UNIVERSITY OF SOUTHAMPTON

THE EFFECT OF HABITAT FRAGMENTATION ON THE POPULATION GENETIC STRUCTURE OF THE WESTERN EUROPEAN HEDGEHOG (*ERINACEUS EUROPAEUS*)

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ABSTRACT

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THE EFFECT OF HABITAT FRAGMENTATION ON THE POPULATION GENETIC STRUCTURE OF THE WESTERN EUROPEAN HEDGEHOG (*ERINACEUS EUROPAEUS*)

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Habitat fragmentation has the potential to isolate populations from dispersal routes, and to restrict geneflow. This study investigates the genetic effects of habitat fragmentation on populations of the European Hedgehog *Erinaceus europaeus*. This was accomplished using mitochondrial D-loop sequence data and nuclear microsatellites. The markers included six existing microsatellite loci and five new loci developed for this project. Data on genetic mixing from analysis of mtDNA and nDNA were interpreted using information from concurrent studies by others on the dispersal behaviour of individual hedgehogs. Local-scale (<50 km)genetic effects were analysed in the context of the overall genetic diversity within Britain. Potential barriers to dispersal, including ancient features such as estuaries and modern features such as roads, were evaluated according to their effects on population genetic structure. Previous and current local scale dispersal behaviour was inferred using molecular markers, and assessed in light of complementary radio-telemetry studies.

A genetic survey, using nDNA and mtDNA, of hedgehog populations throughout Britain revealed a largely homogeneous national population, with low to moderate levels of genetic diversity. A scale effect was detected in nDNA, with the rate of increase of genetic differentiation between populations increasing significantly with geographical distance. However, analysis of molecular variance (AMOVA) of mtDNA revealed no significant component of co-variation above a regional level (approx. 50-150 km), and no evidence of national scale 'source' and 'sink' areas of genetic diversity. Preliminary analysis detected a putative nuclear insertion of mtDNA D-loop sequence.

Mantel analysis of genetic distance values, based on microsatellite data, revealed an effect of isolation by distance at a local scale (<50 km) within the Southampton study area. No significant relationship was detected between the level of habitat fragmentation within 5×5-km squares and the relatedness value R_C, or inbreeding coefficient F_{IS}, of the population inhabiting the habitat patch. Extended Mantel analysis revealed a near significant barrier effect exerted by the M3 motorway at Eastleigh. This effect became significant when related to overall road width, suggesting that barrier width may be more important than barrier frequency. No significant barrier effects were detected for any other barrier type, including minor roads and railways (relatively modern features), or the River Test and Southampton Water Estuary (ancient features).

AMOVA of molecular data, from populations within the Southampton study area, yielded a significantly higher proportion of genetic variation between populations attributed to mtDNA than to nDNA. This suggests a long-standing dispersal gender bias towards male hedgehogs. In contrast assignment testing of individuals, based on microsatellite data, revealed no significant difference between male and female dispersal frequencies over the last three generations. This finding is in agreement with data collected by others on the dispersal behaviour by these same hedgehogs. It implies a recent equalisation of successful dispersals between gender. This is hypothesised to be a result of reduced probability of dispersal success in a fragmented habitat.

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LIST OF DEFINITIONS AND ABBREVIATIONS

AI_C	Corrected assignment index
AMOVA	Analysis of molecular variance
D_c	Cavalli-Sforza and Edwards (1967), chord distance (genetic distance)
DNA	Deoxyribonucleic acid
F_{IS}	Wright's inbreeding coefficient
F_{ST}	Wright's fixation index
GIS	Geographical information systems
H_e	Expected heterozygosity
HFI	Habitat fragmentation index
H_o	Observed heterozygosity
IAM	Infinite allele model (model of microsatellite mutation)
k	Number of alleles
mtDNA	Mitochondrial DNA
nDNA	Nuclear DNA
N_e	Effective population size
N_m	Mean number of immigrants, per population, per generation
R_c	Relatedness value
R_{ST}	Slatkin's R_{ST} (analogous to Wright's F_{ST})
SMM	Stepwise mutation model (model of microsatellite mutation)
TPM	Two-phase model (model of microsatellite mutation)
VNTR	Variable number tandem repeat
Φ_{ST}	Phi-ST, an estimate of genetic differentiation based on AMOVA. Analogous to F_{ST}.
σ_a^2, σ_b^2	Components of co-variance

1.0 Introduction

1.1 *Introduction*

Any organism that consumes resources and reproduces must have the capacity to disperse. This ability is essential in order to escape environments with insufficient resources and to take advantage of new opportunities and resources (food, shelter, mates) elsewhere

The spatially and temporally heterogeneous nature of many environments requires that populations of inhabiting species be flexible and dynamic in their dispersal capacity (Cockburn, 1992). At any given time some habitat patches are more suitable for habitation than others. Consequently some habitat patches will be made use of by certain species, and some, for reasons of lack of resources, or access (Lawton and Woodroffe, 1991), will not. Population structure becomes relative to the scale at which it is observed, as individuals from local population centres interact with other population centres. This dynamic interaction within a larger network of populations has given rise to theories regarding "source" and "sink" populations (Pulliam, 1996), and the formalising of this patch network hypothesis as a metapopulation (Hanski, 1999).

In this context, dispersal throughout a metapopulation becomes a major structuring factor of population genetics (Real, 1994). Rates of dispersal and accessibility of suitable habitat patches define the viability of the metapopulation as a whole. A free flow of genes from one patch to another allows molecular mixing to take place, and individual offspring to be genetically diverse, thus maintaining the ability of the population to cope with disease and environmental change. However, if access between these patches becomes restricted or prevented, individuals within each patch will tend to become genetically more similar to each other with each generation. In cases of extreme isolation, within a small population, subsequent generations may begin to exhibit adverse physical effects as a result of their lack of genetic diversity. These effects are largely an accumulation of deleterious recessive mutations occurring within individuals.

In real populations, heterogeneous environments will vary between these two extremes. This process is dynamic. Patch occupancy and population size is governed by the resource richness of the available habitat patches, and by accessibility (Beier and Noss, 1998), which itself is dictated by population size and proximity to neighbouring patches (Andreassen and Ims, 1998).

The dispersive and reproductive characteristics of the organism itself also affect the metapopulation structure. Species that reproduce sexually have the added requirement of gaining access to a sexual partner to whom they are not closely related. Populations of individuals that cannot disperse from the natal area rapidly become inbred (Gompper *et al.*, 1998; Perrin and Mazalov, 1999). Most sexually reproducing species that employ juvenile or adult dispersal rather than gametic or larval dispersal exhibit a tendency to disperse that is biased toward one gender (Greenwood, 1980). A hypothetical organism that has a dispersive bias towards the non-reproductive gender will tend to colonise unoccupied patches at a slower rate than an organism with less of a gender bias, or a gender bias towards the reproductive gender.

Given the prevalence of this type of environmental heterogeneity in the natural world, many species have developed ecological strategies that favour the persistence of metapopulations by adapting to spatial and temporal habitat variation. However, the large scale urbanisation and industrialisation of many former wildlife habitats that has taken place over the last century goes beyond a natural level of fragmentation. Levels of habitat fragmentation have increased throughout the world, and populations of many species appear to have been forced towards extreme isolation. The effect that this may have on a given species depends on several factors e.g. what are its resource requirements? What are its dispersive capabilities? What effect does this level of fragmentation have on its predators/competitors/prey? Does the fragmentation feature constitute a barrier to gene flow, or a potential new wildlife corridor?

For the impact of habitat fragmentation to be assessed it is necessary that the general effects of population isolation on population genetic structure, and the consequent effects

of genetic change on the individual be well understood. However, for reasons of civil and conservation planning, it is also vital that the true nature of potential barriers to geneflow, or wildlife corridors, be identified and quantified (Ruefenacht and Knight, 1995; Forman and Alexander, 1998).

This project seeks to investigate the genetic structure of hedgehog populations within parts of the United Kingdom. Specifically, I wish to quantify the effects on population genetic structure of urbanisation and other anthropogenic factors leading to habitat fragmentation. I will compare such effects on population genetic structure believed to result from naturally occurring habitat fragmentation. In order to fulfil this objective, the level of variation present prior to modern landscape changes should be established. It would also be helpful to be aware of the historical events that have ultimately shaped the landscape of modern Britain. In this chapter I will provide this background.

The unpredictable and infrequent nature of individual dispersal events means that direct observation involves considerable logistical difficulty for studying dispersal in many species (Beier, 1995; Ims and Yoccoz, 1997). Mark-recapture and radio-telemetry are labour intensive techniques, which are often impractical for use in cryptic species or in inaccessible environments (McShea and Madison, 1992). Consequently much of the data collected on dispersal of vertebrates has been at a population level (Thomas and Harrison, 1992; Desrochers and Hannon, 1997), often employing molecular genetic markers, which are used as a basis to infer previous dispersal (Thompson and Goodman, 1997).

The statistical requirements necessary for analysis of allele frequency type molecular data have usually meant that only metapopulations with large subpopulations ($n > 50$) have been analysed. This frequently rules out the possibility of inferring the movements of individuals, although new analytical techniques are beginning to emerge which allow the assignment of individuals to specific populations of origin (Paetkau *et al.*, 1995; Mossman and Waser, 1999).

Studies on fine scale analysis of the genetic effects of specific fragmentation features in animals are rare (Wauters *et al.*, 1994; Becher and Griffiths, 1998; Cunningham and Moritz, 1998: Dallas *et al.*, 1999; Lugon-Moulin *et al.*, 1999), and studies that quantify the effects of specific barriers, even less so (Hitchings and Beebee, 1997; Gerlach and Musolf, 2000). This has led to a lack of understanding of the immediate effects of specific barrier features. Studies that have concentrated on the behavioural response of dispersing organisms to landscape features, particularly potential barriers (Mader, 1984; Beier, 1995; Richardson *et al.*, 1997; Mulder, 1999), provide information on specific cases, but are unable to relate this to the consequent genetic population structure effects. Studies which combine a population genetic structure approach with an individual-behavioural response approach are required for understanding of the effects of specific barrier features.

The European hedgehog (*Erinaceus europaeus* L.) provides a useful model with which to study both individual level dispersal and the consequent effects on population genetic structure. This species is a nocturnal insectivore found throughout the United Kingdom and western mainland Europe. They are of a predominantly sedentary nature, inhabiting a home range averaging approximately 32.4 ± 17.2 ha (Reeve, 1982; Boitani and Reggiani, 1984; Kristiansson, 1984). However, their capacity to disperse over longer distances has been illustrated by translocated individuals found to disperse up to 3.8 km from their release point (Doncaster, 1992; Doncaster *et al.*, 2001).

Hedgehog populations appear to be distributed within a metapopulation of habitat fragments, which make them a suitable model for this type of study. The distribution of habitable patches appears to be determined by the presence of food resources and predators. Subpopulations within these patches fluctuate from year to year, and can undergo local extinctions (Doncaster *et al.*, 2001). Previous studies of genetic population structure of hedgehogs have established that gene flow does occur between populations, but differing levels of genetic differentiation between populations suggest asymmetry in geneflow via dispersal (Johnson, 1996; Becher and Griffiths, 1998).

The aims of this project are: to establish a baseline of hedgehog national genetic diversity within the UK, as a context to local scale analysis; to identify potential barrier features, ancient and modern, and quantify their impact on population genetic structure; to highlight possible wildlife corridors; to trace current dispersal patterns within a hedgehog metapopulation, with particular reference to any gender bias that may occur. These aims are to be achieved by a spatially hierarchical analysis of nuclear and mitochondrial molecular markers. What strengthens this project in comparison to purely molecular projects is that it draws on results generated from parallel studies of individual hedgehog movements in response to potential barriers and other landscape features, which were done within the same study areas used for the molecular analysis (Doncaster *et al.*, 2001; Rondinini and Doncaster, submitted). This approach allows a better understanding of both the behavioural response to dispersal barriers, and of the population genetic consequences of the resulting in restriction in gene flow.

In this chapter, I begin by setting out the recent evolutionary history of *E. europaeus* and, where relevant, that of other closely related hedgehog species. I describe the current hypotheses about postglacial recolonisation of mainland Europe and the UK by mammals, and how these hypotheses relate to genetic diversity and relatedness in these areas. This will help to put the genetic effects of recent habitat fragmentation into the context of underlying population genetic structure, and diversity. I then describe molecular markers and techniques in current use. I then review the primary literature on molecular population ecology of vertebrates, with emphasis on dispersal behaviour and the genetic effects of habitat fragmentation. I end by stating of the specific objectives of this project.

1.1.1 Postglacial Recolonisation of the UK

The recolonisation of Britain and mainland Europe by many plant and animal species following the Pleistocene glaciations has been well documented (Collura and Stewart, 1995; Greenwood and Pääbo, 1999). During periods of glaciation many species were

forced into southerly refugia such as Iberia, Italy, the Balkans and the Caucasus and were then isolated there for thousands of years (Hewitt, 1993). The final Pleistocene glaciation receded 10,000 years ago allowing these isolated species to recolonise former habitats.

Three species of hedgehog are extant within Europe: the Western European Hedgehog (*E. europaeus*), the Eastern European Hedgehog (*Erinaceus concolour*), and the Algerian Hedgehog (*Atelerix algirus*). The Algerian hedgehog is found only on the Iberian Peninsula. This species is not native to Europe, and its presence is attributed to human introductions from North Africa (Dobson, 1998). *E. europaeus* and *E. concolour* are both native European species that have largely exclusive ranges. Limited natural hybridisation occurs in the small areas of range overlap underlining their genetic similarity (Geisler and Gropp, 1967; Mandal, 1978; Corbet, 1988). It is thought that the two species diverged from a single ancestral species during a period of glacial isolation in the late Miocene to early Pliocene, approximately 5.8 million years ago (Santucci *et al.*, 1998). The UK has only one native species: *E. europaeus*.

Allozyme and mitochondrial DNA analysis of hedgehogs throughout Europe suggests that Britain was recolonised by populations from Spain (Figure 1.1) (Filippucci and Simson, 1996; Santucci *et al.*, 1998). Both of these studies describe a high level of genetic differentiation within the species *Erinaceus europaeus*. Mitochondrial DNA analysis of a 383 bp fragment of the cytochrome *b* gene indicates that two major clades exist within Europe, one consisting of Spanish, French and UK populations and the other of the remaining Italian/German/Swedish populations (Santucci *et al.*, 1998).

One of the consequences of rapid range expansion following the release of climate constraints is a reduction in genetic diversity due to serial bottlenecking (Hewitt, 1989; Hewitt, 1996). This is a progressive effect that is more pronounced in areas most recently colonized. Considering the route of hedgehog colonization of Britain, one might have expected levels of genetic diversity to be low at this time, in comparison to diversity in Spain and Southern France. The exact extent of hedgehog genetic diversity in Britain would depend on the speed of range expansion from their glacial refugia. Another factor

could be the time period between initial recolonisation of Britain and the submersion of land bridges to mainland Europe by rising sea levels. Once isolated from mainland Europe, gene flow would no longer be possible and genetic diversity levels could increase only by mutation.

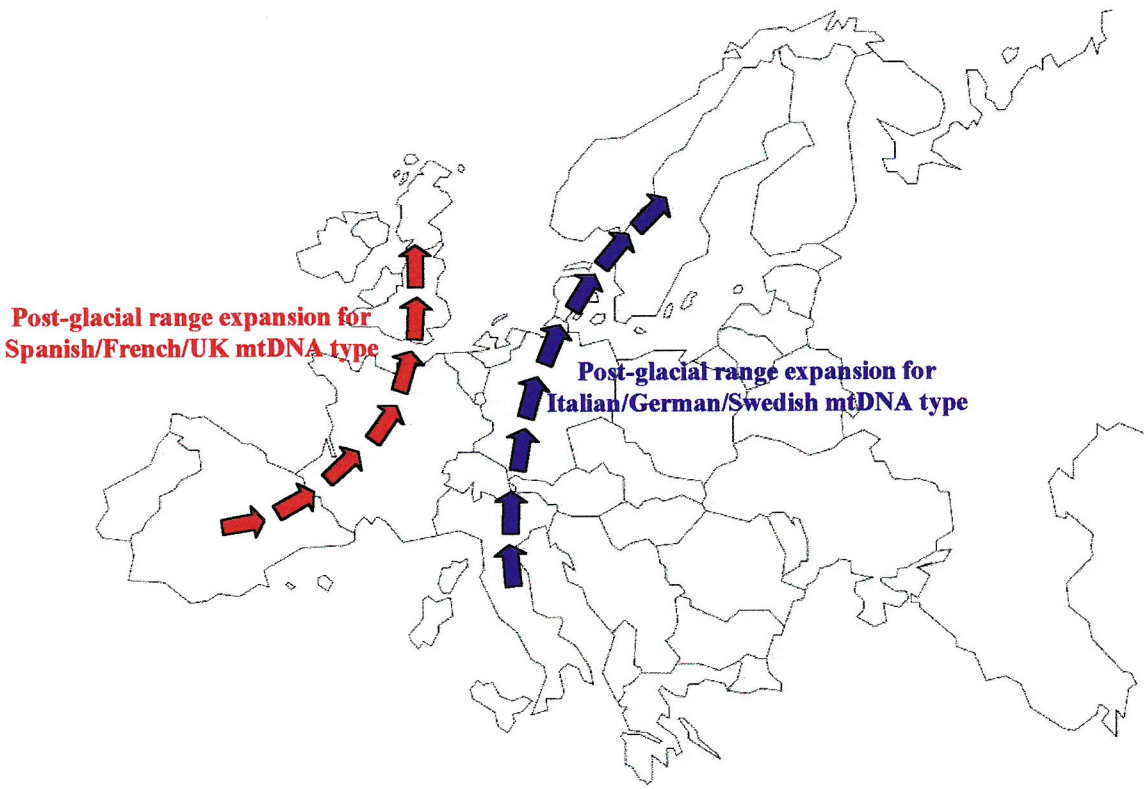

Figure 1.1 Recolonisation routes of *Erinaceus europaeus* following the end of the last Pleistocene glaciation 10,000 years ago. Coloured arrows differentiate between two major clades detected by Santucci *et al.* following phylogenetic analysis of mtDNA cytochrome *b* sequence data (Santucci *et al.*, 1998).

Given that microsatellite DNA sequences have some of the fastest mutation rates of any region of nuclear DNA (estimated in mammals to be between 10^{-5}-10^{-2} per locus per generation (Dallas, 1992; Weber and Wong, 1993)) we can make a generous estimate of the level of mutation that may have occurred since postglacial recolonisation. Assuming an average generation time of two years it can be estimated that 5000 generations have elapsed since the end of the last ice age. This gives a range of 0.05-50 mutations per microsatellite locus.

Initial assumptions about genetic diversity levels in recolonised Britain coupled with the rough estimate above might suggest that current diversity levels would be low. Add to this the effects of selection and random genetic drift, and the levels of allelic diversity may possibly be lower still. But is this really the case? Before making this assumption it is necessary to survey the current levels of genetic diversity at a local and national level. Therefore, data collection for this thesis will be performed throughout the UK, but comparisons will be made to data collected from hedgehog populations from mainland Europe.

1.2 *Landscape Change in Historical Britain*

1.2.1 10,000 BC to 1900 AD

Prior to large scale farming and urbanisation, the natural landscape of Britain would have consisted largely of deciduous woodland. This would have presented adequate nesting and foraging opportunities for hedgehog populations.

The first change to this type of habitat would have occurred around 3800 BC when neolithic man began small scale forest clearance, land cultivation and animal rearing. This type of development expanded slowly over the next 5000 years, but it wasn't until the late 18th century that major landscape changes occurred throughout Britain in the form of the "Enclosure Acts" (Slater, 1907). These acts of parliament reallocated farmers lands and divided them up using hedgerows.

Farming methods and consequent land use has had both positive and negative effects on hedgehog populations. Livestock farmers benefited hedgehogs by providing common fields in which grazing animals kept grass short. Grazed fields with adjacent scrub and woodland is ideal territory for hedgehogs. The woodland provides cover and nest material by day, and the grazed fields provide large areas of easily negotiable foraging space by night. Conversely, arable farmland, whilst potentially rich with prey, often

proves too difficult to cross and tends to be avoided by hedgehogs (Doncaster *et al.*, 2001).

1.2.2 1900 AD to Present Day

Perhaps the largest change that has been made to the hedgehog's environment has come about in the 20th century with the advent of the internal combustion engine. Motor vehicles and the roads on which they travel now cover the countryside, erecting potential barriers where hedgehogs once had unimpeded access. Large-scale urbanisation has also done much to redefine the landscape that hedgehogs inhabit. Developments of this type have the potential to fragment and isolate previously contiguous hedgehog populations. Over time, inbreeding and population differentiation could have resulted from this urbanisation and transport network expansion.

What will be the long term effect of this environmental change on hedgehog population genetic structure? It has been suggested that, as well as acting as barriers, many urban roads/road verges form wildlife corridors. These help individuals to move from sub-population to sub-population, maintaining geneflow within the urban population as a whole. Hedgehogs certainly seem to favour private gardens, sports fields etc. that provide them with easy foraging opportunities and nearby nesting sites. In order to gain a clearer understanding of the effects of anthropogenic habitat fragmentation I will test for evidence of significant genetic differentiation between populations separated by putative barriers. I will also look for evidence of increased inbreeding, and genetic isolation in populations that inhabit areas that are highly fragmented by urbanisation.

1.3 *What can the use of molecular probes add to the study of mammalian population ecology?*

Until comparatively recently, population ecology studies on organisms and assemblages of different species (communities) were performed by direct observation of behaviour and impact on habitat. While this approach has provided most of the knowledge of population and community structures and their underlying dynamic processes, it has often been of rather limited usefulness in determining the genetic structure of populations, and how they might be affected by changes in dispersal behaviour and population size.

Direct observation usually provides a snapshot that is assumed to represent longer term processes. Long term studies are not often attempted owing to practical considerations (funding, manpower, researchers unprepared to commit themselves to long term projects), and these rarely extend beyond the lifetime of the researcher (Ehrlich, 1994). Even long term studies represent only a small part of the story over evolutionary time scales.

Recent developments in the field of biochemical and molecular biology have provided us with new techniques for revealing ecological patterns and processes (Hoelzel, 1998). Statistical analysis of population genetics allows researchers to make inferences about relationships between individuals and populations, both over the short term (within the lifetime of the individual), and the long term (the evolution of the species and its relationship to other species).

However, viewed in isolation these molecular and statistical techniques can also provide misleading results at odds with observed behaviour or circumstances. For example, Balloux *et al.*, (2000) report a significant underestimation of migration rates between populations of *Sorex araneus* when using autosomal (non sex-linked) microsatellite loci; and an allozyme study of 4 populations of *Metepeira* spiders indicated high levels of geneflow, although they appeared morphologically and behaviourally distinct and

subsequent analysis of the 12S mtDNA ribosomal subunit showed them to belong to three different species (Piel and Nutt, 2000).

What can ecologists gain from these techniques beyond what can be drawn from observations and experiments on whole organisms? Biochemical and molecular techniques essentially examine the level of similarity and divergence between sequences within the DNA of individuals and groups of individuals. By analysis of appropriate regions of DNA, different questions can be addressed regarding the historical and current processes affecting the study organism. Over the last twenty years there has been an explosion of ecological studies using molecular techniques and in the number of species subjected to molecular analysis. New journals have appeared dedicated to work performed using molecular techniques (e.g. *Molecular Ecology*; *Molecular Entomology*). The field is already large and growing rapidly, and while one could not hope to compile an exhaustive review of all the work performed, I shall attempt to achieve 3 things with this review.

1) Set the aims of this Ph.D. project within the wider context of molecular approaches to population ecology of mammals.

2) Review the principal molecular techniques currently available to ecologists including a brief description of the principles on which they are based, and an assessment of their particular strengths and limitations.

3) Review the molecular techniques that have been employed by others on similar ecological themes to this thesis.

1.3.1 Molecular Approaches to Population Ecology of Mammals

Whilst mankind develops ever more efficient technologies for exploiting the natural environment, many species find themselves under increasing pressure in terms of the

amount of habitat left for them in which to survive. It is quite often the case that some species have very specific habitat requirements, which preclude them from moving on to other areas. In these situations, a population may find itself forced into an area too small to service the needs of any of the individuals within it (Doncaster *et al.*, 1996). It may also happen that a once contiguous habitat becomes divided into a number of smaller habitats, each of which contains a number of individuals from the original population (Mader, 1984). In this fragmented landscape there may be opportunity for individuals from one subpopulation to travel to another, or this may be effectively impossible (Mann and Plummer, 1995).

If local extinctions occur and some degree of dispersal or migration between patches is possible, then the balance of recolonisation and extinction describe a metapopulation (Levins, 1970). The metapopulation concept attempts to explain population structure in fragmented populations (Hanski and Gilpin, 1997; Hanski, 1999). The dynamic factors controlling metapopulation structure are the level of gene flow and rates of extinction and of recolonisation events. It is worth noting that the existence of a metapopulation structure is not always an outcome of human habitat intrusion. There are examples of naturally occurring metapopulations such as shrews (*Sorex araneus*, *S. caecutiens*, and *S. minutes*) on Finnish lakeland islets (Peltonen and Hanski, 1991), and Danish house mice (*Mus musculus*) in Jutland (Dallas *et al.*, 1995).

The actual dynamics of this system are in dispute, and a number of models have been proposed to attempt to describe the processes involved. These models are not all equally applicable nor mutually exclusive, but tend to be fall into one of three categories.

i) Reaction diffusion: this model simply assumes a random dispersal, making all patches equally likely to be connected (Reeve and Usher, 1989).

ii) Stepping-stone: where each patch is accessible to some local populations but not to others. Total connectivity of patches is achieved in a stepwise manner (Schippers *et al.*, 1996). The "rescue effect" of neighbours controls the dynamics of recolonisation.

iii) Incidence-function: where the likelihood of extinction is related to the size of the habitat patch, and the likelihood of recolonisation is related to the degree of its isolation (Hanski, 1994).

The third type of model has produced convincing analogues to patterns found in nature. Whilst habitat patch size can be estimated fairly easily, isolation of the patch is not simply a function of the distance from other patches. Physical and ecological barriers to dispersal and gene flow often isolate a habitat patch to a far greater extent than could be attributed to geographical distance alone. Ascertaining whether this sort of isolation is going on is in itself a difficult task. It is even more difficult to identify specific barriers in a heterogeneous environment, and assess the effectiveness of the individual barrier. The isolation may result from multiplicity of individual barriers, preventing a clear cut evaluation of their individual effectiveness. The barriers may be transient, such as the presence of predators. Clearly a full knowledge of the ecology of the organism is a prerequisite for recognising barriers. In this respect the study of hedgehogs described in this thesis has some advantages as much prior work has been performed on their ecology (Doncaster, 1992; 1993; 1994; 1996, Micol *et al.*, 1994; Ward *et al.*, 1997), and specifically that of the behavioural responses of dispersing hedgehogs to landscape features (Doncaster *et al.*, 2001; Rondinini and Doncaster, submitted).

The genetic consequences of a population existing in this sort of structure depends on a number of ecological questions related to dispersal and reproductive strategy. Firstly, is the species in question mobile in the adult form, or is gene flow carried out by a dispersal of gametes? If the latter, recolonisation of totally extinct population, although possible, is unlikely. In species that disperse as juveniles or adults the mobility of reproductively active individuals, as well as their susceptibility to topographical and ecological barriers, is of great importance.

A defining influence on the genetic structure of sexually reproducing populations is the extent to which the different sexes disperse. This will be dealt with in greater detail in the final part of this review. However, for the time being it is sufficient to say that the

model with which this project is concerned is mammalian. In the majority of mammalian species there is a disparity in dispersal patterns between males and females. Male mammals tend to disperse over a wider area than do females (Greenwood, 1980; Melnick and Hoelzer, 1992). The specific case being studied in this project is that of *Erinaceus europaeus* or the European hedgehog, which follows this general pattern. The European hedgehog generally inhabits a comparatively small home range, but as with most mammals, they display a pattern of predominantly male dispersal. Estimated values of mean home range vary, but usually show a significant difference between sexes i.e. adult males and females, respectively, 32 and 10 ha (Reeve, 1982), 46.5 and 19.7 ha (Kristiansson, 1984) and 57.13 and 29.08 ha (Boitani and Reggiani, 1984).

1.3.2 Review of Molecular Techniques Available

This section is not intended to be an exhaustive review of all the different molecular techniques available to ecologists. My intention is to give a brief description of the most widely used techniques, and their strengths and limitations. This will reveal the most appropriate molecular markers for studying *E. europaeus*.

1.3.2.1 Allozymes/Isozymes

Allozymes and isozymes were the first molecular/biochemical markers to be used to answer ecological questions. Whilst not strictly categorised as molecular markers, they have been used extensively on a number of different organisms.

These types of markers are, in fact, proteins, and as such they infer variation in the genome of an organism as opposed to showing it directly. Both allozymes and isozymes are usually active enzymes. Allozyme refers to charge/size variant enzymes coded for by alleles at the same locus i.e. a diploid organism may be homozygous (have two copies of the same allozyme variant) or heterozygous (have two different allozyme variants) for that particular locus. Isozyme refers to enzymes that are functionally the same, but are coded for by different loci.

Allozymes are the most useful of the protein markers for ecologists as they can be used to assess variation at a single protein coding locus. Isozymes encompass all variants of a particular enzyme whether the are produced at single or multiple loci, consequently they are less useful for assessing sequence evolution (Hoelzel, 1998). Allozymes are codominant, and they usually assort in a classical Mendelian manner. They are comparatively cheap to isolate and manipulate (separated on starch gels, on the basis of charge to mass ratio, using electrophoresis), and are identified using enzyme specific, colour change reactions.

Where protein markers fail in comparison to DNA markers is that they can give at best two-thirds of the information that is present in the DNA sequence. The "Wobble Hypothesis" (Crick, 1966) states that, of the three bases that are required to code for every amino acid, the first two are always the same for a given amino acid, but the third shows a degree of degeneracy. In practice a mutation leading to a change in any of the first two bases in each codon would result in a change of base which might well have an altered charge. But a mutation leading to a change in the third base would frequently produce a protein with the same amino acid sequence.

In addition, allozymes cannot be described as selectively neutral. Given that they are all functional enzymes, it follows that any mutation that leads to a reduction in efficiency would be selected against, and presumably eradicated quickly.

1.3.2.2 Randomly Amplified Polymorphic DNA (RAPD)

RAPD employs a technique known as polymerase chain reaction (PCR) (as do a number of the techniques described below), which was developed in the mid 1980s (Saiki *et al.*, 1988). To put it simply, PCR is used to amplify regions of DNA exponentially. The technique employs thermostable DNA polymerase enzymes, and short, complementary pieces of DNA (primers) that flank the area to be amplified. This reaction can be performed on very small amounts of genomic DNA template.

The RAPD technique uses a single short primer, about 10 base pairs in length, which are scattered randomly throughout the genome. They adhere to areas of similarity that have a sequence complementary to that of the primer; the degree of homology required being determined by the annealing temperature of the PCR reaction. Opposing primers, annealing within approximately 3kb of each other, allow the intervening DNA sequences to be amplified. This technique produces a number of fragments, which can be separated on an agarose or polyacrylamide gel using electrophoresis. The visible fragments or bands should be the same for an individual run under the same reaction conditions. Some of these bands may differ between individuals however, reflecting polymorphism in the size of the amplified fragments of DNA.

The main benefit of this technique is that it requires no previous knowledge of the organism's DNA sequence (Hadrys *et al.*, 1992). The primers are short enough to be non species-specific. The drawbacks are that the origin of these bands within the genome remain unknown without much extra work. This means that no inference can be made as to whether the individual is heterozygous or homozygous for any locus (Lynch and Milligan, 1994), or about the nature of the region of DNA from which the fragment was amplified. It is therefore impossible to tell whether the area mutates quickly or slowly or whether it is selectively neutral. It is also easy to gain or lose bands if the concentration of the template DNA varies substantially. From a practical point of view RAPD presents difficulties in so far as the lack of specificity of the primers means that great care has to be taken not to introduce contamination into the sample. RAPD primers will amplify fragments from almost any template DNA, and it is thus virtually impossible to differentiate contamination from sample polymorphism.

1.3.2.3 Microsatellite DNA

Microsatellite loci are short, tandemly repeating, usually non-coding regions of DNA (Ashley and Dow, 1994; Jarne and Lagoda, 1996). Members of a microsatellite "family" of sequences are usually scattered throughout the genome and have been found to be

highly polymorphic by length (Litt and Luty, 1989; Weber and May, 1989; Bruford and Wayne, 1993). Several microsatellite loci used in conjunction will give a unique genotype of each individual (Hughes and Queller, 1993). They have been used successfully in population studies (Ashley and Dow, 1994), to assess kinship and degrees of relatedness in a number of animal species (Queller *et al.*, 1993), such as the swallow (Primmer *et al.*, 1995) and deer (Purvis, 1997), as well as humans (Bowcock *et al.*, 1994).

These loci usually contain repeating units of between one and five base pairs. They occur in one, or a combination of three structures, pure, compound or interrupted, as displayed in Figure 1.2, (Jarne and Lagoda, 1996).

PURE CACACACACACACACACACA

COMPOUND CACACACA**GAGAGAGAGAGA**

INTERRUPTED CACA**TT**CACACA**TT**CA**TT**CA

Figure 1.2 Three possible microsatellite sequences, showing the three main types of structure. This could occur as a combination of any nucleotide, between two and five nucleotides per repeating unit, and also in any combination of the three types within one microsatellite region (Jarne and Lagoda, 1996). **Bold type** is included for clarity, and simply indicates the cessation of the basic initial repeating unit (in this case CA).

A convenient shorthand to express the structure of a microsatellite is the sequence of one repeat unit and a subscripted number of repeats. For example, a pure trinucleotide repeat may look like this:

$$CAGCAGCAG = (CAG)_3$$

and a compound dinucleotide repeat may take this form:

$$TATACGCG = (TA)_2/(CG)_2$$

It remains unclear by which mechanisms microsatellites have developed this high level of polymorphism. There is speculation that it is as a result of natural 'slippage' of polymerase during DNA replication (Levinson and Gutman, 1987; Kunkel, 1993; Strand *et al.*, 1993), this being supported experimentally by the observation that DNA polymerase tends to make mistakes in tracts of repetitive DNA (Schloetterer and Tautz, 1992), or through unequal exchange during meiosis (Valdez *et al.*, 1993).

It is thought that the majority of mutation events consist of the addition or deletion of single or double repeat units (Weber and Wong, 1993; Amos *et al.*, 1996). However, there is evidence that occasional large mutations occur which must be accounted for (Goldstein *et al.*, 1995). It also seems to be the case that there is asymmetry in the rates of addition versus deletion mutations. Both allele frequency distribution patterns and experimental observations suggest that many microsatellite loci are subject to an expansion bias i.e. a tendency to increase rather than decrease in size (Weber and Wong, 1993; Ashley and Warren, 1995; Rubinsztein, 1995; Amos *et al.*, 1996; Primmer *et al.*, 1996).

In order to be useful for this type of study, each locus must exhibit some degree of polymorphism. At present there are few data to suggest that one repetitive sequence is any more or less variable than another, but it would appear that interrupted and compound sequences are less polymorphic than pure sequences (Goldstein and Clark, 1995; Pepin *et al.*, 1995). The bases interrupting the sequence seem to stabilise the microsatellite by supplying a more distinctive binding site for the DNA polymerase, and so reducing the chance of polymerase slippage. What is more, there has been speculation that mutation rates at loci for which the individual is heterozygous may be proportionally higher as the size difference between alleles increases (Amos *et al.*, 1996).

There is little point however in trying to predict whether a particular locus will be polymorphic by looking at its repetitive motif and structure. Each locus must be screened in a number of individuals in order to establish its suitability for use as a molecular marker.

The extent of polymorphism, or in effect the number of alleles at each locus, depends on its mutation rate. This is estimated to be between 10^{-5}-10^{-2} per locus, per generation, according to direct counting of mutations from pedigrees in humans and mice, and inference of mutations in recombinant inbred (RI) strains of mice (Dallas, 1992; Weber and Wong, 1993). They often show higher levels of polymorphism than other comparable nuclear markers such as allozymes (Hughes and Queller, 1993). This high level of mutation is ideal for investigating close relationships, but rules out the use of microsatellite DNA as a means to infer phylogenetic relationships over long periods of time, i.e. more than 10^2-10^5 generations. Although there is evidence of the flanking regions of some microsatellite loci being conserved between closely related species (Pemberton *et al.*, 1995; Engel *et al.*, 1996), and that they may even occur in orthologous positions in diverse mammalian genomes (Stallings, 1995) the greater the genetic distance between individuals, the greater the chance of homoplasy occurring. Homoplasy (convergent evolution) occurs when two alleles appear to be the same, but each has arrived at this state via a different mutational pathway (Jarne and Lagoda, 1996), i.e. they are not identical by descent.

One cautionary note in the use of microsatellite DNA in population studies is the occurrence of "null" or non-amplifying alleles (Pemberton *et al.*, 1995; Primmer *et al.*, 1995). The use of PCR to amplify microsatellite loci identified by flanking primers can only work so long as there is no mutation in the flanking areas themselves. If these areas mutate, then amplification of the allele may not occur. Microsatellites are inherited according to Mendelian principles, so a heterozygote with a null allele will appear as a homozygote. This could potentially distort any analysis of results, and potentially greatly reduces the power of the technique.

1.3.2.4 DNA Sequencing & Restriction Fragment Length Polymorphism (RFLPs)

Whilst unrelated in terms of procedure and technique, DNA sequencing and RFLPs have been included together as they are often used to achieve the same ends. DNA sequencing techniques can reveal the order of the bases from fragments of DNA hundreds of nucleotides in length. Obviously this is the most direct way to examine genetic variation between individuals. The data can be statistically analysed, and is flexible enough to be used in a number of different ways (e.g. phylogenetically; haplotyping; designing primers; assessing variation between populations etc.). Unfortunately, DNA sequencing is time consuming and expensive to perform, which makes its use inappropriate for studies involving large numbers of samples.

Restriction Fragment Length Polymorphism is often used as a cheap alternative to large scale DNA sequencing. Although it does not provide the amount of data produced by sequencing, it can be used to target certain areas of DNA and detect mutations. It is comparatively quick and cheap to perform, and is ideally suited to large scale sample screening.

To examine an RFLP, the region of DNA to be assessed (usually greater than 1 kb) is digested using what are known as restriction endonucleases. Restriction endonucleases are a suite of enzymes, produced by bacteria and yeast, which cut nucleic acids. Each restriction endonuclease recognises a specific sequence of bases (usually between 4 and 8), at which the enzyme attaches and then cuts both DNA strands. Any region of DNA undergoing restriction analysis will be cut in a predictable manner, giving a characteristic restriction pattern when separated by gel electrophoresis. If a mutation occurs within a restriction recognition site, then the site will be lost and the restriction pattern will differ. Careful selection of DNA region, and restriction enzymes, can effectively type large numbers of individuals to the required level of resolution.

1.3.2.5 Mitochondrial DNA (mtDNA)

This is not a molecular technique as such; rather it is a region of DNA. The special circumstances that surround mtDNA make it particularly useful in ways that nuclear DNA cannot be.

All eukaryotic cells contain mitochondria, which are responsible for respiration. Each mitochondrion contains its own DNA, which is distinct from the DNA of the cell nucleus. In most diploid, sexually reproducing organisms, nuclear DNA is a mixture of the parents DNA, but mitochondria are usually only passed on maternally within the egg. Sperm also carry one or a small number of mitochondria, but these are eliminated during early embryogenesis, paternal mitochondrial leakage occurring only in some interspecific crosses (Kaneda *et al.*, 1995). There are examples of species that exhibit doubly uniparental inheritance (DUI) of mitochondrial DNA, such as *Mytilus edulis* (Hoeh *et al.*, 1991; Stewart *et al.*, 1995). This describes a genetic system where female parents pass on their mitochondrial DNA to their female offspring, and male parents, to their male offspring.

This method of mitochondrial inheritance means two things, firstly that mtDNA does not undergo meiosis, so any change in sequence between generations is purely as a result of mutation, and not recombination. Secondly, that any individual will inherit mtDNA from an uninterrupted maternal lineage.

Animal mitochondrial DNA is also known to be the region of DNA with the fastest rate of mutation, except microsatellite DNA, particularly in the D-loop region or origin of replication (Brown *et al.*, 1979; Brown, 1983). This is thought to be a result of inefficient DNA repair mechanisms following replication together with the free oxygen radicals often generated during mitochondrial metabolism.

This method of inheritance, coupled with the assumption of a "molecular clock" i.e. a constant fixation rate (Zuckerkandl, 1991) makes it ideal for recreating phylogenetic trees

and studying the occurrence of speciation and introgression (Wayne and Jenks, 1991; Ruedi *et al.*, 1997; Thulin *et al.*, 1997). It can also be used to guide managers in deciding conservation and stock management priorities (Moritz, 1994; Bernatchez and Osinov, 1995; Brown Gladden *et al.*, 1997).

It is widely accepted that any one individual will contain only one type of mtDNA, which they will share with all their full-siblings and maternal half-siblings. However, there is some evidence of HETEROPLASMY, i.e. that individuals in certain species may contain a number of different types of mtDNA, even within a single cell (Krettek *et al.*, 1995; Lunt *et al.*, 1998). Obviously, this could create some problems in terms of defining levels of variability within populations, as well as determining strict haplotypes.

1.3.3 Ecological patterns & processes revealed by molecular techniques, with particular reference to mammals

1.3.3.1 Sex Biased Dispersal

Much literature has been published on this subject generally, and there is a growing series of studies focusing on mammals. The literature dealing with molecular investigations of landscape fragmentation, dispersal and barriers to gene flow in mammals is largely restricted to cetaceans (Hoelzel, 1992; Baker *et al.*, 1994; Brown Gladden *et al.*, 1997; O'Corry-Crowe *et al.*, 1997), primates (Melnick and Hoelzer, 1992; Goldberg, 1997; Keane *et al.*, 1997), and rodents and marsupials (Waser and Elliott, 1991; Ishibashi *et al.*, 1997; Moncrief *et al.*, 1997; Moritz *et al.*, 1997; Petri *et al.*, 1997; Stacy *et al.*, 1997; Taylor *et al.*, 1997).

A study of Serengeti lions by Packer *et al.* (1991) used DNA fingerprinting (similar to microsatellite DNA), to assess kinship within the prides. They found that the majority of female lions are recruited into their natal pride, whereas most of the males are forced to leave and find a new pride. As mentioned previously, male mammals do tend to disperse to a much greater extent than do the females (Greenwood, 1980), but because of the

infrequency of these dispersal events, they have often been missed by conventional radio tracking or capture-release-recapture studies (Koenig *et al.*, 1996). The implementation of molecular techniques and subsequent statistical analysis has allowed a more accurate estimation of dispersal and its consequences for gene flow to be determined (Slatkin and Barton, 1989; Slatkin, 1994; Paetkau *et al.*, 1995; Koenig *et al.*, 1996; Favre *et al.*, 1997; Mossman and Waser, 1999).

Another example of this male/female dispersal bias, and probably the most extreme case of this phenomenon to be published, is that of the macaque (Melnick and Hoelzer, 1992). This study compares mtDNA variation, to that of nuclear DNA (using allozymes), throughout a number of macaque populations in Southeast Asia and Indo-China. They found that while nuclear DNA was largely homogeneous throughout the region, the vast majority of variation in mtDNA occurred between groups. A large degree of mtDNA population subdivision, contrasted with uniform variation in the nuclear genome, suggested extreme natal philopatry in female macaques, and virtually universal dispersal of the males. Male biased dispersal in adult beluga whales was found using mtDNA, although this showed population stratification which separated adult males from both females and juvenile males (O'Corry-Crowe *et al.*, 1997).

While this dispersal pattern is predominantly characteristic of mammals, the same system does occur in other types of animals. For example populations of the endangered green turtle were found to be highly structured with regards to mtDNA, but RFLP analyses of anonymous nuclear loci showed only moderate population substructure, suggesting female philopatry and moderate rates of male-mediated gene flow (Karl *et al.*, 1992).

1.3.3.2 Barriers and corridors

To what extent is dispersal possible in a fragmented and isolated population? How is gene flow defined in this situation, and what will be the effect of isolation or barriers to gene flow? Also, what are the implications, with regards to recolonisation of habitat

patches following local extinctions, of a species with limited female dispersal? These are questions that underly the subjects of chapters 3 and 4 of my thesis.

