highlighted in light grey

	EU, UK & non-EU dispersal models				
	0%	**1%**	**2%**	**3%**	**4%**
EEP	W=221061 P=0.0414	W=227936 P=0.6828	W=250176 P=0.0002	W=244618 P=0.0050	W=249104 P=0.0002
0%		W=221063 P=0.1752	W=241923 P<0.001	W=236903 P<0.001	W=240432 P<0.001
1%			W=235537 P=0.0002	W=230728 P=0.0029	W=234407 P=0.0001
2%				W=227703 P=0.4250	W=232072 P=0.8682
3%					W=230984 P=0.3548

	EU, UK & non-EU dispersal models					
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=249029 P=0.0001	W=244194 P=0.0047	W=249667 P<0.001	W=244527 P=0.0077	W=241773 P=0.0122	W=241791 P=0.0027
0%	W=240508 P<0.001	W=235722 P<0.001	W=240591 P<0.001	W=236236 P<0.001	W=233697 P<0.001	W=233268 P<0.001
1%	W=234333 P=0.0001	W=230147 P=0.0030	W=234882 P<0.001	W=230540 P=0.0048	W=227995 P=0.0072	W=227995 P=0.0015
2%	W=232031 P=0.7875	W=227763 P=0.5008	W=233239 P=0.3097	W=228107 P=0.4160	W=225412 P=0.3151	W=226316 P=0.7330
3%	W=231101 P=0.2854	W=226707 P=0.9306	W=232155 P=0.0736	W=226938 P=0.9342	W=224491 P=0.8314	W=225132 P=0.6909
4%	W=227689 P=0.9253	W=223549 P=0.4082	W = 228944 P=0.3953	W=224076 P=0.3565	W=221386 P=0.2629	W=222263 P=0.6429
5%		W=221775 P=0.3618	W=227111 P=0.4509	W=222071 P=0.2891	W=219687 P=0.2344	W=220508 P=0.5841
6%			W=230265 P=0.0926	W=225370 P=0.9079	W=222823 P=0.7852	W=223654 P=0.7016
7%				W=213432 P=0.0754	W=211014 P=0.0532	W=212007 P=0.2080
8%					W=226208 P=0.8863	W=226759 P=0.6550
9%						W=221621 P=0.5648

N. 6 Bluetongue dispersal model (Tables N.18 – N.21)

Table N,18 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean year extinct for the 12 scenarios of the bluetongue dispersal model. Pairwise differences between scenarios are highlighted in light grey

	Bluetongue dispersal models					
	0%	**1%**	**2%**	**3%**	**4%**	
EEP	W=153593 P<0.001	W=73882 P=0.0002	W=65591 P=0.4397	W=64477 P=0.0013	W=63685 P=0.0303	
0%		W=103079 P<0.001	W=95759 P=0.0093	W=94763 P<0.001	W=93940 P=0.0006	
1%			W=5546 P=0.2666	W=5190 P=0.1143	W=4977 P=0.7490	
2%				W=506 P=0.0601	W=456 P=0.3203	
3%					W=564 P=0.3035	

	Bluetongue dispersal models					
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=63379 P=0.0004	W=63104 P=0.1900	W=63674 P=0.0043	W=64234 P=0.0536	W=63080 P=0.0009	W=63739 P=0.0003
0%	W=93646 P<0.001	W=93440 P=0.0178	W=93842 P<0.001	W=94347 P=0.0004	W=93376 P<0.001	W=93995 P<0.001
1%	W=4838 P=0.0307	W=4737 P=0.9955	W=4956 P=0.2255	W=5205 P=0.6811	W=4763 P=0.0658	W=4995 P=0.0666
2%	W=420 P=0.0152	W=403 P=0.5205	W=448 P=0.0839	W=503 P=0.5409	W=402 P=0.0253	W=453 P=0.0250
3%	W=526 P=0.5546	W=486 P=0.2012	W=586 P=0.3889	W=641 P=0.0663	W=494 P=0.6053	W=586 P=0.9824
4%	W=236 P=0.1290	W=217 P=0.9790	W=258 P=0.4478	W=310 P=0.4868	W=229 P=0.2949	W=265 P=0.2318
5%		W=294 P=0.1192	W=372 P=0.2293	W=419 P=0.0209	W=307 P=0.9856	W=381 P=0.5731
6%			W=127 P=0.4014	W=160 P=0.6629	W=103.0 P=0.1416	W=131 P=0.2204
7%				W=400 P=0.0806	W=272 P=0.2190	W=332 P=0.4385
8%					W=281 P=0.0769	W=306 P=0.0237
9%						W=293 P=0.6303