Clearly, the extent of dispersal that occurs depends on the capabilities of the species attempting to disperse, and the level of heterogeneity of the habitat it inhabits. It should also be pointed out that dispersal of sexually active individuals does not necessarily constitute gene flow. An individual dispersing must breed and produce viable offspring. This has been defined as the movement of genes and their subsequent incorporation into new gene pools (Endler, 1977; Gaines and McClenaghan, 1980), and more stringently, with the addition of the caveat that once having entered the population, the gene must not be selected against. However, Barton (1992) found this interpretation unsatisfactory as it relies on the behaviour of particular alleles, rather than on any property affecting the whole genome, and suggested that gene flow be defined as the entry of selectively neutral alleles into a new gene pool.

There is a growing body of research that makes use of molecular techniques to deal with the effect of barriers to gene flow and their effect on the genetic population structure. A study of the prickly skink in Queensland, Australia (Cunningham and Moritz, 1998), used mtDNA and allozymes to study the effects of isolation in recently fragmented rainforest. The isolation of patches appeared to be virtually absolute, and inbreeding levels were high. However, the results were inconclusive in regard to recent habitat changes as the genetic structure appeared to be dominated by historical (natural), and not current (anthropogenic) fragmentation.

A similar picture was found in the eastern massasauga rattlesnake (Gibbs *et al.*, 1997). Fine scale genetic structuring was found using microsatellite DNA amongst both isolated populations, and perhaps more unusually within continuous populations. The authors suggested that this might have resulted from an inherently limited dispersal capability, leaving them vulnerable to genetic drift. They were unable to clarify their findings further, because of a lack of relevant ecological data.

More convincing results were gained with studies on *Rana temporaria* (common frog) around Brighton, Sussex (Hitchings and Beebee, 1997). The common frog essentially lives its life in and around ponds, but migrates between them, often over comparatively large distances. It might be imagined that the opportunity to migrate between ponds would be lower in an urban area than an equivalent rural area. Using allozymes and mtDNA, Hitchings & Beebee (1997) found this to be the case: genetic differentiation between subpopulations in urban areas (separated by an average of 2.3 km) was twice that of the rural populations (separated by an average of 41 km).

The complex topology of the study site meant that no specific barriers were defined by Hitchings and Beebee, (1997). In some studies initial identification of putative barriers are easier to achieve. For instance, although nuclear variation in the Asian macaques studied by Melnick and Hoelzer (1992) was consistent throughout the populations with very high levels of mtDNA substructuring, the one type of feature that did show a significant differentiation in nuclear variation was the major river. It appeared that despite the wide dispersal of the male macaques, these broad stretches of water presented a significant barrier to gene flow. This would not have been apparent had mtDNA been used alone, as the differences resulting from presence of the rivers would have been non-significant as compared to the differences attributed to female sedentism. Presumably, were the levels of female migration to increase, then mtDNA differentiation, as a result of the river barriers, would become increasingly significant.

Gerlach and Musolf, (2000) found significant, and measurable, barrier effects between populations of bank vole (*Clethrionomys glareolus*) separated by a natural barrier, the River Rhine at Lake Constance, Germany, and artificial barriers, major motorways. However, this effect was not detected across minor roadways or railways suggesting that barrier size or traffic density may play some part in the features effectiveness as a barrier to gene flow.

Historical barrier effects were found to be in evidence in populations of the common shrew (*Sorex araneus*) in the Swiss Alps (Lugon-Moulin *et al.*, 1999). The presence of

multiple riverine barriers, intersecting populations sampled in a linear manner, did not produce significant barrier effects over and above those related to geographical distance alone. Hybridisation of chromosome races, historically isolated by glacier movement, was found to have a more significant effect on contemporary genetic structure than did current topographical features.

Significant isolation by distance effects, and an associated reduction of nuclear genetic diversity, was found in Scottish otters (*Lutra lutra*) inhabiting the Orkney and Shetland islands, as compared to populations on the mainland (Dallas *et al.*, 1999). However, levels of genetic diversity were found to be comparable between fragmented and continuous mainland populations. Presumably, the potentially highly mobile nature of the otter reduces the effect of barriers that would be intransigent to less mobile organisms.

European hedgehog populations within Oxfordshire were found to show a degree of genetic differentiation disproportional to geographic distance, when separated by major roads, railways or waterways (Johnson, 1996). This study was performed using nuclear DNA only (microsatellites), so no reference can be made to the level of genetic substructuring within the mtDNA. The author also points out that multiple barriers usually existed between the populations. This means that it is virtually impossible to attribute the degree to which each individual barrier is preventing or reducing gene flow. There are also a number of barriers which are more ecological than topological in nature, and are harder to identify, such as the presence of predators. Hedgehogs are known to be competitors to, and prey of, the European badger (*Meles meles*) (Doncaster, 1992). Although they compete for the same food they are known to actively avoid areas of high badger density (Doncaster, 1993; Doncaster, 1994; Micol *et al.*, 1994; Ward *et al.*, 1996). Once again this would produce a significant barrier to hedgehog movement, but would be difficult to identify or quantify without detailed knowledge of badger distributions.

A reduction in general dispersal opportunity (effectively gene flow) may lead to a more rapid rate of population differentiation, as well as an increased level of inbreeding. A

species with limited female migration may find that habitat patches that have undergone local extinctions cannot be re-colonised. Even if males are wide ranging, they cannot re-establish a population in a patch with no females, and are unlikely to stay in a territory where there are no females to mate with. The same is not true of a female, as a single, pregnant female can be the progenitor of a new population (Peltonen and Hanski, 1991; Brookes *et al.*, 1997).

A study of mtDNA of Australian feral rabbit populations sought to identify persistence in, and recolonisation of, habitat patches following local eradication measures (Fuller *et al.*, 1997). They compared the results for populations with a high level of connectivity between patches, and populations that are more isolated, in order to decide on effective control measures for these feral populations. The mtDNA data suggested that the isolated populations show a higher degree of population subdivision presumably as a result of a corresponding reduction in gene flow. The control regime that was recommended for populations in arid areas suggested local exterminations in the required areas. However, for semiarid areas, large scale extermination was recommended in order to prevent recolonisation. This suggests that even in this mammalian system a moderate amount of female dispersal must occur.

1.3.4 Conclusions

It would seem that the advent of reliable molecular techniques has provided population ecologists with an invaluable tool with which to study the more cryptic and infrequent processes which define population structure of genetic patterns. I have demonstrated that a number of different techniques are available with which to carry out this type of work. However, this in itself can lead to some problems, as a lack of understanding of the processes that underlie these techniques, or failure to apply these techniques judiciously, can lead to incomplete or misleading interpretations.

The statistical techniques used to analyse molecular data are also becoming increasingly sophisticated. As such, they are reflecting real processes more closely all the time, and are absolutely essential to this type of work. The systems being studied at the population level are themselves extremely complex, and under the influence of many external and internal factors, some of which have been identified and some of which have not.

Evolution and the formation of complex biotic systems are ultimately driven by the interaction of environmental factors with molecular level processes. Whilst molecular data cannot make up for a lack in ecological knowledge, it can be used to assess priorities of study and probabilities in both well understood and less well understood systems.

It's also becoming evident that ecological data cannot make up for a lack of molecular data, and vice-versa. Without full phylogenetic information, ecological patterns may be attributed to ecological dynamics (competition, predation, dispersal etc.) without appreciating the evolutionary lineages that determine which groups of organisms possess which attributes. Thus, for example, hedgehogs may be nocturnal because of ecological pressures faced by their ancestors, not because of any current pressures from diurnal predators etc.

1.3.4.1 Specific objectives of the project

This project employs the molecular techniques described in sections 1.3.2.3-1.3.2.5 to investigate the current genetic structure of hedgehog populations in the United Kingdom. My specific objectives are as follows.

1) Using nuclear and mitochondrial DNA, I will carry out a hierarchical survey of genetic variability from local to international levels. Nuclear microsatellite data, from hedgehog populations within the UK and Finland, will be used to obtain estimates of the rate of increase of genetic differentiation vs. geographical distance between populations (section 2.3.1). A spatially hierarchical analysis of molecular

variance (AMOVA) will be used on mtDNA D-loop sequence data to gauge the scale at which most variation occurs, and hence assess the degree of resolution that can be expected when asking ecological questions (section 2.3.4). Finally, the relationship between mitochondrial haplotypes will be established using neighbour-joining (NJ) methods based on genetic distances (section 2.3.5)

2) At a local scale, target populations separated by putative barriers to gene flow will be compared using nuclear microsatellite DNA. Correlation analysis of genetic distance values vs. geographical distance will be used to establish whether isolation distance is a significant factor in shaping local population genetic structure (section 3.3.5). Habitat fragmentation levels will be tested for a significant relationship with genetic population parameters estimates for relatedness and inbreeding (section 3.3.6). The genetic effects of composite and individual barriers will be tested and quantified using an extended correlation analysis, AMOVA, and direct comparison of population differentiation based on genetic parameter estimators (sections 3.3.7 and 3.3.8). This will be combined with radio-tracking data from animals within these populations. Statistical analysis of these data will identify legitimate barriers and quantify the degree to which they impede gene flow.

3) Nuclear and mitochondrial DNA will be compared at a local scale in order to detect differences in the dispersal potential of male and female hedgehogs, which will provide an insight into recolonisation rates following previous local extinction events. Hierarchical AMOVA will be performed at a local scale on nuclear microsatellite data (bi-parentally inherited), and on mitochondrial microsatellite allele data (strictly maternally inherited), to test whether a long standing male dispersal bias is in evidence (section 4.3.2). Nuclear microsatellite data will be analysed for population assignment of all sampled hedgehogs in order to test whether a male dispersal bias is in evidence over the last three generations (section 4.3.3). Overall migration rates will be assessed from nuclear microsatellite data, and compared for consistency with results generated by assignment testing (section 4.3.4). Finally, phylogenetic relationships between subpopulations, based on genetic distances generated from

nuclear microsatellite data, will be tested using NJ methods and bootstrap support (section 4.3.5). This will be examined in the context of radio-tracking results and geographical features, for ecological meaning relevant to existing knowledge of hedgehog dispersal behaviour.

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2.0 Assessment of current genetic variability of populations of *E. europaeus* in the UK: A hierarchical analysis

2.1 *Introduction*

2.1.1 Contemporary genetic structure and post-glacial recolonisation

Contemporary patterns of genetic population structure can be shaped by recent events, such as anthropogenic habitat fragmentation as a result of urbanisation (Hitchings and Beebee, 1997; Becher and Griffiths, 1998) or deforestation (Beier, 1995; Cunningham and Moritz, 1998), as well as by more historic events such as glacier movement (Lugon-Moulin *et al.*, 1999). In this chapter I aim to investigate at which geographical scales maximum diversity occurs, and identify areas of potential 'source' and 'sink' populations from contemporary genetic population structure in *E. europaeus* in the UK. I will use this information to draw historical inferences pertaining to dispersal, recolonisation, and other possible genetic population structuring factors. The way these events affect existing population structure depends largely on the patterns of initial colonisation and population establishment.

Extant populations of *Erinaceus europaeus* are descendents of populations that colonised or recolonised the UK from glacial refugia in mainland Europe at the end of the last ice age, 10,000 years ago (Hewitt, 1993). Many plant and animal species native to northern Europe and the UK underwent similar range expansions, from southerly refugia, following Pleistocene glaciations. The genetic consequences of this comparatively rapid expansion have been extensively studied (Hewitt, 1989; Avise, 1994; Hewitt, 1996). One important consequence was the progressive loss of alleleic diversity, as a result of serial bottlenecking, at the leading edge of recolonising populations (Hewitt, 1996; Ibrahim *et al.*, 1996). With the UK being close to the northern limit of the post-glacial expansion of *E. europaeus* it is reasonable to assume that British hedgehogs will have low levels of genetic diversity as compared to former glacial refugia such as southern Spain and Italy, as appears to be the case (Santucci *et al.*, 1998).

The western European hedgehog, *E. europaeus*, is one of two species of hedgehog recognised as being native to Europe, the other being *Erinaceus concolour*, the eastern

European hedgehog. *E. concolour* is believed to be the closest living relative of *E. europaeus* (Geisler and Gropp, 1967; Mandal, 1978; Corbet, 1988). Genetic data indicate that they diverged at some point during periods of increased glaciation in the late Miocene to early Pliocene, approximately 5.8 million years ago (Santucci *et al.*, 1998). The distributions of these two species are largely separate, although an area of overlap does occur which stretches from the Baltic to the Adriatic and is about 200 km wide in the Czech Republic. It is believed that limited hybridisation may occur within this area, although each species is known to exhibit a distinct karyotype (Geisler and Gropp, 1967; Mandal, 1978; Searle and Erskine, 1985; Sokolov *et al.*, 1991).

Studies of morphological and molecular characteristics have determined that each species is composed of a number of subspecies (Corbet, 1988; Reeve, 1994; Filippucci and Simson, 1996; Santucci *et al.*, 1998). Filippucci and Simson (1996) and Santucci *et al.* (1998) suggest that *E. europaeus* diverged into two major clades, which appear to be distributed into eastern and western populations. Based on evidence from the mitochondrial cytochrome *b* gene Santucci *et al.* (1998) estimate a divergence time of approximately 3 million years ago. They also find molecular evidence to suggest that this coincides with divergence of the major clades within *E. concolour*, leading them to speculate that this was also a result of the Pleistocene glaciation (Eyles, 1993).

The available sequence data for the entire mitochondrial genome of *E. europaeus* is based on an individual from Kävlinge, Sweden (Krettek *et al.*, 1995). According to Santucci *et al* (1998) this associates with the eastern clade of *E. europaeus*, which also includes German and Italian populations. The western clade includes populations from Spain, France and the UK which, based on the evidence of the mitochondrial cytochrome *b*, should exhibit significant genetic differentiation from the eastern clade.

The recolonisation of the UK appears to have been by representatives of the western clade. The submersion of land bridges between the UK and mainland Europe prevented any further breeding between subspecies within the UK itself. This was not the case on mainland Europe where the possibility of geneflow between subspecies could not be

ruled out. Therefore it is possible that the level of allelic diversity in the UK is lower than that of populations at the equivalent latitude in mainland Europe.

2.1.2 Widespread translocation of *E. europaeus*

In order to establish the level of underlying genetic diversity within the UK it is important to consider the effects of natural recolonisation. However other factors may have played a part in defining local, and possibly national diversity. The western European hedgehog is unusual in the sense that its colonisation has often been aided, if not directed, by man. Successful introductions have been made into areas in which *E. europaeus* is not native. Hedgehogs were introduced into Dunedin, New Zealand in 1870 to control agricultural pests, which had been brought by settlers. Such is the success of the hedgehog it is claimed that there are now more individuals of *E. europaeus* in New Zealand than there are in the UK.

Many of the islands around the coast of Britain do not have native populations of *E. europaeus*. Harvie-Brown (1892) and Evans and Buckley (1899) both note the introduction of *E. europaeus* to the Shetland Islands around 1860, although the origins of the translocated individuals are not recorded.

The most widespread evidence of hedgehog introductions can be found in Scandinavia. The range of *E. europaeus* has rapidly expanded northward in Scandinavia during the 20th century (Kristiansson, 1981). Although common in southern Sweden, it was not until the end of the nineteenth century that hedgehog populations began to appear in the central and northern regions (Ekman, 1922). This is known to be a result of human introductions (Brehm and Ekman, 1938; Notini and Haglund, 1948). The situation in Finland is similar to that in Sweden, except that range expansion occurred even more rapidly, appearing to be most rapid between 1952 and 1975 (Kristoffersson *et al.*, 1966; Kristoffersson *et al.*, 1977). It is assumed that the animals introduced were taken from

areas that now make up the southern part of their range in these countries, however their origins do not appear to have been recorded

2.1.3 Genetic diversity within the UK

Reconstruction of post-glacial recolonisation routes in a number of species (Hewitt, 1993; Nachman *et al.*, 1994; Ferris *et al.*, 1995; Santucci *et al.*, 1998) would suggest that the most likely route taken by *E. europaeus* into the UK was from the south and southeast via existing land bridges. Given that the UK lies to the extreme north-west of the distribution of *E. europaeus* it is possible, but highly unlikely that populations of the eastern clade, Germany/Italy/Sweden, would have had a chance to reach this area before land bridges were submerged. It is likely that genetic diversity amongst colonising populations was low as a result of serial bottlenecking leading to a genetically homogeneous population establishing itself within the UK. The comparatively short period of time since the end of the last ice age, about 10,000 years, suggests that genetic mutation may not have yet lead to a significant increase in diversity.

The contemporary landscape has changed drastically over the last century for much of the wildlife of Britain. Genetic population structure in British hedgehogs, which may have remained largely unchanged since the end of the last ice age, is almost certainly undergoing some degree of change in response to changing environmental factors. There is currently no widespread genetic survey of *E. europaeus* in the UK, and previous studies of British hedgehogs population genetics have focused on specific local study sites. Therefore, it is necessary to determine the current genetic status of *E. europaeus* in the UK in order to put local scale population genetic structure studies into context. Other factors which should be considered in order to predict the outcome of future urban planning, and allow effective wildlife management schemes to be employed include knowledge of general levels of genetic diversity, and identification of local areas of high and low genetic diversity.

An understanding of the underlying genetic diversity within the UK is essential prior to local scale analysis of potentially fragmented populations. Populations subject to genetic isolation, as a result of restricted geneflow, face a potentially serious reduction in genetic diversity. Should these dispersive constraints be lifted then population recovery can only proceed if geneflow can be re-established with populations with higher diversity levels. Therefore it is important that potentially "at risk" populations (sink populations) e.g. urban populations, be identified as being within dispersal range of reservoirs of potential diversity (source populations) e.g. rural populations. In the context of this study, it is also important that genetic diversity levels are established in order to give a baseline comparison for the studies of populations within fragmented urban areas. For instance, should levels of genetic diversity be found to be low within urban environments, this may be less significant against a background of low national diversity levels.

Another factor relevant to the distribution of genetic diversity throughout the country is the original dispersal routes. As reduction of allelic diversity accompanied northward postglacial recolonisation, the route of recolonisation of the UK will may have a bearing on the distribution of diversity. For example, if initial colonisation occurred in the south and progressed in a northerly direction, genetic diversity would be expected to be at its greatest in the south and its lowest in the north. If, however, initial colonisation occurred from the east and spread from there, genetic diversity would be at its greatest in the centre of the UK, decreasing the further north and the further south you go from this area. Therefore, one aspect of this study will be to relate levels of existing genetic diversity to the current hypothesis about post-glacial recolonisation routes (Santucci *et al.*, 1998).

This study uses mitochondrial and nuclear molecular markers to seek answers to the following questions.

i. At what scale does most genetic variation occur in British hedgehogs: national, regional (between counties) or local (within counties)?

ii. Is there evidence of source and sink population centres for genetic diversity at a national scale?

iii. Are patterns of national genetic diversity consistent with the current hypothesis about post-glacial recolonisation routes (Santucci *et al.*, 1998)?

These aims will be achieved partly by the use of nuclear microsatellite data to compare genetic divergence between populations separated at different spatial scales. The primary focus of the study will be a hierarchical analysis of molecular variance (AMOVA) of mitochondrial D-loop sequence data.

2.2 *Methods*

2.2.1 Sample acquisition

2.2.1.1 Sampling strategy

Sample tissue from individuals of *E. europaeus* was sought from as wide an area within the UK as possible, as well as from comparative populations from mainland Europe (Figure 2.1 and Table 2.1). Tissue samples were collected from a single population in Dorset from Brownsea Island, in Poole Harbour (a total of 33 samples), and from 24 populations in and around Southampton, Hampshire (a total of 376), between 1996-1999. These samples were collected by the author as well as A. Davey, C.P. Doncaster, and T. Taylor. Tissue samples and extracted DNA of hedgehogs from sites in Oxford (n = 98), Grampian/Aberdeenshire (n = 25), the Shetland Islands (n = 6) and Finland (n = 5), which had been used in previous studies, were kindly supplied by C.P. Doncaster (Southampton University), P.C.D. Johnson (Cambridge University) and S. Piertney (Aberdeen University).

Subsequent hierarchical analysis was based on geographical comparison of molecular data generated from these samples. The geographical scales/ hierarchical levels are defined thus:

Local: Populations separated by less than 50 km

Regional: Populations separated by 50 –150 km

National: Populations separated by 150-1000 km

International: Populations separated by greater than 1000 km, or by a body of water in excess of 5 km wide at the narrowest point

2.2.1.2 *Sampling method*

A total of 409 tissue samples were collected from hedgehogs from sites throughout Hampshire & Dorset under Home Office Licence (Project licence number: PPL 30/1618) and English Nature Licence (Licence number: 19991166). These tissue samples were taken from living (88%) and road killed (12%) individuals between 1996 and 1999, and consisted mainly of disks of skin 3-5 mm in diameter clipped from the ear, but also included hair and spine material.

Samples were placed immediately into 95% ethanol and stored at -70°C until DNA extraction was performed. At the time of sampling a record was taken of the individuals sex, weight, and details of its geographical position and length of left hind leg, an indicator of approximate age.

Live hedgehogs were tagged in order to prevent re-sampling. The tags used were subcutaneous Radio Frequency Transponders supplied by AVID plc. (Oak Hall Manor, Sheffield Park, Uckfield, East Sussex, TN22 3QY), which gave each tagged individual a unique code, readable using a hand held scanner. Subsequent encounters with tagged hedgehogs were recorded in order to assist in population size assessment.

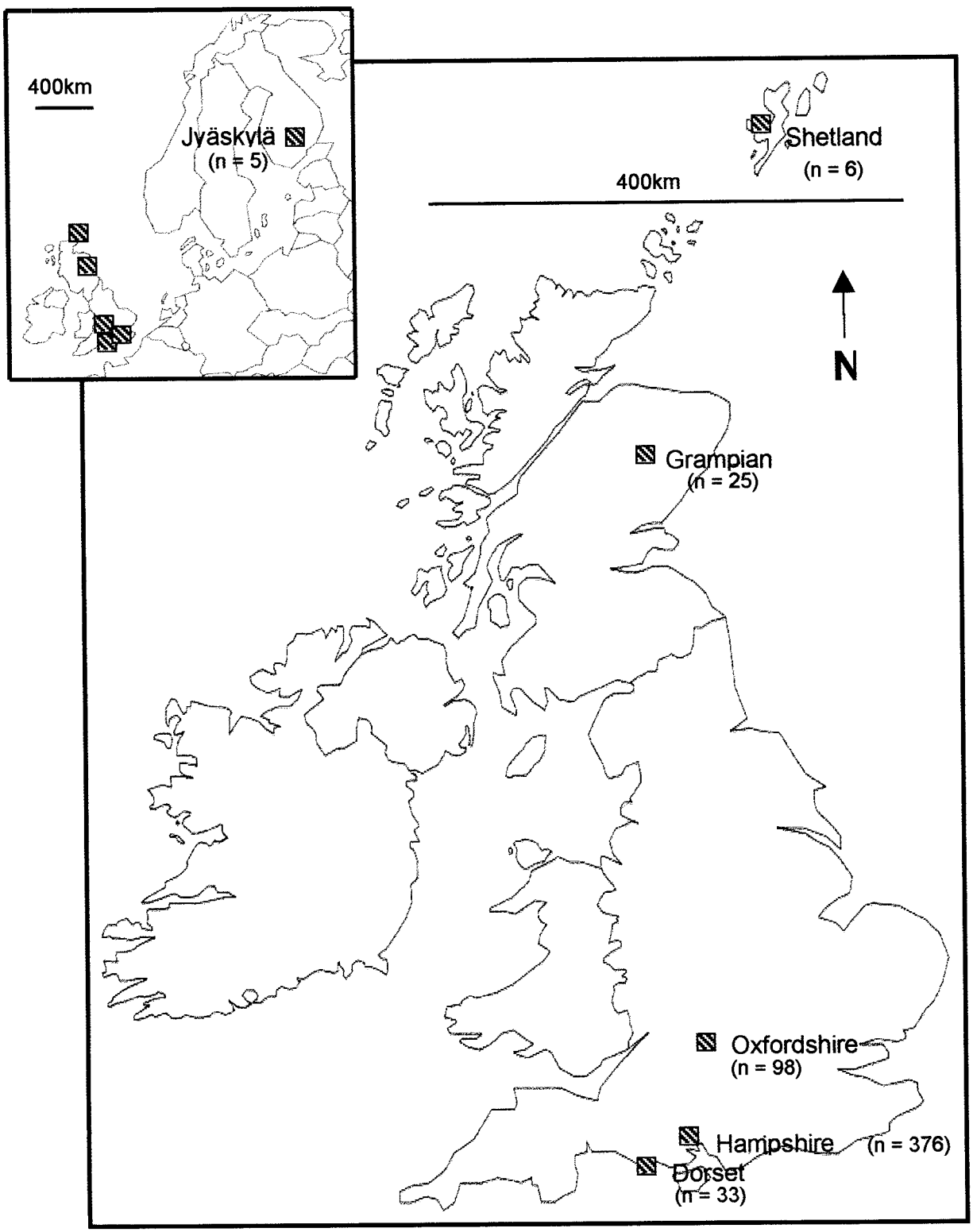

Figure 2.1 Sites from which *E. europaeus* collections were made on a regional and international scale. Sites are shown as crosshatched squares, and the number of individuals used in analyses from each site is bracketed below. Samples from Oxford, Grampian/Aberdeenshire and Finland were provided by Mr P. Johnson, Dr S. Piertney and Dr C. P. Doncaster

Table 2.1 Regional locations of sample sites from which *E. europaeus* samples were obtained. The geographical locations of sampling regions are shown in Figure 2.1. Sample sites are denoted by unique three letter codes

Country	Region	Sample site	Code
England,	*Dorset,*	Brownsea Island,	BSI
	Hampshire,	Blackfield,	BLA
		Colden Common,	COL
		Eastleigh 1,	ECL
		Eastleigh 2,	ENE
		Eastleigh 3,	ENW
		Eastleigh 4,	ESE
		Eastleigh 5,	ESH
		Eastleigh 6,	ESW
		Fawley,	FAW
		Hamble,	HAM
		Hedge End,	HED
		Holbury,	HOL
		Hythe,	HYT
		Hursley,	IBM
		Mottisfont Abbey,	MOT
		Marchwood,	MWD
		Nettley Abbey,	NET
		Redbridge,	RBR
		Romsey,	ROM
		Rownhams,	ROW
		Totton 1,	TON
		Totton 2,	TOS
	Oxfordshire,	Ditchley,	DIT
		Marston,	MAR
		Kirtlington,	KIR
		Rousham,	ROU
Scotland,	*Grampian*	Aberdeen,	ABE
	Shetland Islands,	Shetland,	SHE
Finland,	*Jyäskylä,*	Jyäskylä,	FIN

2.2.2 Molecular methods

2.2.2.1 DNA extraction

Extraction of DNA was performed using a protocol based on the "salting-out" procedure (Bruford *et al.*, 1998) according to S. Piertney's modifications described in Appendix I, section 6.1. Extracted DNA was then stored in T.E. buffer (pH 8) at -20°C.

2.2.2.2 Microsatellite development and typing

At the outset of this project, oligonucleotide primers were available for six polymorphic microsatellite loci in *E. europaeus* (Becher and Griffiths, 1997). Primers for five new polymorphic microsatellite loci were designed for this project to supplement the loci already available (Henderson *et al.*, 2000). Table 2.2 shows the new primers, which were designed using the online software PRIMER3.0 (http://www.genome.wi.mit/edu/cgi-bin/primer/primer3_www.cgi) (Rozen and Skaletsky, 1998), and were based on sequences supplied by Dr Anette Becher, from her Ph.D. Thesis (Becher, 1994). These sequences were obtained from a partial genomic library enriched for microsatellite DNA, as described in Becher and Griffiths (1997).

All hedgehog DNA samples were typed using primers incorporating the fluorescent dyes FAM, HEX and TET (Genosys Biotechnologies (Europe) Ltd.). Each sample underwent polymerase chain reaction (PCR) for each of the twelve loci. Optimal reaction conditions for the six existing primer sets given by Becher and Griffiths (1997), were further optimised to increase product yield and minimise stutter under lab conditions at Southampton University. All PCR was carried out on a HYBAID "Touchdown" thermo-cycler.

Allele sizes were ascertained using a P.E. Biosystems ABI PRISM® 377 Auto-Sequencer, employing GENESCAN® 3.1 and GENOTYPER® 2.5 software.

Table 2.2 Primer sequences for five new microsatellite loci in *Erinaceus europaeus*. Allele numbers were calculated from a sample of 132 individuals, and observed heterozygosity values from a population of 39 individuals. Observed heterozygosities did not differ significantly from expected values. All samples were drawn from populations within Southampton. Table taken from (Henderson *et al.*, 2000).

Locus	GenBank accession no.	Primer sequence (5' to 3')	Repeat type	Annealing temp	Size (bp)	No. of alleles	H_o
EEU12H	AF276820	F: CTGCATGTACCTCTCCTCTACCTC R: TTTTCTTTTTCCACCGGTGTTATC	$d(CT)_{15}$	65.2°C	100	7	0.62
EEU36H	AF276822	F: GACTCTGGAACTCAAAACCAGG R: GGTAGACAGAGAGATCAAAAGGGA	$d(CT)_{21}$	64.0°C	150	5	0.15
EEU37H	AF276823	F: ATGAGGTGAGGCTTACCAAAAA R: GGAATCTCACAGATGTAAAGTTCTAGC	$d(GT)_{23}$	64.0°C	260	6	0.71
EEU43H	AF276824	F: CCATGTACAGTGGATTTACCTGC R: ACCCTAGGAGCAACTTGGAGAT	$d(GT)_{25}(GA)_6$	65.2°C	159	9	0.84
EEU54H	AF276825	F: CATCGGCTCAGCATTCTCTT R: ATCCCCAGAGTTGTGTTTGC	$d(GA)_{31}$	56.2°C	284	8	0.70

Microsatellite data were then analysed using a number of software packages, freely available via the Internet.

2.2.2.3 *Amplification of mtDNA D-loop*

The D-loop, or Control Region, was selected as one of the mitochondrial markers for this project. The D-loop is a non-coding region of the mitochondrial genome, involved in the initiation of replication (Brown, 1983). It is known to be the most variable region of the mitochondrial genome (Moritz *et al.*, 1987). It is also known to contain a variable number tandem repeat (VNTR), which has exhibited heteroplasmy in some individuals of certain species (Hoelzel *et al.*, 1994; Krettek *et al.*, 1995). Mitochondrial D-loop primers, based on conserved sequences described in Kocher *et al.* (1989), were designed for this project from comparisons and sequence alignments performed between the hedgehog mitochondrial genome (Krettek *et al.*, 1995) and other published mammalian mtDNA sequences (tRNA-pro 5'-cctaccatcaacacccaaag-3'; tRNA-phe 5'-cccttctaagcattttcagtgc-3'). These searches were performed using Internet online BLAST searches of GENBANK and EMBL nucleic acid sequence databases. The use of these primers for sequencing was subsequently discontinued as a result of the discovery of a nuclear insertion, detailed in section 2.3.2.

All sequencing was performed using a second set of primers based on sequences supplied by F. Santucci and J. Seddon of the University of East Anglia (PHE-Hedg 5'-ataggcccttctaagcattttcag-3'; DLL-Hedg 5'-gacatctggttctttcttcaggac-3'). Primers were supplied by Genosys Biotechnologies (Europe) Ltd. All PCR was performed on a HYBAID "Touchdown" thermocycler under the following reaction conditions. Each 100µl reaction 40µL HPLC water, 10µl 10x PCR buffer (Perkin Elmer), 2µl (10 mM) dNTPs (ABgene), 24µl (25 mM) $MgCl_2$ solution (Perkin Elmer), 2µl each of (10µM) primer and 0.4µl of (5u/µl) *Taq* DNA Polymerase (Perkin Elmer). PCR took place at 94°C for 5 mins (1 cycle); 94°C 40 secs, 60°C for 2 mins and 72°C for 30 secs (30 cycles); 72°C for 10 mins (1 cycle).

PCR product was subjected to electrophoresis on a horizontal 1% agarose gel, at 150 mV for 20 minutes. The 1Kb fragment was visualised using an ultra-violet transilluminator, then excised from the gel using a sterile scalpel blade. This was necessary to ensure accurate sequencing of mtDNA, for reasons outlined in section 2.3.2. The PCR product was then recovered and purified using QIAquick Gel Extraction Kit (Qiagen), following the protocol supplied, and suspended in 30µl of Qiagen 'EB' buffer.

The sequence extension reaction was performed in both directions for each sample using Perkin Elmer 'BigDye'™ sequencing kits. Opposing sequences were used to check the accuracy of data. The manufacturer's protocol & recommended reaction conditions were followed, apart from halving all reaction volumes. All sequence visualisation was performed using a P.E. Biosystems ABI PRISM® 377 Auto-Sequencer. Sequence data were aligned using CLUSTAL W (Thompson *et al.*, 1994), and by hand.

2.2.3 Data analysis

2.2.3.1 *International comparisons using nuclear microsatellites*

Nuclear microsatellite data, from all populations at all eleven loci, were used to estimate genetic distances between all population pairs. The genetic estimators employed were Wright's F_{ST} (Wright, 1969), calculated as Weir and Cockerham's analogue value θ (Weir and Cockerham, 1984), using FSTAT version 2.9.1, and Slatkin's R_{ST} (Slatkin, 1995) which was calculated using the software RST Calc. Version 2.2 (Goodman, 1997).

The value F_{ST} gives accurate estimations of genetic distance when allelic distributions between population pairs are defined predominantly by genetic drift (Takezaki and Nei, 1996; Ruzzante, 1998) i.e. when populations are recently divergent. The value R_{ST} allows for mutation, in a stepwise manner characteristic of microsatellite data, between allelic distributions (O'Connell *et al.*, 1997). Consequently, R_{ST} may be more appropriate for populations separated over longer distances and time periods which may contain a higher number of new alleles as a result of microsatellite mutation. As this study considers

population comparisons over a range of geographical and temporal scales both F_{ST} and R_{ST} values were employed.

Genetic distance estimates between population pairs were compared to the natural logarithm (ln) of geographical distances, in kilometres. A standard Mantel test (Mantel, 1967) was used to determine whether a relationship existed between the sets of data and to assess the value and significance of the correlation coefficient, *r*.

2.2.3.2 *Hierarchical AMOVA of mtDNA D-loop*

The microsatellite repeat region of the D-loop was deemed to be too variable to be useful at the scale employed by this study. Consequently, 218 bp fragments of the available data were used, corresponding to positions 17368-17436 in the published sequence (Krettek *et al.*, 1995), GenBank accession number X88898. These data were analysed for all populations within the study using a hierarchical analysis of molecular variance (AMOVA) approach. Populations were partitioned, at five hierarchical levels, based on geographic position. The hierarchical regime employed is outlined in Table 2.3.

AMOVA analysis was conducted using the software ARLEQUIN version 2.000 (Schneider *et al.*, 2000). The approach is based on the method outlined in Excoffier *et al.*, (1992) which performs an analysis of variance of gene frequencies within populations, to produce a set of F-statistic analogues (Weir and Cockerham, 1984), as defined in Excoffier *et al.* (1992), where the relationship between

$$\sigma_a^2 + \sigma_b^2 + \sigma_c^2 = \sigma_T^2 \qquad (2.1)$$

Table 2.3 Geographical partition regime employed for hierarchical analysis of molecular variance (AMOVA) of mtDNA performed by ARLEQUIN 2.000 (Schneider *et al.*, 2000). Each three letter code corresponds to a single sampling site. The code and location of each site is detailed in Table 2.1. Each hierarchical level includes the same individuals/populations as the last. With each successive level the populations are partitioned into more, and smaller groups.

Hierarchical Levels	Population Groupings
Level 1 (All pops)	{BLA, COL, ECL, ENE, ENW, ESE, ESH, ESW, FAW, HAM, HED, HOL, HYT, IBM, MOT, MWD, NET, RBR, ROM, ROW, TON, TOS, BSI, DIT, MAR, KIR, ROU, ABE, SHE, FIN}
Level 2 (UK & Finland)	{BLA, COL, ECL, ENE, ENW, ESE, ESH, ESW, FAW, HAM, HED, HOL, HYT, IBM, MOT, MWD, NET, RBR, ROM, ROW, TON, TOS, BSI, DIT, MAR, KIR, ROU, ABE, SHE} {FIN}
Level 3 (England, Scotland & Finland)	{BLA, COL, ECL, ENE, ENW, ESE, ESH, ESW, FAW, HAM, HED, HOL, HYT, IBM, MOT, MWD, NET, RBR, ROM, ROW, TON, TOS, BSI, DIT, MAR, KIR, ROU} {ABE, SHE} {FIN}
Level 4 (Regions)	{BLA, COL, ECL, ENE, ENW, ESE, ESH, ESW, FAW, HAM, HED, HOL, HYT, IBM, MOT, MWD, NET, RBR, ROM, ROW, TON, TOS, BSI} {DIT, MAR, KIR, ROU} {ABE, SHE} {FIN}
Level 5 (Regions & Islands)	{BLA, COL, ECL, ENE, ENW, ESE, ESH, ESW, FAW, HAM, HED, HOL, HYT, IBM, MOT, MWD, NET, RBR, ROM, ROW, TON, TOS} {BSI} {DIT, MAR, KIR, ROU} {ABE} {SHE} {FIN}

and the F-statistic analogues are

$$\Phi_{CT} = \frac{\sigma_a^2}{\sigma_T^2} \tag{2.2}$$

$$\Phi_{SC} = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_c^2} \tag{2.3}$$

and

$$\Phi_{ST} = \frac{\sigma_a^2 + \sigma_b^2}{\sigma_T^2} \tag{2.4}$$

where σ_a^2, σ_b^2 and σ_c^2 are the components of covariance at the level of group (*a*), population (*b*) and haplotypes within a population, within a group (*c*), respectively, and σ_T^2 is the total molecular variance. The analysis was performed for both haplotypic frequency data alone, and for haplotypic frequency data including molecular sequence divergence estimations. Significance levels were calculated for σ_a^2 and Φ_{CT} by permuting populations among groups; for σ_b^2 and Φ_{SC} by permuting haplotypes among populations within groups; and for σ_c^2 and Φ_{ST} by permuting haplotypes among populations among groups. Each test was subject to 10,000 permutations.

2.2.3.3 *Haplotype relationships*

Each distinct mitochondrial haplotype, plus the corresponding sequence from the published data for *E. europaeus* (Krettek *et al.*, 1995), GenBank accession number X88898, were aligned using the program CLUSTAL W (Thompson *et al.*, 1994) and realigned by eye. Phylogenetic relationships were determined by constructing a neighbour joining (NJ) tree based on gamma corrected genetic distances (Tamura and Nei, 1993). This model was designed using nucleotide substitution data from the mammalian mitochondrial D-loop, and as such is appropriate to use in this situation. The

genetic distance estimation and tree construction was performed using a beta test version of the program PAUP version 4.0 (Swofford, 1998), and the trees robustness was tested using 1000 bootstrap replicates. The outgroup used for this analysis was sequence data from *Erinaceus concolour*, supplied by J. Seddon of the UEA.

2.3 *Results*

2.3.1 Nuclear genetic scale effects of geographical distance

A comparison of genetic distances, based on nuclear microsatellite data to geographical distances for all of the populations in this study gives a clear indication of scale related differences in the relationships between these variables. For the full set of population comparisons, the genetic distance correlates positively with geographical distance for all (Figure 2.2 and Figure 2.3), for both F_{ST}, $r = 0.78$ ($p < 0.001$), and R_{ST}, $r = 0.76$ ($p < 0.001$), employing a standard Mantel test (Mantel, 1967). However, the relationships are clearly not linear, but become more pronounced at larger scales.

The genetic and geographic distances of local to regional comparisons, where populations are separated by less than 150 km (local and regional hierarchical levels, as detailed in section 2.2.1.1), display a significant relationship where $r = 0.39$ ($p < 0.01$) for F_{ST} values, and $r = 0.30$ ($p < 0.05$) for R_{ST} values. Compare these to the values for 'regional to international' comparisons, where populations are separated by a minimum of 50 km (i.e. incorporating all hierarchical levels except local, as detailed in section 2.2.1.1). These exhibit a correlation with r = 0.90 ($p < 0.001$) for F_{ST} values, and r = 0.86 ($p < 0.01$) for R_{ST} values, and 12.0x (F_{ST}), and 9.7x (R_{ST}) the rate of change in genetic distance with geographic distance than at the local to regional scale

The local to regional comparisons consist of population pairs located within Hampshire, or within Oxford. In other words, local scale barrier effects notwithstanding, geographical distance alone separates these populations. Therefore it is hard to assess the degree of geneflow, or the timescale over which it may have been possible, between these

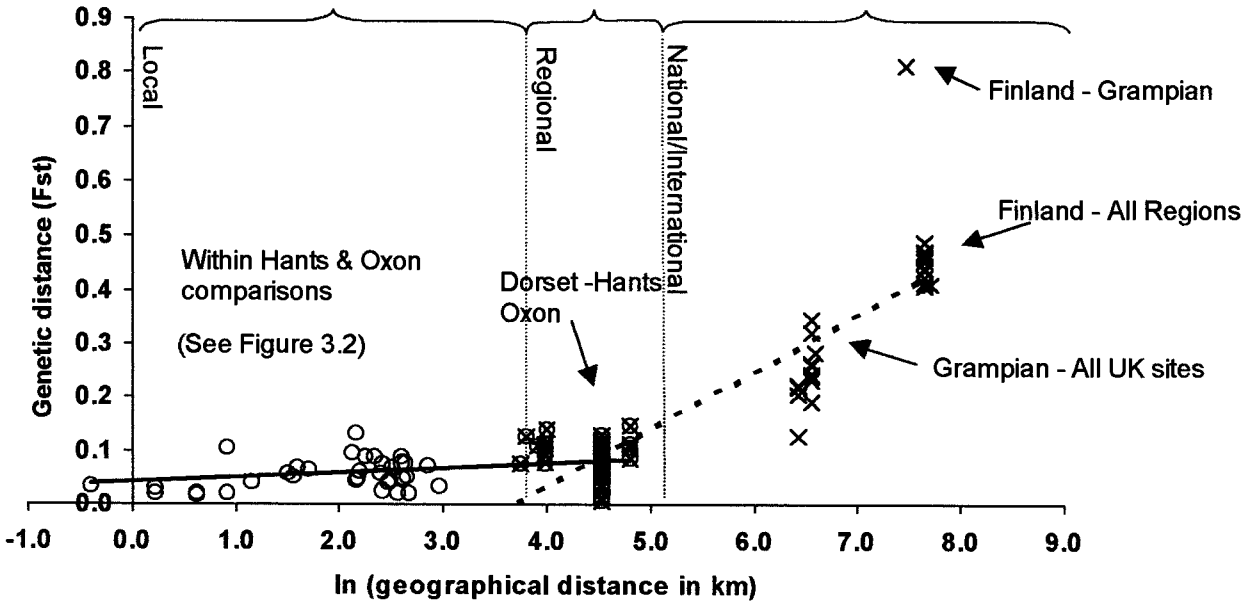

Figure 2.2 Plot of F_{ST} versus the natural log of pairwise geographical distance between regional populations. 'Local to regional' comparisons appear as empty circles, and 'regional to international' comparisons are shown as crosses. Trendlines are included for both of these groupings, and appear as a solid line and a broken line respectively. The overall $r = 0.78$, (Mantel test, $p < 0.0004$) for 105 data points. For the local/regional comparisons $r = 0.39$ ($p < 0.01$) for 78 data points, and for the regional/international comparisons $r = 0.9$ ($p < 0.001$) for 71 data points.