Table N.19 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean population size of extant populations for the 12 scenarios of the bluetongue dispersal model. Pairwise differences between scenarios are highlighted in light grey

	Bluetongue dispersal models				
	0%	**1%**	**2%**	**3%**	**4%**
EEP	W=17311 P=0.0553	W=17958 P<0.001	W=14276 P<0.001	W=13094 P<0.001	W=13518 P<0.001
0%		W=5094 P<0.001	W=3690 P<0.001	W=3256 P<0.001	W=3423 P<0.001
1%			W=152567 P<0.001	W = 139487 P<0.001	W=139616 P<0.001
2%				W=209422 P=0.0001	W=209009 P<0.001
3%					W=225614 P=0.5780

	Bluetongue dispersal models					
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=13197 P<0.001	W=13715 P<0.001	W=12988 P<0.001	W=13641 P<0.001	W=13935 P<0.001	W=13201 P<0.001
0%	W=3294 P<0.001	W=3526 P<0.001	W=3196 P<0.001	W=3469 P<0.001	W=3563 P<0.001	W=3248 P<0.001
1%	W=137101 P<0.001	W=140538 P<0.001	W=139970 P<0.001	W=148503 P<0.001	W=150745 P<0.001	W=149147 P<0.001
2%	W=205819 P<0.001	W=210987 P<0.001	W=210539 P<0.001	W=222403 P=0.1902	W=225688 P=0.4475	W=223830 P=0.3856
3%	W=222239 P=0.2163	W=228171 P=0.7720	W=227465 P=0.9889	W=239290 P=0.0049	W=242713 P=0.0008	W=240973 P=0.0009
4%	W=230941 P=0.4785	W=237171 P=0.7810	W=2366020 P=0.5480	W=248402 P=0.0007	W=252047 P=0.0001	W=250290 P=0.0001
5%		W=238735 P=0.3325	W=238253 P=0.1883	W=250018 P<0.001	W=253371 P<0.001	W=251765 P<0.001
6%			W=239858 P=0.7365	W=252169 P=0.0013	W=255616 P=0.0002	W=253749 P=0.0002
7%				W=244829 P=0.0038	W=248305 P=0.0005	W=246678 P=0.0006
8%					W=235047 P=0.5669	W=233290 P=0.6137
9%						W=233479 P=0.9019

Table N.20 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean gene diversity of extant populations for the 12 scenarios of the bluetongue dispersal model. Pairwise differences between scenarios are highlighted in light grey

	Bluetongue dispersal models				
	0%	**1%**	**2%**	**3%**	**4%**
EEP	W=18073 P=0.0003	W=18538 P<0.001	W=15967 P<0.001	W=14688 P<0.001	W=14333 P<0.001
0%		W=4122 P<0.001	W=3321 P<0.001	W=3058 P<0.001	W=2923 P<0.001
1%			W=160480 P<0.001	W=150954 P<0.001	W=147783 P<0.001
2%				W=214595 P=0.0047	W=209910 P<0.001
3%					W=221468 P=0.1278

	Bluetongue dispersal models					
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=14406 P<0.001	W=14854 P<0.001	W=13982 P<0.001	W=14718 P<0.001	W=15134 P<0.001	W=14724 P<0.001
0%	W=3000 P<0.001	W=3027 P<0.001	W=2838 P<0.001	W=2973 P<0.001	W=3083 P<0.001	W=2969 P<0.001
1%	W=147169 P<0.001	W=152285 P<0.001	W=147430 P<0.001	W=151604 P<0.001	W=155422 P<0.001	W=152008 P<0.001
2%	W=209386 P<0.001	W=216049 P=0.0011	W=210447 P<0.001	W=215425 P=0.0033	W=220341 P=0.0452	W=215910 P=0.0064
3%	W=220930 P=0.1229	W=227769 P=0.7021	W=222124 P=0.2065	W=226954 P=0.9377	W=231958 P=0.3865	W=227419 P=0.8868
4%	W=233980 P=0.9937	W=240918 P=0.2578	W=235434 P=0.7435	W=239835 P=0.1614	W=245142 P=0.0169	W=240797 P=0.0819
5%		W=239585 P=0.2446	W=234051 P=0.7311	W=238560 P=0.1485	W=243898 P=0.0146	W=239277 P=0.0840
6%			W=234697 P=0.3994	W=239411 P=0.7712	W=244742 P=0.2010	W=240196 P=0.5590
7%				W=237138 P=0.2647	W=242561 P=0.0327	W=238038 P=0.1499
8%					W=236793 P=0.3294	W=232355 P=0.7740
9%						W=230992 P=0.4847