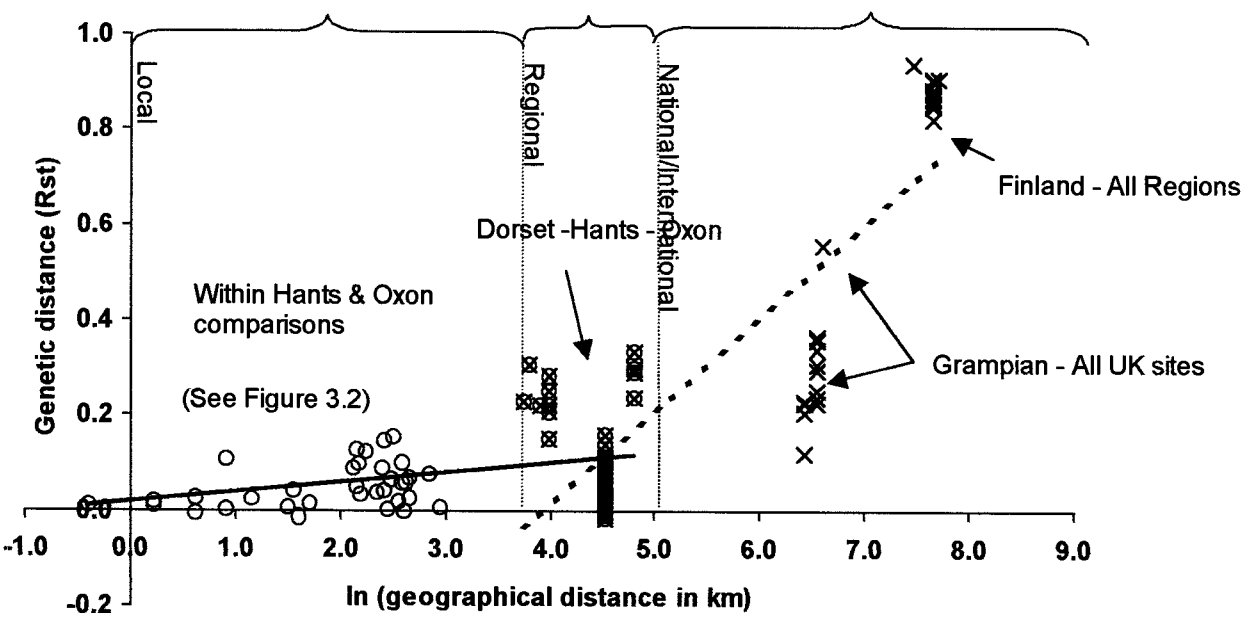

Figure 2.3 Plot of R_{ST} versus the natural log of pairwise geographical distance between regional populations. 'Local to regional' comparisons appear as empty circles, and 'regional to international' comparisons are shown as crosses. Trendlines are included for both of these groupings, and appear as a solid line and a broken line respectively. The overall $r = 0.76$, (Mantel test, $p < 0.0002$) for 105 data points. For the local/regional comparisons $r = 0.30$ ($p < 0.05$) for 78 data points, and for the regional/international comparisons $r = 0.86$ ($p < 0.009$) for 71 data points.

populations. However, all of the international population pair comparisons are separated by the North Sea, a body of water that is known to have had no land bridge since the end of the last Pleistocene glaciation 10,000 years ago. This puts a fixed minimum time limit on any potential indirect geneflow between certain population pairs. Whilst in reality this may be comparable to the situation between distant populations within the UK, e.g. a population within Southampton compared to a Scottish population, it is possible that this ancient and intractable barrier may exaggerate the scale effect of geographical distance alone. Nevertheless these results strongly indicate that genetic distance increases significantly with geographical distance, and that the rate of increase depends on the scale

2.3.2 Discovery of a putative nuclear insertion of mtDNA

Initial amplification of the mitochondrial D-loop using primers tRNA-pro and tRNA-phe (see section 0) consistently produced two bands when the PCR product was visualised on electrophoretic gels (Figure 2.4). The upper band was of approximately the size expected, given the sequence of the published data for *E. europaeus* D-loop (Krettek *et al.*, 1995). It also appeared to be the most variable in terms of size polymorphism (2.0 ± 0.1 Kb), which would be consistent with the presence of a hypervariable microsatellite repeat. The lower band was approximately 500 bp smaller than expected (1.5 Kb), based on the published sequence data, but did not exhibit any discernable size polymorphism.

Further optimisation and increased stringency of PCR conditions did nothing to improve the specificity of the PCR product. Its apparently more conserved nature, and its persistence despite increasingly stringent PCR conditions leads me to speculate that the lower band may be the incidental amplicon of a nuclear insertion. This refers to a section of mitochondrial DNA that has at some time in the past been excised from the mitochondrial genome and incorporated into the nuclear genome. This is a phenomenon, which is being uncovered in a growing number of taxa (Zhang and Hewitt, 1996; Lunt *et al.*, 1998; Zischler *et al.*, 1998).

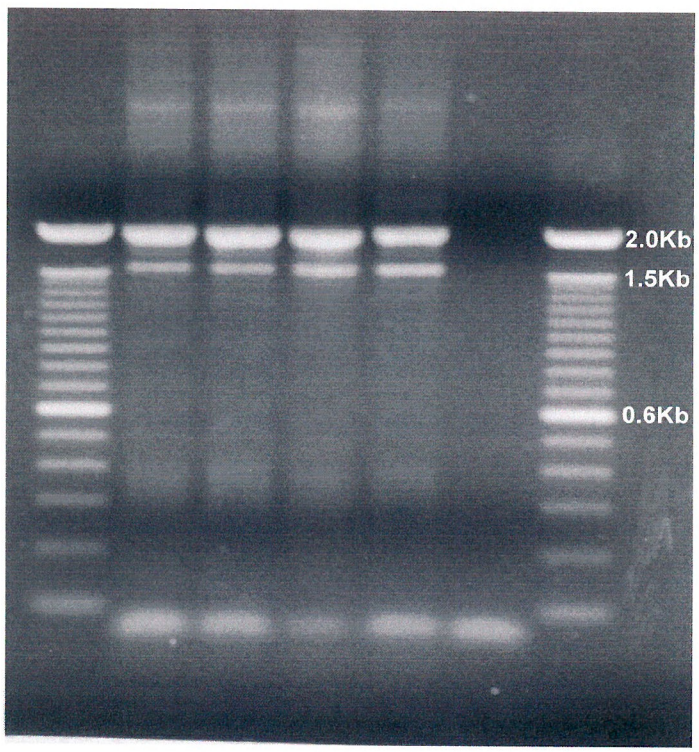

Figure 2.4 PCR product following amplification of the mtDNA Control Region (primers tPRO-For/tPHE-Rev). Lanes 1 & 7, Gibco 100bp DNA ladder; lanes 2-5, Southampton individuals; lane 6, negative control. Upper bands show putative "target" DNA. Lower bands show unidentified DNA fragments, approximately 500bp smaller than "target" fragment.

Several attempts were made to investigate the nature of this DNA region without success. Putative nuclear PCR fragments were excised directly from agarose gels followed by gel purification. However, subsequent sequencing failed to produce readable sequences for comparison to the homologous mitochondrial sequence. This was followed by an attempt to produce mitochondrial enriched DNA template (enrichment protocol outlined in Appendix II, section 6.2) from hedgehog liver tissue. PCR could have confirmed the nature of the putative nuclear insert by the absence or reduction of the non-target DNA fragment following amplification using enriched mtDNA template. Unfortunately mtDNA enrichment was unsuccessful, and time constraints prevented further investigation into the true nature of this putative nuclear insert.

2.3.3 Mitochondrial nucleotide diversity

Sequence data for a 218 bp fragment of mtDNA D-loop from 110 individuals generated a total of 6 haplotypes (Figure 2.5). Haplotypes exhibited between 0.46 % and 6.42 % sequence divergence, indicating a high level of genetic diversity between all populations studied. From the 18 polymorphic sites determined by sequence comparison 4 were indels (insertion/deletion mutations) 3 of which being contiguous, 2 were transversions (purine to purine, or pyrimidine to pyrimidine substitution mutations), and 12 were transitions (purine to pyrimidine, or pyrimidine to purine substitution mutations). This represents a transition: transversion ratio of 6:1 that is consistent with some of the published data for mammals e.g. 5.2:1 in the red squirrel, *Sciurus vulgaris*, (Barratt *et al.*, 1999). However, this appears quite low for the mammalian mtDNA D-loop in general, which has been estimated as having a transition: transversion ratio of between 15:1 (Vigilant *et al.*, 1991) and 20:1 (Brown *et al.*, 1982). Given the low number of mutations detected overall this comparatively low ratio may simply be a sampling artefact.

								1	1	1	1	1	1	1	1	1	2	2
	6	6	8	8	8	9	9	0	0	0	2	3	4	6	7	8	0	1
Haplotype	5	7	3	4	5	2	4	2	3	7	3	4	4	1	9	5	1	5
H1	A	G	-	-	-	A	T	T	G	A	C	A	G	T	T	T	A	G
H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H3	C	T	T	A	T	.	C	.	A	-	T	.	A	C	C	.	G	A
H4	C	T	T	A	T	.	C	.	A	-	T	.	A	C	.	.	G	A
H5	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
H6	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.
H7	C	T	T	A	T	.	.	C	A	-	T	.	A	C	.	.	.	A

Figure 2.5 Variable sites in the DNA sequence of the mitochondrial D-loop of *E. europaeus*. Six haplotypes were determined from a total of 110 individuals from Finland and throughout the UK. Haplotype 'H7' is taken from sequence data in Krettek *et al.* (1995), GenBank accession number X88898.

2.3.4 Hierarchical AMOVA of mtDNA

The hierarchical AMOVA of mtDNA initially partitioned total molecular variance into two regions (level 2: UK and Finland) shown in Table 2.4, exhibits a high Φ_{ST} value of 0.4, a low Φ_{CT} value of -0.6 ($p > 0.6$) and a correspondingly high Φ_{SC} value of 0.6, revealing no significant structure. This should come as no surprise given the limited number of samples available for comparison in the Finnish population, coupled with the fact that all the Finnish samples possessed the haplotype that predominates in the UK samples.

Table 2.4 Analysis of molecular variance (AMOVA) of mtDNA for populations of *E. europaeus* from the UK and Finland, with and without the inclusion of sequence divergence data. The hierarchical partition regime employed is outlined in Table 2.3. The 7th and 8th columns in this table indicate *p* values (where significance is $p < 0.05$) relating to genetic differentiation between 'groups'.

Level	No. of population groups	Molecular data	ΦST	ΦSC	ΦCT	P VarA & ΦST	P Var & ΦCT
1	1	yes	0.611	-	-	<0.001	-
		no	0.611	-	-	<0.001	-
2	2	yes	0.386	0.616	-0.599	-	0.580
		no	0.386	0.616	-0.599	-	0.558
3	3	yes	0.870	0.373	0.793	-	0.008
		no	0.870	0.373	0.793	-	0.010
4	4	yes	0.811	0.317	0.723	-	0.001
		no	0.811	0.317	0.723	-	<0.001
5	6	yes	0.792	0.219	0.733	-	0.003
		no	0.792	0.219	0.733	-	0.003

More fine grained hierarchical levels all show similarly high Φ_{ST} values between 0.8 and 0.9, and Φ_{CT} values ranging from 0.7 to 0.8, all of which exhibit significant genetic divergence. This indicates that genetic differentiation amongst 'groups' (i.e. hierarchical association of populations, as specified in Table 2.3) differs little after partition between broad national groupings, i.e. England, Scotland and Finland. High and significant Φ_{SC} values indicate high levels of homogeneity within groups following partition at hierarchical levels 3-5. The similarity between results gained from the inclusion and

exclusion of sequence divergence data reflects the modest scale of sequence mutation, and also suggests their comparative recentness.

2.3.5 Phylogeny of mitochondrial haplotypes

The relationship between six haplotypes determined from this study, plus the published haplotype, H7, from Sweden (Krettek *et al.*, 1995), are shown in Figure 2.6. The phylogram diverges into two clades that are well supported with 100% bootstrap values. Sequence divergence within these two clades ranges from 0.46-1.83 % in the clade containing haplotypes H3, H4 and H7, and at 0.46 %, between all haplotypes, in the clade containing haplotypes H1, H2, H5 and H6. Sequence divergence between these two clades ranges from 5.50-6.42 %. Given the apparently low mutation rate of this region in *E. europaeus*, this bifurcation represents a fundamental genetic divergence between these groups of haplotypes.

The operational taxonomic units (OTUs) in the first clade, in this case mitochondrial haplotypes, are also well supported by high bootstrap values. Surprisingly two haplotypes from the same site in Oxford, H3 and H4, associate most strongly with the published haplotype (H7) originating from Sweden.

The second clade is not as well supported internally, haplotypes H1, H2, H5 and H6, and both unsupported nodes have been effectively collapsed leaving the relationship between the 4 OTUs unresolved. Haplotype H1 predominates appearing in 89.9% of all samples typed. This particular haplotype is found throughout all of the English sites, Southampton, Dorset (Brownsea Island) and Oxford, but not in the Scottish samples from Aberdeenshire. Haplotype H1 also occurs outside its expected range in the Shetlands i.e. beyond the apparent interface of the predominant English (H1) and Scottish (H6) haplotypes. The Scottish haplotype, differs from haplotype H1 by a single base change (an A to G transition at position 92, see Figure 2.5), and is consistent in all of the Scottish

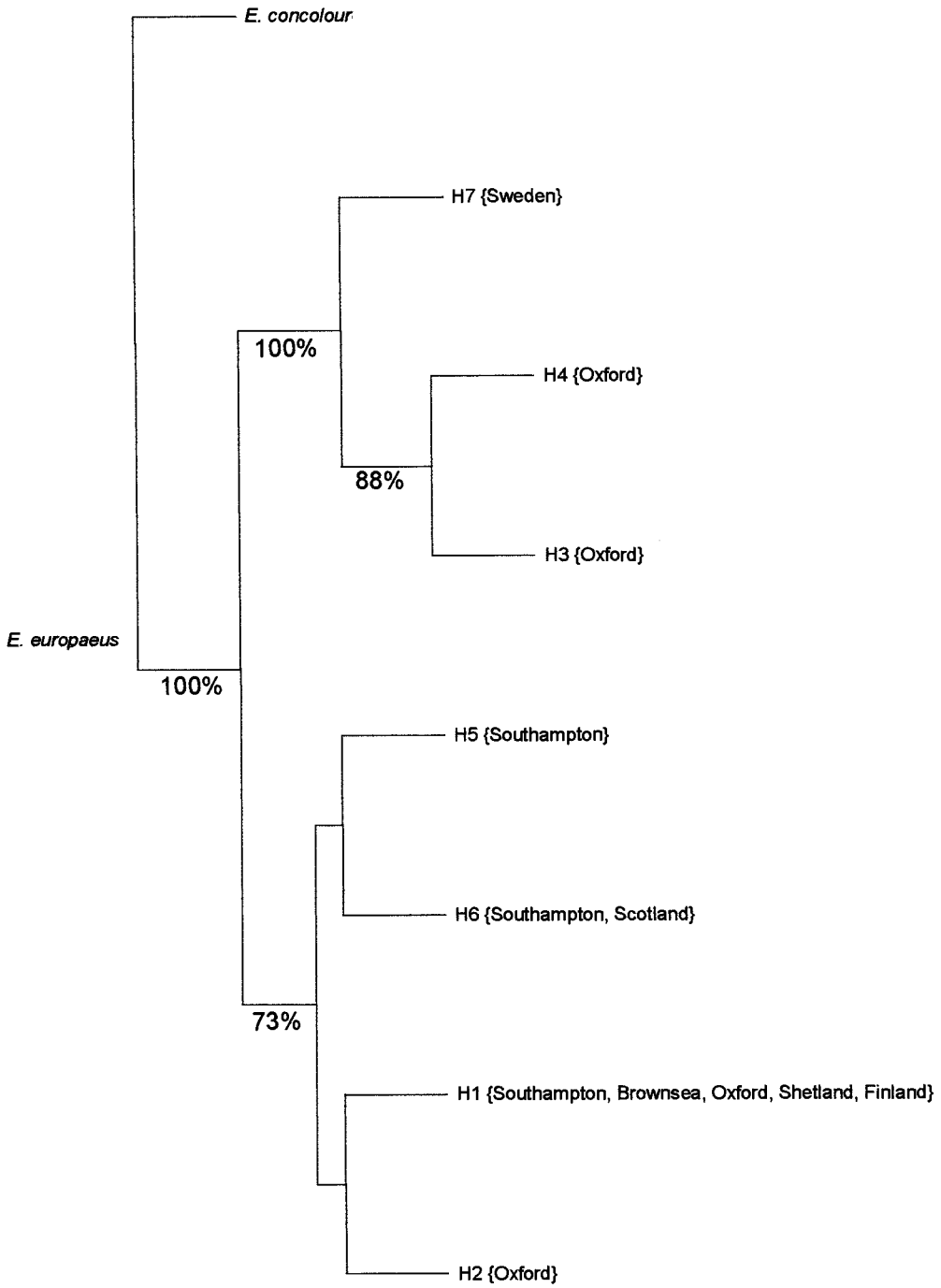

Figure 2.6 Neighbour-joining (NJ) tree describing the relationship between the 7 defined mtDNA D-loop haplotypes, including data from the published haplotype, H7 (Krettek *et al.*, 1995). The phylogenetic tree is based on gamma corrected genetic distances (Tamura and Nei, 1993). Analysis of data, and tree construction was performed using PAUP* 4.0 (Swofford, 1998). Robustness of each node was assessed over 1000 bootstraps, only values over 50% are shown.

samples. However, this haplotype also occurs in two of the individuals sampled at a site in Southampton.

The remaining haplotypes within this clade, H2 and H5, differ from the English haplotype by only one base change (a T to C transition at position 185, and an A to G transition at position 134, respectively, see Figure 2.5). Neither of these haplotypes were found in more than one individual and so, presumably, represent recent mutations of haplotype H1.

2.4 *Discussion*

2.4.1 The effects of scale on genetic diversity

The results of the analysis of nuclear data show a different spatial relationship to those gained from the mitochondrial data. The nuclear microsatellites, shown in section 2.3.1, present a relationship that predicts a higher rate of genetic differentiation over increasing geographical distance (an in-depth analysis of local scale 'genetic vs. geographical distance' is presented in Chapter 3). On the other hand, the hierarchical AMOVA of mitochondrial data, section 2.3.4, suggests that genetic variation is at its peak between a regional scale i.e. comparing between regions such as Southampton, Oxford and Aberdeenshire, and a national scale i.e. comparing between England, Scotland & Finland. An international scale comparison of mtDNA, such as the UK to Finland, does not display the high levels of differentiation predicted by the nuclear microsatellite data. Bearing in mind that the sole representative of mainland Europe is one small population from Finland, conclusions drawn about comparisons at an international scale must be treated with caution. It is likely that international scale comparisons made with nuclear microsatellites data are less biased, as they reflect information from 11 loci rather than a single locus as in the case of the mtDNA.

According to mtDNA data, genetic variation within the UK appears to remain approximately constant at all of the hierarchical levels studied. This would support the

initial assumption that levels of genetic diversity within hedgehog populations in the UK are low. The low number of mitochondrial haplotypes found within the UK, 6 in this study, also support the suggestion that the populations recolonising the UK following the end of the last ice age were extremely homogeneous, and that extant populations have not yet had time to diversify genetically.

The distribution of the different mitochondrial haplotypes, with regards to their phylogenetic relationship, would broadly support the existing hypotheses regarding post-glacial recolonisation routes. The presence of a single mutation common to all samples taken from Scotland, which is absent from English and Finnish individuals, suggests range expansion in a northerly direction from initial colonisation in the south or southeast.

For a more comprehensive answer to the question of recolonisation routes it would be desirable to sample individuals and populations from other areas of Scotland and the north of England, to see if the 'Scottish' haplotype is ubiquitous within Scotland, and whether it extends down into northern England. Samples from the far west of the UK, e.g. the West Country and Wales, could possibly elucidate dispersal routes, given the presence of region specific haplotypes.

2.4.2 Anomalous individuals: translocations or natural occurrence

One of the most curious aspects of this study has been the occurrence of individuals exhibiting the 'inappropriate' haplotype for the area they were sampled in. In some cases this is understandable, for instance, individuals sampled from the Shetland Islands exhibit the English haplotype (H1) when one might expect them to have the Scottish haplotype (H6). Section 2.1.2 describes how *E. europaeus* is a recent introduction to the Shetlands, and whilst it might have been assumed that the origin of the translocated individuals would be mainland Scotland, this study would suggest that they are more likely to have come from England or the west of Scotland (from which no samples were available).

Another example of this unusual distribution of haplotypes is found in Scandinavia. From the cytochrome *b* region of mtDNA, Santucci *et al.* (1998) defined two clades within *E. europaeus*, one in which samples from the UK, France and Spain associated, and one in which Italy, Germany and Sweden associated. The sequence data from which the Swedish individual was included was that of Krettek *et al.* (1995). The sequence data generated by this study, from the mtDNA D-loop, also defines two major clades, one containing the UK and one containing Sweden and the two samples from Oxford. I would cautiously suggest that the UK western clade and the Swedish eastern clade of this study are analogous to the UK/France/Spain western clade and the Swedish/German/Italian eastern clade of Santucci *et al.* (1998).

The unusual feature of this situation is that the Finnish population (Jyväskylä) exhibits the English haplotype (H1), which associates in the western clade. However, the Finnish population is situated approximately 750 km east and 250 km north of the Swedish individual (Kävlinge) sequenced by Krettek *et al.* (1995), which exhibited haplotype H7 that is associated with the eastern clade. Presumably this inversion is a result of human translocation in an area where this is known to have been commonplace. Without a wider selection of samples it is difficult to say for certain which is the 'appropriate' haplotype/clade for this region. However, the individual from Sweden comes from a location very close to the southernmost tip of Sweden, an area that was known to have native hedgehog populations. It is therefore likely that the Swedish individual represents a native matriline, and that the more northerly Finnish population are offspring of hedgehogs previously translocated from populations outside Scandinavia, probably Spain, France or the UK.

It is possible that the Finnish population represents the descendents of individuals of the western clade that, having recolonised Spain, France and the UK, continued along the western edge of Norway eventually moving southwest into northern Sweden and Finland. Consequently, at these higher latitudes, the western clade may be found to the west and the east of the eastern clade. This would explain both the comparative locations of the sampled haplotypes, and the very low level of genetic diversity within the Finnish

population i.e. the result of serial population bottlenecking. However, for this situation to have occurred recolonisation rates at higher latitudes would have to be considerably higher in the western clade than the eastern clade. This would also be inconsistent with the findings of Kristoffersson *et al.* (1966, 1977) who reported that hedgehogs were largely absent from the north of Finland until the latter part of the 20th century.

Some of the results from 'inappropriate' haplotypes offer less chance of explanation, for example, the Southampton individual exhibiting the Scottish haplotype (H6). There is no evidence of occurrence of this haplotype in between Southampton and Aberdeenshire, and the sites are separated by a distance of approximately 890 km, therefore the most likely explanations of this are that either an identical mutation recently occurred within an individual from the Southampton site, or that the appearance of this mutation within Southampton was a result of translocation of a Scottish female. It is unlikely that this was the result of a simple mix up with the Scottish and Southampton samples as the samples themselves were stored separately, and haplotype analysis was performed independently on separate occasions.

An even more puzzling situation occurs in a population within Oxford. Two of the individuals within this population exhibit haplotypes (H3 and H4) that associate within the eastern clade of *E. europaeus*. These haplotypes are found nowhere else in the samples taken from within the UK. Whilst it is conceivable that some representatives of the eastern clade may have arrived here at the same time as the western clade it is unlikely that the individuals are a relic population of original recolonists. A population of this sort would most likely have managed to spread the mtDNA haplotype out of one small area over the last 10,000 years. The only conditions under which such a population could thrive in isolation would be strict female philopatry, and an extreme selective advantage to the area in which they live. This would not appear to be the case, as at least half of the population exhibit a different haplotype i.e. the English haplotype, H1. It can only be assumed therefore that these individuals exhibiting mtDNA haplotypes H3 and H4, are the offspring of individuals translocated from the eastern part of the range of *E. europaeus*. The apparently narrow containment of the non-native haplotypes suggest that

the introductions were comparatively recent. This question could possibly be resolved by sampling more individuals from along the presumptive recolonisation route, such as East Anglia and the East Midlands, although Santucci *et al.* (1998) sequenced several individuals from East Anglia and found only western clade haplotypes.

Most of the samples that produced anomalous results were collected by proxy and posted to the author. Although all of these samples were obtained from reliable sources, and mislabelling of samples is unlikely, it is possible that these anomalous results were a result of accidental re-labelling or mix-ups whilst the samples were in transit. Therefore, the possibility that some of these results are erroneous cannot be absolutely ruled out.

2.4.3 Conclusions

Despite the high level of apparent intentional translocation of individual hedgehogs throughout Europe, the situation within the UK is fairly clear. The low level of genetic diversity predicted by (Ibrahim *et al.*, 1996) and (Hewitt, 1996) appears to have been born out in this analysis. The 10,000 years since the end of the last Pleistocene glaciation has not yet led to significant levels of genetic diversification.

Assuming that the increase in diversity provided by a single population in Oxford, detailed in section 2.2.3.3, is anomalous, there is little to indicate that there are diversity 'hotspots', or 'source' and 'sink' populations (Pulliam, 1996) for *E. europaeus* within the UK. What genetic diversity there is seems to be spread evenly throughout an essentially homogeneous island population.

Finally, this study revealed nothing that contradicts the existing hypotheses regarding post-glacial recolonisation routes. Although an analysis of populations from other areas of the UK may provide more fine scale information about recolonisation, the low level of diversity may make it difficult to obtain region specific haplotypes.

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3.0 Isolation and quantification of the effects of specific barriers to gene flow in a highly fragmented environment

3.1 *Introduction*

Concern has been expressed over the status of many species subject to accelerating levels of habitat fragmentation (Levins, 1970; Mader, 1984; Hanski, 1994). It is feared that populations undergoing high levels of habitat, and consequently population, fragmentation risk reduction of effective population size (N_e), and isolation from other proximate breeding units. This, in turn, may lead to higher levels of inbreeding, reduced population fitness and a greater likelihood of local extinction events (Soulé, 1987). These assertions beg several questions. What constitutes a barrier to gene flow? To what extent does a particular class of barrier affect the genetic isolation of the populations it intersects, and over what time scale? What can be done to mitigate the effects of a barrier? In this chapter I will seek answers to these questions using hedgehogs as a model organism.

Although it is widely accepted that a reduction in genetic diversity leads to reduced fitness of a population i.e. inbreeding depression (Shultz and Willis, 1995; Hedrick and Kalinowski, 2000), it has proved extremely difficult to estimate the exact cost of inbreeding depression (Crnokrak and Roff, 1999), or indeed to conclusively prove a causal link in wild populations (Wauters *et al.*, 1994). It has been proposed that a reduction in fitness associated with a reduction in genetic diversity may only be expressed under certain environmental conditions, for instance, situations of intense competition (Jimenez *et al.*, 1994; Keller *et al.*, 1994; Pray *et al.*, 1994). Another situation in which inbreeding depression may occur is the reduction of habitat size. Hanski *et al.* (1995) managed to show that the probability of extinction in the Glanville fritillary butterfly (*Melitaea cinxia*) increased in small isolated habitat patches. Whether inbreeding depression occurs as a result of the reduction of available genetic diversity within a small isolated habitat patch, or whether it occurs as a result of increased competition, a possible corollary of decreasing habitat size, is open to speculation. An experimental reduction of absolute patch size in the root vole, *Microtus oeconomus*, did not appear to reduce fitness parameters, although individuals attempting inter-patch dispersal suffered greater mortality at the hands of predators (Andreassen and Ims, 1998).

In this case it would seem that empirical evidence of inbreeding depression could not be directly ascribed to increased competition or other intra-species interactions.

To define what constitutes a barrier to dispersal, and potentially to gene flow, we must first specify the organism susceptible to the barrier effects. The size and means of locomotion of the organism may determine the physical nature of a barrier. A number of studies have sought to identify barriers using behavioural data from particular species, or specific groups of organisms such as, carabid beetles and forest dwelling mice (Mader, 1984), carabid beetles and spiders (Mader *et al.*, 1990), carabid beetles (Joyce *et al.*, 1999), field voles *Microtus agrestis*, bank voles *Clethrionomys glareolus*, and wood mice *Apodemus sylvaticus* (Richardson *et al.*, 1997), hedgehogs (Mulder, 1996; Huijser and Bergers, 2000; Doncaster *et al.*, 2001), and a range of vertebrates (Holisova and Obrtel, 1986) were assessed using radio-tracking techniques and recapture techniques. However, these studies only give accounts of the short term behavioural consequences of dispersal barriers on particular individuals.

An increasing number of researchers have performed studies attempting to quantify the longer term effects of these barriers by employing molecular analyses of population genetic structure. Distinct population genetic substructure has been detected in a number of species inhabiting fragmented landscapes. Urban populations of the common frog (*Rana temporaria*) exhibited genetic distances twice that of their rural counterparts separated by an equivalent distance (Hitchings and Beebee, 1997). Populations of bank vole (*Clethrionomys glareolus*) separated by the River Rhine at Lake Constance in Germany showed a degree of genetic subdivision equivalent to an additional geographical distance of 7.7 km (Gerlach and Musolf, 2000). The same study presented evidence indicating a significant barrier effect from major highways, although not from more minor roads or railways.

Several studies have detected genetic effects of non-contemporary factors. The historical progression of an alpine glacier is thought to have led to the formation of several "chromosome" races of common shrew *Sorex araneus* (Lugon-Moulin *et al.*, 1999).

Subsequent introgression between these races is believed to have had a more significant effect than the current landscape on population genetic structure. The prickly skink *Gnypetoscincus queenslandiae* in Australia was found to exhibit high levels of genetic subdivision which did not appear to correspond with current fragmentation patterns (Cunningham and Moritz, 1998). The authors concluded that historical factors, rather than anthropogenic fragmentation, were responsible for the genetic population structure (Cunningham and Moritz, 1998).

The European hedgehog is currently extremely abundant in the United Kingdom (Reeve, 1994; Reeve and Huijser, 1999). A highly visual victim of the effects of anthropogenic fragmentation, frequent road mortalities may simply reflect an abundant population. Hedgehog populations appear to thrive in urban and particularly suburban areas (Kristiansson, 1984; Doncaster, 1994; Doncaster *et al.*, 2001), and whilst urban environments often tend to exhibit higher levels of anthropogenic development than equivalent rural areas, it may be more accurate to describe these urban areas as being 'fine-grained' rather than fragmented. The finely grained nature of these environments describes a rapid change in habitat 'types' over small geographical areas, sometimes only a matter of metres e.g. interconnected suburban gardens. While these fine grained environments have an effect on habitat use and dispersal (Doncaster *et al.*, 2001; Rondinini and Doncaster, submitted) they should not necessarily be construed as barriers, nor render the environment 'fragmented'.

Despite their sedentary habits, hedgehogs have the potential for long distance migration events. Translocation experiments have shown that individual hedgehogs can travel distances of up to 3.0-3.8 km from their release point, a total distance of up to 9.9 km, over a 10-20 day period to find suitable habitats (Doncaster, 1992; Doncaster *et al.*, 2001). To put this into context the latter study estimated the average span (maximum distance between two radio-locations) of a non-dispersing hedgehog over the same time period was 0.8 km, and previous estimations of hedgehog home range, for adult males and females respectively, are 32 and 10 ha (Reeve, 1982), 46.5 and 19.7 ha (Kristiansson, 1984) and 57.13 and 29.08 ha (Boitani and Reggiani, 1984). It has also been

demonstrated that migrating hedgehogs will utilise particular landscape features, particularly within urban areas, and will actively avoid other features such as arable farmland (Doncaster *et al.*, 2001).

It has been suggested that hedgehogs may be attracted by road-killed carrion, and use the road surface as a foraging area (Poduschka, 1971). It has also been suggested that the heat retaining properties of road surfaces attract large numbers of insects at night, which in turn attract foraging hedgehogs (Holisova and Obrtel, 1986). If this is true, the extra time spend on the road itself may lead to an increase in hedgehog casualties. However, the effect it would have on the number of successful road crossings is not so clear. If more hedgehogs are attracted to roads as foraging areas, even if the ratio of successful to unsuccessful road crossings remained the same, the absolute number of successful road crossings may well increase. In contrast Rondinini and Doncaster (submitted) found that hedgehogs actively avoided contact with roads going so far as to adopt an uncharacteristic 'legs-extended' posture on occasions when road crossings were performed. This type of behaviour was also observed by Reeve (1994) and Mulder, (1999), and led Rondinini and Doncaster (submitted) to speculate that this may be a result of an aversion to synthetic road surface, and possibly to illumination by artificial lighting.

The European hedgehog provides an ideal model organism for a study of this type, firstly, because of its abundance and ease of handling, but also for the following reasons concerning population structure and dispersal capability. I have established that the normal range of a non-dispersing hedgehog is small enough that areas of population abundance may be identified and revisited for repeat sampling. I have also established that individual hedgehogs, of both sexes, are capable of comparatively long distance dispersal, which would allow for migration from one population centre to another. This suggests the possibility of a metapopulation structure reliant on free dispersal between populations. A population such as this should ideally illustrate the genetic effects of dispersal barriers between sub-populations.

Previous molecular genetic studies of hedgehog populations have established that significant population substructuring is present, possibly a result of habitat fragmentation, but have stopped short of implicating specific barrier features (Johnson, 1996; Becher and Griffiths, 1997; Becher and Griffiths, 1998). These studies are valuable as far as they go, but rely on assumptions made about the genetic consequences of the actual behaviour of individuals relative to larger breeding units. These assumptions were often justified by studies of other species, which exhibit fundamental behavioural differences and often inhabit entirely different environments. Subsequent conclusions drawn from the results of molecular surveys of, for instance, hedgehog populations could not be backed up by relevant whole animal/behavioural studies. Recent reviews have highlighted the need for studies employing a combined approach to population genetics, which use molecular and whole organisms results in a complimentary way (Koenig *et al.*, 1996; Thompson and Goodman, 1997).

This study uses molecular data generated from populations of hedgehogs within a study site in and around Southampton in combination with whole organism radio-telemetry data generated by two studies within the Southampton study site (Rondinini and Doncaster, submitted), and a similar site in Oxfordshire (Doncaster *et al.*, 2001). This approach was adopted to enable investigation of hedgehog populations, in fine grained and fragmented environments, at a much closer scale than previously possible. It allows questions such as, what are the effect of different types of potential barrier on the behaviour and genetics of hedgehog individuals and populations, to be answered. It also allows quantification of the comparative effects of various classes of barriers; and what kind of impact the topography, habitat-grain, and level of urbanisation of an environment has on the effectiveness of the barriers within it.

The aims of this section are to use molecular nuclear microsatellite data and radio-telemetry results to address the following issues.

1. Is there a relationship between the geographic and genetic distance among hedgehog populations at a local scale?

2. If a relationship is detected is it a straightforward linear relationship, or are other factors impinging upon it?

3. Does the nature of the environment (i.e. level of fragmentation, scale of 'grain') make a difference to the genetic population structure of the populations inhabiting it?

4. How is the genetic variation apportioned throughout the study site? Is it consistent with the relative positions of the sub-populations to the major barrier features?

5. Do intervening geographical features affect genetic population structure of compared populations i.e. do they act as barriers to gene flow?

6. Do the nature and size of the geographical feature affect its effectiveness as a barrier to gene flow, and to what extent?

3.2 *Methods*

3.2.1 Sample collection

3.2.1.1 Sampling strategy

Hedgehog ear skin samples were collected within a 32 kilometre radius of the centre of Southampton, Hampshire, Figure 3.1. Certain areas were targeted as suitable sampling sites to allow comparisons of genetic population data. These sites were in close proximity to potential barriers that were to be investigated. The data collection design allowed sampling of replicate sub-populations bordering, and either side of, ancient barriers, such as the Rivers Test and Itchen, and more recent barriers, such as the M3 and M27 motorways. Populations either side of the barrier in question provided the 'barrier-effect' comparison, whilst the populations bordering the barrier provided a control comparison.

Hedgehog populations are known to inhabit many areas within the region shown in Figure 3.1. Following extensive survey work the areas that were selected for intensive sampling were those identified as population centres exhibiting the greatest abundance of individuals. Hedgehogs were sought as intensively as possible with a view to sampling a large proportion of each local population. Recapture data was taken for each sample site on subsequent visits. These data were analysed using the Lincoln index and Jolly-Seber method (Southwood, 1978) to estimate the proportion of each population sampled. Short grass habitats, such as park land, school and sports fields, were targeted as they are known to be favoured by hedgehogs for nocturnal foraging.

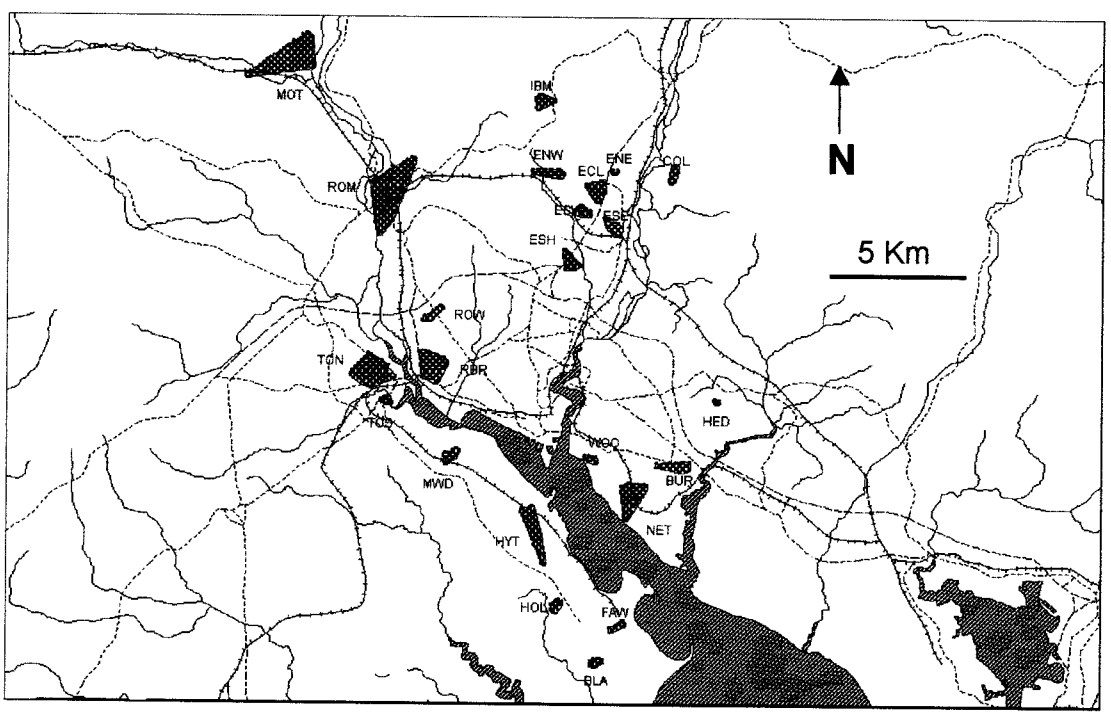

Figure 3.1 Location of 24 sample sites throughout Southampton (grey hatched polygons) and their unique three letter codes (see Table 2.1). Although hedgehogs can be found throughout this region, extensive surveying revealed these to be the principal sites of local abundance. The map also shows the position of the main barrier types in this area: motorways and A roads (broken lines); waterways (unbroken lines); railways (crossed lines).

A total of 376 skin samples from individual hedgehogs were collected from 24 sample sites, Figure 3.1. Sampling was performed over four collecting seasons (June to October, 1996-1999). Each site was revisited, on average, 1-2 times per week for the duration of the collecting season, or until no new individuals had been sampled over three consecutive visits for that collecting season. In this way the probability of sampling all

individuals present at a site was maximised. Sampling was focused around three groups containing a total of 18 sample sites, targeting three different potentially major barriers. Those barriers were, the M3 motorway, Compton to Bassett section, which was the most modern of the potential barriers having been opened in January 1992 after being built on the site of a major trunk road, that had been used in excess of 40 years. The second major barrier targeted was the River Test, near Totton, which is a heavily developed area with numerous road and rail bridges, crossing tidal flats; and the final barrier was the Southampton Water estuary, which was the most ancient, and the widest, of the barriers. Group 1 (M3) consisted of sample sites ECL, ENE, ENW, ESE, ESH & ESW. Group 2 (River Test) consisted of MWD, RBR, ROW, TON & TOS. Group 3 (Southampton Water) consisted of BLA, BUR, FAW, HOL, HYT, NET & WOO, Figure 3.1. Other sample sites were included for comparison between habitat types, and to increase the number of control comparisons available.

Where possible, population sampling was performed to replicate barrier comparisons (e.g. the waterways Southampton Water and the River Test). However, the lack of population centres spanning the M27 prevented replication of a dedicated motorway comparison such as the M3. Limited replication was achievable using populations bordering the Test (TON, TOS, RBR and ROW), which are also intersected by a motorway (M271).

Table 3.1 Annual numbers of hedgehog samples collected from each of the sample sites, and their locations, between 1996 and 1999. The column on the far right indicates the total number of individuals available for analysis having detected no significant temporal variation in allele frequency ($p = 0.05$, Bonferroni corrected value $p = 0.000044$), by *G*-test. The *G*-test was performed using FSTAT v. 2.9.1 (Goudet, 1995).

Site Code	*Location*	*1996*	*1997*	*1998*	*1999*	*Total n*
BLA	Blackfield	-	-	5	2	7
BUR	Bursledon	-	-	8	3	11
COL	Colden Common	-	-	-	16	16
ECL	Eastleigh central	-	-	32	4	36
ENE	Eastleigh north-east	-	-	6	3	9
ENW	Eastleigh north-west	5	-	7	1	13
ESE	Easteigh south-east	-	-	8	18	26
ESH	Eastleigh south	2	-	6	-	8
ESW	Eastleigh south-west	2	-	25	12	39
FAW	Fawley	-	-	-	10	10
HAM	Hamble	-	-	4	1	5
HED	Hedge End	-	-	3	-	3
HOL	Holbury	-	-	20	-	20
HYT	Hythe	-	-	3	5	8
IBM	IBM grounds/Hursley	12	3	2	-	17
MOT	Mottisfont Abbey	18	-	-	-	18
MWD	Marchwood	-	-	7	7	14
NET	Netley Abbey	-	-	2	7	9
RBR	Redbridge	-	-	14	6	20
ROM	Romsey	9	-	-	26	35
ROW	Rownhams	7	-	-	2	9
TON	Totton north	-	-	16	8	24
TOS	Totton south	-	-	6	3	9
WOO	Woolston	-	-	6	4	10

3.2.1.2 Sampling method

The methods used for sampling individual hedgehogs is outlined in section 2.2.1.2.

3.2.2 Analysis of environmental features using GIS

All ancillary sampling data (location, sex etc.) were analysed using the Geographical Information Systems (GIS) software packages MapInfo Professional, version 6.0 (MapInfo Corporation, Troy, NY) & Idrisi (Clark Labs, Mass.). Minimum area population polygons were constructed to encompass all sampled individuals at each site.

Geographical distances, in kilometres, were calculated from the polygon centroid of each sample site.

Commercially available maps of landscape features were imported into the GIS software, and potential barriers were classified. The following classifications of barriers were employed: roadways (motorway, 'A' road dual carriageway, 'A' road single carriageway, 'B' road, minor road); railways, and waterways (sea, river, secondary river, tertiary river, minor stream). Road width and age data were obtained from Hampshire County Council's County Surveyor's office.

For each sample site comparison barrier separation was deemed to occur when the line connecting polygon centroids intersected any barrier specified in the previous paragraph.

In order to classify the habitat in which each sample site was located a Habitat Fragmentation Index (HFI) was constructed. This was achieved by counting the numbers of discrete land areas, bordered on all sides by barriers of all classification, within a 5 x 5 km square surrounding each sample area. This index was then used to assess whether the genetic structure of the sub-population inhabiting that sample site was affected by the level of fragmentation or 'grain'. The HFI values for each sample area were expressed as a function of the inbreeding coefficient F_{IS}, and relatedness value R_C, calculated from microsatellite allele frequencies, of the sub-population inhabiting that area.

The hypothesis is that sub-populations within a more fragmented area will tend to exhibit higher levels of allelic fixation as a result of genetic drift. Populations suffering from allelic fixation would give a more positive F_{IS} value. Associated with this is the increased likelihood of breeding with a closely related mate. The degree to which this has occurred can be determined using the relatedness constant R_C (Queller and Goodnight, 1989). Negative F_{IS} values indicate an excess of heterozygotes either as a result of outbreeding, the Wahlund effect i.e. the merging of previously isolated sub-populations (Wahlund, 1928), or random differences in female and male allele frequencies when number of breeders is small (Luikart and England, 1999).

3.2.3 DNA Extraction

DNA extraction methods are outlined in section 2.2.2.1, and Appendix 1.

3.2.4 Microsatellite development and typing

Microsatellite development and typing methods are outlined in section 2.2.2.2.

3.2.5 Validity of Microsatellite Data

Before the microsatellite allele data could be used to make any inferences about population substructure they were checked for independence. The presence of a "null" or non-amplifying allele at a locus could lead to biased results. For instance, a heterozygote possessing a null allele could be mistaken for a homozygote. The bias would increase at loci where the null allele was more common, or the range of alleles smaller. In principle, the presence of null alleles can be detected. This is done by comparing the number of heterozygotes vs. homozygotes at any one locus in the sample population against the number of heterozygotes vs. homozygotes expected for a given set of allele frequencies, assuming Hardy-Weinberg equilibrium:

$$(p+q)^2 = p^2 + 2pq + q^2 \qquad (3.1)$$

where p is frequency of allele 'A' and q is the frequency of allele 'a'.

The presence of a null allele would present a heterozygote deficit compared to Hardy-Weinberg equilibrium (Brookfield, 1996). This was tested for using the Markov chain method of Guo and Thompson (1992) to calculate an exact p value. The population analysis software used was GENEPOP 3.1d (Raymond and Rousset, 1995) and ARLEQUIN (Schneider *et al.*, 2000).

Another possible source of bias is linkage between loci in which two or more loci are found close together on the same chromosome. The effect of linkage is that the two loci do not assort independently. The two linked loci give the same information as a single unlinked locus. Consequently bias may occur as statistical analysis gives an undue weight to the information from both loci.

Statistical evidence of linkage between loci was sought by testing that alleles at all loci assort independently. This was also carried out using GENEPOP 3.1d and ARLEQUIN.

3.2.6 Genetic analyses

3.2.6.1 Preliminary data testing

Most of the samples were collected over a period of 2-4 years, and although they were collected from the same sites it is possible that genetic drift, extinction or recolonisation could have substantially altered allele frequencies (Viard *et al.*, 1997). In order to establish whether data from individuals collected at the same site over different years could legitimately be pooled, an exact *G*-test for genetic differentiation was performed (Goudet *et al.*, 1996). This test involves the random permutation of genotypes amongst samples (in this case 112800 permutations, as recommended by the computer program employed based on the number of populations being compared), in order to assess the significance of genotypic variation from year to year.

Other tests performed included the detection of recent 'effective population size' (N_e) reduction, using BOTTLENECK version 1.2.02 (Cornuet and Luikart, 1996). In a recently bottlenecked population allele number (k) would tend to fall faster than the observed gene diversity value, H_o (observed heterozygosity). Consequently, if H_e (expected heterozygosity) was calculated based on the value k in a recently bottlenecked population, it would be somewhat lower than the actual gene diversity value H_o. Values were calculated using a Two Phase Mutational model (TPM), based on a Stepwise Mutation Model with (SMM) 5% multi-step mutations, as recommended within the

software's literature. Significance was assessed using a Wilcoxon signed-ranks test (Sokal and Rohlf, 1995).

The sampling method of this study presents the risk of taking samples from related individuals. To measure this effect potential family relationships between individuals, and overall relatedness within sample sites (R_c) were estimated using the program KINSHIP version 1.3.1 (Queller and Goodnight, 1989). The inbreeding coefficient F_{IS} and associated p values were estimated for all sample sites using the software FSTAT version 2.9.1 (Goudet, 1995).

An Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992) was employed to test the hypothesis that sample sites separated a major barrier contribute a significantly larger proportion of the total genetic variation than those that are not. The AMOVA was performed on the microsatellite data of the 18 selected sample sites around the 3 specified barriers, the M3, River Test and Southampton Water estuary (Section 3.2.1.1). This was performed using the software ARLEQUIN version 2.000 (Schneider *et al.*, 2000). The aim of the AMOVA was to investigate whether barrier induced variation was greater than background levels. Therefore the hierarchical levels employed were i) variance amongst groups, ii) variance across barriers, and iii) variance within groups.

3.2.6.2 *Genetic distance estimation*

Estimates of genetic distance were calculated from allele frequencies using three different approaches. The chord distance (D_C) (Cavalli-Sforza and Edwards, 1967) was calculated using the software MICROSAT version 1.5b (Minch, 1997). This is based on the principles of Euclidean geometry, where the square roots of the allele frequencies of a sample are plotted as points on a hypersphere, and D_C is the chord distance between the points. This is a purely geometric distance, and as such it takes no account of any particular mutational model.

Wright's F_{ST} (Wright, 1969) was calculated, as Weir and Cockerham's statistic θ (Weir and Cockerham, 1984), using FSTAT version 2.9.1. Statistical significance was obtained using an exact *G*-test by randomising genotypes among samples. The assumption of the Infinite Allele Model (IAM) is implicit in the calculation of F_{ST} (θ). The IAM predicts that every time a mutation occurs at a microsatellite locus a new allele is created. As the mutation may involve the addition of single or multiple repeat units no inferences can be drawn on the allele's former state based on its current state. Wright defined F_{ST} as:

$$F_{ST} = \frac{H_T - H_S}{H_T} \tag{3.2}$$

where H_T is the expected heterozygosity of the whole population and H_S is the average expected heterozygosity of the sub-populations being compared (Wright, 1969). The algorithm used to calculate θ (F_{ST} analogue) within FSTAT is based on the following equation:

$$\theta = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_b^2 + \sigma_w^2} \tag{3.3}$$

where σ_a^2 is the among sample variance component, σ_b^2 is the between individual within sample variance component, and σ_w^2 is the within individual variance component.

Slatkin's R_{ST} (Slatkin, 1995) was calculated using the software RST Calc. Version 2.2 (Goodman, 1997). Slatkin's R_{ST} is also an analogue of Wright's F_{ST}, but it has been adapted for use with microsatellite data. It is based upon the assumption of the Stepwise Mutation Model (SMM). This model differs from the IAM as it predicts mutation events to occur in single repeat unit steps. Consequently the model assumes that a pair of alleles that differ by one repeat unit are more closely related than a pair of alleles that differ by two or more repeat units. Slatkin defined R_{ST} as:

$$R_{ST} = \frac{(\overline{S} - S_W)}{\overline{S}} \tag{3.4}$$

where S_W is the sum over all loci of twice the weighted mean of the within-population variances σ_a^2 and σ_b^2, and $\overline{S}$ is the sum over all loci of twice the variance $\sigma_{(a+b)}^2$ of the combined population.

3.2.6.3 *Mantel analysis*

Isolation by distance and barrier effects were evaluated using a combination of standard Mantel tests and partial Mantel tests. A standard Mantel test is a method of assessing the degree of correlation between two matrices (Mantel, 1967) these representing a matrix of geographical distance, and a matrix of genetic distance between pairs of sites. A percentage p value is derived by ranking the observed correlation coefficient r value against r values recalculated following randomisation of column and row for one of the matrices. Mantel analyses were performed using a Basic program MANTEL written by C. P. Doncaster.

A partial Mantel test is a method of comparing three or more matrices. It evaluates the extra variation explained by a third matrix once the second has been explained (Manly, 1986; Smouse *et al.*, 1986). Barrier effects were estimated by comparing matrices of genetic distance to matrices of quantitative barrier data (barrier intersection frequencies and barrier width) having explained the variation of matrices of geographical distance. Partial Mantel analyses were performed using MANTEL3, a Pascal program, based on a FORTRAN code given in Manly (1991), written and freely available from Jérôme Goudet (Université de Lausanne).

All genetic distance matrices were constructed from Cavalli-Sforza & Edwards chord distance D_C, as this estimator has been found to produce the most accurate phylogenetic reconstructions between closely related populations (Takezaki and Nei, 1996). All

significance tests were performed over 20,000 randomisations, for Mantel and partial Mantel analysis. Where multiple Mantel or partial Mantel tests were considered the significance levels ($p < 0.05$) were corrected using the standard Bonferroni method, which is slightly more conservative than the Bonferroni sequential correction, or Dunn-Šidák method in reducing the likelihood of Type I errors (Sokal and Rohlf, 1995).

3.3 *Results*

3.3.1 Animal captures

Approximately 85% of individuals were recaptured on subsequent sampling sessions. Population estimates based on recapture rate data predict that 70-95% of individuals were sampled over all populations. The average distance travelled from the hedgehog's first sampling encounter was 0.18 km. The average distance travelled by females was 0.16 km, and by males 0.22 km, however this difference was not found to be significant according to a two tailed t-test.

A total of 14 individuals were found to have crossed some kind of barrier over the course of the study. Of these individuals 9 were female and 5 were male. To explain the position of all of these individual, following subsequent recaptures, a minimum of 19 barrier traversals must have occurred. Barrier class traversal frequencies were as follows, minor roads (10), B roads (1), A roads (5), streams (2), railways (1). No individual was found to have traversed motorway or riverine barriers over the full duration of sampling.

3.3.2 Allelic content

Of the twelve loci employed two were found to be in significant linkage disequilibrium. Therefore one of the new loci was excluded from further analysis. The remaining loci exhibited a minimum of 6 and a maximum of 11 alleles per locus. Observed heterozygosities (H_o) within each sample site were very similar to expected (H_e) (Table 3.2). In addition Hardy-Weinberg equilibrium was tested for each sample site using the

Markov chain method (Guo and Thompson, 1992) employed in GENEPOP (Raymond and Rousset, 1995). None of the sample sites tested were found to differ significantly from Hardy-Weinberg equilibrium. A total of 10 alleles from 7 different loci were found to occur at one sample site only (*private alleles:* alleles exclusive to one sample site or population). However, each of these sites was different and no sample site had more than one private allele (except COL which had 2), so on this basis no conclusions were drawn about individual population isolation.

Table 3.2 Expected (H_e) and observed (H_o) heterozygosities, allelic variance and average number of alleles per locus, per sample site.

Population	H_e	H_o	*Variance*	*Average number of alleles*
BLA	0.58	0.58	14.10	3.64
BUR	0.54	0.60	14.24	4.27
COL	0.62	0.64	16.50	4.36
ECL	0.64	0.65	13.11	5.45
ENE	0.61	0.62	17.67	4.00
ENW	0.62	0.59	13.99	4.55
ESE	0.65	0.62	17.47	5.36
ESH	0.61	0.56	13.14	3.73
ESW	0.64	0.69	16.57	5.00
FAW	0.60	0.54	16.29	4.27
HAM	0.64	0.59	11.81	3.55
HED	0.68	0.70	13.98	3.18
HOL	0.63	0.54	16.79	5.09
HYT	0.60	0.61	17.03	3.73
IBM	0.55	0.62	14.70	4.55
MOT	0.64	0.62	16.67	5.00
MWD	0.61	0.65	18.33	4.27
NET	0.63	0.61	17.05	4.18
RBR	0.58	0.56	13.58	4.18
ROM	0.66	0.61	16.06	5.73
ROW	0.58	0.52	17.54	3.55
TON	0.58	0.59	16.02	4.91
TOS	0.52	0.51	12.90	3.09
WOO	0.55	0.57	15.94	3.91

3.3.3 G-test for annual pooling

The *G*-test performed on the discrete annual population data found allelic differentiation to be non-significant for all year to year comparisons ($p = 0.05$, Bonferroni corrected value $p = 0.000044$). Consequently all further analysis was performed on the total number of individuals collected from a given site over the duration of the project, Table 3.1.

3.3.4 Population size fluctuations, inbreeding, and within sample relatedness

BOTTLENECK analysis (Cornuet and Luikart, 1996) gave no indication of significant excess of heterozygosity at any sample site. Hence we can infer that it is unlikely that any population substructure is a result of recent reductions in effective population size (N_e).

Mean relatedness values (R_c) and inbreeding coefficients (F_{IS}), for all sample sites are shown in Table 3.3. F_{IS} values appear to be quite low, and the only sample site which has an inbreeding coefficient which differs significantly from zero is HOL ($F_{IS} = 0.152$, $p = 0.0002$), a significant positive value indicating high levels of inbreeding.

High *Rc* values indicate that a large number of family, or partial family groups were sampled at many sample sites. KINSHIP analysis estimated that the percentage of individuals at each sample site who were at least half-siblings ranged from 9.0% to 61.1%. It is possible that this level of sibling co-capture could bias results to indicate higher levels of population differentiation than is actually the case. However, it could simply be a reflection of the inbred nature of the populations that are being sampled from.

Table 3.3 F_{IS}, *Rc* and HFI values for all sample sites. Significant inbreeding coefficients (F_{IS} values) are denoted by an asterisk. Relatedness values (*Rc*) were calculated for all individuals using KINSHIP (Queller and Goodnight, 1989) then averaged over sample sites. Habitat Fragmentation Index (HFI values) indicates the number of discrete, barrier bordered land polygons within 5 km^2 of each sample site polygon centroid (see Figure 3.3)

Sample site	*F IS*	*p-value*	*Rc*	*HFI*
BLA	0.015	0.468	0.165	14
BUR	-0.115	0.981	0.268	54
COL	-0.045	0.804	0.146	39
ECL	-0.019	0.728	0.070	58
ENE	-0.03	0.699	0.167	46
ENW	0.057	0.143	0.094	26
ESE	0.042	0.117	0.042	65
ESH	0.083	0.130	0.151	56
ESW	-0.078	0.996	0.047	55
FAW	0.104	0.058	0.077	14
HAM	0.081	0.188	0.034	21
HED	-0.034	0.704	-0.004	72
HOL	0.152	<0.001*	0.070	15
HYT	-0.022	0.634	0.101	24
IBM	-0.122	0.995	0.261	15
MOT	0.036	0.208	0.076	36
MWD	-0.076	0.942	0.099	43
NET	0.033	0.295	0.063	31
RBR	0.031	0.247	0.171	81
ROM	0.073	0.010	0.016	54
ROW	0.107	0.076	0.158	59
TON	-0.016	0.649	0.162	65
TOS	0.002	0.506	0.319	61
WOO	-0.041	0.750	0.222	49

3.3.5 Isolation by distance

In order to identify any genetic isolation by distance effects, matrices of Cavalli-Sforza & Edwards chord distance, D_c and geographical distance between all of the Hampshire sample sites were analysed for degree of correlation using a Mantel test. This initial test compared only genetic and geographical distance, and made no allowance for the presence of barriers.

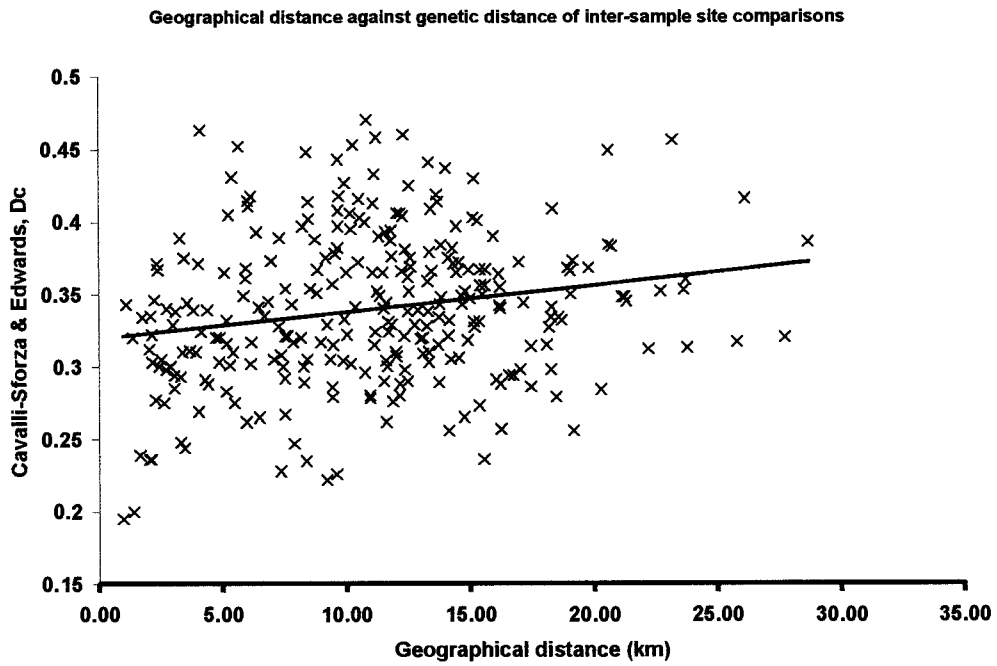

Figure 3.2 Genetic distance as a function of geographical distance for pairs of sample sites. There is a significant increase in genetic distance with geographic distance for pairs of sample sites in and around Southampton city (continuous line shows regression: Mantel $r = 0.196$, $p = 0.041$).

Figure 3.2 shows genetic distance as a function of the geographic distance between sub-populations in the Southampton study area, and describes a significant positive relationship between the two (Mantel $r = 0.196$, $p = 0.041$). A general isolation by distance effect is therefore detectable within the study area. Without further analysis it is impossible to say whether this is a result of distance alone, or of barrier mediated reduction in geneflow.

3.3.6 General effects of habitat fragmentation

The *habitat fragmentation index* (HFI) results, based on areas defined in Figure 3.3, are displayed in Table 3.3. The HFI values for each sample area are compared to the inbreeding coefficient F_{IS} of the sub-population inhabiting that area, Figure 3.4, and also to the relatedness value R_C (not shown).

The data do not exhibit the positive correlation that would support the hypothesis that populations within highly fragmented habitats tend to suffer from higher levels of inbreeding. When analysed using a Pearson Product Moment Correlation, no significant relationship could be detected (HFI vs. F_{IS}: $r = -0.17$, and $p = 0.42$; HFI vs. R_C: $r = 0.07$, and $p = 0.75$). However, the lack of a positive correlation is not necessarily surprising when it is considered that the F_{IS} values used were quite low (minimum -0.122, maximum 0.152), and only one of the values differed significantly from zero for all of the sites sampled. The HFI used was based on fragmentation by barriers of all classes, ranging from motorways and major watercourses to minor roads and streams. The result may simply reflect the lack of impact of the most common, minor barrier features on geneflow between sample areas.

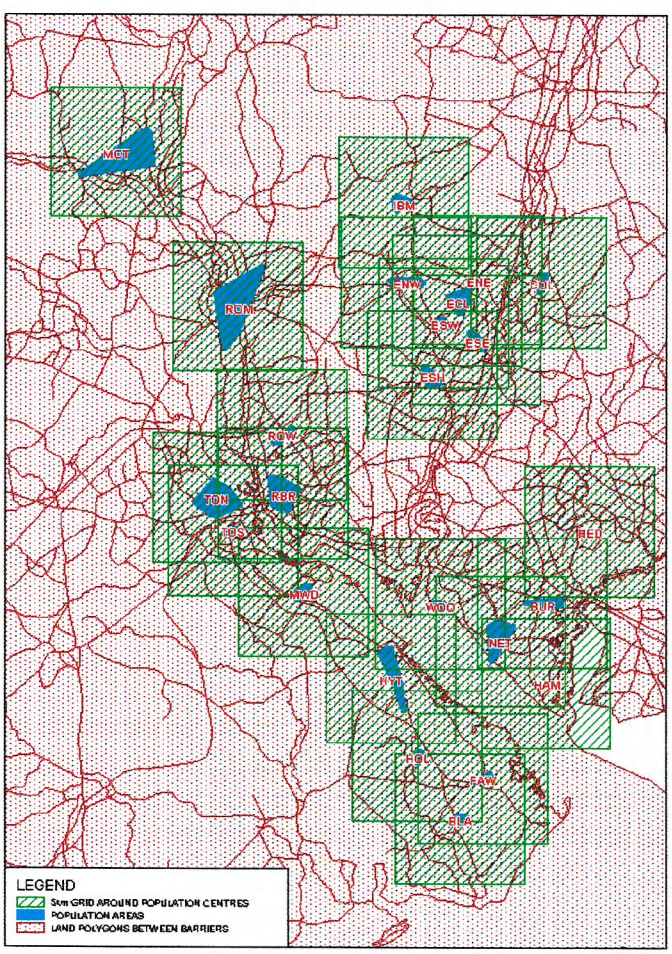

Figure 3.3 shows 5 km^2 grids around sample site polygon centroids from which a Habitat Fragmentation Index (HFI) was constructed. HFI values are presented in Table 3.3

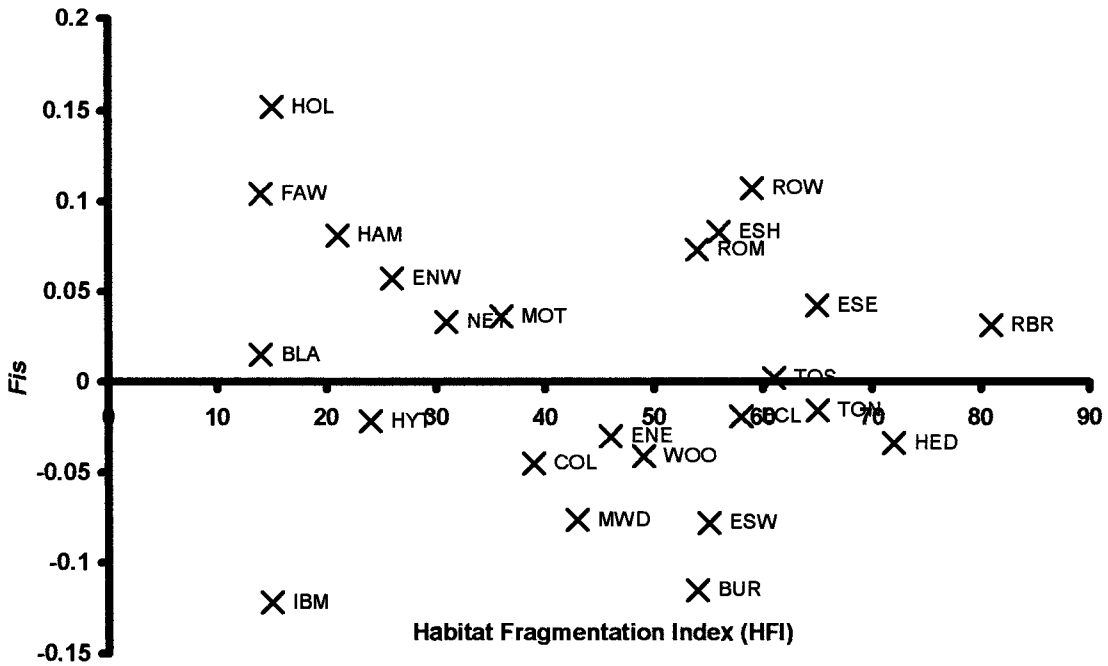

Figure 3.4 Inbreeding coefficient as a function of Habitat Fragmentation Index (HFI) versus inbreeding coefficient (F_{IS}). It was hypothesised that an increase in fragmentation level may lead to an increase in inbreeding (a more positive F_{IS} value). No significant relationship was detected (Pearson Product Moment Correlation r = -0.17, p = 0.42).

3.3.7 Composite barrier effects

Partial Mantel tests were employed in order to gauge the potential genetic isolating effects of multiple barriers, within the study area. Matrix A consists of genetic distances between sample sites, chord distance, D_C (Cavalli-Sforza and Edwards, 1967); Matrix C consists of geographical distance in kilometres between sample sites. Matrices B^1 to B^8 contain barrier frequency data and other quantitative barrier data. A total of eight analyses were performed each of which assessed the level of genetic variation in matrix 'A' explained by different 'B' matrices once the effects of matrix 'C' had been accounted for. A key to the contents of all of the B matrices is given in Table 3.4.

Table 3.4 Description of the nature of the values that comprise the contents of 'B' matrices. These matrices are substituted between subsequent partial Mantel analyses in order to assess the proportion of genetic variation (matrix A) they explain once the effects of geographic distance (matrix C) have been accounted for.

Matrix number	*Matrix description*	*Matrix value contents*
B1	All barriers	Frequency of barriers of all class separating populations
B2	All roads	Frequency all roads separating populations
B3	Motorways	Frequency of all motorways separating populations
B4	Total road width	Cumulative width of all roads separating populations
B5	All waterways	Frequency of all rivers and estuaries separating populations
B6	Sea & major rivers	Frequency of major rivers (e.g. Test, Itchen & Hamble) and estuaries separating populations
B7	Sea	Frequency estuaries separating populations
B8	All railways	Frequency all railway lines separating populations

Table 3.5 Results of partial Mantel analyses for comparisons between all sample sites. Each test estimates the amount of extra variation in genetic distance (D_c) explained by different barriers types, once geographical distance has been accounted for. The significance level has been corrected for multiple tests using the Bonferroni method, Bonferroni-corrected alpha probability level ($p = 0.00625$) for $p < 0.05$. No significant relationships were found between genetic variation and any of the barrier features identified.

Matrices (AB.C)	*Extra variance explained*	*p*
Genetic distance. Sea & major rivers. Geo	0.008	0.959
Genetic distance. Motorways. Geo	0.012	0.910
Genetic distance. All waterways. Geo	0.059	0.723
Genetic distance. All railways. Geo	0.058	0.623
Genetic distance. Total road width. Geo	0.104	0.561
Genetic distance. All barriers. Geo	0.205	0.401
Genetic distance. All roads. Geo	0.187	0.386
Genetic distance. Sea. Geo	0.230	0.082

Neither the combined barrier frequency matrices, nor the single class barrier matrices showed a significant relationship before or after Bonferroni correction (Table 3.5). The only comparison that approached significance was that of populations separated by the sea or Southampton Water estuary ($r = 0.2296, p = 0.0824$). Having performed a multiple barrier analysis, lack of a significant relationship, above that of geographical distance alone, suggests one of two things. Firstly it is possible that none of the stipulated barriers have a significant effect on movement and consequent gene flow between hedgehog populations. Secondly, and more likely, the barriers that have been included in the analysis are not the only factors, above geographical distance, causing restriction to gene flow or influencing genetic distance. It is also worth considering that

all of the frequency matrices employed make no prior assumptions about the comparative nature of the specified barriers i.e. a motorway crossing carries as much weight in the analysis as a minor road or minor stream crossing. It is possible that this lack of barrier weighting, over large geographical distances, is skewing the analysis and masking significant relationships. This problem will be addressed in the following sections by restricting the analyses to individual barrier features.

3.3.8 Single and small scale barrier effects

3.3.8.1 AMOVA

Three groups of sample sites focused on barriers of the following types, a motorway (M3, at Eastleigh. Figure 3.5); a major river (River Test, at Totton, Figure 3.6) and an estuary (Southampton Water, Figure 3.7). The groups contained six, five and seven sample sites respectively, and were arranged with no less than two sample sites on each side of the specified barrier.

An AMOVA analysis was performed on raw allelic data from these three groups of sample sites, to determine how the components of variance are partitioned. The first level of hierarchy was determined as within group variation. The second level was variation between sample sites separated by the specified barrier, and the third level was variation within sample sites. The results of the AMOVA, for each group, are shown in Table 3.6.

The majority of the variation occurs between individuals, an average value of 91.8%. The remainder of the variation is partitioned almost equally between i) variation among sample sites within groups (average 4.7%), and ii) variation across specified barrier (average 3.6%). These averaged results suggest that the barriers that have been specified are unlikely to have lead to extreme population subdivision, and that any substructure that may be detected is likely to be subtle.

Table 3.6 shows the partitioning of the percentage of variation at different hierarchical levels, in three key groups. Most allelic variation occurs amongst individuals. Variation within groups and across barriers is approximately equal, with a slightly larger portion of variation within groups than across barriers.

	Percentage of variation		
Groups/Specified Barrier	*Between individuals*	*Within groups*	*Across barrier*
Motorway (M3)	95.6	3.0	1.4
River (River Test)	88.7	7.0	4.4
Estuary (Southampton Water)	91.1	4.0	5.0
Average values	91.8	4.7	3.6

3.3.8.2 Overall F_{ST} and R_{ST} results

Values for F_{ST} (based on IAM mutational model) and R_{ST} (based on SMM mutational model), and their associated p values (derived from 30,000 permutations) for the three barrier targeted groups are shown in Table 3.7. The average F_{ST} value was 0.071, whilst the lowest F_{ST} value recorded (0.014) was found amongst the Southampton Water sample sites, and the highest (0.212) amongst the River Test sample sites. Average R_{ST} value was 0.061, whilst the highest (0.181) and lowest (-0.023, effectively an R_{ST} of 0.000) were both found amongst the Southampton Water sample sites.

In total 70% of F_{ST} comparisons and 15% of R_{ST} comparisons showed a significant genetic differentiation following Bonferroni correction (Sokal and Rohlf, 1995). Of the F_{ST} values from comparisons spanning specified barriers 79% showed a significant genetic differentiation, as opposed to 56% in comparisons not separated by the specified barrier. The corresponding values for R_{ST} were 25% and 0% respectively. The average F_{ST} values for comparisons not separated by the specified barrier, and those that were, were 0.051 and 0.084 respectively. The corresponding values for R_{ST} were 0.046 and 0.070.

The specific F_{ST} and R_{ST} averages for each barrier are given in the following three sections, but the overall results show that the barriers identified and specified seem to be

having an effect on genetic isolation above that of isolation by geographical distance alone.

Table 3.7 Estimates of genetic differentiation between sample sites at the three key barriers. Values which differ significantly from zero are indicated by **bold type**. Significance corrected to $p = 0.0011$ ($p = 0.05/46$) using Bonferroni method. "# " indicates comparisons separated by specified barrier.

Barrier/Group	*Sample sites*	*FST*	*p value*	*RST*	*p value*
Motorway	ECL /ENE#	0.079	**0.001**	0.070	0.006
(M3)	ECL /ESE#	0.045	**0.001**	0.046	**0.001**
	ECL /ESH#	0.054	**0.001**	0.085	**0.001**
	ENE /ENW#	0.074	0.005	0.032	0.129
	ENE /ESH#	0.113	**0.001**	0.104	0.027
	ENE /ESW#	0.065	**0.001**	0.051	0.011
	ENW /ESE#	0.030	**0.001**	0.036	0.030
	ENW /ESH#	0.055	0.007	0.086	0.020
	ESE /ESW#	0.022	**0.001**	0.026	**0.001**
	ESH /ESW#	0.050	**0.001**	0.047	0.013
	ECL /ENW	0.026	0.029	0.011	0.236
	ECL /ESW	0.022	**0.001**	0.015	0.007
	ENE /ESE	0.021	0.003	0.024	0.124
	ENW /ESW	0.031	**0.001**	0.044	0.007
	ESE /ESH	0.056	0.006	0.042	0.037
River	MWD /RBR#	0.080	**0.001**	0.010	0.182
(River Test)	MWD /ROW#	0.113	**0.001**	0.026	0.115
	RBR /TON#	0.093	**0.001**	0.105	**0.001**
	RBR /TOS#	0.170	**0.001**	0.157	**0.001**
	ROW /TON#	0.099	**0.001**	0.028	0.095
	ROW /TOS#	0.212	**0.001**	0.127	0.009
	MWD /TON	0.077	**0.001**	0.042	0.007
	MWD /TOS	0.097	**0.001**	0.077	0.006
	RBR /ROW	0.090	**0.001**	0.054	0.040
	TON /TOS	0.083	**0.001**	0.081	0.004
Estuary	BLA/BUR#	0.116	**0.001**	0.157	0.002
(S'ton Water)	BLA/NET#	0.101	0.002	0.172	0.002
	BLA/WOO#	0.135	**0.001**	0.181	**0.001**
	BUR /FAW#	0.055	**0.001**	0.058	0.063
	BUR /HOL#	0.116	**0.001**	0.117	0.063
	BUR /HYT#	0.075	**0.001**	0.022	0.126
	FAW /NET#	0.042	**0.001**	0.031	0.190
	FAW /WOO#	0.066	**0.001**	0.030	0.162
	HOL /NET#	0.068	0.004	0.068	0.011
	HOL /WOO#	0.119	**0.001**	0.098	**0.001**
	HYT /NET#	0.033	0.481	-0.003	0.346
	HYT /WOO#	0.058	0.104	0.001	0.259
	BLA/FAW	0.055	**0.001**	0.154	0.010
	BLA/HOL	0.031	0.056	0.071	0.025
	BLA/HYT	0.080	**0.001**	0.109	0.009
	BUR /NET	0.043	**0.001**	0.024	0.149
	BUR /WOO	0.047	0.003	0.046	0.040
	FAW /HOL	0.031	0.008	0.028	0.120
	FAW /HYT	0.014	0.276	-0.011	0.538
	HOL /HYT	0.062	**0.001**	0.031	0.081
	NET /WOO	0.043	0.033	-0.023	0.732

3.3.8.3 M3 motorway comparison

This analysis compared six study sites separated by a major motorway, Table 3.7. Even at this scale, where the average distance between sites is 2.3 km and the maximum distance between sites is 4.1 km, other barriers did pass between some of the control comparisons. This was an unfortunate necessity given the location of apparent hedgehog subpopulations, and has been addressed within the analysis.

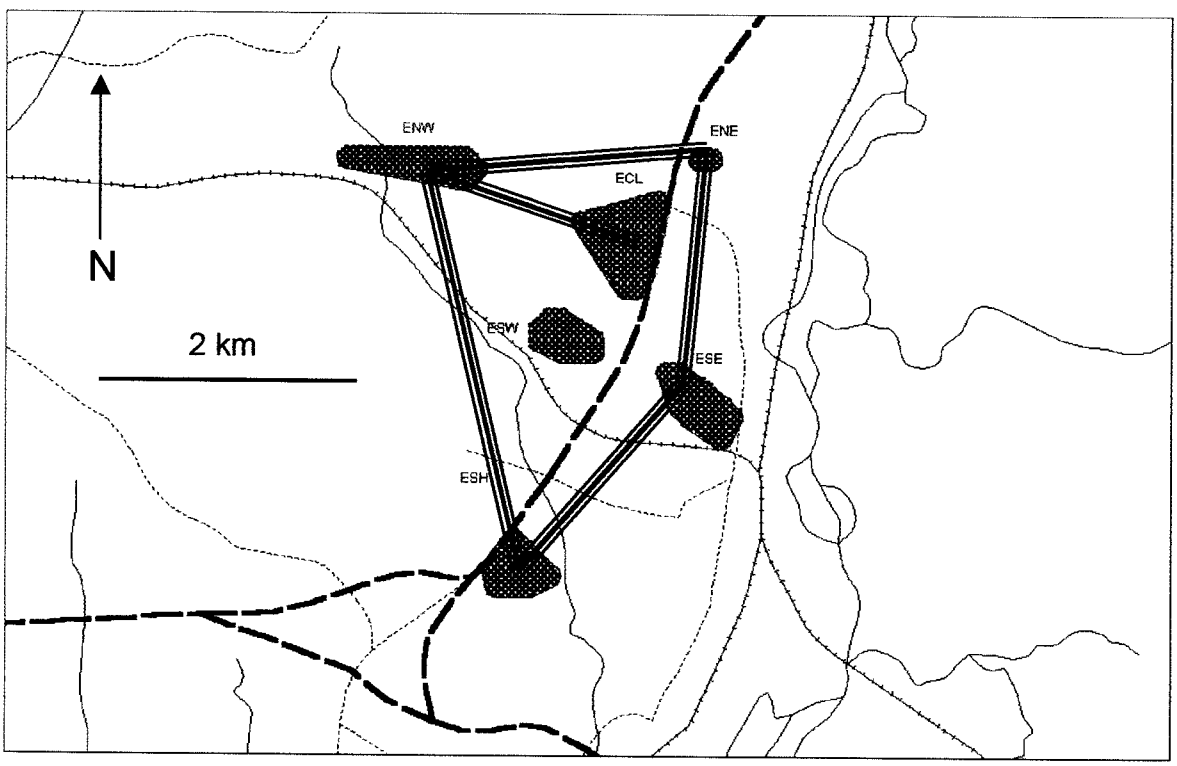

Figure 3.5 Map showing the position of sample sites compared within Group 1: M3 motorway barrier. Motorways are represented by thick broken lines, and A roads by thin broken lines; railways are represented by crossed lines, and waterways by unbroken lines. Sample sites which do not exhibit significant genetic differentiation (F_{ST}) are denoted with connection by a triple line. All other relationships between sample sites were found to exhibit significant genetic differentiation, although these are not marked for the sake of clarity.

The low average F_{ST} and R_{ST} values (0.050 and 0.048, for an average geographical distance between sample sites of 2.3 km, Table 3.7) of the comparisons between these sample sites suggest a low level of genetic differentiation. This may be because the perceived barriers within the area were all built within the last 50 years. However, the

average F_{ST} and R_{ST} values of motorway separated comparisons are almost twice those of non-motorway separated comparisons i.e. motorway separated F_{ST} and R_{ST} = 0.059 and 0.058; non-motorway separated F_{ST} and R_{ST} = 0.031 and 0.027.

Partial Mantel tests were employed on a subset of the total data in order to isolate the effects of specific barriers. Genetic distance D_C was related to quantitative barrier data having allowed for variance components as a result of geographical distance. Table 3.8 shows the results of the partial Mantel analyses that test for the effects of the frequency of road intersections, motorway intersections and the total width of all roads on genetic variation between populations. Their significance is determined against a Bonferroni corrected p value of $p = 0.0167$ (p =0.05/3).

Table 3.8 Results of partial Mantel analyses for group 1 (M3) sample sites, corrected for multiple tests using Bonferroni method. Bonferroni-corrected alpha probability level $p = 0.0167$ (p = 0.05/3). Significant results are shown in **bold type**.

Matrices (AB.C)	*Extra variance explained*	*p*
Genetic distance. All roads. Geo	0.274	0.532
Genetic distance. Motorway. Geo	0.338	0.047
Genetic distance. Total road width. Geo	0.483	**0.014**

A partial Mantel test of all road barriers was performed which showed no significant relationship ($r = 0.2741, p = 0.5318$). However, when road barrier intersection frequencies were combined with road class width data, effectively giving a comparative weighting to different barrier types, a significant proportion of the extra genetic variance was explained ($r = 0.4832, p = 0.0142$). This would suggest that the width of a road has more of an effect on restricting geneflow than simply the presence of the road itself This would also agree with (Huijser and Bergers, 1998) who found that successful hedgehog road crossings were inversely proportional to the width of the road. It is also consistent with the findings of Rondinini and Doncaster, (submitted) who state that hedgehogs are more likely to cross small roads (< 4 m wide) than large roads.(≥ 4 m wide) or motorways (2-3 traffic lanes, recovery lanes, central reservation and scrub verges).

The motorway was found to have no significant effect on genetic variation following Bonferroni correction ($r = 0.3383, p = 0.0466$). Given that road width has already been found to have a significant effect on restriction of geneflow when all road barriers were considered, the result of the motorway alone test might well have been confounded by the presence of the other barriers in the area.

3.3.8.4 River Test comparison

Further partial Mantel tests were performed on a group of five sites spanning the river Test, Figure 3.6. The results are shown in Table 3.9, and their significance is determined against a Bonferroni corrected probability value of $p = 0.0167$ (p =0.05/3).

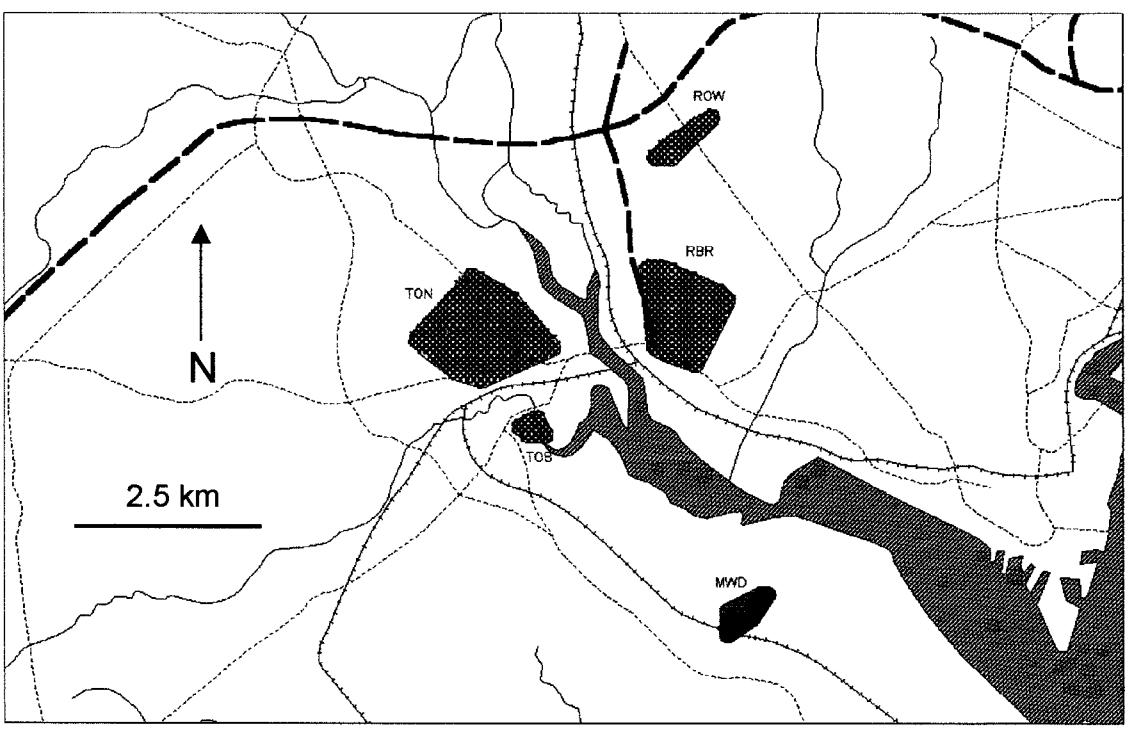

Figure 3.6 Map showing the position of sample sites compared within Group 3: River Test barrier. The most northerly portion of Southampton Water estuary and the mouth of the Test is represented by the grey, hatched area; motorways are represented by thick broken lines, and A roads by thin broken lines; railways are represented by crossed lines, and waterways by unbroken lines. All relationships between sample sites were found to exhibit significant genetic differentiation.

Table 3.9 Results of partial Mantel analyses for group 2 (River Test) sample sites, corrected for multiple tests using Bonferroni method. Bonferroni-corrected alpha probability level $p = 0.0167$ ($p = 0.05/3$). No significant relationships were found between genetic variation and any of the barrier features identified.

Matrices (AB.C)	*Extra variance explained*	*p*
Genetic distance. Sea. Geo	0.380	0.258
Genetic distance. Total road width. Geo	0.573	0.080
Genetic distance. All barriers. Geo	0.841	0.060

The primary comparison, in this case, concerns the presence of the river Test itself. However, in addition to the river itself the study area contains a number of roads, railways and minor waterways, the average HFI for samples sites in this area being 62. This may have a bearing on the high proportion of significant genetically differentiated relationships between sample sites in this area (Table 3.7). The average F_{ST} and R_{ST} values for this set of sample sites were 0.111 and 0.071 respectively, for an average geographical distance of 3.4 km. This shows a disproportionately large increase in genetic differentiation for the average comparative geographical distance. Average F_{ST} and R_{ST} values for sub-populations separated by the river were 0.128 and 0.075 respectively, and the values were 0.087 and 0.064 for those not separated by the river. Again this shows an average increase in genetic differentiation across the specified barrier, but what we see for the first time is a disagreement between F_{ST} and R_{ST} values. Given that these two genetic estimators are based on principles of two different mutational models, the discrepancy suggests that the individuals within the sample sites compared have diverged not only by changes in allele frequencies but also by the presence of new mutations. Whether F_{ST} is overestimating genetic differentiation or R_{ST} is underestimating it depends on whether the stepwise mutation model (SMM) or the infinite allele model (IAM) most closely resembles actual mutation at these sample sites.

No significant relationship between genetic variation and the presence of the river could be detected with partial Mantel tests when analysing populations across the river. Given the large number of bridges that cross the river (1 two lane road bridge, 1 railway bridge, and one footbridge) it is possible that the hedgehogs may use the bridges as a form of

wildlife corridor. River crossing is unlikely as it remains a substantial barrier even where it narrows further to the north.

Neither of the other comparisons showed a significant relationship between genetic variation and barrier feature but the probability values were closer to significance when considering all barriers and total road width, than they were when considering the River Test in isolation. This suggests that any effect the river Test may have on the genetic substructure of the surrounding populations is minor in comparison to other local barriers.