Table N.21 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean inbreeding of extant populations for the 12 scenarios of the bluetongue dispersal model. Pairwise differences between scenarios are highlighted in light grey

	Bluetongue dispersal models				
	0%	**1%**	**2%**	**3%**	**4%**
EEP	W=16140 P=0.4629	W=54222 P<0.001	W=66319 P<0.001	W=67697 P<0.001	W=70127 P<0.001
0%		W=22670 P<0.001	W=27884 P<0.001	W=28356 P<0.001	W=29382 P<0.001
1%			W=204447 P<0.001	W=210441 P<0.001	W=220888 P<0.001
2%				W=233122 P=0.1223	W=246014 P=0.0001
3%					W=238900 P=0.0110

	Bluetongue dispersal models					
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=69964 P<0.001	W=70052 P<0.001	W=70241 P<0.001	W=69249 P<0.001	W=69791 P<0.001	W=69440 P<0.001
0%	W=29348 P<0.001	W=29481 P<0.001	W=29432 P<0.001	W=29081 P<0.001	W=29295 P<0.001	W=29141 P<0.001
1%	W=222218 P<0.001	W=219510 P<0.001	W=224076 P<0.001	W=218546 P<0.001	W=219989 P<0.001	W=220595 P<0.001
2%	W=248083 P=0.0000	W=244661 P=0.0008	W=251122 P<0.001	W=244889 P=0.0001	W=246048 P=0.0001	W=248093 P<0.001
3%	W=241460 P=0.0011	W=237800 P=0.0530	W=244661 P=0.0001	W=238209 P=0.0105	W=239369 P=0.0096	W=241364 P=0.0006
4%	W=236881 P=0.5085	W=233255 P=0.5393	W=240401 P=0.1408	W=234270 P=0.9086	W=234994 P=0.9536	W=237791 P=0.2969
5%		W=229232 P=0.2293	W=236408 P=0.3741	W=230400 P=0.6558	W=231061 P=0.6081	W=233925 P=0.6283
6%			W=247433 P=0.0387	W=241493 P=0.4430	W=242255 P=0.4759	W=244900 P=0.0961
7%				W=226786 P=0.1994	W=227434 P=0.1776	W=230301 P=0.7202
8%					W=232310 P=0.9534	W=234943 P=0.3737
9%						W=238133 P=0.3405

N.7 Countries dispersal model (Table L.22)

Table N.22 Results of the pairwise Mann-Whitney U tests with Bonferroni correction for the mean year extinct for the 12 scenarios of the countries dispersal model. Pairwise differences between scenarios are highlighted in light grey

	Countries dispersal models				
	0%	**1%**	**2%**	**3%**	**4%**
EEP	W=228280 P=0.1339	W=342044 P<0.001	W=357017 P<0.001	W=361060 P<0.001	W=362474 P<0.001
0%		W=332295 P<0.001	W=344706 P<0.001	W=347456 P<0.001	W=348255 P<0.001
1%			W=314466 P<0.001	W=348809 P<0.001	W=364128 P<0.001
2%				W=308500 P<0.001	W=341666 P<0.001
3%					W=293514 P<0.001

	Countries dispersal models					
	5%	**6%**	**7%**	**8%**	**9%**	**10%**
EEP	W=362867 P<0.001	W=363105 P<0.001	W=363021 P<0.001	W=363097 P<0.001	W=363116 P<0.001	W=363168 P<0.001
0%	W=348466 P<0.001	W=348555 P<0.001	W=348520 P<0.001	W=348559 P<0.001	W=348567 P<0.001	W=348567 P<0.001
1%	W=369784 P<0.001	W=372994 P<0.001	W=371940 P<0.001	W=373046 P<0.001	W=373352 P<0.001	W=373772 P<0.001
2%	W=357696 P<0.001	W=367234 P<0.001	W=364239 P<0.001	W=367622 P<0.001	W=368509 P<0.001	W=370516 P<0.001
3%	W=324527 P<0.001	W=344837 P<0.001	W=338144 P<0.001	W=346927 P<0.001	W=348343 P<0.001	W=357187 P<0.001
4%	W=290278 P<0.001	W=318549 P<0.001	W=308712 P<0.001	W=322293 P<0.001	W=323900 P<0.001	W=340697 P<0.001
5%		W=280041 P<0.001	W=269448 P<0.001	W=285453 P<0.001	W=287816 P<0.001	W=311194 P<0.001
6%			W=239526 P=0.0189	W=256715 P=0.1569	W=259344 P=0.0465	W=287708 P<0.001
7%				W=267392 P=0.0002	W=270118 P<0.001	W=296702 P<0.001
8%					W=253354 P=0.4968	W=281474 P<0.001
9%						W=277615 P<0.001