3.3.8.5 *Southampton Water comparison*

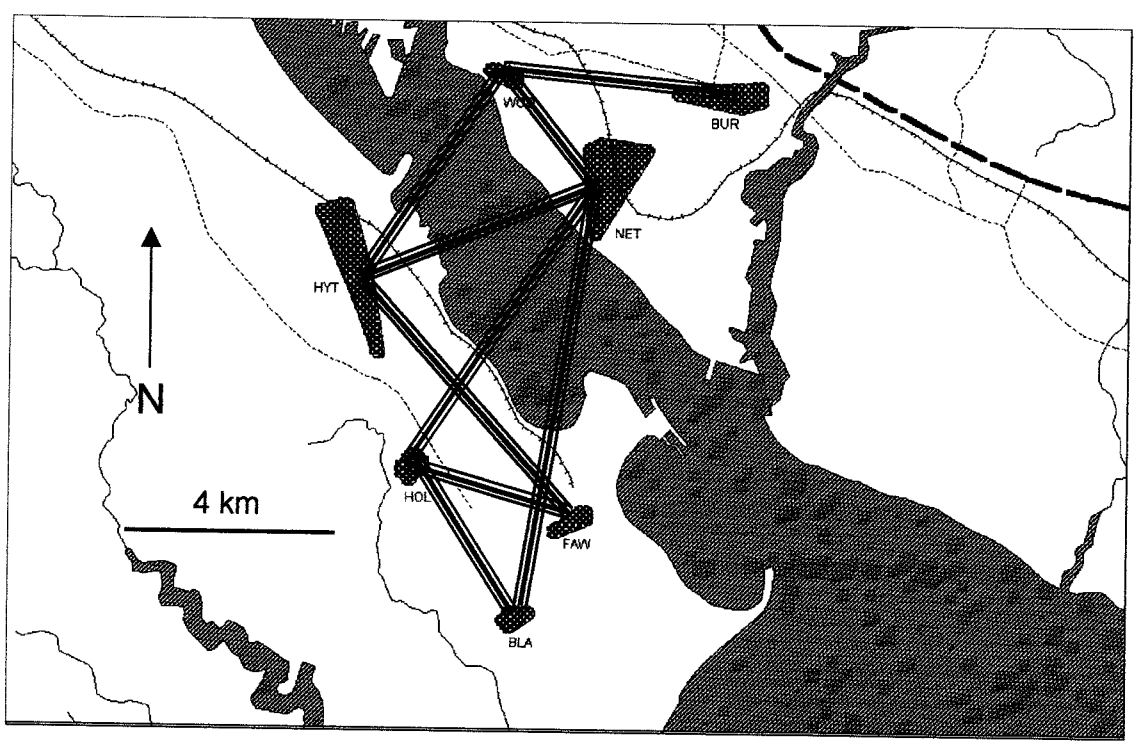

Figure 3.7 Map showing the position of sample sites compared within Group 3: Southampton water estuary barrier. Southampton Water estuary is represented by the grey, hatched area; motorways are represented by thick broken lines, and A roads by thin broken lines; railways are represented by crossed lines, and waterways by unbroken lines. Sample sites which do not exhibit significant genetic differentiation (F_{ST}) are denoted with connection by a triple line. All other relationships between sample sites were found to exhibit significant genetic differentiation, although these are not marked for the sake of clarity.

Table 3.10 Results of partial Mantel analyses for group 3 (Southampton Water) sample sites, corrected for multiple tests using Bonferroni method. Bonferroni-corrected alpha probability level $p = 0.0167$ ($p = 0.05/3$).

Matrices (AB.C)	*Extra variance explained*	*p*
Genetic distance. All barriers. Geo	0.001	0.998
Genetic distance. Total road width. Geo	0.120	0.707
Genetic distance. Sea. Geo	-0.203	0.536

The sites compared in this analysis are shown in Figure 3.7, and the results of the partial Mantel tests in Table 3.10. Significance levels are again corrected to $p = 0.0167$ ($p = 0.05/3$) using a Bonferroni correction.

Despite the presence of the Southampton Water estuary, a barrier that at first site appears to be larger and more long standing than previous barriers we have considered, this study area is a great deal less fragmented than the others are. The average *HFI* for the sites included in this part of the analysis is 29, as compared to those for the M3 motorway (51) and the River Test (62).

Average F_{ST} and R_{ST} values for this area are 0.066 and 0.065 respectively, for an average geographical distance of 5.1 km. Whilst F_{ST} and R_{ST} values across the estuary are considerably higher than those which don't cross the estuary (estuary separated F_{ST} and $R_{ST} = 0.082$ and 0.078 respectively; non-estuary separated F_{ST} and $R_{ST} = 0.045$ and 0.048 respectively) the comparison is less valid, in this case, as the average geographical distance of comparisons across the estuary is 6.4 km as compared to 3.4 km for comparisons not crossing the estuary. It would appear that the genetic estimator value roughly doubles for a doubling in geographical distance.

The results of the partial Mantel tests show that all r values are low or negative, and p values are high. It might be concluded that this implies that the specified barriers in this area have no effect on geneflow. Alternatively the larger areas of habitat between barrier constraints may have allowed populations to maintain a larger effective population size (N_e). If this is the case, then these populations should have been less sensitive to genetic

drift and to the effects of allelic loss following reduction of N_e. Consequently populations on either side of Southampton Water, although isolated from each other for longer, may have retained ancestral allele frequencies. This may have led to them being more similar to each other than to many populations, more recently isolated, inhabiting smaller habitat patches.

3.4 *Discussion*

In choosing to perform a small scale analysis of genetic population structure in a highly fragmented, or fine grained, environment several experimental design problems presented themselves. The greatest problem to overcome was to be able to select sample sites and sub-populations which could be used to compare genetic variation over geographical features whilst being able to isolate the effect of the putative barrier, and provide adequate control comparisons. Hedgehog population locations, individual abundance and the location of putative barrier features meant that some compromises had to be made. If absolute isolation of barrier features was impossible, attempts were made to reduce the number of other factors which may affect the potential relationship between genetic variation between sub-populations and the barrier feature being tested.

Nevertheless, some significant relationships were determined. A significant isolation by distance effect was established across all of the sample sites employed in the study. Although significant the relationship between genetic variation and geographic distance was quite weak, which suggested that other factors may be mediating the relationship, or that the relationship was based on factors which correlate to geographic distance rather than on geographical distance itself

Whilst the level of fragmentation of the habitat surrounding a sub-population did not appear to have a consistent affect on the level of inbreeding within that population, there did appear to be a positive relationship between the level of genetic divergence between sub-populations and the fragmentation index of their surroundings.

In terms of specific barrier types, the frequency of roadway, railway, and waterway intersections showed no significant effect on genetic variation between populations. However, in areas where roadways were the predominant barrier feature, total road width was determined to have a significant effect on genetic variation.

3.4.1 Fragmentation: the effects of spatial complexity

A number of previous studies have attempted to assess the effects of individual barriers within areas of low to moderate fragmentation (Piertney *et al.*, 1998; Stewart *et al.*, 1999; Gerlach and Musolf, 2000). What I have attempted to do is to measure the effects of different classes of barrier within a highly fragmented and complex environment. Similar studies have investigated the effects of habitat fragmentation on hedgehogs inhabiting urban and suburban areas, however, the effects of specific barriers was not examined (Becher and Griffiths, 1998).

Performing this type of analysis within, and on the margins of, a busy urban area such as Southampton presented a number of logistical problems, primarily ones of access to sampling areas. The territorial and predominantly sedentary nature of the hedgehog, coupled with the public's attitude towards them meant that remote trapping was never an option. Therefore a strategy of opportunistic sampling had to be employed. These constraints meant that ideal sample sites were not always available, and estimates of population size difficult to make.

Wherever possible "internal" controls were attempted i.e. if we consider three proximate populations two of which are separated by a barrier, then the comparison between the two not separated by the barrier would be deemed the control. Within a study area as fragmented as the urban environment selected for this project it was often difficult to find suitable controls. Some of the areas within the study site were so fragmented that it was impossible to sample from sites that had no potential barriers separating them. To an extent this was overcome by making allowances for all types of barriers in the partial

Mantel analyses. However, this presented the problem of barrier weighting i.e. how do we differentiate between the effects of differing classes of barrier when we have no *a priori* method of comparatively assessing the impact a barrier may have (see section 3.4.2).

Often, when barrier effects have been tested in the past, control comparisons have been made between populations geographically distant from the barrier separated populations. This raises a number of questions with regards to the legitimacy of the control comparison. For instance (Hitchings and Beebee, 1997) found significant differences in the level of genetic substructure between populations of *Rana temporaria* (common frog) in urban and rural areas. However they were attempting to look at the effects of urban fragmentation as a whole, and were not trying to assess the individual effects of specific barriers.

A loss of genetic variation is thought to be a consequence of reduction in effective population size, then leading to an increase in differentiation. This was shown to be the case in South African buffalo, where these three factors were shown to be significantly correlated (O'Ryan *et al.*, 1998). When compared to the levels of genetic differentiation, measured with F_{ST} and R_{ST}, the increase in perceived fragmentation within this study, as quantified using the HFI, supports this view.

3.4.2 Types of barrier

3.4.2.1 Linear features

Potential barriers such as roads, rivers & railways present different problems to different organisms wishing to negotiate a crossing. To a small mammal, such as a vole, the road itself presents an often intractable problem. The road surface itself may provide an unmanageable crossing surface. A small woodland mammal may be unwilling to attempt a crossing as it would risk exposure to predators for an unacceptably long period (Richardson *et al.*, 1997). This would not appear to be the case for the hedgehog, with

few natural predators and being predominantly nocturnal (Morris, 1991). Hedgehog road casualties will testify to the fact that they are frequently willing to attempt road crossings. Anecdotal evidence of hedgehogs feeding on road-killed carrion, or on the insects drawn to it, suggests that hedgehogs may actually be drawn to the roads for foraging opportunities (Mulder, 1996). However, although there is evidence that dispersing hedgehogs preferentially associate with road verges (Doncaster *et al.*, 2001) non-dispersing or foraging individuals do not do so, and would appear to actively avoid contact with artificial road surfaces (Rondinini and Doncaster, submitted).

Width would seem to be implicated in the degree to which a road acts as a barrier to geneflow. Huijser and Bergers (1998) found this to be the case, and this study found genetic effects that elicit a tentative agreement. But is it the case that the width of a road, hence an increase in average crossing time, simply increases the chance of being struck by traffic. Or is the increase in road width inversely proportional to the chances of the animal making the attempt.

Hedgehogs are known to be both excellent swimmers and climbers (Reeve, 1994). Potential barriers such as small streams and railways would not, in themselves, present a significant problem to a hedgehog. It is possible that factors such as increased rail traffic density may affect crossing success, or more likely discourage attempts by creating unfavourable conditions of high noise and pollution levels. Waterways present the additional problems of current speed, and unmanageably steep banks when attempting to leave the water. These factors would suggest that a hedgehog is unlikely to attempt to negotiate all but the most minor linear water features, although Doncaster (1992) records indirect evidence of one individual having swum the River Thames at Oxford.

3.4.2.2 Non-linear features

Whilst this study has concerned itself primarily with linear barrier features, several types of non-linear and ecological features have been shown to be actively avoided by

dispersing hedgehogs. Arable farmland is known to be avoided by a number of small animals (Mader, 1984). Hedgehogs were found to actively avoid arable farmland in preference to more urbanised environments in radio-tracking experiments by Doncaster *et al.*, (2001). Hedgehogs have also been shown to avoid areas where badgers (*Meles meles*) are common (Micol *et al.*, 1994). Their response is known to be influenced by a physiological response to badger odour (Ward *et al.*, 1996; Ward *et al.*, 1997).

Neither of these factors has been a feature of this analysis. The presence of arable farmland is unlikely to have played a part in the contemporary genetic structure of the populations studied, as most of the arable land within the study area is located outside the periphery of the populations examined in this study. However, there are areas within the study site where urban badger populations are known to be present (i.e. Southampton Common and Chilworth Public Golf Course) and it is possible that this has contributed to the patterns that have been found.

3.4.3 Historical factors

The historical presence and abundance of hedgehog populations in this area has not been well chronicled. The history of Southampton as a port has meant that it would have been one of the earliest settlements to be connected by busy trade and transportation routes, such as railways and then roads. Whilst current road and rail usage is far in excess of these early transport routes, local wildlife may have been suffering from the effects of urban fragmentation since the industrial revolution.

Initial levels of genetic diversity would have been dictated by the rate of recolonisation from southern Europe, at the end of the last ice age (approximately 10,000 years ago). Generally, levels of genetic diversity, based on observed heterozygosity, would appear to be moderately high. At a regional scale there is no evidence to indicate a significant reduction in connectivity between historical populations, or any far reaching population

crashes. The assumption must be made that current population genetic structure can be ascribed to comparatively recent, i.e. the last 200 years, geographical changes

3.4.4 Barriers or wildlife corridors?

The assumption has been made that habitat fragmentation leads to a reduction in connectivity between potentially interbreeding sub-populations. It is possible that in a species such as the hedgehog, which appears to thrive in a more urbanised environment, habitat fragmentation simply rearranges normal dispersal routes. Urban and suburban spatial organisation can offer many foraging and hibernating advantages to a hedgehog (Kristiansson, 1984; Reeve, 1994). It has also been shown that dispersing hedgehogs tend to associate with road verges for significantly longer periods of time than would be expected under a "random walk" (Doncaster *et al.*, 2001). These type of linear features appear to be actively used as dispersal routes.

Once these features, formerly perceived only as barriers, are seen from a different perspective a number of possibilities arise. Bridges and crossings have the potential to act as wildlife corridors. Whilst simply mediating the effect of a new barrier, a bridge over an ancient barrier, such as a wide stretch of river, may allow geneflow into areas previously isolated from each other.

A factor which may have some bearing on genetic structure in urban populations is that of human mediated translocation. Given the public's interest and sympathetic attitude towards hedgehogs a number of individuals and organisations, who care for injured animals, regularly take in between 10-50 animals per year from various parts of the region. Whilst some of these organisations endeavour to return the animals to the same area in which they were found, others simply release the recovered animal at sites that are perceived to be suitable for hedgehog habitation. Many of these animals will die without having mated in their new habitat. However, one successful male, from a genetically distinct stock, may sire a great number of offspring. It is unlikely that local populations

have evolved to be so specialised to their local environment that this outbreeding would have deleterious effects on the offspring. From the perspective of monitoring the genetic population structure, this arrangement may significantly misrepresent the true effects of population isolation in that area.

Recent changes in hedgehog and human behaviour are bound to have effects on the genetic structure of urban populations. It is unclear at present whether these effects will prove to be deleterious or beneficial for these animals. These effects will certainly be unpredictable, and may prove difficult to quantify. This has been compounded by the lack of baseline data with regards to previous hedgehog status. Therefore, further investigations into the plight of hedgehogs inhabiting urban and suburban populations are warranted.

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4.0 Gender specific dispersal, and habitat recolonisation

4.1 *Introduction*

4.1.1 The advantages and disadvantages of dispersal

Most individuals are subject to dispersal at some stage of their life cycle. A definition of dispersal, in this context, is an event in which permanent departure from the natal range, or home site, into an area where they will reproduce, or would have reproduced if they had survived and found a mate (Howard, 1960). In general the dispersal stage is embryonic in marine animals, and juvenile or adult for terrestrial animals. The stage at which this occurs differs among species, gender, age and environmental factors. Neither zygotic nor gametic dispersal, of the type typical of many invertebrates and terrestrial plant species, will be considered in this chapter.

Vertebrate dispersal often displays a marked gender bias. Within bird species the dispersal bias is towards the female, who will leave the natal area and mate with a male which defends it's own territory (Greenwood and Harvey, 1982). The general trend in mammals is the reverse, where young males leave the natal area to find, comparatively, sedentary females (Greenwood, 1980). The main aim of this chapter is to seek the differences between male and female dispersal patterns in hedgehogs, and how they impact on mixing between sub-populations of hedgehogs. The secondary aims will be to assess the effects of population level factors, such as recent bottlenecks; to establish connectivity between sub-populations, and relate this to previous extinction and recolonisation events. Hedgehogs are a good model for studying sex biases in dispersal because their movements are not constrained by territories. Also, we have a lot of information on their ecology from radio-tracking studies e.g. Doncaster *et al.*, (2001) and Rondinini and Doncaster, (submitted).

Dispersal can improve the reproductive potential of an individual by facilitating association with different conspecifics, or to improve the quality of its environment (Cockburn, 1992). The main reasons advanced for the prevalence of dispersal are the avoidance of inbreeding (Pusey, 1980; Clutton-Brock, 1989), and the reduction of

competition for resources (Greenwood, 1980; Dobson, 1982). While these would appear to be universal themes, in any given situation, the actual driving force will depend on the countermanding costs of dispersal (Bengtsson, 1978; Bateson, 1983; Shields, 1983). These costs may be a result of a number of different factors, such as dominance status (in gregarious organisms), fitness effects, or increased intrasexual competition (Waser, 1996).

Dispersal can incur considerable costs. Individuals that disperse are at increased risk of predation, increased parasite load, and poor condition as a result of increased stress and unfamiliarity with their surroundings (Lidicker, 1975). It must, therefore, be borne in mind that any potential benefits that dispersal could bring to the reproductive success an individual are negated if the individual is unfit to mate, or dies before mating following dispersal.

4.1.2 Types of dispersal

The dominant forms of dispersal in vertebrates are *natal dispersal* and *breeding dispersal* (Greenwood and Harvey, 1982). Natal dispersal refers to a permanent movement away from the natal site by juveniles, usually subsequent to the cessation of parental investment i.e. following weaning in mammals, and fledging in birds. Breeding dispersal refers to any movement between sites between periods of reproductive activity (Greenwood, 1980). Of the two strategies the former appears to be the most common in vertebrates (Baker, 1978). Simulation modelling has shown that natal dispersal appears to be favoured over a range of dispersal frequency distributions and age classes, either as a single strategy or in combination with breeding dispersal (Johst and Brandl, 1999).

There is another distinction which must be made when considering the circumstances of dispersal, and that is whether it is *saturation* or *presaturation* dispersal (Lidicker, 1975). This refers to the 'population density vs. resource availability' status of the source population. These terms differentiate between situations where individuals disperse

because the site they inhabit has reached its population carrying capacity, and situations where dispersal occurs before this point is reached. The distinction is important as it bears relevance to the relative benefits an individual may gain in deciding to disperse, and possibly to the ability of the individual to successfully disperse.

4.1.3 Gender biased dispersal

A large number of vertebrate species exhibit gender biased dispersal. This is particularly true of mammals and birds where one sex tends to disperse and the other tends to philopatry (Greenwood, 1980). An inversion occurs between these two groups, as mammals tend to exhibit predominantly male dispersal (Clutton-Brock, 1989) and birds, female dispersal (Greenwood and Harvey, 1982; Clarke *et al.*, 1997).

A number of theories have been advanced to explain gender bias in dispersal (Motro, 1982; Gandon, 1999; Perrin and Mazalov, 1999; Julliard, 2000), which are usually based around the search for a genetically distinct mate. However, they make no assumption about the sex that should benefit the most from dispersal. Three predictive models have been suggested to explain gender of the dispersive sex.

i) The resource competition model (Greenwood, 1980; Greenwood, 1983) assumes a benefit to the philopatric gender or genders. The assertion being that familiarity with the natal area allows optimal resource usage. In a polygynous species females find themselves putting the greatest investment into the rearing of offspring. Therefore they gain the most by remaining philopatric. For example, in polygynous mammals, females are the limiting resource so males disperse in search of females. In a monogamous species the male makes the greatest investment in defending and maintaining a territory, so here he has the greatest incentive to remain philopatric. In monogamous birds, food is the limiting resource so females disperse in search of males that hold resource rich territories.

ii) The local mate competition model (Dobson, 1982) is based on the idea that philopatry increases competition for mates between kin, thus leading to inbreeding and its associated fitness costs. It predicts that the sex with the greatest reproductive potential gains the greatest benefit from dispersal. Therefore in a polygynous species males would disperse, and in a monogamous species there would be no difference in dispersal between sexes.

iii) The inbreeding model (Wolff, 1993; Wolff, 1994) suggests that philopatry leads to the increased likelihood of mating with the opposite sex parent. Therefore the sex at greatest risk should be the one to disperse. In polygynous species only the mother would remain in proximity to her offspring. Female offspring do not run the risk of breeding with their mothers, so male offspring should disperse. In a monogamous species inbreeding risk is equal in both sexes, therefore no sex bias in dispersal should be evident.

It is not always clear, however, that inbreeding is the negative, dispersal driving force that it has been assumed to be. Inbreeding is not necessarily an infrequent situation (Woolpy and Eckstrand, 1979; Keane *et al.*, 1996), neither does it have to lead to negative fitness effects (Bonnell and Selander, 1974; Gilbert *et al.*, 1990; Reeve *et al.*, 1990; Caro and Laurenson, 1994). Male biased dispersal has been reported in species that still mate exclusively with females from their natal groups, which suggests that dispersal is driven by foraging competition rather than inbreeding avoidance (Gompper *et al.*, 1998).

4.1.4 Methods of detecting and measuring dispersal

4.1.4.1 Direct observation

Because it usually only happens once in an individuals lifetime, dispersal is very difficult to detect and measure (Ims and Yoccoz, 1997). Direct observation of natural dispersal, using techniques such as capture-mark-recapture and radio-telemetry, is fraught with

logistical difficulties, and have their own inherent analytical limitations (Koenig *et al.*, 1996). It is difficult to predict which individuals will disperse, or of what age they will disperse. As most individuals will only disperse once in their lifetime, (Beier, 1995), the odds are against it being observed. In the case of the European hedgehog dispersal events are more difficult to detect than in many mammals, as they seem to have no clearly defined dispersal phase or sex bias (Reeve, 1994).

Emigration rates are sometimes estimated, at least partially, by the absence of individuals that were previously counted as part of a local population (Favre *et al.*, 1997; Mossman and Waser, 1999). In its basic form this method will tend to overestimate emigration rates, as it makes no distinction between individuals that are absent by emigration and those that are absent by mortality. This type of error can usually be corrected for by including a term to account for mortality (Barrowclough, 1978; Waser *et al.*, 1994). This also holds true for studies that classify dispersal by mark-recapture, especially when trying to detect long distance dispersal events. Koenig *et al.* (1996) suggest that the majority of mark-recapture studies have underestimated dispersal levels by failing to take into consideration that long distance dispersers are more likely to be missed during recapturing sessions.

A few studies have made inferences about dispersal potential and habitat usage based on movement following artificial translocation of individuals (Doncaster, 1992; Doncaster, 1994; Doncaster *et al.*, 2001). Whilst these types of study produce valuable information on dispersive mechanisms and dispersal vectors they do not directly address factors such as frequency of dispersal in unmanipulated animals.

4.1.4.2 Inferred dispersal: the use of molecular genetic data

Molecular techniques and statistical genetic analysis appear to offer some considerable benefits over direct observation when it comes to studying dispersal. Besides the comparative ease of data generation (Avise, 1994), theoretically molecular data provides

a temporal aspect to population structure (Koenig *et al.*, 1996), and also reflects only that dispersal which led to successful reproduction and gene inheritance.

A rapidly expanding range of molecular techniques are available for use in this type of study. Whilst many researchers previously used protein electrophoresis, allozymes and isozymes, to make estimations of dispersal rates and nuclear gene flow (Melnick and Hoelzer, 1992; Bernatchez and Osinov, 1995; Hitchings and Beebee, 1997; Cunningham and Moritz, 1998; Seppä and Laurila, 1999), polymorphic microsatellite markers have largely supplanted their use in recent years. Microsatellite DNA loci are regions of tandemly repeating sequences, with a repeat unit of less than 6 base pairs (Jarne and Lagoda, 1996). These regions usually develop within non-coding regions of the genome, and are generally considered to be selectively neutral (Ashley and Dow, 1994). Their abundance, (Lagercrantz *et al.*, 1993) and their high level of polymorphism, due to their selective neutrality, make them a better choice of marker for dispersal studies than isozymes and allozymes, which are functional enzymes and consequently subject to selective pressures. Nuclear microsatellites have been used successfully to study dispersal in a wide range of organisms e.g. in walrus (Anderson *et al.*, 1998), voles (Ishibashi *et al.*, 1997), rattlesnakes (Gibbs *et al.*, 1997), red grouse (Piertney *et al.*, 1999) etc.

Mitochondrial DNA has also been used extensively, although more commonly for phylogenetic reconstructions over an evolutionary time scale (O'Corry-Crowe *et al.*, 1997; Santucci *et al.*, 1998). This being said, Moritz (1994) describes how mitochondrial sequence data can be employed for the reconstruction of phylogenetic lineages, whilst mitochondrial haplotype frequencies can be used for population studies over an ecological time-scale. Two fundamental properties of mitochondrial DNA which distinguish it from nuclear DNA are 1) it is inherited purely maternally, except for some exceptional situations i.e. paternal leakage in mice (Kaneda *et al.*, 1995), and doubly uniparental inheritance in certain molluscs (Hoeh *et al.*, 1991; Hoffmann *et al.*, 1992; Stewart *et al.*, 1995), and 2) it does not undergo recombination, so any sequence variation is purely a result of mutation (Brown, 1983).

Combinations of nuclear and mitochondrial data have been used successfully to infer population structure, and gender differences in dispersal and recolonisation (Melnick and Hoelzer, 1992; Bernatchez and Osinov, 1995; Brookes *et al.*, 1997; Anderson *et al.*, 1998; Waits *et al.*, 2000). The results of female mediated gene flow are reflected in the mitochondrial DNA, and male mediated gene flow can be inferred from the nuclear DNA data.

Most of the statistical tools used to analyse molecular data are based on the assumptions that gene frequency distributions are dictated by gene flow (Cockerham and Weir, 1993; Gaggiotti *et al.*, 1999), and that gene flow is representative of dispersal. However, Bossart and Prowell, (1998) warn that gene flow is only one of a number of contributory factors shaping population genetic structure. They go on to assert that current analytical techniques and models for the analysis of gene frequencies make too many assumptions about temporal environmental homogeneity and break down under the assumption of stochastic environmental events.

New analytical techniques, such as 'assignment testing' (Paetkau *et al.*, 1995; Waser and Strobeck, 1998; Cornuet *et al.*, 1999), provide a new approach to detection of dispersal. They take a probabilistic approach to the likelihood of the individual's genotype originating from the populations in which the individual was sampled, and are powerful and sensitive enough to detect the offspring of immigrants for up to two generations (Rannala and Mountain, 1997). This technique has been used successfully in a number of organisms, such as humans (Rannala and Mountain, 1997), microtine rodents (Favre *et al.*, 1997; Mossman and Waser, 1999) and ursids (Paetkau *et al.*, 1995).

The strength of this study is its use of molecular and 'whole-animal' dispersal data, an approach that has been recommended in previous reviews of this subject (Koenig *et al.*, 1996; Thompson and Goodman, 1997). An in-depth analysis of the differing dispersal strategies employed by either sex of the hedgehogs in this study may allow a greater understanding of the dynamics of a metapopulation, and the relationships between the component sub-populations. This will be achieved primarily by the use of the molecular

data generated for this study in combination with whole-animal data on dispersal of individuals within this study site.

Using a molecular approach, and employing some of the techniques described, I intend to achieve the following aims in this chapter. (i) Reconstruct phylogenies from mtDNA (strict maternal inheritance) and nDNA (inherited by both sexes), and use these to (ii) identify sex differences in molecular mixing between sub-populations, (iii) seek effects of other factors such as previous population bottlenecks and differences in effective population size; (iv) identify sources of colonisation events and to seek evidence for specific source populations.

4.2 *Methods*

4.2.1 Sample collection

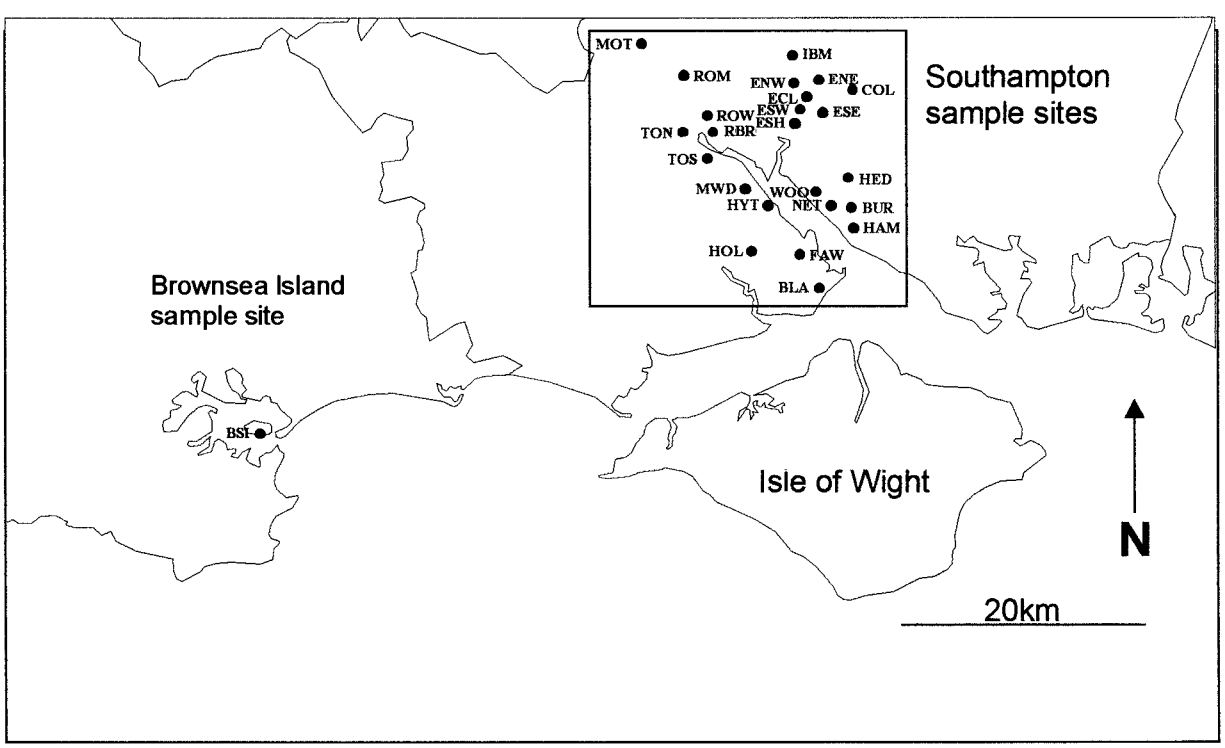

Figure 4.1 Location population BSI in relation to the sample sites within the Southampton area. Collections were made in the summer and autumn of 1996 and 1997, and a total of 33 individuals were sampled at BSI. A more detailed map of the position of populations within Southampton can be found in section 3.2.1.1, Figure 3.1

Details of sample collection from Southampton sites are outlined in, section 3.2.1, Figure 3.1. Samples were also acquired from a site on Brownsea Island, situated in the middle of Poole harbour, Dorset, between 1996 and 1997, for comparison as a totally isolated population.

4.2.2 Isolation of microsateilite DNA and typing of samples

Details of microsatellite isolation and sample typing are given in section 3.2.4.

4.2.3 Amplification of mtDNA

The methods used for the amplification of mtDNA D-loop are outlined in section 0.

4.2.4 Data analysis

4.2.4.1 AMOVA and overall genetic differentiation

Mitochondrial haplotype data, and microsatellite data were analysed using a hierarchical analysis of molecular variance (AMOVA), as described in Excoffier *et al.* (1992), with variance being partitioned amongst individuals and between sample sites. The analysis was performed using the program Arlequin version 2.000 (Schneider *et al.*, 2000). Significance values for the covariance components, σ_a^2 and σ_b^2, and for the fixation index (F_{ST} analogue) Φ_{ST}, were calculated using 10,000 permutations (of haplotypes with mitochondrial data, and of individual genotypes with microsatellite data) to produce a null distribution to which the values were compared.

4.2.4.2 Assignment testing

Genotypic assignment was performed using microsatellite data, following the method described in Paetkau *et al.* (1995; 1997) Rannala and Mountain (1997) and Favre *et al.* (1997). An assignment index (*AI*) was calculated (Paetkau *et al.*, 1995) for each individual, the index being the probability that it came from this population. The probability that individual *k* originates from population *l* is calculated from the frequencies of all alleles within that reference population. Assuming Hardy-Weinberg equilibrium, an individual's probability values at each locus are represented by p_{il}^2 in a homozygous individual, and $2p_{il}p_{jl}$ in a heterozygous individual, where p_{il} and p_{jl} are the frequencies of alleles *i* and *j* in population *l*, including the individual being assigned. These values are then multiplied over all loci, and log-transformed.

Assignment was performed using the program GeneClass 1.0.02 (Cornuet *et al.*, 1999), which calculates an assignment probability for each individual of each population, thus providing a probability value for the population in which it was sampled, as well as suggesting which population it is most likely to originate from. Assignment index values, *AI*, were corrected by subtracting the population mean from each individual giving the population an overall mean of zero. Negative corrected assignment values (AI_C) indicate individuals that are likely to be immigrants or first and second generation offspring of immigrants. Gender differences in AI_C value distributions were tested for significance using a Mann-Whitney *U*-test.

Assignment testing was performed on all individuals for all populations, in the manner described above. The population giving the highest probability value for an individual was nominally defined as its 'assigned population'. The composition of each population was calculated in terms of percentage of individuals assigned to their 'sampled population', and those assigned to different populations. Direct estimates of dispersal distances in field experiments have suggested that natural dispersals of hedgehogs in this area are unlikely to exceed a straight line distance of 4 km (Doncaster *et al.*, 2001).

Therefore all individuals assigned to a population greater than 4 km from the population in which they were sampled were assumed to have been mis-assigned, or individuals that had been translocated by members of the public (see section 3.4.4).

4.2.4.3 Genetic differentiation and estimation of migration rates

Global genetic differentiation was estimated as θ, which is an F_{ST} analogue proposed by Weir and Cockerham (1984), and is calculated from microsatellite allele frequencies using the program FSTAT version 2.9.1 (Goudet, 1995). Confidence intervals were calculated to 95% by bootstrapping over loci (1000 bootstraps), and significance of θ was determined by *G*-test (Goudet *et al.*, 1996) employing 5000 permutations of individual genotypes.

Global F_{ST} (θ) was used to calculate overall migration rates (*Nm*) between populations analysed. The value of *Nm* relates to the population size (*N*), and the mutation rate (*m*), and is measured in number of migrants per population per generation. Two versions of *Nm* were calculated, firstly, using the 'island model' of Wright (1943), based on the following equation:

$$Nm = \frac{1 - F_{ST}}{4F_{ST}} \qquad (4.1)$$

Confidence intervals were deduced by calculating *Nm* for the upper and lower 95% CI produced for F_{ST} (θ).

The second estimation of *Nm* is the 'private allele' method, which gives a multilocus estimate based on regression data published in Barton and Slatkin (1986). This value was calculated using the program GENEPOP version 3.2a (Raymond and Rousset, 1995).

4.2.4.4 Phylogenetic reconstructions

Phylogenetic reconstructions were attempted for nuclear microsatellite data, and also for mitochondrial haplotype data. Neighbour-joining (NJ) trees were constructed using Cavalli-Sforza & Edwards chord distance D_C (Cavalli-Sforza and Edwards, 1967). This is a geometric genetic distance, which has been found to be one of the most accurate metrics for reconstructing phylogenies between closely related groups (Takezaki and Nei, 1996). Genetic distances were calculated from microsatellite data using MICROSAT (Minch, 1997), creating 1000 distance matrices from resampled datasets bootstrapped over loci. Mitochondrial haplotype data was bootstrapped over populations to produce 1000 datasets, which was performed using a Microsoft EXCEL macro. Genetic distances were then calculated using the subroutine GENDIST within PHYLIP version 3.57c (Felsenstein, 1995). Bootstrapped distance matrices from both GENDIST and MICROSAT were then used as input for the NJ tree constructing subroutine NEIGHBOUR, invoking option 'J' to randomise sample input to prevent their order affecting the topology of the final tree. The 1000 NJ trees from mtDNA and microsatellite data were inputted into the CONSENSE subroutine of PHYLIP to produce two consensus trees giving bootstrapped robustness estimations at each node.

4.3 *Results*

4.3.1 Allelic distribution and mutational inferences from mtDNA VNTR

4.3.1.1 General comments

Individuals from populations BUR and WOO, Figure 3.1, failed to generate readable sequences. For the sake of a balanced comparison between mitochondrial and nuclear data these populations were also excluded from further microsatellite analysis.

4.3.1.2 Non-VNTR region

The 560 bp fragment of mitochondrial D-loop was partitioned into two sections: the VNTR region (position 218-551), and the non VNTR region (position 1-217 and 552-560). Of the individuals sequenced, only 4 contained any mutations within the non-VNTR region. All of these mutations were A to G transition mutations, which occurred at position 89 (2 individuals), position 131 (1 individual) and position 217 (1 individual), giving a sequence variation of between 0.4-0.9%.

4.3.1.3 VNTR region

The VNTR region of the mitochondrial D-loop exhibited high levels of polymorphism. It consisted of an 8 bp repeat unit with a 'TATTATTA' motif. Repeat numbers were found to range from 10 to 41 giving a total of 16 haplotypes (Table 4.1). Despite the findings of Krettek *et al.* (1995) none of the individuals sampled exhibited heteroplasmy, allowing unambiguous assignment of haplotypes to each individual.

D-loop VNTR haplotype frequencies are shown in Figure 4.2. A normal distribution would indicate that stepwise mutation had been the predominant allele frequency shaping factor (Rand and Harrison, 1986). A left-handed skew to a normal distribution would suggest that the locus shows an expansion bias (Wierdl *et al.*, 1997), i.e. alleles have a tendency to add repeat units rather than lose them (Ashley and Warren, 1995). A right-handed skew would imply selection for smaller allele sizes (Cesaroni *et al.*, 1997). The allele frequencies exhibited in Figure 4.2 show little evidence of a normal distribution or skew, and thus it is likely that the mechanism of mutation is not strictly stepwise and that the allele sizes have no expansion bias. Whilst some stepwise mutation may have occurred to produce the allele frequency distribution from 23 to 32 repeat units it is unlikely that this is the only significant mutational mechanism in play. Consequently a strict stepwise mutation model, SMM, (Ohta and Kimura, 1973) based analysis is ruled out. There is no clear indication as to whether an infinite allele model, IAM, (Estoup *et*

al., 1995) or a two phase mutation model, TPM, (Di Rienzo *et al.*, 1994) would be the appropriate analytical approach, genetic distance was estimated on the basis of allele frequencies and not repeat number divergence.

Table 4.1 Distribution of mtDNA VNTR haplotypes amongst 23 sample sites grouped geographically. See Figure 3.1 for comparative geographical location of sample sites.

Geographical Locations	*Population*	*Haplotype*
Northwest:	MOT	7, 10
Romsey area	ROM	1, 6, 8, 10, 13, 13, 14
	RBR	9, 9, 11, 12, 12
	ROW	6, 8, 9, 9, 11
Northeast:	IBM	9, 12, 12, 12
Eastleigh area	ECL	1, 1, 1, 7
	ENE	1, 13, 15
	ENW	1, 1, 6, 10
	ESE	1, 3, 13, 13, 13
	ESH	11, 11, 11, 12
	ESW	8, 14, 14
	COL	13, 13, 14, 16
Southeast:	HAM	2, 6
North Southampton Water	HED	4, 7
	NET	6, 10
Southwest:	TON	5, 7, 13
South Southampton Water	TOS	9, 11, 11, 11
	MWD	8, 9, 11, 14
	HYT	11, 13
	HOL	1, 3, 10
	FAW	3, 3, 3, 3, 5
	BLA	3, 3, 3, 5, 8
Far Southwest:	BSI	13, 13, 13, 13, 14
Brownsea Island		

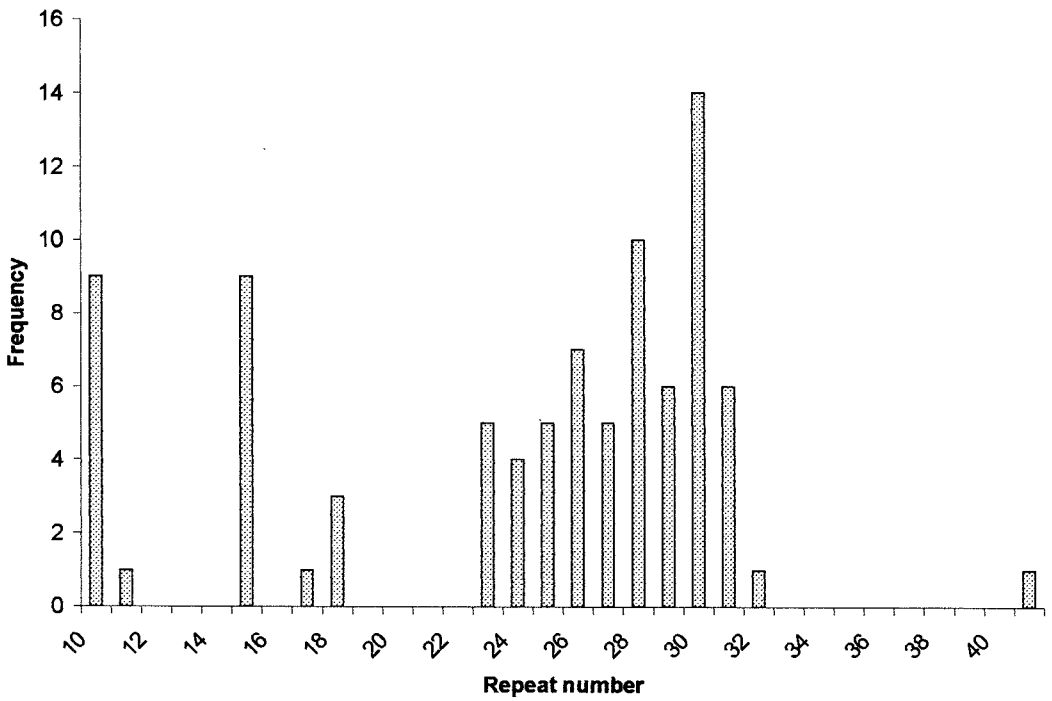

Figure 4.2 Mitochondrial D-loop VNTR allele frequency distribution. Repeat units consist of an 8bp motif 'ATATTATT', which vary in repeat number from 10 to 41. Outlying allele sizes suggest that mutation is not occurring in a strict stepwise manner (Ohta and Kimura, 1973). It is more likely that mutation conforms to a two phase model (Di Rienzo *et al.*, 1994).

4.3.2 AMOVA

Analysis of molecular variation revealed a disparity between levels of genetic differentiation inferred from mtDNA VNTR haplotypes and microsatellite markers. Overall Φ_{ST} results indicate a greater level of differentiation between populations when estimated using the mtDNA VNTR where Φ_{ST} is 0.177, than from nuclear microsatellite loci where Φ_{ST} is 0.058 (Table 4.2). Despite this difference both Φ_{ST} values are significantly different from zero, and imply that significant population structure has been detected by both markers in these populations.

Table 4.2 Analysis of molecular variance (AMOVA) that partitions variance between individuals, and between populations (Excoffier *et al.*, 1992). Covariance components (Va = σ_a^2 and Vb = σ_b^2) reveal that the majority of variation is explained by differences in individuals. However, when comparing mtDNA to nDNA the proportion of the variation between populations is larger by approximately 10%. This effect is shown in Figure 4.3. PhiST (Φ_{ST}) and associated *p* values describe significant genetic differentiation across the populations analysed, which again appears to be considerably higher in mtDNA than nDNA estimates.

		Among populations			*Within populations*		
	Φ_{ST}	*v.c*	*%age var.*	*p value*	*v.c*	*%age var.*	*p value*
Microsatellite data	0.058	0.181 Va	5.8	<0.001	2.934 Vb	94.2	<0.001
Mitochondrial data	0.177	0.082 Va	17.7	<0.001	0.383 Vb	82.3	<0.001

The covariance components, σ_a^2 and σ_b^2, explain the partitioning of allelic variation between populations, and between all individuals within all pooled populations respectively. The percentage of total variation ascribed to 'between population' differences is greater from the mtDNA data than from the microsatellite data (Table 4.2 and Figure 4.3).

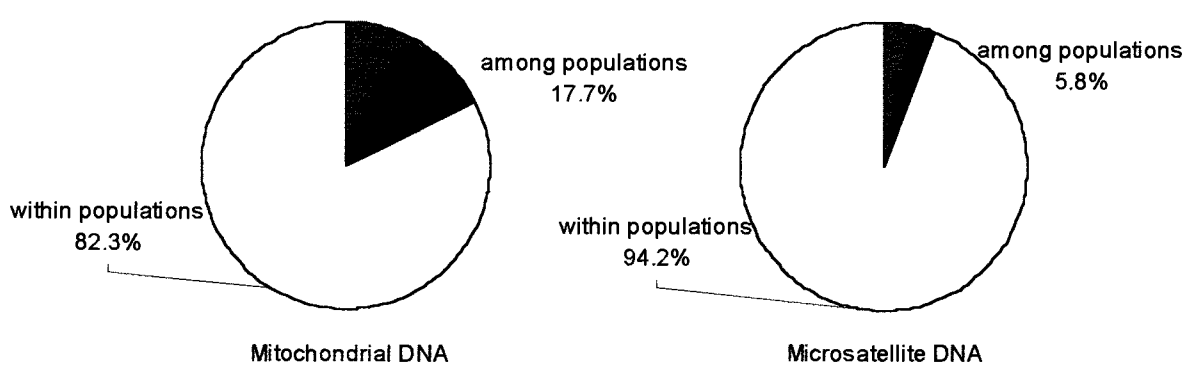

Figure 4.3 Each pie chart represents the total variation, based on metric distances, between individuals and populations. Hierarchical analysis by AMOVA partitioned the percentage of variation as shown above.

Mitochondrial DNA reflects population genetic structure as a result of gene flow via female dispersal, whilst nuclear microsatellite DNA reflects population genetic structure as a result of gene flow mediated by both sexes. This shows a typical mammalian sex

bias where female philopatry has lead to greater differentiation of mtDNA between populations than observed in nuclear DNA that is predominantly a result of male dispersal. It should be acknowledged that the results of the microsatellite DNA are calculated over a number of diploid loci, whereas the results of the mitochondrial DNA are effectively calculated over one haploid locus. The effective populations size (N_e) of the mitochondrial dataset is effectively half that of an equivalent nuclear dataset. The smaller N_e of the mtDNA dataset, coupled with the single locus nature of the marker, means that it is likely to be far more sensitive to bias introduced by genetic drift and bottleneck effects than is the nuclear data.

4.3.3 Population assignment testing

Of the 388 individuals that underwent assignment testing, 301 (77.6%) were assigned to the population they were found in. The percentage of individuals, by gender, assigned to populations other than the ones they were found in was approximately equal (males 11.4% and females 11.0%). In order to estimate the average distance travelled by dispersing individuals of each sex the following calculation was performed. The mean average distances between the population an individual was sampled in and the population to which it was assigned, using data from mis-assigned individuals only, were 9.96 ± 1.51 (SE) km in females, and 8.73 ± 1.46 (SE) km in males. The difference between distance distributions was found to be non-significant ($p < 0.88$, Mann-Whitney *U*-test).

Gender differences of corrected assignment index value (AI_C) frequencies are shown in Figure 4.4. The gender difference in AI_C value frequencies was not found to be significant ($p < 0.30$, Mann-Whitney *U*-test). Nevertheless, some interesting qualitative differences are worth noting. Individuals with an AI_C value lower than zero have a probability of being immigrants, or offspring of immigrants, to the population they were sampled in which is inversely proportional to their AI_C value. The majority of individuals have an AI_C of –5.0 or greater, however the distribution of males appears to show a

limited bimodal distribution with a three individuals with AI_C values of less than –4.0. This bimodality could suggest two distinct groups within the males tested; one group that have a tendency to philopatry, and one group that have a tendency to disperse (Favre *et al.*, 1997). The small proportion of individuals belonging in this 'low value AI_C' mode would suggest that the group with the tendency to be philopatric predominate.

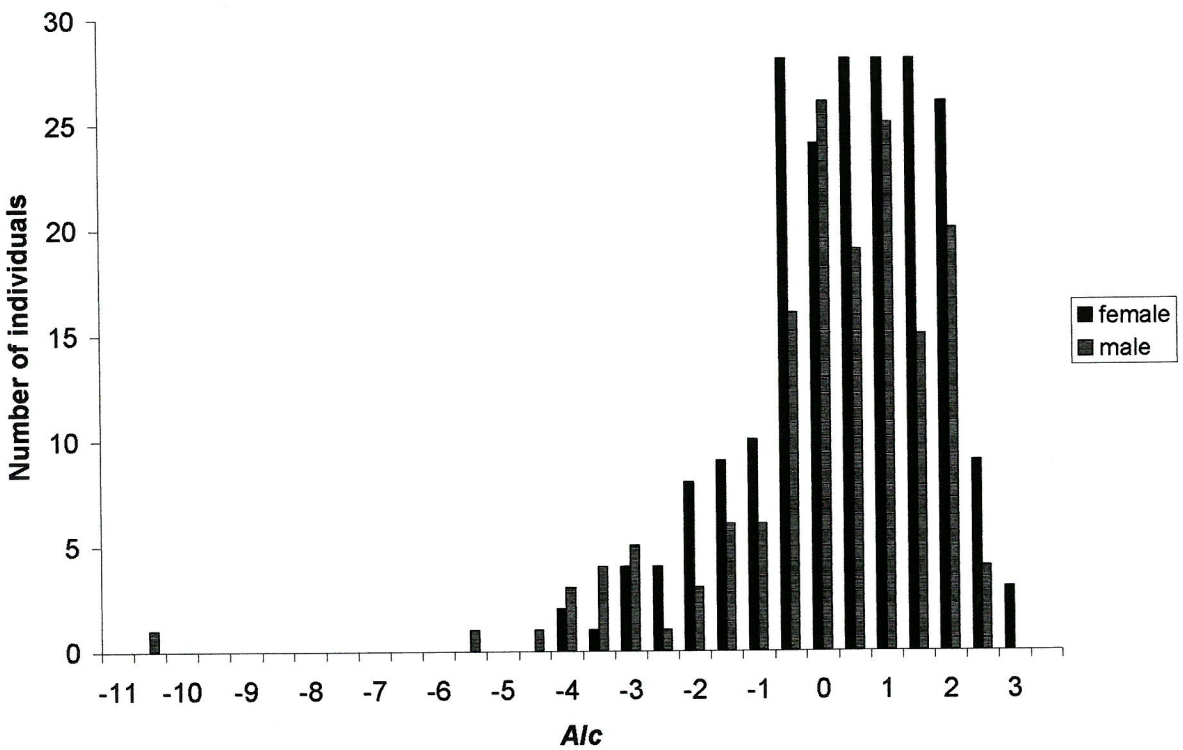

Figure 4.4 Distribution of corrected assignment index (AI_C) frequencies for males and females, with reference to the population in which they were sampled, based on a log likelihood test using allele frequencies, see section 4.2.4.2, (Paetkau *et al.*, 1995). The corrected *AI* value gives each population a mean AI_C value of zero, therefore individuals with AI_C values of less than zero are increasingly less likely to have originated from the population in which they were sampled. Despite some outlying, extreme negative values in males, no significant difference could be detected between distributions of male and female AI_C value frequencies using a Mann-Whitney *U*-test ($p < 0.30$).

Contrast this with the female distribution, which has no individuals with an AI_C of less than –4.0. Whilst there is no apparent bimodality in the female distribution, there does appear to be a left-handed skew that would not be expected in individuals that do not undergo dispersal.

The results of the population assignment and percentage composition are shown in Table 4.3. Dispersal between populations around Eastleigh appears to be endemic. Populations ECL, ENE, ENW, ESE, ESH, and ESW appear to exhibit multiple interpopulation dispersals. There is also some evidence of individuals from these populations dispersing to two other nearby sites, IBM and COL. Of these eight populations only ENE and ESH appear to have no potential immigrants, although individuals assigned to these populations were found in four of the other Eastleigh populations (COL, ENW, ESE and ESW). Whilst it is possible that this represents 'one-way dispersal', it is more likely that there have not been any immigrants within the last two generations, or that they were simply not sampled.

Other populations that exhibited recent emigration/immigration were TOS/MWD, RBR/TON, TOS/TON and FAW/HOL.

Table 4.3 Percentage composition of each population, based on log likelihood assignment test predictions of which population each individual originated from. These percentages exclude any individual who was assigned to a population more than 4 km away from the population they were sampled in. Top left to bottom right diagonal values give the percentage of each population that were accepted as having originated from that population i.e. 100% means that no immigrants were detected. The populations are listed in geographical order (see Table 4.1), and intra-region comparisons are enclosed within boxes.

		Assigned population																					
Sampled population	*Regions*	*MOT*	*ROM*	*RBR*	*ROW*	*IBM*	*ECL*	*ENE*	*ENW*	*ESE*	*ESH*	*ESW*	*COL*	*HAM*	*HED*	*NET*	*TON*	*TOS*	*HYT*	*MWD*	*HOL*	*FAW*	*BLA*	*BSI*
MOT	Northwest: Romsey area	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ROM		-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RBR		-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ROW		-	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IBM	Northeast: Eastleigh area	-	-	-	-	86.7	-	-	13.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ECL		-	-	-	-	-	68.8	-	3.1	3.1	-	18.8	6.3	-	-	-	-	-	-	-	-	-	-	-
ENE		-	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ENW		-	-	-	-	-	8.3	-	75.0	-	8.3	8.3	-	-	-	-	-	-	-	-	-	-	-	-
ESE		-	-	-	-	-	4.8	-	4.8	81.0	4.8	4.8	-	-	-	-	-	-	-	-	-	-	-	-
ESH		-	-	-	-	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-
ESW		-	-	-	-	-	5.7	2.9	2.9	2.9	2.9	82.9	-	-	-	-	-	-	-	-	-	-	-	-
COL		-	-	-	-	-	-	13.3	-	-	-	-	86.7	-	-	-	-	-	-	-	-	-	-	-
HAM	Southeast: North Southampton Water	-	-	-	-	-	-	-	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-	-
HED		-	-	-	-	-	-	-	-	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-
NET		-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-
TON	Southwest: South Southampton Water	-	-	9.5	-	-	-	-	-	-	-	-	-	-	-	-	85.7	4.8	-	-	-	-	-	-
TOS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.0	-	-	-	-	-	-
HYT		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.0	-	-	-	-	-
MWD		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.1	-	92.9	-	-	-	-
HOL		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	88.2	11.8	-	-
FAW		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.0	-	-
BLA		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.0	-
BSI	Far Southwest: Brownsea Island	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.0

4.3.4 Population genetic differentiation (F_{ST}), and migration rates (*Nm*)

Genetic differentiation, as estimated by Weir and Cockerham (1984) F_{ST} analogue θ, gives a low but significant ($p < 0.0002$) global value of 0.068 (0.055 – 0.080 95% CI). The two methods of calculating the overall migration rate *Nm* produced roughly comparable estimates. The Barton and Slatkin private allele model (Barton and Slatkin, 1986) gave an estimate of 4.86 migrants per generation per population. The Wright's island model (Wright, 1943) based on equation (4.1), gave a slightly lower estimation of 3.44 (2.88-4.30 95% CI) migrants per generation per population. Both of these values are marginally higher than, but comparable to those estimated from hedgehog populations in Oxford (Becher and Griffiths, 1998).

These results would appear to be broadly consistent with the results of the assignment test. If the total number of individuals with an AI_C of less than zero are designated as immigrants to the population they were sampled in, and that total divided by the number of populations sampled, the mean number of immigrants per population is 4.91. This rough estimate appears to be very close to the value of *Nm* derived using the private allele method, however it should be regarded with some caution for the following reasons. The mean average number of immigrants, based on assignment index values, assumes that all individuals with sub-zero AI_C values are true immigrants having arrived within a single generational time span. It is most likely that this is not the case, and that a proportion of these individuals are offspring of immigrants, and immigrants who arrived prior to the current generation. The migration rate estimates of *Nm* describe the number of 'new' migrants entering the population, and as such are not directly compatible to the mean number of sub-zero AI_C individuals. To use the assignment test as a representative measure of migration rates would require information pertaining to survivial and reproductive success of migrants, and average reproductive life span of an individual.

4.3.5 Phylogenetic reconstructions

Attempts at phylogenetic reconstruction using microsatellite data produced a neighbour joining (NJ) consensus tree that is poorly supported. All nodes with less than 50% bootstrap value have been collapsed leaving only two nodes intact (Figure 4.5). The only populations that show a robust relationship are ECL and ESW. Relating this to the results of population composition, based on assignment test results (Section 4.3.3), 18.8% of the sampled individuals from ECL were classified as belonging to ESW, and 5.7% of population ESW classified as ECL. This suggests that these two sites have a free flow of dispersal between populations.

Most of the populations from north of the Southampton Water estuary appear in a monophyletic group, with the exception of HAM and NET, whereas the populations from south of the estuary form their own clade which associate with the individuals sampled from Brownsea Island (BSI).

The results of the phylogenetic reconstruction based on chord distance (D_C) calculated from mitochondrial haplotype frequencies is shown in Figure 4.6. Again bootstrap values are low indicating little support for the tree, and again all nodes with less than 50% bootstrap support have been collapsed.

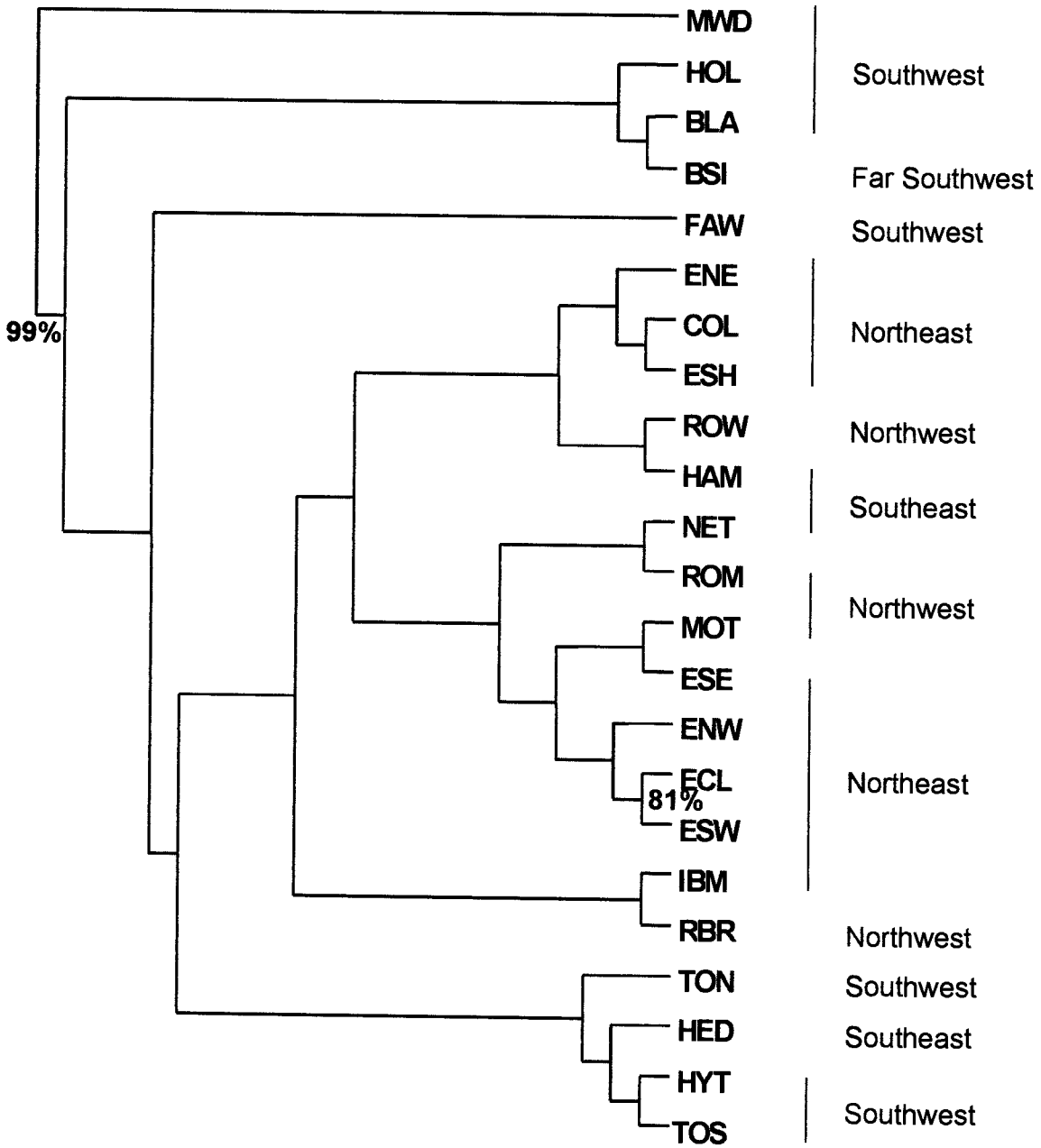

Figure 4.5 Consensus neighbour-joining tree (NJ) based on the geometric chord distance D_C (Cavalli-Sforza and Edwards, 1967) calculated from nuclear microsatellite data at 11 loci. Support for this tree was calculated from 1000 bootstraps over loci. Only bootstrap values of over 50% have been displayed, all other nodes should be considered to be collapsed. Bootstrapped datasets and genetic distance metrics were calculated using the program MICROSAT (Minch, 1997), NJ and consensus trees were constructed using the NEIGHBOUR and CONSENSE subroutines of the PHYLIP 3.57c package (Felsenstein, 1995).

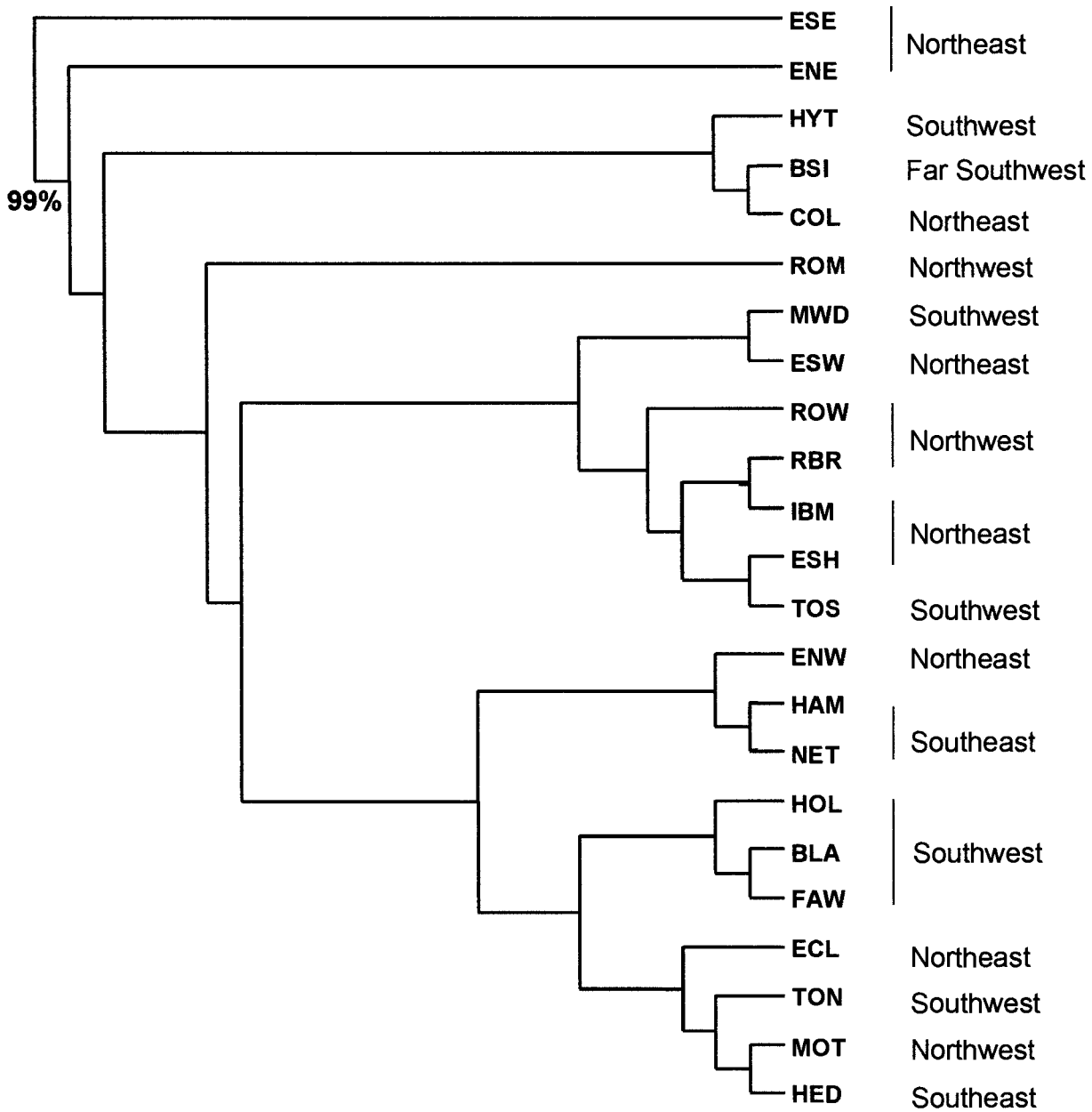

Figure 4.6 Consensus neighbour-joining tree (NJ) based on the geometric chord distance D_C (Cavalli-Sforza and Edwards, 1967) calculated from mitochondrial haplotype data. Consensus trees were constructed using the, GENDIST, NEIGHBOUR and CONSENSE subroutines of the PHYLIP 3.57c package (Felsenstein, 1995).

4.4 *Discussion*

4.4.1 Population genetic differentiation

The results of the three sets of tests performed fall into two apparently contradictory groups, those consistent with gender biased dispersal (i.e. comparative AMOVAs), and those consistent with gender neutral dispersal (i.e. assignment testing and comparative phylograms). Comparison of AMOVAs of nuclear and mitochondrial DNA suffers for the fact that the mitochondrial DNA represents one haploid locus making it far more susceptible to bias as a result of stochastic events than the eleven diploid nuclear loci. This is not to say that the mitochondrial data is flawed only that a comparative analysis is less reliable than an analysis of based solely on nuclear data.

The assignment test offers the greatest degree of sensitivity and reliability of the analyses in this chapter. To an extent the sensitivity of the test makes it difficult to differentiate actual migrants from the offspring of recent migrants, but this does not necessarily represent a major problem so long as adequate sample sizes are maintained so as to minimise possible immigrant allele bias within sampled populations. However, it must be added that in theory the assignment test may overestimate the results of short distance dispersal in population distributions exhibiting significant isolation by distance. In this situation spatially proximate populations will also be genetically more similar than population pairs separated by longer distances. This type of test calculates the probability an individual coming from a particular population based on the similarity of the genotype the individual in reference to the allele frequencies of the population. Consequently assignment testing between genetically similar populations is more likely to give an erroneous result, appearing to show short distance dispersal, than between more genetically distinct populations.

Comparison of phylograms based on mitochondrial and nuclear data share some of the uncertainties outlined in the comparisons between AMOVAs. This is offset by the facility of analysing multiple resampled datasets (bootstraps). However, the nature of the

two types of data required differences in the bootstrapping variable i.e. over loci for the nuclear dataset, and over populations in the mitochondrial dataset. However, this inconsistency should not invalidate the comparisons.

Despite these problems, some connectivity between populations was established, broadly in agreement with the results of the previous chapter. Although a significant isolation by distance effect was detected for microsatellite results neither of the phylograms showed very high degrees of clustering between geographically proximate populations. Levels of genetic differentiation, as explained by F_{ST} (θ) and Φ_{ST}, indicate significant, but low level sub-structuring, which does not significantly contradict the lack of robustness of the phlyograms. The overall migration rate (*Nm*) values generated by both Wright's model (Wright, 1943) and Barton and Slatkin's model (Barton and Slatkin, 1986) were quite high (Section 4.3.4) which would be consistent with a highly mobile metapopulation, such as appears to be the case in the phylograms. The *Nm* values appear to be higher than the mean and mode average values quoted from Oxford hedgehog populations in Becher and Griffiths (1998). Whilst the study sites in Oxford and Southampton are comparable in terms of geography and urbanisation the populations sampled in Southampton are closer to each other and more numerous than those in Oxford. This proximity may produce an analytical artefact i.e. higher apparent migration rates in Southampton than in Oxford. On the other hand, urbanisation and the creation of potential new wildlife corridors (e.g. road verges, bridges etc.) may facilitate movement between otherwise isolated patches.

Given that the significant isolation by distance effect is rather weak ($r = 0.20$, $p = 0.04$) compared to that of the F-statistics and their analogues I would suggest that geographic distance is a minor factor in the shaping of population structure in this area. The almost random nature of the relationships between populations may reflect the effects of comparatively recent localised genetic drift overlaid onto a composite population, or metapopulation, which was historically panmictic at this scale. Recent events and developments, leading to habitat fragmentation may have reduced metapopulation

connectivity to the extent that genetic drift has caused populations, which were previously genetically similar, to diverge in an unpredictable manner.

4.4.2 The effects of gender biased dispersal

The hypothesis stated above could explain the apparent random association of populations indicated by both microsatellite and mitochondrial data. Closer comparison of both of these phylograms shows that there is a limited degree of connectivity between certain populations. For instance, populations HOL, BLA and FAW are geographically close, they exist in an area of comparatively lower fragmentation, and they cluster on the mtDNA phylogram, and to a lesser extent on the nDNA derived phylogram. This would imply that dispersal is not limited to males in this area. The comparatively unfragmented nature of this particular area allows for dispersal of both sexes.

It is possible that all of these populations share a common recent female ancestor, and suggest previous recolonisation between these populations. However, the 'open' nature of this area would tend to suggest that localised extinction is an unlikely scenario.

Areas such as those in which the Eastleigh populations are found show clustering in the nDNA derived phylogram, but not in the mtDNA. This group of populations exhibit a degree of dispersal, but this appears to be almost exclusively male. The results of the AMOVA, support the other evidence (Reeve, 1994) that hedgehogs employ predominantly male dispersal. This is a common feature of genetic variance partitioning in mammals as a result of gender biased dispersal patterns. This indicates that the ratio of male to female dispersal in these populations is high enough to produce significant differences in the population genetic structure. Statistically male mammals will tend to disperse more frequently or further than females (Greenwood, 1980; Clutton-Brock, 1989). The genetic consequences of this are that nuclear DNA tends to exhibit lower levels of differentiation over distance, or between populations, than does mtDNA. This occurs as a result of higher levels of male mediated geneflow, allowing mixis to occur

over a wider area, whilst strictly maternally inherited mitochondrial DNA receives less opportunity to disperse. These results show a less extreme version of the type of genetic patterns found in Cercopithecine monkeys (Melnick and Hoelzer, 1992).

However the results of the phylograms suggest that limited female dispersal does occur within habitats with a minimum of barriers to dispersal. However, no significant difference was found in AI_C values between males and females i.e. the number of male immigrants was not significantly different to the number of female immigrants within the populations studied.

Radio-tracking studies of individual hedgehogs found no significant gender difference in dispersal capacity in dispersing (Doncaster *et al.*, 2001) or foraging (Doncaster, 1992; Doncaster *et al.*, 1996; Rondinini and Doncaster, submitted) individuals. Although females tend to inhabit smaller home ranges, an average of 50% smaller by area according to the findings of Doncaster *et al.*, (2001), and are less likely to disperse, their capacity is no less than that of the male hedgehog. It must be borne in mind, however, that the individuals being tracked during dispersal following translocation were adults of both sexes, but there is evidence to suggest that the majority of spontaneous dispersal activity may be carried out by males (Reeve and Huijser, 1999; Smit and Meijer, 1999) and juveniles before their first winter hibernation (Berthoud, 1978; Reeve, 1994). It is likely, therefore, that the frequency and gender bias of dispersal events is different in a natural setting than in the response of translocated individuals. The results of assignment testing reflects dispersal frequencies in unmanipulated animals, therefore the situation within the Southampton study site appears inconsistent with the published literature. This suggests that a recent change in dispersal behaviour may have occurred.

In light of the gender effect detected in the AMOVA results (Section 4.3.2), the non-significance of the gender based assignment test (Section 4.3.3) could be explained in one of three ways.

Firstly, limited female dispersal may result in the left-handed skew (Figure 4.4) masking the effects of male dispersal.

Secondly, the assignment test employed gives probabilities of immigration status, and is sensitive enough to detect offspring of immigrant ancestors for up to two generations (Rannala and Mountain, 1997). Clear distinctions between immigrants and their offspring are not always forthcoming, thus allowing the possibility of daughters of immigrant fathers to be assigned to distant populations. Therefore, one possibility to explain females with sub-zero AI_C values is that they are second or third generation offspring of immigrant fathers. Of course the same argument can be used for the distribution of both male and female AI_C values. The probability of an immigrant or its offspring being assigned to a population other than the one in which the individual was sampled decreases with passing generations, leading generations to exhibit moderately low AI_C values. The individuals exhibiting sub '–4.0' AI_C values may represent true immigrants, whilst the more moderate sub-zero individuals represent the second or third generation offspring of a reproductively successful male immigrant, and true immigrants from genetically similar populations.

Thirdly. the genetic structure represented by the results of the AMOVA reflect population structure based on long term dispersal patterns. Assignment testing of this type gives reliable information on the dispersal events of only three generations. The absence of a significant sex-bias detected by assignment testing may reflect recent changes in the dispersal behaviour of these populations, as a result of barriers to geneflow, or reduction in habitat connectivity. These changes would include the building of potential barriers such as major roadways, like the M3 and M25. Although the construction of minor roads are unlikely to produce barriers to dispersal (Rondinini and Doncaster, submitted) road verges and urbanisation would almost certainly affect dispersal routes and habitat usage (Doncaster *et al.*, 2001).

Even with the limitations of the comparisons between nuclear and mitochondrial AMOVA analysis, outlined in section 4.3.2, it would be unwise to dismiss the results generated. Female dispersal almost certainly occurs as part of 'local extinction' and 'recolonisation' cycles of metapopulation dynamics, but it is unlikely that it would mask a significant gender bias, if that were the case. It is also unlikely that assignment test

sensitivity is responsible for giving a false view of gender biased dispersal as the results appears to be in concordance with those of the radio-tracking studies. It is quite possible that recent environmental changes have initiated changes in behaviour that are only now beginning to impact on the genetics of the populations being studied. Therefore, I would propose that hedgehogs inhabiting increasingly fine grained habitats find that rapid habitat change and reduction in long distance dispersal opportunity has led to an equilibration of dispersal between sexes.

4.4.3 Conclusions

The analysis of the molecular data and the results of the individual radio-tracking (Doncaster *et al.*, 2001; Rondinini and Doncaster, submitted) appear largely in agreement. Whilst conventional mammalian dispersal gender differences appear when using an AMOVA approach none of the other molecular analyses detected a significant difference between sexes. This was reflected in radio-tracked individuals, where no significant gender difference was detected. An important distinction must be made between normal foraging behaviour, which an individual spends much time repeatedly engaged in, and dispersal behaviour, which probably occurs only once in an individuals lifetime. Males may be found to range wider than females during foraging, but dispersal distances were found to be comparable between sexes (Doncaster *et al.*, 2001).

The apparent contradiction between, on the one hand, the radio-tracking results and the analysis of the majority of the molecular data, and, on the other hand, the contrasting AMOVA results from the nuclear and mitochondrial data poses some problems in making firm conclusions with regards to current and historical gender biased dispersal. A possible conclusion drawn from the patterns of genetic differentiation observed is that these populations are currently undergoing changes to their environment that have lead to changes in their dispersal behaviour and population structure. These changes almost certainly are a result of habitat fragmentation as a result of the building of multiple roads, walls, railways etc. At this scale, an approximately 35 km diameter circle, historical

populations may well have had a high level of connectivity, and been essentially panmictic. Current population genetic structure represents a transitional period that may lead to populations that become increasingly inbred as selection and periodic bottlenecks cause alleles to become fixed. Without the remediating effect of unfettered dispersal localised extinctions may become more frequent, and the opportunity for recolonisation more remote.

5.0 General discussion

5.1 *Overview*

This study has examined the effects of habitat fragmentation primarily from a molecular genetics perspective, and has interpreted the results in conjunction with the results of complementary studies by others of individual dispersal behaviour. Throughout this thesis I have employed a spatially hierarchical approach to the analysis of molecular data, generated from populations of *E. europaeus* in the UK and Finland. This chapter will focus on the underlying themes that unite the previous chapters, and will attempt to put the overall results into the context of the existing literature. First I will briefly summarise the main findings of this thesis (section 5.2), before proceeding with the main body of the discussion (section 5.4). I will conclude by outlining the limitations of the studies, and providing suggestions for future work.

5.2 *Summary of results*

In chapter 2, I sought to establish current levels of genetic diversity in hedgehogs from across Britain. The purpose of this analysis was to establish a baseline level of diversity, and provide a context in which to view subsequent analyses performed at a smaller spatial scale (chapters 3 and 4). A comparison of genetic distance, based on nuclear microsatellite data, vs. geographical distances between hedgehog populations, detected an increase in the rate of genetic differentiation between populations separated by increasing geographical distance. Mitochondrial sequence data indicated a lower level of differentiation, and an AMOVA of these data suggested that genetic variation between populations did not increase significantly from a "within county", to a "within the Britain" scale. Phylogenetic analysis of mtDNA types showed several consistent regional variations, but also uncovered several individuals exhibiting mitochondrial haplotypes inconsistent with their location, which could not be readily accounted for.

Chapters 3 and 4 examined the genetic effects of habitat fragmentation and dispersal behaviour at a local scale. The study area for both chapters was located within a 32 km radius of Southampton, Hampshire. In Chapter 3, evidence of an "isolation by distance"

effect was found when comparing genetic differentiation values, based on nuclear microsatellites. No relationship could be found between apparent habitat fragmentation levels and subpopulation relatedness and inbreeding parameters, at very small scale. Examination of individual barriers showed that motorways have a near significant impact on genetic differentiation levels. Further investigation suggested that total road width showed a significant effect more closely correlated to the barrier effect than the presence of a road of a particular class. These results compared well with data collection by others of individual movements of these same hedgehogs.

Spatially hierarchical AMOVA of nuclear microsatellites vs. mitochondrial allele frequencies, in chapter 4, detected higher levels of "between-population" variation in mtDNA than nDNA. This was attributed to a lower level of maternal, than paternal dispersal, and hence gender-biased geneflow. Assignment analysis of individuals, based on nuclear microsatellites, found no significant gender bias in dispersal numbers over the last three generations. The results of the assignment test viewed against that of the AMOVAs suggest that differences in dispersal behaviour have occurred within recent generations.

5.3 *Limitations of the study*

The various studies conducted involved two main areas that could potentially lead to misleading results. The first, experimental limitations, can be broken down into sources of error stemming from sampling; and sources of error derived from assumptions made about methods of molecular evolution and mutation.

These potential shortcomings were identified at the time of designing data collection protocols. However, full resolution of these limitations were not possible for reasons of time, finance or from gaps in current understanding of the systems being studied.

5.3.1 Experimental limitations

5.3.1.1 Sources of error: Sampling

Chapter 2 attempts to present a picture of genetic diversity across the entire UK using a hierarchical approach. A more comprehensive survey could be gained by the inclusion of samples throughout areas not covered in the initial sampling regime.

Whilst its results stand as a 'thumbnail-sketch' of current diversity levels, it would have been desirable to include samples from the west of Scotland, the northeast and northwest of England and also from the west country (Devon, Cornwall, East Anglia, Suffolk etc.).

The inclusion of the Finnish population into the AMOVA may have given a falsely homogeneous impression of the European mainland. The results of both nuclear and mitochondrial DNA analysis showed this population to be highly inbred, thus unlikely to be representative of levels of European genetic diversity in *E. europaeus*. This may have in turn created the impression that increase in genetic diversity effectively peaks at a lower geographical scale than is the case. A broader selection of European samples would have helped to put the genetic diversity within the UK into a wider perspective

However, these factors do not impact on the main conclusion drawn from this chapter, that being that the hedgehog populations within the UK exhibit a low level of genetic diversity. AMOVA results were consistent at all hierarchical levels within the UK, and varied only when international comparisons were specified.

Sampling for chapters 3 and 4 was performed intensively over a period of 4 years. The requirement of population comparisons across nominated barriers, and of control comparisons not separated by barriers, meant that sample number was often traded off against location. A number of thriving populations, from which great numbers of individuals could potentially be sampled, were not in ideal locations for comparisons to other populations. Many of the populations finally chosen for intensive sampling were

found to contain only small numbers of individuals. At any sample site recapture data were recorded between subsequent sampling dates. These data were used to estimate the percentage of population sampled. Whilst all efforts were made to ensure that the samples taken constituted the majority of the individuals present, it is impossible to say if this is the case. It is unlikely that this would affect data generated from large populations, although the effect of a sampling omission on the analysis of a small number of individuals could be significant. Bias introduced in this manner could lead to a random under- or over-estimation of genetic differentiation. Most genetic differentiation estimators will tend to overestimate, rather than underestimate at low sample numbers, dependent on the estimator employed e.g. approximately 80% of errors using R_{ST} will be overestimates; approximately 66%, using F_{ST}; and approximately 55%, using D_C (Ruzzante, 1998).

Attempting to sample within a highly fragmented habitat posed the problem of an excessive number of barriers. This made it very difficult to locate viable populations for comparison as legitimate controls, since populations not separated by any barriers were very hard to find. In cases where multiple barriers passed between populations *a priori* assumptions were made about the isolated effects of individual minor barriers. It was assumed that potential minor barriers, such as streams and minor roads, presented no barrier effect at a local scale. This was largely vindicated at a later stage when partial Mantel tests were performed on genetic distance against multiple barriers once geographical distance had been taken into account (see section 3.3.7, Table 3.5). Any effect produced by a minor barrier such as a B road, minor road, stream etc. is unlikely to be detectable next to the effect generated by the specified barrier, which in each case was a major landscape feature.

5.3.1.2 Sources of error: Molecular markers

All attempts were made to ensure accuracy and reduce any bias that may be present in the molecular data. Chapters 3 and 4, in particular, may have been prone to sample size bias given the necessity to obtain representative allele frequencies for microsatellite analysis.

Ruzzante (1998) showed that bias increased at small sample numbers for a number of genetic estimators e.g. D_C chord distance (Cavalli-Sforza and Edwards, 1967), but not in others e.g. F_{ST} (Wright, 1951), and R_{ST} (Slatkin, 1995). However, he also showed that confidence intervals for all genetic estimators tested grew considerably larger at low sample number. A sample size of 50-100 individuals was suggested for optimum reliability of genetic estimator, dependent on number of loci, number of alleles and range in allele size (Ruzzante, 1998). However, it must be stated that the numbers and range of allele sizes used in this study were much smaller than those in Ruzzante's study, and the number of loci was twice that used by him.

Further justification of the comparatively small sample sizes used in this study relates to the number of individuals present, and the large proportion of the individual populations that I believe were sampled. The sample sizes used in this study are of the same order of magnitude to those employed in similar studies into population fragmentation in mammalian systems and in some cases bigger (Lugon-Moulin *et al.*, 1999).

In order to check whether small sample numbers biased the results of individual analyses, sub-set analysis was performed for each analysis. At each repetition of an analysis populations were gradually excluded, based on their population number, until only populations with an $n > 30$ remained. In no instance did the overall results vary significantly between repetitions. Therefore I would maintain that the results of analyses comparing the genetic population structure, within this thesis, are valid.

5.3.2 Human mediated translocation

In each section of this study it has become apparent that frequent translocations of individuals and groups of hedgehogs have occurred both historically and contemporarily. The effect this has had on population genetic structure is hard to assess. Whilst long distance translocation may generate incongruous results in a study of this type, it is unlikely that local scale or even international scale translocations will have much of an impact on the ecology of *E. europaeus* or its surroundings. The low level of diversity found in hedgehogs throughout Europe by this and other studies (Filippucci and Simson, 1996; Santucci *et al.*, 1998), coupled with the short time period since recolonisation suggest that national and regional variants are unlikely to be highly differentiated. Consequently, regional adaptation would not be thought to be a community-shaping factor at this time.

Despite these limitations, some interesting general conclusions can be drawn about dispersal and habitat fragmentation.

5.4 *Dispersal and habitat fragmentation*

5.4.1 Combining a population genetic and individual behaviour approach

The analysis of the population genetic data in this thesis has been compared to the results of extensive complementary studies of individual hedgehog dispersal behaviour in relation to general landscape features (Doncaster *et al.*, 2001), and to specific barrier features (Rondinini and Doncaster, submitted). The results of this thesis were consistent with these studies with regard to the barrier effect exerted by major roads on hedgehog dispersal, and also on the inverse relationship of road width to successful crossing attempts. This approach has been advocated by a number of recent reviews (Koenig *et al.*, 1996; Thompson and Goodman, 1997). The molecular methods employed in the studies contained within this thesis have been used successfully to study other organisms

and population systems e.g. the AMOVA of mtDNA D-loop haplotypes in chapter 2, in populations of humans (Excoffier *et al.*, 1992), and in the red squirrel (Barratt *et al.*, 1999); partial Mantel analysis of nuclear microsatellites to quantify barrier effects in chapter 3, in the common shrew (Lugon-Moulin *et al.*, 1999), and bank voles (Gerlach and Musolf, 2000); and assignment testing of individuals based on microsatellite distribution frequency probabilities to determine general dispersal, and gender biased dispersal in chapter 4, in Canadian polar bears (Paetkau *et al.*, 1995), in microtine rodents (Favre *et al.*, 1997; Mossman and Waser, 1999).

All of the studies listed above developed or employed one or other of these techniques. However, by combining the results of a number of these techniques I have been able to draw conclusions regarding changes in behaviour over a period of time. For instance, a comparison of mtDNA and nDNA AMOVAs to gender specific assignment testing, as described in sections 4.3.2 and 4.3.3, suggested that while male biased dispersal, typical of mammals (Greenwood, 1980), had been evident in the past, dispersal rates between sexes had recently equalised. I would suggest that this might either be a result of increased perturbation within habitat patches causing females to increase their dispersal rate to male-comparable levels in search of less disturbed habitats, or that habitat fragmentation has increased the chances of male hedgehogs being killed while dispersing, thus reducing the level of successful dispersals to a female-comparable level. As hedgehogs are known to thrive within urban areas, often as a result in part to the absence of badgers (Doncaster, 1992; Doncaster, 1994; Micol *et al.*, 1994; Ward *et al.*, 1996) it seems unlikely that females would continue to leave patches once development has been completed. In fact hedgehogs have been shown to be drawn to the presence of road edges (Doncaster *et al.*, 2001), which often coincide and converge within urban habitats. Another explanation of this reduction in dispersal, which is associated with apparent increased foraging opportunities in urban areas, is that genetic mixing may decrease if population carrying capacities go up leading to more mating opportunities locally, i.e. a reduction in the Allee effect (Allee, 1931) (an inverse density-dependent effect that describes, for example, the increased difficulty associated with finding potential mates at low population densities, (Courchamp *et al.*, 1999)). Be it a result of a change in

behaviour, or simply a reduction the probability of a successful dispersal, it seems more likely that this change reflects the relative lack of male dispersals. Although it was not possible to put a specific date to this change it must have been recent enough so that the differences in mitochondrial and nuclear markers have not been masked by an equality in dispersal rates. However, this change must not have been so recent that gender bias still appeared in the results of the assignment test, its sensitivity being up to 3 generations (Paetkau *et al.*, 1995; Cornuet *et al.*, 1999).

Is it valid, however, to make the assumption that changes in dispersal behaviour/success are a result of habitat fragmentation? Hedgehogs are known to modify their behaviour as a result of their surroundings. During periods of dispersal they tend to gravitate towards, and follow, linear features such as grass verges, road edges etc. (Doncaster *et al.*, 2001; Rondinini and Doncaster, submitted). From this perspective the lack of a significant linear relationship between habitat fragmentation and population relatedness and inbreeding parameters (section 3.3.6) is less surprising. A complex, or fine grained, habitat may provide equal opportunities for dispersal and isolation. Within this type of habitat one might assume that dispersal vectors may be more strictly defined, although given the complexity and small scale at which these features are exerting an influence, prediction of immediate dispersal, and consequent genetic effects may prove problematic.

Many of the putative barrier features investigated in this thesis did not appear to significantly reduce geneflow to the extent that it could be measured using molecular markers. Even the presumptive effects of "ancient" barriers seem to have had little impact on population genetic structure (sections 3.3.8.4 and 3.3.8.5). In a study of bank voles Gerlach and Musolf, (2000) found significant barrier effects produced by an ancient barrier, the Rhine at Lake Constance, and also by major roadways (a 4 lane highway, 50 m in width). In this location, the River Rhine represents a significantly more complete barrier than any of the ancient barriers included in this thesis (i.e. the River Test at Totton, and Southampton Water estuary), which may explain why no similar effect was detected within Southampton. However, the barrier effect produced by total road width was also detected in this study (section 3.3.8.3). This is consistent with the results of

Rondinini and Doncaster, (submitted), who found that although hedgehogs were drawn to road edges they showed a statistical and observed reluctance to move onto the road surface. An associated barrier effect, detailed in section 3.3.8.3, was that genetic differentiation between populations was significantly related to total intervening road width, once geographical distance had been accounted for. This would suggest that while the effects of smaller roads may be small enough to be non-significant cumulative effect may have an overall influence by restricting geneflow. This is also consistent with the findings of Huijser and Bergers, (1998) and Rondinini and Doncaster, (submitted) who found that road width was a factor determining attempted and successful crossings in hedgehogs.

5.4.2 The wider implications of habitat fragmentation

Hedgehogs appear to use dispersal to successfully take advantage of much of the suitable habitat around them. Micol *et al.*, (1994;) and Doncaster *et al.*, (1996) determined that the hedgehogs in their study area used greater than 90% of the suitable habitat available. In terms of populations, hedgehogs seem to be highly mobile and opportunistic, with individuals actively seeking out good quality foraging areas (Doncaster *et al.*, 2001). The lack of significant genetic differentiation around substantial ancient barriers, such as Southampton Water estuary, would suggest that, historically at least, individual and population mobility was of a high enough level that geneflow around the margins of this barrier was enough to maintain genetic homogeneity.