Glossary of Terms

Allele retention (r_i): the proportion of a founder's genes surviving to the living descendant population (Ballou *et al.* 2010a).

Carrying Capacity (*K*): the maximum population size (*N*) that can be supported by the environment**.** For captive populations this may be the number of available spaces for a species (Ballou *et al.* 2010a).

Census Population Size (*N*): The total number of individuals in the population

Delta (Δ): Change in value

Deterministic or Intrinsic Growth Rate (*r*): the rate of change in population size at any instant in time. It is calculated by solving the Euler equation:

$$1 = \sum(l_x m_x e^{-rx})$$

in which l_x and m_x are the age specific survivorship and fecundity rates respectively for age class x to x+1. The summation is over all age classes (Thompson 2004), and is centred around 0.00 (Miller & Lacy 2005; Thompson 2004).

Effective Population Size (N_e): the size of a randomly mating population of constant size with an equal sex ratio and a Poisson distribution of family sizes that would either result in the same rate of genetic drift, or result in the same mean rate of inbreeding, as that observed in the population under consideration. These two definitions are equal only if the population is demographically stable (Ballou *et al.* 2010a).

Fecundity (*Mx*): The age specific fecundity (fertility) is the expected number of same sex offspring produced by a parent in age class x. Values range from 0 to the maximum number of offspring produced by an individual (Pollak *et al.* 2007).

Founder Contribution (p_i): the percentage of a living population's genes that have descended from each founder according to the rules of Mendelian inheritance (Thompson 2004; Wilcken & Lees 1998).

Founder Genome Equivalents (*FGE*): the theoretically expected number of equally represented founders with no loss of alleles (retention = 1) that would provide the same gene diversity as that observed in the living descendant population (Ballou *et al.* 2010a). The value of FGE can be estimated by:

$$FGE = \frac{1}{\sum_{i=1}^{N_f} (p_i^2 / r_i)}$$

where N_f is the number of founders, p_i is the founder contribution defined as the expected proportion of the population's gene pool that is descended from founder i, and r_i is allele

retention defined as the expected proportion of founder ***i***'s alleles that have survived to the living descendant population (Pollak *et al.* 2007).

Founder Genome Surviving (*FGS*): is defined as the upper limit of FGE retention summed over all founders **(Σr_i)** in a population, where r_i is the allele retention of founder ***i*** (Ballou & Lacy 1995; Ballou *et al.* 2010a).

Founder Importance Coefficient (*FIC*): is the degree to which an individual ***i*** is descended from under- or over-represented founders, and be summarised as:

$$FIC_i = \sum_{j=1}^{N_f} (p_{j.} \times p_{ji})$$

in which $p_{j.}$ is the founder contribution of founder j to the population's gene pool; p_{ji} is the contribution of founder ***j*** to individual ***i***; and N_f is the number of founders contributing to the living descendant population (Ballou & Lacy 1995).

Gene Diversity (*GD*): the heterozygosity expected in a population if the population were in Hardy-Weinberg equilibrium:

$$GD = 1 - \Sigma [q_i^2]$$

Where q_i is the frequency of allele ***i*** (Ballou & Lacy 1995). Gene diversity is the heterozygosity expected in progeny produced by random mating. The proportional gene diversity (as a proportion of the source population) is the probability that two alleles from the same locus sampled at random from the population will be identical by descent (Ballou & Lacy 1995), so gene diversity can be represented by:

$$GD = 1 - \frac{1}{[2*FGE]}$$ (Lacy 1995; Pollak *et al.* 2007)

Gene Drop Simulation: a computer simulation of the transmission of alleles from founders to the living descendants. Gene drop simulations provide approximate values for the genetic variation retained and the probabilities of alleles being represented in any living animal (Ballou & Lacy 1995).

Gene Value (*GV*): the gene diversity that would be expected in the next generation if all animals bred at random and produced a number of progeny for the next generation equal to their reproductive values Vx so:

GV = 1- mean KV (Ballou *et al.* 2010a; Pollak *et al.* 2007)

Generation Length (*T*): the time elapsing from reproduction in one generation to the time the next generation reproduces, or the mean age at which a male or female produces offspring (Pollak *et al.* 2007). It is also the ratio of the natural log of the net reproductive, to the intrinsic, rate of increase (Pollak *et al.* 2007)

Genome Uniqueness (*GU*): the probability that any one allele from an individual is unique within the living population. It can be defined as:

$$GU_i = \frac{\sum_{j=1}^{N_{SIM}} a_j}{2*N_{SIM}}$$

where a_j is the number of individual ***i***'s alleles at a given locus that are present in no other living animal in simulation ***j***; N_{SIM} is the number of simulations (Gage 1995).

Inbreeding: the mating of related animals resulting in a non-zero probability that alleles at a particular locus are identical by descent. The inbreeding coefficient *F* of an offspring is equal to the kinship between its parents based on pedigree analysis (Ballou & Lacy 1995).

Inbreeding Coefficient (*F*): the probability that the two alleles at a genetic locus are identical by descent from a common ancestor. The mean inbreeding coefficient of a population is the proportional decrease in observed heterozygosity relative to the expected heterozygosity of the founder population (Pollak *et al.* 2007).

Kinship Value (*KV*): The weighted mean kinship coefficients between an individual and all members of the population (including itself), with the weights being the reproductive values (***Vxj***) for the age class (***x***) of which the individual (***j***) is a member. The mean kinship value of a population predicts the loss of gene diversity expected in the subsequent generation if all animals were to mate randomly and all were to produce the numbers of offspring expected for animals of their age (Pollak *et al.* 2007)

$$KV_i = \frac{\sum_{j=1}^{N} f_{ij} v_{xj}}{\sum_{j=1}^{N} v_{xj}}$$

(Ballou & Lacy 1995; Pollak *et al.* 2007)

Lambda (λ): the annual multiplicative growth rate, or the rate of change per year (Miller & Lacy 2005; Thompson 2004). Lambda for an individual year is calculated by:

$$\lambda = \frac{N_t}{N_{t-1}}$$

(Ballou & Lacy 1995)

λ is related to r by $\lambda = e^r$ (Ballou *et al.* 2010a), and centered around 1.00 (Miller & Lacy 2005; Thompson 2004)

Mean Kinship (*MK*): the mean kinship of a population is equal to the proportional loss of gene diversity of the descendant (captive-born) population relative to the founders. Mean kinship is also the reciprocal of 2*FGE (Ballou *et al.* 2010a). The relationship between a pair of individuals can be measured with the kinship coefficient f_{ij} which is the probability that alleles randomly selected from two individuals (***i*** and ***j***) are identical by descent. The

mean kinship of individual ***i*** (***mk***$_i$) is the average of the kinship coefficients between that individual and all individuals (including itself) in the living, captive-born population:

$$mk_i = \frac{\sum_{j=1}^{N} f_{ij}}{N}$$

Where ***N*** is the number of living individuals in the population (Pollak *et al.* 2007).

Mortality (*Qx*): the proportion of animals alive at age x that are expected to die during that age class (Ballou & Lacy 1995).

Phenotypic Variance (V_P): the total phenotypic variability for a trait (Thompson 2004)

Reproductive Value (*Vx*): Expected future reproduction of an individual at age x during its lifetime:

$V_x = (\lambda/Lx)\ \Sigma\lambda_{-t}\ L_tM_t$

with the summation over all values of t from x to the maximum age. Vx is used to calculate Kinship Value (Allendorf & Luikart 2007a).

Stable Age Distribution: the age distribution at which the relative proportions of each age class remain stable (change at the same rate) and the population growth rate remains constant. The stable age distribution, or the proportion of the population at each class c_x, is given by:

$$c_x = \frac{l_x e^{-rx}}{\sum(l_x e^{-rx})}$$

If the mortality schedules are different between males and females, female life history tables are used to calculate *r* (as females control population growth), but the l_x values are male. The life table calculation assumes that there is no limitation of mates (Thompson 2004).

Survival Rate (*Px*): The age specific survival rate is defined as the probability that an animal alive at age x will survive to age x+1. The values range between 0 and 1 (Thompson 2004).

Survivorship (*Lx*): Age specific survivorship is the probability that a newborn individual will be alive at age x. The range of values are between 0 and 1 (Thompson 2004; Wilcken & Lees 1998).

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