The effects of range-expansion bottlenecking, and the comparatively short period of time since the recolonisation of the UK by the hedgehog has produced a national population with a moderately low level of genetic diversity (section 2.3). Whilst its dispersal capability cannot be compared to that of some of the larger and more far ranging mammals, such as wolves (Boyd and Pletscher, 1999) bears (White *et al.*, 2000) and buffalo (O'Ryan *et al.*, 1998), the hedgehogs capacity for effective local scale dispersal and habitat usage has made it a persistent and robust species. By employing this strategy

of small scale dispersal following linear landscape features the hedgehog has generally managed to reduce the risk of encountering natural dispersal barriers. However, as the grain of the habitat types becomes finer, and the likelihood of encountering a barrier feature increases, what effect will this have on the persistence of hedgehog populations?

The scale at which fragmentation is occurring is likely to make a difference to the level of dispersal. Contained areas with high levels of fragmentation (e.g. the River Test at Totton, see section 3.3.8.4) may reduce dispersal between patches within the fragmentation matrix, although it is unlikely to act as a barrier to geneflow between populations lying on its margins. More extensive areas of fragmentation, such as Southampton city itself, may act as a more effective area of friction slowing down the rate at which geneflow occurs. However, the complexity and fine grain of an urban environment of this type does not seem to impede geneflow to the extent of extensive linear barrier such as the M3 or, presumably, the M25 motorways (sections 3.3.7 and 3.3.8.3). It is possible that the increased number of linear features which may act as wildlife corridors, general absence of badgers within and urban environment, and the presence of ideal foraging areas (e.g. sporting fields, school grounds, parks, private gardens etc.) has gone some way to mitigate against the effects of increased numbers of barriers.

The levels of genetic differentiation between hedgehog populations recorded in these studies have been low to moderately significant. Evidence of recent behavioural change has been detected by molecular methods, which is consistent with observed behaviour Rondinini and Doncaster, (submitted) and environmental change. Some studies have detected significant high levels of genetic differentiation in urbanized wildlife, such as the common frog (Hitchings and Beebee, 1997). The results of the studies in this thesis suggest that the fragmentation, or grain, of an urban habitat is affecting hedgehog dispersal. However, either the low background levels of genetic diversity in UK hedgehogs are making the results of this restriction appear non-significant, or the hedgehogs ability to capitalise on environments like an urban habitat and still maintain

some of its dispersive capability has meant that behavioural changes have not yet significantly affected population genetic structure.

5.5 *Future work*

5.5.1 Genetic diversity within the UK

As discussed in section 5.3.3.1, a possible expansion of the survey of genetic diversity within the UK could be performed with the inclusion of samples from a broader study area. Ideally, samples should be taken from as wide a spread of areas within the UK as possible.

An intensive sampling strategy, of the type employed in chapters 3 and 4, would not be necessary for representative populations of mitochondrial haplotype populations. The low level of overall genetic diversity suggests that only small numbers of individuals would be required from each sample site/population in order to ensure that the majority of haplotypes are represented in subsequent analysis. Sample numbers required for the inclusion of rare haplotypes can be calculated using available methods and software, such as GENESAMP (Sjögren and Wyöni, 1994). However, some knowledge of local population numbers is required for this type of calculation.

A fuller analysis, using the same methods employed in chapter 2, could confirm initial results, with regards to overall diversity levels, the origin of apparently translocated individuals and post-glacial migration routes within the UK itself.

5.5.2 Habitat fragmentation

Attempts to improve barrier isolation and control comparisons within a study area as fragmented as the one employed in chapter 3 are likely to encounter the same difficulties as this study. The nature of highly fragmented urban and suburban environments reduces the likelihood of identifying sites that are, firstly, in close proximity to a suitable barrier

feature; secondly, approximately equidistant to a control population and a 'barrier-separated' population; and thirdly, each of the populations contains between 50-100 individuals.

The European hedgehog is virtually ubiquitous within the UK, so the identification of such a site, or group of sites, is possible. As a species they seem to thrive in populated areas, and are known to inhabit most city suburbs. Given adequate time and resources for preliminary study site survey, a site that allows unequivocal assessment of genetic barrier effects may be identified.

5.5.3 Putative nuclear insertion

Despite attempts to characterise the putative nuclear insert outlined in chapter 2 (Section 2.3.2) no firm conclusions could be drawn. Sequence data generated by the presence of a nuclear insert is potentially misleading if mistaken for mtDNA. Any future work performed on the mitochondrial D-loop of *E. europaeus* must take into consideration the possibility of a similar sequence, amplified using the same primers, which may be evolving at a different rate.

Confirmation of the status of the 'incidental' fragment amplified by PCR, described in section 0, could be confirmed following mitochondrial enrichment of hedgehog tissue. It may be possible to perform this enrichment using the protocol outlined in appendix II, however, this protocol is unlikely to provide a separation of nuclear and mitochondrial DNA clean enough to prevent amplification of the putative nuclear insert.

A more difficult and time consuming procedure may be employed, which could provide a more reliable separation of genomic mtDNA and nDNA. This procedure employs a caesium chloride (CsCl) gradient through which nuclear DNA and intact mitochondria are spun. High speeds and long run times are required to separate the nDNA and

mitochondria, but separation and yields are better than the basic enrichment procedure attempted in this study.

5.6 *Final comment*

Whilst this project has focused primarily on a molecular approach to the population ecology of *E. europaeus,* relevant non-molecular data have been employed whenever possible. The molecular work relating to dispersal has been supplemented by available radio tracking data, which was performed within the same study area (Doncaster *et al.*, 2001; Rondinini and Doncaster, submitted). These two approaches can successfully complement each other e.g. 'whole animal' ecological data providing a realistic context to the more abstract aspects of genetic analysis; or genetic approach confirming or refuting the historical impact of observed contemporary behaviour.

It is clear that the statistical analysis of genetic data, the understanding of the mutational processes underlying genetic evolution and mixing and, to a lesser extent, the molecular techniques used to generate the genetic data still require refining. However, there are a large number of studies currently dealing with many of these aspects, and many groundbreaking refinements are coming to light. Therefore, I would suggest that future evolutionary and particularly ecological studies will benefit greatly from the combination of established non-molecular research techniques with a rapidly advancing knowledge of molecular techniques and their underlying genetic processes.

6.0 Appendices

6.1 *Appendix I: DNA Extraction Protocol*

DNA extraction protocol based on the "salting-out" procedure of Bruford *et al.*, (1998). The protocol has been modified and supplied by S. Piertney (University of Aberdeen).

Hedgehog DNA Extraction Protocol

1. To an Eppendorf tube add:

   300µl 1x TNE (10mM Tris.HCl pH 8.0; 1mM EDTA pH 8; 0.1M NaCl)
   30 µl 1M Tris-HCl (pH 8.0)
   2 µl Proteinase K (tube stock)
   8 µl 25% SDS
   Ear punch sample

2. Mix well

3. Incubate overnight at 55°C, re-mixing the tube every 15 minutes or so, for the first couple of hours.

4. Add 170 µl 5M NaCl and shake hard for 20 seconds.

5. Add 1x volume of chloroform (preferably in fume hood, or well ventilated area), and vortex vigorously for 10 seconds.

6. Overend turn for 30 minutes to 1 hour.

7. Centrifuge at 1700xg for 15 minutes.

8. Pipette off the top aqueous layer to a fresh Eppendorf tube. Try to avoid taking any precipitate from the interface of the two layers.

9. Add 1x volume of ice cold isopropanol. Mix by inversion. DNA should appear as white strands. Don't worry if it doesn't.

10. Centrifuge at high speed to pellet the DNA. Make sure the hinge to the Eppendorf points up.

11. Remove the absolute alcohol, being careful not to dislodge the DNA pellet.

12. Add 1000 µl of ice cold 70% ethanol. Mix by inversion and leave for 30 minutes (preferably on the rotator).

13. Centrifuge at high speed to re-pellet the DNA. Ensure the Eppendorf hinge points down.

14. Remove the 70% ethanol.

15. Allow the pellets to dry by leaving the tube open at RTP for 15 minutes.

16. Re-suspend the DNA in 100 µl of TE buffer (10mM Tris.HCl pH 7.4, 1mM EDTA pH 8.0).

17. Leave the tubes overnight on the bench, then store at 4°C subsequently.

6.2 *Appendix II: Mitochondrial Enrichment Protocol*

Protocol presented verbatim, as provided by Douda Bensasson (University of East Anglia, UK).

Enriching for mitochondrial DNA

This protocol was adapted from (Lansman *et al.*, 1981) by DeXing Zhang. It has been shown that this technique is capable of increasing the proportion of mitochondrial DNA extracted, relative to nuclear, to such an extent that nuclear DNA will not be amplified by subsequent PCRs (Zhang and Hewitt, 1996). For this procedure to succeed there needs to be plenty of undamaged mitochondria (membranes intact) within the sample tissue being used. For this reason, enriching for mitochondria was only possible for samples that were stored fresh at -80°C.

Cell and tissue debris are removed; intact mitochondria are pelleted (by spinning above 15 000g), washed, then resuspended in a solution that should be relatively free of nuclear DNA. The solution is then treated with 0.2% Nonidet P40 (Sigma). This will break down mitochondrial membranes but not nuclear membranes; any intact nuclei or any debris that has been contaminating the mitochondria by also pelleting above 15 000g will be removed by spinning again at 15000g; the mtDNA will remain free in solution.

Care was taken to keep samples free of foreign DNA and negative controls were set up to check that this had been successful (see PCR contamination prevention).

Protocol

The tissue was kept as cold as possible (all work was done on ice) at least until the mitochondrial membranes were dissolved. This was to minimise the activity of any enzymes that may degrade the mitochondrial membranes.

A whole grasshopper was cut in half using a new disposable razor blade, each half was put into a pre-chilled 2 ml microfuge tube and kept on dry ice (the tissue was split in this way to facilitate grinding). Each tube was held in liquid N_2 for about a minute. The

tissue was ground into a fine powder using a clean pre-chilled glass rod. It is critical that the tissue is ground into a fine powder and that this powder is not allowed to dry out. In this step lumps of tissue need to be ground up enough for cells to be released and broken so that their contents (including intact mitochondria) are freed into solution. Homogenising the tissue in this way was the most difficult step in the protocol when done manually. An electric microhomogeniser was never used in this project but it is recommended.

From this stage until the mitochondrial membranes were deliberately broken down, samples were handled with great care (in order to keep mitochondrial membranes intact) i.e. no vortexing; pipetting (except gently with cut tips).

As soon as the liquid N_2 had evaporated 0.75 ml of ice cold sucrose buffer was added (15% sucrose, 5 mM Tris-HCl, 10 mM EDTA, pH 7.6). The samples were gently swirled to suspend the tissue and the two grasshopper halves were pooled.

Total genomic DNA was extracted from a 100µl aliquot of this suspension (see previous section). This is the unenriched sample necessary for later comparisons to the enriched DNA.

The rest of the suspension was spun at 2 000g for 10 minutes at 4°C to remove unbroken tissue or cells, cell debris and nuclei. The supernatant was spun until no pellet was visible and then one more time. The smallest bit of debris carrying over to the DNA extraction can yield surprisingly large amounts of total genomic DNA.

The supernatant was then spun at 20 000g for 40 minutes; to pellet the mitochondria. A pale yellow - orange pellet was visible. This is the colour that you would expect mitochondria to be. Cytochromes, incorporated into mitochondrial membranes (in different proportions in different organisms) contain haem groups (which contain iron) giving characteristic colours from pale yellow through to dark brown. However it could be that the observed colour is caused by something else (a dye incorporated into some other substance perhaps) that pellets at the same speed as mitochondria.

The pellet was washed twice in 1.5 mls of ice cold buffer. Increasing the number of washes at this stage will minimise carry-over of nuclear DNA but will also decrease yields of mtDNA.

The mitochondrial pellet was then lysed in 0.2% of Nonidet P40 in ice cold sucrose buffer. Any remaining debris or nuclei were pelleted by spinning at 20 000g for 10 minutes again. The supernatant was then incubated at 40°C in 50µl/ml of Proteinase K and cleaned using the Wizard™ DNA Clean-Up System (Promega) as described in the previous section. Aliquots of the unenriched and enriched DNA were run out on an agarose gel to check yields and possible carryover of total genomic DNA.

Comments

It was shown that the technique was successful in decreasing the relative amounts of nuclear DNA to levels at which at least some of it will no longer amplify (see Chapter 4). However yields of mtDNA were very low; so low that when 20% of all the enriched DNA obtained was run on an agarose gel no bands were visible. As it was never visualised it was difficult to know if the enrichment had worked or not.

Several attempts were made to obtain enriched mtDNA and most of these were unsuccessful. Frequently, total genomic DNA would be carried over to such an extent that it would be visible on an agarose gel (DNA of higher molecular weight than the mitochondrial genome was obtained). It is therefore likely that isolation of mtDNA has not been complete. However to purify the mtDNA further by CsCl / ethidium bromide gradient ultracentrifugation (as described by Lansman et al. 1981) would require a much greater yield from this protocol. If the DNA obtained from it cannot even be seen on an agarose gel it is unlikely to be visible on the gradient.

Yields may have been increased through the use of an electric homogeniser, or possibly by phenol-chloroform extraction instead of extraction using the Promega Clean-Up System. Carry-over of nuclear DNA could have been reduced further by using larger centrifuge tubes allowing washes to be made using larger volumes of buffer and thereby reducing carry over of genomic DNA.

6.3 *Appendix III: Raw Data*

6.3.1 Nuclear Microsatellite Allele Frequencies

Pops.	Allele Sizes										
EEU1	131	133	135	137	139	141	143	145	147	149	151
BLA	-	-	42.86	-	-	-	-	7.14	-	35.71	14.29
BSI	-	-	44.64	17.86	1.79	1.79	-	16.07	-	17.86	-
BUR	-	-	36.36	-	-	-	-	31.82	-	22.73	9.09
COL	-	-	46.88	6.25	-	-	18.75	-	-	25.00	3.13
DIT	-	-	56.82	2.27	-	-	2.27	18.18	-	20.45	-
ECL	-	-	57.14	2.86	2.86	2.86	1.43	1.43	-	31.43	-
ENE	-	-	38.89	11.11	-	-	11.11	11.11	-	27.78	-
ENW	-	-	61.54	-	-	-	11.54	7.69	-	19.23	-
ESE	-	-	46.15	15.38	-	-	7.69	1.92	-	21.15	7.69
ESH	-	-	42.86	-	-	-	21.43	7.14	-	28.57	-
ESW	-	-	48.65	5.41	-	-	5.41	-	-	40.54	-
FAW	-	-	60.00	-	-	-	5.00	10.00	-	20.00	5.00
FIN	-	100.00	-	-	-	-	-	-	-	-	-
GRA	-	-	100.00	-	-	-	-	-	-	-	-
HAM	-	-	30.00	-	-	-	-	20.00	10.00	40.00	-
HED	-	-	16.67	-	-	-	16.67	33.33	-	33.33	-
HOL	-	-	63.16	2.63	-	-	-	7.89	-	21.05	5.26
HYT	-	-	25.00	-	-	6.25	6.25	18.75	-	43.75	-
IBM	-	-	44.12	5.88	-	-	2.94	29.41	-	14.71	2.94
KIR	-	-	50.00	7.69	-	-	-	15.38	-	26.92	-
MAR	-	-	31.25	31.25	-	-	-	6.25	12.50	18.75	-
MOT	-	-	42.31	3.85	-	-	15.38	11.54	11.54	11.54	3.85
MWD	-	-	23.08	3.85	-	-	7.69	38.46	7.69	19.23	-
NET	-	-	50.00	-	-	-	-	38.89	-	11.11	-
RBR	-	-	15.00	-	-	-	15.00	22.50	-	47.50	-
ROM	1.56	-	51.56	1.56	-	-	-	17.19	-	21.88	6.25
ROU	-	-	64.29	17.86	-	-	-	12.50	3.57	1.79	-
ROW	-	-	64.29	-	-	-	-	-	7.14	28.57	-
TON	-	-	56.25	-	-	-	4.17	8.33	-	31.25	-
TOS	-	-	11.11	-	-	-	16.67	38.89	-	33.33	-
WOO	-	-	50.00	-	-	-	-	-	-	50.00	-

Pops.	Allele Sizes								
EEU2	258	260	262	264	266	268	270	272	274
BLA	-	7.14	7.14	-	14.29	14.29	28.57	28.57	-
BSI	-	16.67	-	2.78	-	-	50.00	25.00	5.56
BUR	-	22.22	-	5.56	27.78	-	22.22	11.11	11.11
COL	-	15.63	3.13	-	46.88	-	21.88	12.50	-
DIT	-	34.78	-	2.17	21.74	2.17	26.09	13.04	-
ECL	-	19.35	-	3.23	37.10	-	19.35	20.97	-
ENE	-	16.67	-	-	38.89	-	5.56	38.89	-
ENW	-	50.00	-	3.85	11.54	-	11.54	23.08	-
ESE	-	22.73	-	6.82	31.82	-	6.82	31.82	-
ESH	-	57.14	-	-	7.14	-	14.29	21.43	-
ESW	2.70	21.62	-	13.51	18.92	-	17.57	25.68	-
FAW	-	45.00	10.00	5.00	-	10.00	15.00	15.00	-
FIN	-	-	-	-	-	-	85.71	14.29	-
GRA	-	100.00	-	-	-	-	-	-	-
HAM	10.00	40.00	-	20.00	20.00	10.00	-	-	-
HED	-	16.67	-	-	33.33	-	33.33	16.67	-
HOL	-	18.42	10.53	-	7.89	-	52.63	10.53	-
HYT	-	35.71	-	-	14.29	-	50.00	-	-
IBM	-	27.27	-	-	9.09	-	-	63.64	-
KIR	-	43.14	-	-	25.49	-	11.76	19.61	-
MAR	-	43.75	-	-	37.50	-	6.25	12.50	-
MOT	-	50.00	-	-	11.11	-	5.56	16.67	16.67
MWD	-	50.00	7.14	-	-	-	32.14	10.71	-
NET	6.25	37.50	12.50	-	6.25	-	37.50	-	-
RBR	-	57.89	-	2.63	13.16	-	21.05	5.26	-
ROM	-	22.58	1.61	1.61	24.19	-	41.94	6.45	1.61
ROU	-	51.79	-	-	17.86	-	17.86	10.71	1.79
ROW	-	50.00	-	12.50	12.50	-	-	25.00	-
TON	-	37.50	-	-	10.42	-	43.75	8.33	-
TOS	-	6.25	-	-	6.25	-	37.50	50.00	-
WOO	-	61.11	-	-	33.33	-	5.56	-	-

Pops. **Allele Sizes**

EEU3	155	159	161	163	165	167	169	171	173	175
BLA	-	7.14	-	-	21.43	-	7.14	64.29	-	-
BSI	-	9.38	-	-	26.56	-	6.25	43.75	3.13	10.94
BUR	-	13.64	-	-	13.64	-	13.64	54.55	-	4.55
COL	3.13	-	-	-	28.13	6.25	21.88	25.00	-	15.63
DIT	-	19.05	-	-	21.43	-	-	35.71	2.38	21.43
ECL	-	2.78	-	-	22.22	9.72	12.50	37.50	11.11	4.17
ENE	-	-	-	-	61.11	-	-	22.22	-	16.67
ENW	-	3.85	-	-	7.69	3.85	26.92	53.85	-	3.85
ESE	-	9.62	-	-	40.38	3.85	9.62	28.85	-	7.69
ESH	-	-	-	-	43.75	-	12.50	31.25	-	12.50
ESW	-	12.82	-	-	30.77	7.69	3.85	39.74	1.28	3.85
FAW	-	-	-	-	30.00	-	10.00	60.00	-	-
FIN	100.00	-	-	-	-	-	-	-	-	-
GRA	-	41.67	-	-	-	-	-	58.33	-	-
HAM	-	-	-	-	10.00	-	20.00	40.00	-	30.00
HED	-	-	-	-	-	-	16.67	50.00	-	33.33
HOL	-	10.00	-	-	20.00	2.50	10.00	55.00	2.50	-
HYT	-	-	-	-	31.25	-	6.25	50.00	-	12.50
IBM	-	-	-	-	32.35	17.65	11.76	32.35	-	5.88
KIR	-	3.85	-	-	59.62	-	-	15.38	-	21.15
MAR	-	6.25	-	-	25.00	-	-	37.50	6.25	25.00
MOT	-	11.11	-	-	52.78	5.56	5.56	25.00	-	-
MWD	-	7.14	-	-	25.00	-	21.43	39.29	7.14	-
NET	-	5.56	-	5.56	33.33	-	5.56	33.33	5.56	11.11
RBR	-	-	-	-	25.00	-	37.50	32.50	-	5.00
ROM	-	14.29	1.43	1.43	38.57	2.86	7.14	27.14	-	7.14
ROU	-	21.43	-	-	41.07	-	-	33.93	-	3.57
ROW	-	27.78	-	-	-	-	27.78	22.22	-	22.22
TON	-	10.42	-	-	45.83	6.25	4.17	31.25	-	2.08
TOS	-	-	-	-	50.00	33.33	5.56	11.11	-	-
WOO	-	10.00	-	-	20.00	-	15.00	45.00	-	10.00

Pops.	Allele Sizes										
EEU4	149	151	153	155	157	159	161	163	165	167	169
BLA	-	-	-	-	7.14	14.29	35.71	-	-	42.86	-
BSI	-	-	-	-	-	-	28.79	-	3.03	50.00	18.18
BUR	-	-	5.00	-	20.00	-	50.00	5.00	10.00	10.00	-
COL	3.13	-	-	-	37.50	12.50	31.25	-	6.25	9.38	-
DIT	-	-	6.52	-	2.17	6.52	67.39	-	10.87	6.52	-
ECL	-	-	-	2.86	10.00	10.00	41.43	-	11.43	21.43	2.86
ENE	-	-	6.25	-	-	18.75	43.75	-	-	31.25	-
ENW	-	-	-	-	9.09	22.73	22.73	-	9.09	36.36	-
ESE	-	-	2.00	-	28.00	6.00	18.00	-	2.00	42.00	2.00
ESH	-	-	-	-	12.50	37.50	43.75	-	-	6.25	-
ESW	-	-	6.58	-	15.79	2.63	17.11	-	18.42	39.47	-
FAW	-	-	15.00	-	10.00	5.00	50.00	-	-	20.00	-
FIN	-	70.00	-	-	30.00	-	-	-	-	-	-
GRA	-	-	-	-	-	-	100.00	-	-	-	-
HAM	-	-	-	-	-	10.00	40.00	-	-	40.00	10.00
HED	-	-	16.67	-	-	16.67	-	16.67	16.67	33.33	-
HOL	-	-	5.00	-	20.00	5.00	55.00	5.00	2.50	7.50	-
HYT	-	-	31.25	-	12.50	12.50	12.50	-	-	31.25	-
IBM	-	-	-	-	2.94	8.82	38.24	-	23.53	26.47	-
KIR	-	-	8.33	-	12.50	10.42	35.42	2.08	2.08	29.17	-
MAR	-	-	43.75	-	-	-	37.50	6.25	6.25	6.25	-
MOT	-	-	-	-	27.78	-	19.44	-	13.89	36.11	2.78
MWD	-	-	19.23	-	3.85	-	19.23	-	-	57.69	-
NET	-	5.56	-	-	38.89	11.11	22.22	-	-	22.22	-
RBR	-	-	2.63	-	2.63	21.05	28.95	-	13.16	18.42	13.16
ROM	-	2.86	4.29	1.43	5.71	14.29	15.71	5.71	1.43	44.29	4.29
ROU	-	-	26.79	-	1.79	23.21	23.21	1.79	17.86	5.36	-
ROW	-	-	6.25	-	-	12.50	75.00	-	-	6.25	-
TON	-	-	10.87	-	26.09	19.57	26.09	2.17	-	8.70	6.52
TOS	-	-	27.78	-	5.56	-	11.11	-	-	55.56	-
WOO	-	-	5.00	-	45.00	5.00	15.00	-	10.00	20.00	-

Pops.	Allele Sizes									
EEU5	116	118	120	122	126	128	130	132	134	136
BLA	-	28.57	-	-	-	57.14	7.14	-	7.14	-
BSI	8.62	58.62	-	12.07	-	20.69	-	-	-	-
BUR	-	10.00	-	-	-	25.00	35.00	-	30.00	-
COL	-	37.50	-	-	-	21.88	6.25	18.75	15.63	-
DIT	-	22.73	-	4.55	-	40.91	4.55	-	27.27	-
ECL	-	9.72	-	-	-	23.61	37.50	19.44	9.72	-
ENE	-	27.78	-	-	-	-	38.89	22.22	11.11	-
ENW	-	15.00	-	-	-	15.00	25.00	20.00	25.00	-
ESE	-	35.42	-	-	-	18.75	20.83	12.50	12.50	-
ESH	-	18.75	-	-	-	62.50	6.25	-	12.50	-
ESW	-	20.51	-	-	-	32.05	23.08	12.82	11.54	-
FAW	-	10.00	5.00	-	-	20.00	30.00	5.00	25.00	5.00
FIN	-	100.00	-	-	-	-	-	-	-	-
GRA	-	-	-	-	-	66.67	-	-	33.33	-
HAM	-	-	-	-	-	20.00	30.00	10.00	30.00	10.00
HED	-	16.67	-	-	-	33.33	33.33	16.67	-	-
HOL	-	31.58	-	-	-	26.32	5.26	5.26	31.58	-
HYT	-	31.25	-	-	-	6.25	37.50	-	25.00	-
IBM	-	29.41	-	-	-	2.94	26.47	35.29	5.88	-
KIR	-	9.62	-	-	-	30.77	34.62	-	25.00	-
MAR	-	31.25	-	-	-	18.75	25.00	6.25	18.75	-
MOT	-	22.22	-	-	5.56	13.89	25.00	16.67	16.67	-
MWD	-	53.85	-	-	-	11.54	7.69	7.69	19.23	-
NET	-	38.89	-	-	-	11.11	27.78	5.56	16.67	-
RBR	-	57.89	-	-	-	7.89	15.79	18.42	-	-
ROM	-	21.21	-	-	-	21.21	31.82	10.61	15.15	-
ROU	-	24.07	-	-	-	25.93	12.96	-	37.04	-
ROW	-	18.75	-	-	-	37.50	18.75	-	25.00	-
TON	-	38.10	-	-	-	7.14	42.86	9.52	2.38	-
TOS	-	50.00	-	-	-	-	50.00	-	-	-
WOO	-	25.00	-	-	-	10.00	25.00	15.00	25.00	-

Pops.	Allele Sizes								
EEU6	146	154	156	158	160	162	164	166	168
BLA	-	-	-	-	28.57	7.14	64.29	-	-
BSI	-	-	-	15.91	22.73	61.36	-	-	-
BUR	-	10.00	-	5.00	70.00	15.00	-	-	-
COL	-	3.13	-	9.38	34.38	25.00	28.13	-	-
DIT	-	-	2.50	27.50	27.50	40.00	2.50	-	-
ECL	-	-	1.39	13.89	55.56	25.00	4.17	-	-
ENE	-	33.33	-	27.78	5.56	22.22	11.11	-	-
ENW	-	11.54	-	26.92	42.31	7.69	11.54	-	-
ESE	-	6.00	2.00	28.00	26.00	16.00	20.00	-	2.00
ESH	-	-	-	7.14	42.86	35.71	14.29	-	-
ESW	-	-	-	16.67	47.44	16.67	19.23	-	-
FAW	-	-	-	35.00	50.00	10.00	5.00	-	-
FIN	100.00	-	-	-	-	-	-	-	-
GRA	-	-	-	20.00	10.00	70.00	-	-	-
HAM	-	20.00	-	10.00	60.00	10.00	-	-	-
HED	-	16.67	-	-	33.33	16.67	33.33	-	-
HOL	-	-	-	17.50	27.50	17.50	35.00	2.50	-
HYT	-	-	-	18.75	56.25	18.75	6.25	-	-
IBM	-	5.88	-	2.94	67.65	8.82	8.82	5.88	-
KIR	-	11.54	15.38	15.38	23.08	30.77	3.85	-	-
MAR	-	-	-	50.00	18.75	25.00	-	6.25	-
MOT	-	-	-	16.67	19.44	25.00	38.89	-	-
MWD	-	-	-	15.38	73.08	11.54	-	-	-
NET	-	-	-	27.78	33.33	33.33	5.56	-	-
RBR	-	-	-	35.00	5.00	25.00	35.00	-	-
ROM	-	1.43	-	12.86	50.00	31.43	4.29	-	-
ROU	-	18.18	18.18	12.73	14.55	5.45	30.91	-	-
ROW	-	-	-	-	27.78	55.56	16.67	-	-
TON	-	-	4.17	25.00	47.92	18.75	4.17	-	-
TOS	-	5.56	-	-	66.67	27.78	-	-	-
WOO	-	-	-	5.00	70.00	5.00	20.00	-	-

Pops.	Allele Sizes					
EEU36H	144	146	148	150	152	154
BLA	-	-	-	92.86	7.14	-
BSI	-	-	-	93.94	6.06	-
BUR	-	4.55	-	90.91	4.55	-
COL	-	-	-	100.00	-	-
DIT	-	-	-	93.33	-	6.67
ECL	-	1.47	-	86.76	5.88	5.88
ENE	-	5.56	-	94.44	-	-
ENW	-	-	3.85	88.46	3.85	3.85
ESE	-	-	-	92.31	3.85	3.85
ESH	-	-	-	100.00	-	-
ESW	1.28	-	-	93.59	2.56	2.56
FAW	-	-	-	95.00	5.00	-
FIN	-	12.50	-	87.50	-	-
GRA	-	-	-	-	-	-
HAM	-	10.00	-	90.00	-	-
HED	-	-	-	83.33	-	16.67
HOL	-	-	-	80.00	20.00	-
HYT	-	-	-	100.00	-	-
IBM	-	-	-	100.00	-	-
KIR	-	11.36	-	88.64	-	-
MAR	-	8.33	-	83.33	-	8.33
MOT	-	-	-	88.89	11.11	-
MWD	-	-	-	89.29	7.14	3.57
NET	-	-	-	94.44	5.56	-
RBR	-	-	-	94.74	5.26	-
ROM	-	4.41	-	85.29	10.29	-
ROU	-	1.85	-	98.15	-	-
ROW	-	-	-	94.44	5.56	-
TON	-	2.17	-	97.83	-	-
TOS	-	-	-	100.00	-	-
WOO	-	5.00	-	95.00	-	-

Pops.	Allele Sizes						
EEU37H	250	258	260	262	264	266	268
BLA	-	-	-	-	83.33	16.67	-
BSI	-	-	-	-	98.48	1.52	-
BUR	-	9.09	-	-	90.91	-	-
COL	-	23.33	-	-	70.00	6.67	-
DIT	-	3.85	-	-	88.46	7.69	-
ECL	-	33.33	8.33	-	56.94	1.39	-
ENE	-	11.11	-	-	88.89	-	-
ENW	-	15.38	7.69	-	76.92	-	-
ESE	-	1.92	5.77	1.92	88.46	1.92	-
ESH	-	6.25	-	18.75	56.25	18.75	-
ESW	-	28.21	6.41	3.85	61.54	-	-
FAW	-	10.00	10.00	-	80.00	-	-
FIN	66.67	-	-	-	33.33	-	-
GRA	-	-	-	-	-	-	-
HAM	-	20.00	-	-	80.00	-	-
HED	-	-	-	-	83.33	16.67	-
HOL	-	7.50	17.50	-	72.50	2.50	-
HYT	-	-	18.75	-	75.00	6.25	-
IBM	-	-	8.82	2.94	85.29	2.94	-
KIR	-	-	-	-	89.47	-	10.53
MAR	-	-	30.00	-	50.00	-	20.00
MOT	-	5.56	5.56	-	80.56	5.56	2.78
MWD	-	-	17.86	3.57	78.57	-	-
NET	-	5.56	16.67	-	72.22	5.56	-
RBR	-	-	2.50	5.00	90.00	2.50	-
ROM	-	13.24	11.76	4.41	60.29	10.29	-
ROU	-	-	5.77	-	94.23	-	-
ROW	-	11.11	-	-	88.89	-	-
TON	-	2.08	10.42	-	79.17	6.25	2.08
TOS	-	-	38.89	-	61.11	-	-
WOO	-	5.00	15.00	-	80.00	-	-

Pops.	Allele Sizes									
EEU54H	278	280	282	284	286	288	290	292	294	298
BLA	-	-	-	42.86	50.00	-	-	7.14	-	-
BSI	1.52	-	3.03	25.76	18.18	22.73	3.03	25.76	-	-
BUR	-	-	-	9.09	81.82	-	4.55	4.55	-	-
COL	-	-	-	3.13	31.25	12.50	-	53.13	-	-
DIT	-	-	-	3.33	70.00	3.33	-	23.33	-	-
ECL	-	-	-	7.14	65.71	1.43	5.71	20.00	-	-
ENE	-	-	-	5.56	27.78	5.56	-	61.11	-	-
ENW	-	-	9.09	-	50.00	18.18	-	22.73	-	-
ESE	-	-	-	13.46	40.38	15.38	-	30.77	-	-
ESH	-	-	-	-	50.00	31.25	-	18.75	-	-
ESW	-	-	5.13	7.69	35.90	3.85	-	47.44	-	-
FAW	-	-	5.00	10.00	50.00	5.00	-	30.00	-	-
FIN	-	-	-	25.00	25.00	-	25.00	-	-	25.00
GRA	-	-	-	-	-	-	-	-	-	-
HAM	-	-	-	25.00	62.50	-	-	12.50	-	-
HED	-	-	-	-	83.33	-	-	16.67	-	-
HOL	-	-	10.00	22.50	20.00	-	-	40.00	7.50	-
HYT	-	6.25	-	12.50	31.25	12.50	-	37.50	-	-
IBM	-	-	-	8.82	55.88	5.88	-	29.41	-	-
KIR	-	-	-	2.27	65.91	2.27	-	27.27	2.27	-
MAR	-	-	8.33	-	50.00	-	-	41.67	-	-
MOT	-	-	2.78	2.78	13.89	25.00	5.56	50.00	-	-
MWD	-	-	21.43	7.14	21.43	21.43	-	21.43	7.14	-
NET	-	-	-	5.56	61.11	-	-	33.33	-	-
RBR	-	-	-	23.53	38.24	5.88	-	26.47	5.88	-
ROM	-	-	-	5.71	41.43	15.71	4.29	32.86	-	-
ROU	-	-	1.92	11.54	65.38	-	17.31	1.92	1.92	-
ROW	-	-	-	16.67	50.00	11.11	-	22.22	-	-
TON	-	-	14.58	8.33	54.17	2.08	2.08	18.75	-	-
TOS	-	-	-	22.22	38.89	11.11	-	22.22	5.56	-
WOO	-	-	-	-	75.00	-	-	25.00	-	-

Pops.	Allele Sizes						
EEU12H	92	94	96	98	100	102	104
BLA	-	57.14	14.29	-	14.29	14.29	-
BSI	-	68.18	1.52	-	15.15	13.64	1.52
BUR	-	22.73	4.55	-	68.18	4.55	-
COL	-	59.38	3.13	-	37.50	-	-
DIT	3.33	46.67	6.67	3.33	40.00	-	-
ECL	-	47.22	2.78	-	36.11	13.89	-
ENE	5.56	16.67	5.56	5.56	61.11	5.56	-
ENW	-	38.46	-	-	50.00	11.54	-
ESE	-	40.38	-	1.92	36.54	21.15	-
ESH	-	75.00	-	12.50	12.50	-	-
ESW	2.70	54.05	-	-	36.49	6.76	-
FAW	-	50.00	-	-	40.00	10.00	-
FIN	30.00	40.00	-	-	20.00	10.00	-
GRA	-	-	-	-	-	-	-
HAM	-	30.00	-	-	60.00	10.00	-
HED	-	16.67	-	33.33	50.00	-	-
HOL	-	60.00	-	5.00	22.50	12.50	-
HYT	-	31.25	-	6.25	56.25	6.25	-
IBM	-	70.59	2.94	-	20.59	5.88	-
KIR	2.27	20.45	2.27	-	65.91	9.09	-
MAR	16.67	33.33	-	-	33.33	16.67	-
MOT	2.78	27.78	-	-	63.89	5.56	-
MWD	-	53.57	10.71	-	35.71	-	-
NET	-	16.67	-	-	50.00	33.33	-
RBR	-	47.50	-	-	42.50	10.00	-
ROM	-	37.14	-	-	48.57	14.29	-
ROU	1.85	44.44	3.70	3.70	44.44	1.85	-
ROW	-	55.56	-	-	38.89	5.56	-
TON	-	32.61	-	4.35	58.70	4.35	-
TOS	-	38.89	-	-	55.56	5.56	-
WOO	-	5.00	5.00	5.00	70.00	15.00	-

Pops.	Allele Sizes								
EEU43H	155	157	159	161	163	165	167	169	171
BLA	-	-	7.14	50.00	21.43	-	-	21.43	-
BSI	-	-	1.52	68.18	7.58	4.55	-	18.18	-
BUR	4.55	-	4.55	18.18	-	-	-	63.64	9.09
COL	12.50	-	-	6.25	-	3.13	28.13	50.00	-
DIT	-	-	13.33	23.33	30.00	-	6.67	26.67	-
ECL	5.56	-	19.44	31.94	-	19.44	16.67	5.56	1.39
ENE	22.22	-	-	16.67	-	22.22	11.11	27.78	-
ENW	3.85	-	15.38	57.69	3.85	11.54	7.69	-	-
ESE	9.62	-	7.69	21.15	1.92	17.31	23.08	19.23	-
ESH	6.25	-	6.25	25.00	-	18.75	31.25	12.50	-
ESW	1.28	-	20.51	26.92	-	17.95	25.64	7.69	-
FAW	20.00	-	-	55.00	-	-	-	10.00	15.00
FIN	-	-	50.00	50.00	-	-	-	-	-
GRA	-	-	-	-	-	-	-	-	-
HAM	-	-	-	50.00	-	-	10.00	40.00	-
HED	-	-	-	50.00	-	-	-	50.00	-
HOL	10.00	5.00	-	30.00	30.00	12.50	5.00	5.00	2.50
HYT	-	-	-	68.75	6.25	-	-	25.00	-
IBM	8.82	2.94	5.88	52.94	5.88	-	11.76	11.76	-
KIR	-	-	9.09	59.09	4.55	6.82	-	20.45	-
MAR	-	-	16.67	41.67	8.33	8.33	-	25.00	-
MOT	8.33	-	13.89	33.33	-	2.78	22.22	19.44	-
MWD	14.29	-	-	25.00	7.14	-	28.57	25.00	-
NET	-	5.56	16.67	11.11	-	-	16.67	50.00	-
RBR	-	-	5.00	27.50	-	-	60.00	7.50	-
ROM	1.43	-	-	45.71	2.86	12.86	18.57	18.57	-
ROU	-	-	3.70	70.37	3.70	5.56	3.70	12.96	-
ROW	5.56	-	27.78	11.11	-	16.67	22.22	16.67	-
TON	4.17	-	-	58.33	2.08	8.33	4.17	22.92	-
TOS	-	-	-	94.44	-	-	-	5.56	-
WOO	-	5.00	10.00	20.00	5.00	30.00	-	30.00	-

6.3.2 Mitochondrial Sequence Data

6.3.2.1 Non VNTR region

Corresponding to positions 17436-17331 of published sequence (GenBank accession number X88898, Krettek *et al.*, 1995)

Haplotype 1 (Southampton, Brownsea Island, Oxford, Shetland, Finland)
AATAAGTTTAATAATTATAAGGAATATAAATAAAATAGATCTAGTTTAATTAT
TCAGATCTTATATGACGATATAAAATAAA---TGTAAAATTATTAATATGGATA
AAATTTTAATTAAAACTTTATAGATAAATTTTATATGGTTAACTTTTTATAAAT
AAAGTAAGAAAGCAAAATTTAATTTTTAATTATATGGTAAATTATTTGTAATT
GCTA

Haplotype 2 (Oxford)
AATAAGTTTAATAATTATAAGGAATATAAATAAAATAGATCTAGTTTAATTAT
TCAGATCTTATATGACGATATAAAATAAA---TGTAAAATTATTAATATGGATA
AAATTTTAATTAAAACTTTATAGATAAATTTTATATGGTTAACTTTTTATAAAT
AAAGTAAGAAAGCAAAATTTAATCTTTAATTATATGGTAAATTATTTGTAATT
GCTA

Haplotype 3 (Oxford)
AATAAGTTTAATAATTATAAGGAATATAAATAAAATAGATCTAGTTTAATTAT
TTAGATCCTATCTTACGATATAAAATAAATATTGTAAAATCATTAATATAGAT
-AAATTTTAATTAAAATTTTATAGATAAATTTTATATAGTTAACTTTTTATAAA
CAAAGTAAGAAAGCAAAACTTAATTTTTAATTATATGATAGATTATTTGTAAT
TACTA

Haplotype 4 (Oxford)
AATAAGTTTAATAATTATAAGGAATATAAATAAAATAGATCTAGTTTAATTAT
TTAGATCCTATCTTACGATATAAAATAAATATTGTAAAATCATTAATATAGAT
-AAATTTTAATTAAAATTTTATAGATAAATTTTATATAGTTAACTTTTTATAAA
CAAAGTAAGAAAGCAAAATTTAATTTTTAATTATATGATAGATTATTTGTAAT
TACTA

Haplotype 5 (Southampton)
AATAAGTTTAATAATTATAAGGAATATAAATAAAATAGATCTAGTTTAATTAT
TCAGATCTTATATGACGATATAAAATAAA---TGTAAAATTATTAATATGGATA
AAATTTTAATTAAAACTTTATAGATAGATTTTATATGGTTAACTTTTTATAAAT
AAAGTAAGAAAGCAAAATTTAATTTTTAATTATATGGTAAATTATTTGTAATT
GCTA

Haplotype 6 (Scotland, Southampton)
AATAAGTTTAATAATTATAAGGAATATAAATAAAATAGATCTAGTTTAATTAT
TCAGATCTTATATGACGATATAAAATAAA---TGTAAAGTTATTAATATGGATA
AAATTTTAATTAAAACTTTATAGATAAATTTTATATGGTTAACTTTTTATAAAT
AAAGTAAGAAAGCAAAATTTAATTTTTAATTATATGGTAAATTATTTGTAATT
GCTA

6.3.2.2 *VNTR region*

Corresponding to positions 17342-17004 of published sequence (GenBank accession number X88898, Krettek *et al.*, 1995). The repeat region is underlined, and the 8 base repeat unit is shown in **bold type**. Alleles varied from 10 to 41 repeat units.

TTTGTAATTGCT**ATATTATT**ATATTATTATATTATTATATTATTATATTATTAT
ATTATTATATTATTATATTATTATATTATTATATTATTATATTATTATATTATTA
TATTATTATATTATTATATTATTATATTATTATATTATTATATTATTATATTATT
ATATTATTATATTATTATATTATTATATTATTATATTATTATATTATTATATTAT
TATATTATTATATTATTATATTATTATATTATTATATTATTATATTATTATATTA
TTATATTATTATATTATTATATTATTATATTATTATATTATTATATTATTATATT
ATTATATTATTATTATTATTGTCGTTAAA

7.0 References

7.1 References

Allee, W.C. (1931). *Animal Aggregations. A Study In General Sociobiology.* University of Chicago Press, Chicago.

Amos, W., Sawcwe, S.J., Feakes, R.W. and Rubinsztein, D.C. (1996). Microsatellites show mutational bias and heterozygote instability. *Nature Genetics,* **13**: 390-391.

Anderson, L.W., Born, E.W., Gjertz, I., Wiig, Ø., Holm, L.-E. and Bendixon, C. (1998). Population structure and gene flow of the Atlantic walrus (*Odobenus rosmarus rosmarus*) in the eastern Atlantic Arctic based on mitochondrial DNA and microsatellite variation. *Molecular Ecology,* 7: 1323-1336.

Andreassen, H.P. and Ims, R.A. (1998). The effects of experimental habitat destruction and patch isolation on space use and fitness parameters in female root vole *Microtus oeconomus*. *Journal of Animal Ecology,* **67**(6): 941-952.

Ashley, C.T. and Warren, S.T. (1995). Trinucleotide repeat expansion and human disease. *Annual Review of Genetics,* **29**: 703-728.

Ashley, M.V. and Dow, B.D. (1994). The use of microsatellite analysis in population biology: Background, methods and potential applications. In: *Molecular Ecology and Evolution, Approaches and Applications*. B. Schierwater, Streit, B., Wagner, G. P., DeSalle, R. (ed.). Birkhauser Verlag Base, Switzerland, 185-201 pp.

Avise, J.C. (1994). *Molecular Markers, Natural History and Evolution.* Chapman & Hall, London.

Baker, C.S., Slade, R.W., Bannister, J.L., Abernathy, R.B., Weinrich, M.T., Lien, J., Urban, J., Corkeron, P., Calmabokidis, J., V., O. and Palumbi, S.R. (1994). Hierarchical structure of mitochondrial DNA gene flow among humpback whales *Megaptera novaeangliae*, world-wide. *Molecular Ecology,* **3**: 313-327.

Baker, R.R. (1978). *The Evolutionary Ecology of Animal Migration.* Hodder & Stoughton, London, 1012 pp.

Balloux, F., Brunner, H., Lugon-Moulin, N., Hausser, J. and Goudet, J. (2000). Microsatellites can be misleading: An empirical and simulation study. *Evolution,* **54**(4): 1414-1422.

Barratt, E.M., Gurnell, J., Malarky, G., Deaville, R. and Bruford, M.W. (1999). Genetic structure of fragmented populations of red squirrel (*Sciurus vulgaris*) in the UK. *Molecular Ecology,* **8**: S55-S63.

Barrowclough, G. (1978). Sampling bias in dispersal studies based on finite area. *Bird Banding,* **49**: 333-341.

Barton, N.H. (1992). The genetic consequences of dispersal. In: *Animal Dispersal: Small Mammals as a Model.* N. C. Stenseth, Lidicker, W. Z. (ed.). Chapman & Hall, London. p.37-63.

Barton, N.H. and Slatkin, M. (1986). A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity,* **56**: 409-415.

Bateson, P. (1983). Optimal outbreeding. In: *Mate Choice.* P. Bateson (ed.). Canbridge University Press, Cambridge. p.257-277.

Becher, S.A. (1994). Population genetics of the European hedgehog (*Erinaceus europaeus*).thesis, University of Oxford.

Becher, S.A. and Griffiths, R. (1997). Isolation and characterization of six polymorphic microsatellite loci in the European hedgehog *Erinaceus europaeus*. *Molecular Ecology*, **6**(1): 89-90.

Becher, S.A. and Griffiths, R. (1998). Genetic differentiation among local populations of European hedgehog (*Erinaceus europaeus*) in mosaic habitats. *Molecular Ecology*, 7: 1599-1604.

Beier, P. (1995). Dispersal of juvenile cougars in fragmented habitat. *Journal of Wildlife Management*, **59**: 228-237.

Beier, P. and Noss, R.F. (1998). Do habitat corridors provide connectivity? *Conservation Biology*, **12**: 1241-1252.

Bengtsson, B.O. (1978). Avoid inbreeding: at what cost? *Journal of Theoretical Biology*, **73**: 439-440.

Bernatchez, L. and Osinov, A. (1995). Genetic diversity of trout (genus *Salmo*) from its most eastern native range based on mitochondrial DNA and nuclear gene variation. *Molecular Ecology*, **4**: 285-297.

Berthoud, G. (1978). Note préliminaire sur les déplacements du hérisson européen (*Erinaceus europaeus* L.). *Terre Vie*, **32**: 73-82.

Boitani, L. and Reggiani, G. (1984). Movements and activity patterns of hedgehogs *Erinaceus europaeus* in Mediterranean coastal habitats. *Z.Saugetierk*, **49**: 193-206.

Bonnell, M.L. and Selander, R.K. (1974). Elephant seals: genetic variation and near extinction. *Science*, **184**: 908-909.

Bossart, J.L. and Prowell, D.P. (1998). Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Trends in Ecology and Evolution*, **13**(5): 202-206.

Bowcock, A.M., Ruiz-Linares, A., Tonfohrde, J., Minch, E., Kidd, J.R. and Cavalli-Sforza, L.L. (1994). High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, **368**(6470): 455-457.

Boyd, D.K. and Pletscher, D.H. (1999). Characeristics of dispersal in a colonizing wolf population in the central Rocky Mountains. *Journal of Wildlife Management*, **63**(4): 1094-1108.

Brehm, A. and Ekman, S. (1938). *Djurens liv. Däggdjur l*, Stockholm, 640 pp.

Brookes, M.I., Graneau, Y.A., King, P., Rose, O.C., Thomas, C.D. and Mallet, J.L.B. (1997). Genetic analysis of founder bottlenecks in the rare British butterfly *Plebejus argus*. *Conservation Biology*, **11**(3): 648-661.

Brookfield, J.F.Y. (1996). A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology*, **5**(3): 453-455.

Brown Gladden, J.G., Ferguson, M.M. and Clayton, J.W. (1997). Matriarchal genetic population structure of North American beluga whales *Delphinapterus leucas* (Cteacea: Monodontidae). *Molecular Ecology*, **6**: 1033-1046.

Brown, W.M. (1983). Evolution of animal mitochondrial DNA. In: *Evolution of Genes and Proteins*. M. Nei, Koehn, R. (ed.). Sinauer Press, Sunderland. p.62-88.

Brown, W.M., George, M. and Wilson, A.C. (1979). Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Science of the USA*, **76**: 1967-1971.

Brown, W.M., Prager, E.M., Wang, A. and Wilson, A.C. (1982). Mitochondrial DNA sequences of primates: Tempo and mode of evolution. *Journal of Molecular Evolution*, **18**: 225-239.

Bruford, M.W., Hanotte, O., Brookfield, J.F.Y. and Burke, T. (1998). Multilocus and single-locus DNA fingerprinting. In: *Molecular Genetic Analysis of Populations: A Practical Approach*. A. R. Hoelzel (ed.). Oxford University Press, Oxford. p.287-336.

Bruford, M.W. and Wayne, R.K. (1993). Microsatellites and their application to population genetic studies. *Current Opinion in Genetics and Development*, **3**: 939-943.

Caro, T. and Laurenson, K. (1994). Ecological and genetic factors in conservation: a cautionary tale. *Science*, **263**: 485-486.

Cavalli-Sforza, L.L. and Edwards, A.W.F. (1967). Phylogenetic analysis: models and estimation procedures. *American journal of Human Genetics*, **19**: 233-257.

Cesaroni, D., Venanzetti, F., Allegrucci, G. and Sbordoni, V. (1997). Mitochondrial length variation and heteroplasmy in natural populations of the European sea bass, *Dicentrarchus labrax*. *Molecular Biology and Evolution*, **14**: 560-568.

Clarke, A.L., Saether, B.E. and Roskaft, E. (1997). Sex biases in avian dispersal: a reappraisal. *Oikos*, **79**(3): 429-438.

Clutton-Brock, T.H. (1989). Female transfer and inbreeding avoidance in social mammals. *Nature*, **337**: 70-72.

Cockburn, A. (1992). Habitat heterogeneity and dispersal: environmental and genetic patchiness. In: *Animal Dispersal.* N. C. Stenseth, Lidicker, W. Z. (ed.). Chapman & Hall, London. **1st**. p.65-95.

Cockerham, C.C. and Weir, B.S. (1993). Estimation of gene flow from *F*-statistics. *Evolution,* **47**: 855-863.

Collura, R. and Stewart, C. (1995). Insertions and duplications of mtDNA in the nuclear genomes of Old World monkeys and hominoids. *Nature,* **378**: 485-489.

Corbet, G.B. (1988). The family Erinaceidae: a synthesis of its taxonomy, phylogeny, ecology and zoogeography. *Mammal Review,* **18(3)**: 117-172.

Cornuet, J.M. and Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics,* **144**: 2001-2014.

Cornuet, J.M., Piry, S., Luikart, G., Estoup, A. and Solignac, M. (1999). New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics,* **153**: 1989-2000.

Courchamp, F., Clutton-Brock, T. and Grenfell, B. (1999). Inverse density dependence and the Allee effect. *Trends in Ecology and Evolution,* **14**: 405-410.

Crick, F.H.C. (1966). Codon-anticodon pairing: the wobble hypothesis. *Journal of Molecular Biology,* **19**: 548-555.

Crnokrak, P. and Roff, D.A. (1999). Inbreeding depression in the wild. *Heredity,* **83(3)**: 260-270.

Cunningham, M. and Moritz, C. (1998). Genetic effects of forest fragmentation on a rainforest restricted lizard (Scincidae: *Gnypetoscinus queenslandiae*). *Biological Conservation*, **83**(1): 19-30.

Dallas, J.F. (1992). Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. *Mammalian Genome*, **3**(8): 452-456.

Dallas, J.F., Bacon, P.J., Carss, D.N., Conroy, J.W.H., Green, R., Jefferies, D.J., Kruuk, H., Marshall, F., Piertney, S.B. and Racey, P.A. (1999). Genetic diversity in the Eurasian otter, *Lutra lutra*, in Scotland. Evidence from microsatellite polymorphism. *Biological Journal of the Linnean Society*, **68**(1-2): 73-86.

Dallas, J.F., Dod, B., Boursot, P., Prager, E.M. and Bonhomme, F. (1995). Population subdivision and gene flow in danish house mice. *Molecular Ecology*, **4**(3): 311-320.

Desrochers, A. and Hannon, S.J. (1997). Gap crossing decisions by forest songbirds during the post-fledging period. *Conservation Biology*, **11**: 1204-1210.

Di Rienzo, A., Peterson, A.C. and Garza, J.C. (1994). Mutational processes of simple sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences, USA*, **91**: 3166-3170.

Dobson, F.S. (1982). Competition for mates and predominant juvenile male dispersal in mammals. *Animal Behaviour*, **30**: 1183-1192.

Dobson, M. (1998). Mammal distributions in the western Mediterranean: the role of human intervention. *Mammal Review*, **28**(2): 77-88.

Doncaster, C.P. (1992). Testing the role of intraguild predation in regulating hedgehog populations. *Proceedings of the Royal Society of London*, **249**: 113-117.

Doncaster, C.P. (1993). Influence of predation threat on the foraging pattern: The hedgehog's gambit. *Review of Ecology*, **48**: 207-213.

Doncaster, C.P. (1994). Factors regulating local variations in abundance: field tests on hedgehogs, *Erinaceus europaeus*. *Oikos*, **69**: 182-192.

Doncaster, C.P., Micol, T. and Plesner Jensen, S. (1996). Determining minimum habitat requirements in theory and practice. *Oikos*, **75**(2): 335-339.

Doncaster, C.P., Rondinini, C. and Johnson, P.D. (2001). Field tests for environmental correlates of dispersal in hedgehogs. *Journal of Animal Ecology*, **70**: 33-46.

Ehrlich, P.R. (1994). Enhancing the status of population biology. *Trends in Ecology and Evolution*, **9**(4): 157-157.

Ekman, S. (1922). *Djurvärldens utbredningshistoria på den Skandinaviska havlön*, Stockholm, 614 pp.

Endler, J.A. (1977). *Geographic Variation, Speciation and Clines.* Princeton University Press, Princeton, New Jersey, 295 pp.

Engel, S.R., Linn, R.A., Taylor, J.F. and Davis, S.K. (1996). Conservation of microsatellite loci across species of artiodactyls: implications for population studies. *Journal of Mammology*, 77(2): 504-518.

Estoup, A., Garnery, L., Solignac, M. and Cornuet, J.M. (1995). Microsatellite variation in honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the Infinite Allele and Stepwise Mutation Models. *Genetics*, **140**: 679-695.

Evans, A.E. and Buckley, T.E. (1899). *A vertebrate fauna of the Shetland Islands.* Oliver and Boyd, Edinburgh.

Excoffier, L., Smouse, P.E. and Quattro, J.M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. *Genetics,* **131**(479-491).

Eyles, N. (1993). Earth's glacial record and its tectonic setting. *Earth-Science Reviews,* **35**: 1-248.

Favre, L., Balloux, F., Goudet, J. and Perrin, N. (1997). Female-biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns. *Proceedings of the Royal Society of London B,* **264**: 127-132.

Felsenstein, J. (1995). PHYLIP (Phylogeny Inference Package). Seattle, University of Washington.

Ferris, C., Oliver, R.P., Davy, A.J. and Hewitt, G.M. (1995). Using chloroplast DNA to trace postglacial migration routes of oaks into Britain. *Molecular Ecology,* **4**: 731-738.

Filippucci, M.G. and Simson, S. (1996). Allozyme variation and divergence in Erinaceidae (Mammalia, Insectivora). *Israel Journal of Zoology,* **42**: 335-345.

Forman, R.T.T. and Alexander, L.E. (1998). Roads and their major ecological effects. *Annual Review of Ecology and Systematics,* **29**: 207-231.

Fuller, S.J., Wilson, J.C. and Mather, P.B. (1997). Patterns of differentiation among wild rabbit populations *Oryctolagus cuniculus* L. in arid and semiarid ecosystems of north-eastern Australia. *Molecular Ecology,* **6**: 145-153.

Gaggiotti, O.E., Lange, O., Rassmann, K. and Gliddon, C. (1999). A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. *Molecular Ecology*, **8**: 1513-1520.

Gaines, M.S. and McClenaghan, L.R. (1980). Dispersal in small mammals. *Annual Review of Ecology and Systematics*, **11**: 163-196.

Gandon, S. (1999). Kin competition, the cost of inbreeding and the evolution of dispersal. *Journal of Theoretical Biology*, **200**(4): 345-364.

Geisler, M. and Gropp, A. (1967). Chromosome polymorphism in the European hedgehog *Erinaceus europaeus* (Insectivora). *Nature*, **214**: 396-397.

Gerlach, G. and Musolf, K. (2000). Fragmentation of landscape as a cause for genetic subdivision of bank vole populations. *Conservation Biology*, **14**(4): 1066-1074.

Gibbs, H.L., Prior, K.A., Weatherhead, P.J. and Johnson, G. (1997). Genetic structure of populations of the threatened eastern massasauga rattlesnake, *Sistrurus c. catenatus*: evidence from microsatellite DNA markers. *Molecular Ecology*, **6**: 1123-1132.

Gilbert, D.A., Lehman, N., O'Brien, S.J. and Wayne, R.K. (1990). Genetic fingerprinting reflects population differentiation in the California Channel Island fox. *Nature*, **344**: 764-767.

Goldberg, T.L. (1997). Inferring the geographic origins of "refugee" chimpanzees in Uganda from mitochondrial DNA sequences. *Conservation Biology*, **11**(6): 1441-1446.

Goldstein, D.B. and Clark, A.G. (1995). Microsatellite variation in North-American populations of *Drosphila melanogaster*. *Nucleic Acids Research*, **23**: 3882-3886.

Goldstein, D.B., Ruiz Linares, A., Cavalli-Sforza, L.L. and Feldman, M.W. (1995). Genetic absolute dating based on microsatellites and the origin of modern humans. *Proceedings of the National Academy of Science of the USA*, **92**: 6723-6727.

Gompper, M.E., Gittleman, J.L. and Wayne, R.K. (1998). Dispersal, philopatry, and genetic relatedness in a social carnivore: comparing males and females. *Molecular Ecology*, **7**: 157-163.

Goodman, S.J. (1997). RST CALC: A collection of computer programs for calculating unbiased estimates of genetic differentiation and determining their significance for microsatellite data. *Molecular Ecology*, **6**: 881-885.

Goudet, J. (1995). FSTAT (version 1.2), a computer program to calculate F statistics. *Journal of Heredity*, **86**: 485-486.

Goudet, J., Raymond, M., de Meeüs, T. and Rousset, F. (1996). Testing differentiation in diploid populations. *Genetics*, **144**: 1933-1940.

Greenwood, A.D. and Pääbo, S. (1999). Nuclear insertion sequences of mitochondrial DNA predominate in hair but not in blood of elephants. *Molecular Ecology*, **8**: 133-137.

Greenwood, P.J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**: 1140-1162.

Greenwood, P.J. (1983). Mating systems and the evolutionary consequences of dispersal. In: *The Ecology of Animal Movement*. I. R. Swingland and P. J. Greenwood (eds.). Clarendon Press, Oxford. p.116-131.

Greenwood, P.J. and Harvey, P.H. (1982). The natal and breeding dispersal of birds. *Annual Review of Ecology and Systematics*, **13**: 1-21.

Guo, S.W. and Thompson, E.A. (1992). Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics*, **48**: 361-372.

Hadrys, H., Balick, M. and Schierwater, B. (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecology*, **1**: 55-63.

Hanski, I. (1994). Patch-occupancy dynamics in fragmented landscapes. *Trends in Ecology & Evolution*, **9**: 131-135.

Hanski, I. (1999). *Metapopulation Ecology*. Oxford University Press, Oxford.

Hanski, I. and Gilpin, M.E., eds. (1997). Metapopulation Biology: Ecology, Genetics, and Evolution. San Diego, Academic Press.

Hanski, I., Pöyry, J., Pakkala, T. and Kuussaari, M. (1995). Multiple equilibria in metapopulation dynamics. *Nature*, **377**: 618-621.

Harvie-Brown, J.A. (1892). *A vertebrate fauna of Argyll and the Inner Hebrides*. D. Douglas, Edinburgh.

Hedrick, P.W. and Kalinowski, S.T. (2000). Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics*, **31**: 139-162.

Henderson, M., Becher, S.A., Doncaster, C.P. and Maclean, N. (2000). Five new polymorphic loci in the European hedgehog *Erinaceus europaeus*. *Molecular Ecology*, **9**(11): 1949-1950.

Hewitt, G.M. (1989). The subdivision of species by hybrid zones. In: *Speciation and its Consequences*. D. Otte and J. Endler (eds.). Sinauer Associates, Sunderland, MA. p.85-110.

Hewitt, G.M. (1993). Postglacial distribution and species substructure: lessons from pollen, insects and hybrid zones. In: *Evolutionary Patterns and Processes*. D. R. Leeds and D. Edwards (eds.). Academic Press, London. p.97-123.

Hewitt, G.M. (1996). Some genetic consequences of ice ages. *Biological Journal of the Linnean Society*, **58**: 247-276.

Hitchings, S.P. and Beebee, T.J.C. (1997). Genetic substructuring as a result of barriers to geneflow in urban *Rana temporaria* (common frog) populations: implications for biodiversity conservation. *Heredity*, **79**: 117-127.

Hoeh, W.R., Blakley, K.H. and Brown, W.E. (1991). Heteroplasmy suggests limited biparental inheritance of *Mytilus* mitochondrial DNA. *Science*, **251**: 1488-1489.

Hoelzel, A.R. (1992). Conservation genetics of whales and dolphins. *Molecular Ecology*, **1**: 119-125.

Hoelzel, A.R., ed. (1998). Molecular Genetic Analysis of Populations: A Practical Approach. The Practical Approach Series. Oxford, Oxford University Press.

Hoelzel, R.A., Lopez, J.V., Dover, G. and O'Brien, S.J. (1994). Rapid evolution of a heteroplasmic repetitive sequence in the mitochondrial DNA control region of carnivores. *Journal of Molecular Evolution*, **39**: 191-199.

Hoffmann, R.J., Boore, J.L. and Brown, W.M. (1992). A novel mitochondrial genome organization for the Blue Mussel, *Mytilus edulis*. *Genetics*, **131**: 397-412.

Holisova, V. and Obrtel, R. (1986). Vertebrate casualties on a Moravian road. *Acta Sc Nat Brno,* **20**(9): 1-44.

Howard, W.E. (1960). Innate and environmental dispersal of individual vertebrates. *American Midland Naturalist,* **63**(152-161).

Hughes, C.R. and Queller, D.C. (1993). Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Molecular Ecology,* **2**: 131-137.

Huijser, M.P. and Bergers, P.J.M. (1998). Platte egels tellen: resultaten van een VZZ actie. *Zoogdier,* **9**(2): 20-25.

Huijser, M.P. and Bergers, P.J.M. (2000). The effects of roads and traffic on hedgehog (*Erinaceus europaeus*) populations. *Biological Conservation,* **95**: 111-116.

Ibrahim, K.M., Nichols, R.A. and Hewitt, G.M. (1996). Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity,* 77: 282-291.

Ims, R.A. and Yoccoz, N.G. (1997). Studying transfer processes in metapopulations: emigration, migration, and colonization. In: *Metapopulation Biology: Ecology, Genetics and Evolution*. I. A. Hanski and M. E. Gilpin (eds.). Academic Press, London. p.247-265.

Ishibashi, Y., Saitoh, T., Abe, S. and Yoshida, M.C. (1997). Sex-related spatial kin structure in a spring population of grey-sided voles *Clethrionomys rufocanus* as revealed by mitochondrial and microsatellite DNA analyses. *Molecular Ecology,* **6**: 63-71.

Jarne, P. and Lagoda, J.L. (1996). Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution,* **11**: 424-429.

Jimenez, J.A., Hughes, K.A., Alaks, G., Graham, L. and Lacy, R.C. (1994). An experimental study of inbreeding depression in a natural habitat. *Science,* **266**: 271-273.

Johnson, P.C.D. (1996). Genetic structure of Oxfordshire hedgehog populations: an analysis using microsatellites. BSc (Hons) thesis, University of Aberdeen, 37 pp.

Johst, K. and Brandl, R. (1999). Natal versus breeding dispersal: evolution in a model system. *Evolutionary Ecology Research,* **1**(8): 911-921.

Joyce, K.A., Holland, J.M. and Doncaster, C.P. (1999). Influences of hedgerow intersections and gaps on the movement of carabid beetles. *Bulletin of Entomological Research,* **89**: 523-531.

Julliard, R. (2000). Sex-specific dispersal in spatially varying environments leads to habitat-dependent evolutionary stable offspring sex ratios. *Behavioural Ecology,* **11**(4): 421-428.

Kaneda, H., Hayashi, J.I., Takahama, S., Taya, C., Lindahl, K.F. and Yonekawa, H. (1995). Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis. *Proceedings of the National Academy of Science of the USA,* **92**: 4542-4546.

Karl, S.A., Bowen, B.W. and Avise, J.C. (1992). Global population genetic structure and male-mediated geneflow in the green turtle (*Chelonia mydas*): RFLP analyses of anonymous nuclear loci. *Genetics,* **131**: 163-173.

Keane, B., Creel, S.R. and Waser, P.M. (1996). No evidence of inbreeding avoidance or inbreeding depression in a social carnivore. *Behavioural Ecology*, 7: 480-489.

Keane, B., Dittus, W.P.J. and Melnick, D.J. (1997). Paternity assessment in wild groups of toque macaques *Macaca sinica* at Polonnaruwa, Sri Lanka using molecular markers. *Molecular Ecology*, **6**: 267-282.

Keller, L.F., Arcese, P., Smith, J.N.M., Hochachka, W.M. and Stearns, S.C. (1994). Selection against inbred song sparrows during a natural population bottleneck. *Nature*, **372**: 356-357.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X. and Wilson, A.C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Science of the USA*, **86**: 6196-6200.

Koenig, W.D., Van Vuren, D. and Hooge, P.N. (1996). Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends in Ecology and Evolution*, **11**(12): 514-517.

Krettek, A., Gullberg, A. and Arnason, U. (1995). Sequence analysis of the complete mitochondrial DNA molecule of the hedgehog, *Erinaceus europaeus*, and the phylogenetic position of the lipotyphla. *Journal of Molecular Evolution*, **4**: 952-957.

Kristiansson, H. (1981). Distribution of the European hedgehog (*Erinaceus europaeus* L.) in Sweden and Finland. *Ann. Zool. Fennici*, **18**: 115-119.

Kristiansson, H. (1984). Ecology of a hedgehog (*Erinaceus europaeus*) populations in southern Sweden. PhD thesis, University of Lund, 75 pp.

Kristoffersson, R., Soivio, A. and Suomalainen, P. (1966). The distribution of the hedgehog (*Erinaceus europaeus* L.) in Finland in 1964-1965. *Ann. Acad. Scient. Fennicae,* **(A IV) 102**: 1-12.

Kristoffersson, R., Soivio, A. and Suomalainen, P. (1977). The distribution of the hedgehog (*Erinaceus europaeus* L.) in Finland in 1975. *Ann. Acad. Scient. Fennicae,* **(A IV) 209**: 1-6.

Kunkel, T.A. (1993). Slippery DNA and diseases. *Nature,* **365**: 207-208.

Lagercrantz, U., Ellegren, H. and Andersson, L. (1993). The abundance of various polymorphic microsatellite motifs differ between plants and vertebrates. *Nucleic Acids Research,* **21**: 1111-1115.

Lansman, R.A., Shade, R.O., Shapira, J.F. and Avise, J.C. (1981). The use of restriction endonucleases to measure mitochondrial-DNA sequence relatedness in natural-populations .3. Techniques and potential applications. *Journal Of Molecular Evolution,* **17**(4): 214-226.

Lawton, J.H. and Woodroffe, G.L. (1991). Habitat and the distribution of water voles - why are there gaps in a species' range? *Journal of Animal Ecology,* **60**: 79-91.

Levins, R. (1970). Extinction. In: *Some Mathematical Questions in Biology.* J. D. Cowan (ed.). **2**. p.75-107.

Levinson, G. and Gutman, G.A. (1987). High frequency of short frameshifts in poly-CA/GT tandem repeats borne by bacteriophage M13 in *Escherichia coli* K-12. *Nucleic Acids Research,* **15**: 5323-5338.

Lidicker, W.Z. (1975). The role of dispersal in the demography of small mammals. In: *Small Mammals: productivity and dynamics of populations*. K. Petrusewicz, F. B. Golley and L. Ryszkowski (eds.). Cambridge University Press, Cambridge. p.103-128.

Litt, M. and Luty, J.A. (1989). A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics*, **44**: 397-401.

Lugon-Moulin, N., Brünner, H., Balloux, F., Hausser, J. and Goudet, J. (1999). Do riverine barriers, history or introgression shape the genetic structuring of a common shrew (*Sorex araneus*) population? *Heredity*, **83**: 155-161.

Luikart, G. and England, P.R. (1999). Statistical analysis of microsatellite DNA data. *Trends in Ecology & Evolution*, **14**(7): 253-256.

Lunt, D.H., Whipple, L.E. and Hyman, B.C. (1998). Mitochondrial DNA variable number tandem repeats (VNTRs): utility and problems in molecular ecology. *Molecular Ecology*, **7**: 1441-1455.

Lynch, M. and Milligan, B.G. (1994). Analysis of population genetic structure with RAPD markers. *Molecular Ecology*, **3**: 91-99.

Mader, H.J. (1984). Animal habitat isolation by roads and agricultural fields. *Biological Conservation*, **29**: 81-96.

Mader, H.J., Schell, C. and Kornacker, P. (1990). Linear barriers to arthropod movements in the landscape. *Biological Conservation*, **35**: 209-222.

Mandal, N. (1978). Variation in C-stained chromosome regions in European hedgehogs (Insectivora, Mammalia). *Hereditas*, **89**: 107-128.

Manly, B.F.J. (1986). Randomization and regression methods for testing associations with geographical, environmental and biological distances between populations. *Research in Population Ecology,* **28**: 201-218.

Manly, B.F.J. (1991). *Randomization, and Monte Carlo methods in biology.* Chapman & Hall, London, 388 pp.

Mann, C.C. and Plummer, M.L. (1995). Are wildlife corridors the right path? *Science,* **270**: 1428-1430.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research,* **27**: 209-220.

Melnick, D.J. and Hoelzer, G.A. (1992). Differences in male and female macaque dispersal lead to contrasting distributions of nuclear and mitochondrial DNA variation. *International Journal of Primatology,* **13**(4): 379-393.

McShea, W.J. and Madison, D.M. (1992). Alternative approaches to the study of small mammal dispersal: insights from radiotelemetry. In: *Animal Dispersal: Small Mammals as a Model.* N. C. Stenseth and W. Z. J. Lidicker (eds.). Chapman & Hall, London. pp.319-332.

Micol, T., Doncaster, C.P. and Mackinlay, L.A. (1994). Correlates of local variation in the abundance of hedgehogs *Erinaceus europaeus*. *Journal of Animal Ecology,* **63**: 851-860.

Minch, E. (1997). MICROSAT, Version 1.5b. Stanford, Stanford University Medical Centre.

Moncrief, N.D., Cockett, N.E., Neff, A.D., Thomas, W.L. and Dueser, R.D. (1997). Polymorphic microsatellites in the meadow vole *Microtus pennsylvanicus*: conservation of loci across species of rodents. *Molecular Ecology*, **6**: 299-301.

Moritz, C. (1994). Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology*, **3**: 401-411.

Moritz, C., Dowling, T.E. and Brown, W.M. (1987). Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual review of Ecology and Systematics*, **18**: 269-292.

Moritz, C., Heideman, A., Geffen, E. and McRae, P. (1997). Genetic population structure of the Greater Bilby *Macrotis lagotis*, a marsupial in decline. *Molecular Ecology*, **6**: 925-936.

Morris, P.A. (1991). Hedgehogs *Erinaceus europaeus*. In: *The Handbook of British Mammals*. G. B. Corbet and H. Harris (eds.). Blackwell Scientific, Oxford.

Mossman, C.A. and Waser, P.M. (1999). Genetic detection of sex-biased dispersal. *Molecular Ecology*, **8**: 1063-1067.

Motro, U. (1982). Optimal rates of dispersal II. Diploid populations. *Theoretical Population Biology*, **21**: 412-429.

Mulder, J. (1996). Waarom lopen egels op de weg? *Zoogdier*, 7(3): 20-24.

Mulder, J.L. (1999). The behaviour of hedgehogs on roads. *Lutra*, **42**(35).

Nachman, M.W., Boyer, S.N. and Searle, J.B. (1994). Mitochondrial DNA variation and the evolution of Robertsonian chromosomal races of house mice, *Mus domesticus*. *Genetics*, **136**: 1105-1120.

Notini, G. and Haglund, B. (1948). *Svenska däggdur. Däggduren*, Stockholm, 651 pp.

O'Connell, M., Danzmann, R.G., Cornuet, J.M., Wright, J.M. and Ferguson, M.M. (1997). Differentiation of rainbow trout (*Oncorhynchus mykiss*) populations in Lake Ontario and the evaluation of the stepwise mutation and infinite allele mutation models using microsatellite variability. *Canadian Journal of Fisheries and Aquatic Science*, **54**: 1391-1399.

O'Corry-Crowe, G.M., Suydam, R.S., Rosenberg, A., Frost, K.J. and Dizon, A.E. (1997). Phylogeography, population structure and dispersal patterns of the beluga whale *Delpinapterus leucas* in the western Nearctic revealed by mitochondrial DNA. *Molecular Ecology*, **6**: 955-970.

Ohta, T. and Kimura, M. (1973). A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetical Research*, **22**: 201-204.

O'Ryan, C., Harley, E.H., Bruford, M.W., Beaumont, M., Wayne, R.K. and Cherry, M.I. (1998). Microsatellite analysis of genetic diversity in fragmented South African buffalo populations. *Animal Conservation*, **1**: 85-94.

Packer, C., Gilbert, D.A., Pusey, A.E. and O'Brien, S.J. (1991). A molecular genetic analysis of kinship and cooperation in African lions. *Nature*, **351**: 562-565.

Paetkau, D., Calvert, W., Stirling, I. and Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**: 347-354.

Paetkau, D., Waits, L.P., Clarkson, P.L., Craighead, L. and Strobeck, C. (1997). An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. *Genetics*, **147**: 1943-1957.

Peltonen, A. and Hanski, I. (1991). Patterns of island occupancy explained by colonization and extinction rates in shrews. *Ecology,* **72**(5): 1698-1708.

Pemberton, J.M., Slate, J., Bancroft, D.R. and Barrett, J.A. (1995). Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology,* **4**: 249-252.

Pepin, L., Amigues, Y., Lepingle, A., Berthier, J.L., Bensaid, A. and Vaiman, D. (1995). Sequence conservation of microsatellites between *Bos taurus* (cattle), *Capra hircus* (goat) and related species. Examples of use in parentage testing and phylogeny analysis. *Heredity,* **74**: 53-61.

Perrin, N. and Mazalov, V. (1999). Dispersal and inbreeding avoidance. *American naturalist,* **154**(3): 282-292.

Petri, B., Paabo, S., Von Haeseler, A. and Tautz, D. (1997). Paternity assessment and population subdivision in a natural population of the larger mouse-eared bat *Myotis myotis*. *Molecular Ecology,* **6**: 235-242.

Piel, W.H. and Nutt, K.J. (2000). One species or several? Discordant patterns of geographic variation between allozymes and mtDNA sequences among spiders in the genus *Metepeira* (Araneae : Araneidae). *Molecular Phylogenetics and Evolution,* **15**(3): 414-418.

Piertney, S.B., MacColl, A.D.C., Bacon, P.J. and Dallas, J.F. (1998). Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Molecular Ecology,* 7: 1645-1654.

Piertney, S.B., MacColl, A.D.C., Lambin, X., Moss, R. and Dallas, J.F. (1999). Spatial distribution of genetic relatedness in moorland populations of red grouse (*Lagopus lagopus scoticus*). *Biological Journal of the Linnean Society,* **68**: 317-331.

Poduschka, W. (1971). Was kann zur Erhaltung des Igels getan werden? *Natur und Landschaft,* **46**(8): 218-221.

Pray, L.A., Schwartz, J.M., Goodnight, C.J. and Stevens, L. (1994). Environmental dependency of inbreeding depression- implications for conservation biology. *Conservation Biology,* **8**(2): 562-568.

Primmer, C.R., Ellengren, H., Saino, N. and Møller, A.P. (1996). Directional evolution in germline microsatellite mutations. *Nature Genetics,* **13**: 391-393.

Primmer, C.R., Møller, A.P. and Ellengren, H. (1995). Resolving genetic relationships with microsatellite markers: a parentage testing system for the swallow *Hirundo rustica*. *Molecular Ecology,* **4**: 493-498.

Pulliam, H.R. (1996). Sources and sinks: empirical evidence and population consequences. In: *Population Dynamics in Ecological Space and Time*. O. E. Rhodes, R. K. Chesser and M. H. Smith (eds.). University of Chicago Press, Chicago. p.45-69.

Purvis, A.I. (1997). A phylogeographic study of three deer species using polymorphic microsatellites. MSc thesis, University of Leicester.

Pusey, A.E. (1980). Inbreeding avoidance in chimpanzees. *Animal Behaviour,* **28**: 543-552.

Queller, D.C. and Goodnight, K.F. (1989). Estimating relatedness using genetic markers. *Evolution*, **43**(2): 258-275.

Queller, D.C., Strassmann, J.E. and Hughes, C.R. (1993). Microsatellites and kinship. *Trends in Ecology and Evolution*, **8**(8): 285-288.

Rand, D.M. and Harrison, R.G. (1986). Mitochondrial DNA transmission genetic in crickets. *Genetics*, **114**: 955-970.

Rannala, B. and Mountain, J.L. (1997). Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Science of the USA*, **94**: 9197-9201.

Raymond, M. and Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**: 248-249.

Real, L.A., ed. (1994). Ecological Genetics. Princeton N.J., Princeton University Press.

Reeve, H.K., Westneat, D.F., Noon, W.A., Sherman, P.W. and Aquadro, C.F. (1990). DNA fingerprinting reveals high levels of inbreeding in colonies of the eusocial naked mole rat. *Proceedings of the National Academy of Sciences of the USA*, **87**: 2496-2499.

Reeve, N. (1994). *Hedgehogs*. T. & A. D. Poyser Ltd, London, 313 pp.

Reeve, N.J. (1982). The home range of the hedgehog as revealed by a radio tracking study. *Symposium Zoological Society of London*, **49**: 207-230.

Reeve, N.J. and Huijser, M.P. (1999). Mortality factors affecting wild hedgehogs: a study of records from wildlife rescue centres. *Lutra*, **42**: 7-24.

Reeve, S.A. and Usher, M.B. (1989). Application of a diffusion model to the spread of an invasive species: the coypu in Great Britain. *Ecological Modelling*, **47**: 217-232.

Richardson, J.H., Shore, R.F., Treweek, J.R. and Larkin, S.B.C. (1997). Are major roads a barrier to small mammals? *Journal of Zoology, London*, **243**: 840-846.

Rondinini, C. and Doncaster, C.P. (submitted). Roads as barriers to hedgehogs.

Rozen, S. and Skaletsky, H.J. (1998). PRIMER 3.0, Whitehead Institute for Biomedical Research.

Rubinsztein, D.C.e.a. (1995). Microsatellites are generally longer in humans compared to their homologous in non-human primates: evidence for directional evolution at microsatellite loci. *Nature Genetics*, **10**: 337-343.

Ruedi, M., Smith, M.F. and Patton, J.L. (1997). Phylogenetic evidence of mitochondrial DNA introgression amongst pocket gophers in New Mexico (family Geomyidae. *Molecular Ecology*, **6**: 453-462.

Ruefenacht, B. and Knight, R. (1995). Influences of corridor continuity and width on survival and movement of deermice. *Biological Conservation*, **71**: 269-274.

Ruzzante, D.E. (1998). A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. *Canadian Journal of Fisheries and Aquatic Science*, **55**: 1-14.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988). Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, **239**: 487-491.

Santucci, F., Emerson, B.C. and Hewitt, G.M. (1998). Mitochondrial DNA phylogeography of European hedgehogs. *Molecular Ecology*, 7: 1163-1172.

Schippers, P., Verboom, J., Knaapen, J.P. and van Apeldoorn, R.C. (1996). Dispersal and habitat connectivity in complex heterogeneous landscapes: an analysis with a GIS-based random walk model. *Ecography*, **19**: 97-107.

Schloetterer, C. and Tautz, D. (1992). Slippage synthesis of simple sequence DNA. *Nucleic Acids Research*, **20**: 211-215.

Schneider, S., Roessli, D. and Excoffier, L. (2000). Arlequin ver. 2.000: A software for population genetics data analysis. Switzerland, Genetics & Biometry Laboratory, University of Geneva.

Searle, J.B. and Erskine, I. (1985). Evidence for a widespread karyotipic race of hedgehog (*Erinaceus europaeus*) in Britain. *Journal of Zoology of London*, **206**: 276-278.

Seppä, P. and Laurila, A. (1999). Genetic structure of island populations of the anurans *Rana temporaria* and *Bufo bufo*. *Heredity*, **82**: 309-317.

Shields, W.M. (1983). Optimal inbreeding and the evolution of philopatry. In: *The Ecology of Animal Movement*. I. R. Swingland and P. J. Greenwood (eds.). Clarendon Press, Oxford. p.132-159.

Shultz, S.T. and Willis, J.H. (1995). Individual variation in inbreeding depression - The roles of inbreeding history and mutation. *Genetics*, **141**(3): 1209-1223.

Sjögren, P. and Wyöni, P.I. (1994). Conservation genetics and detection of rare alleles in finite populations. *Conservation Biology*, **8**: 267-270.

Slater, G. (1907). *English peasantry and the enclosures of common fields.* Archibald Constable & Co. Ltd., London.

Slatkin, M. (1985). Gene flow in natural populations. *Annual Review of Ecology and Systematics,* **16**: 393-430.

Slatkin, M. (1994). Gene flow and population structure. In: *Ecological Genetics.* L. A. Real (ed.), pp. 3-17, Princeton University Press, Princeton, New Jersey.

Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics,* **139**: 457-462.

Slatkin, M. and Barton, N.H. (1989). A comparison of three indirect methods for estimating average levels of gene flow. *Evolution,* **43**(7): 1349-1368.

Smit, G.F.J. and Meijer, A.J.M. (1999). Experience with the counting of animal road casualties. *Lutra,* **43**: 25-33.

Smouse, P.E., Long, J.C. and Sokal, R. (1986). Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology,* **35**(4): 627-632.

Sokal, R.R. and Rohlf, F.J. (1995). *Biometry : the principles and practice of statistics in biological research.* 3rd edn. W. H. Freeman and company, New York, 887 pp.

Sokolov, V.Y., Aniskin, V.M. and Lukyanova, I.V. (1991). Karyological differentiation of two hedgehog species in genus *Erinaceus* (Insectivora, Erinaceidae) in the USSR. *Zoologichesky Zhurnal,* **70**: 111-120.

Soulé, M.E. (1987). Minimum viable populations: processes of species extinction. In: *Conservation Biology: the Science of Scarcity and Diversity*. M. E. Soulé (ed.), pp. 19-34, Sinauer Associates, Sunderland, MA.

Southwood, T.R.E. (1978). *Ecological Methods.* Chapman and Hall, London, 524 pp.

Stacy, J.E., Jorde, P.E., Steen, H., Ims, R.A., Purvis, A. and Jakobsen, K.S. (1997). Lack of concordance between mtDNA gene flow and population density fluctuations in the bank vole. *Molecular Ecology,* **6**: 751-759.

Stallings, R.L. (1995). Conservation and evolution of (CT)n/(GA)n microsatellite sequences at orthologous positions in diverse mammalian genomes. *Genomics,* **25**: 107-113.

Stewart, D.T., Saavedra, C., Stanwood, R.R., Ball, A.O. and Zouros, E. (1995). Male and female mitochondrial-DNA lineages in the Blue mussel (*Mytilus edulis*) species group. *Molecular Biology and Evolution,* **12**(5): 735-747.

Stewart, W.A., Dallas, J.F., Piertney, S.B., Marshall, F., Lambin, X. and Telfer, S. (1999). Metapopulation genetic structure in the water vole, *Arvicola terrestris*, in NE Scotland. *Biological Journal of the Linnean Society,* **68**: 159-171.

Strand, M., Prolla, T.A., Liskay, R.M. and Petes, T.D. (1993). Destabilization of tracts of simple repetitive DNA in yeasts by mutations affecting DNA mismatch repair. *Nature,* **365**: 274-276.

Swofford, D.L. (1998). PAUP: Phylogenetic Analysis Using Parsimony. Sunderland, MA., Sinauer Associates.

Takezaki, N. and Nei, M. (1996). Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics,* **144**: 389-399.

Tamura, K. and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the controll region of the mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**: 512-526.

Taylor, A.C., Horsup, A., Johnson, C.N., Sunnucks, P. and Sherwin, B. (1997). Relatedness structure detected by microsatellite analysis and attempted pedigree reconstruction in an endangered marsupial, the northern hairy-nosed wombat *Lasiorhinus krefftii*. *Molecular Ecology*, **6**: 9-19.

Thomas, C.D. and Harrison, S. (1992). Spatial dynamics of a patchily distributed butterfly species. *Journal of Animal Ecology*, **61**: 437-446.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**(22): 4673-4680.

Thompson, P.M. and Goodman, S. (1997). Direct and indirect estimates of dispersal distances. *Trends in Evolution & Ecology*, **12**: 195-196.

Thulin, C.-G., Jaarola, M. and Tegelstrom, H. (1997). The occurrence of mountain hare mitochondrial DNA in wild brown hares. *Molecular Ecology*, **6**: 463-467.

Valdez, A.M., Slatkin, M. and Freimer, N.B. (1993). Allele frequencies at microsatellite loci - the stepwise mutation model revisited. *Genetics*, **133**: 737-749.

Viard, F., Justy, F. and Jarne, P. (1997). Population dynamics inferred from temporal variation at microsatellite loci in the selfing snail *Bulinus truncatus*. *Genetics*, **146**: 973-982.

Vigilant, L., Stoneking, H., Harpending, K., Hawkes, K. and Wilson, A.C. (1991). African populations and the evolution of human mitochondrial DNA. *Science,* **253**: 1503-1507.

Wahlund, S. (1928). Composition of populations from the perspective of the theory of heredity. *Hereditas,* **11**: 65-105.

Waits, L., Taberlet, P., Swenson, J.E., Sandegren, F. and Franzén, R. (2000). Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian brown bear (*Ursus arctos*). *Molecular Ecology,* **9**: 421-431.

Ward, J.F., Macdonald, D.W. and Doncaster, C.P. (1997). Responses of foraging hedgehogs to badger odour. *Animal Behaviour,* **53**: 709-720.

Ward, J.F., MacDonald, D.W., Doncaster, C.P. and Mauget, C. (1996). Physiological response of the European hedgehog to predator and nonpredator odour. *Physiology & Behaviour,* **60**(6): 1469-1472.

Waser, P., Creel, S. and Lucas, J. (1994). Death and disappearance: estimating mortality associated with philopatry and dispersal. *Behavioural Ecology,* **5**: 135-141.

Waser, P.M. (1996). Patterns and consequences of dispersal in gregarious carnivores. In: *Carnivore Behaviour, Ecology, and Evolution*. J. L. Gittleman (ed.). Cornell University Press, Ithaca, New York. **2**. p.267-295.

Waser, P.M. and Elliott, L.F. (1991). Dispersal and genetic structure in kangaroo rats. *Evolution,* **45**(4): 935-943.

Waser, P.M. and Strobeck, C. (1998). Genetic signatures of interpopulation dispersal. *Trends in Ecology & Evolution,* **13**(2): 43-44.

Wauters, L., Hutchison, Y., Parkin, D.T. and Dhondt, A.A. (1994). The effects of habitat fragmentation on demography and the loss of genetic variation in the red squirrel. *Proceedings of the Royal Society of London*, **255**(107-111).

Wayne, R.K. and Jenks, S.M. (1991). Mitochondrial DNA analysis implying extensive hybridisation of endangered red wolf *Canis rufus*. *Nature*, **351**: 565-568.

Weber, J.L. and May, P.E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics*, **44**: 388-396.

Weber, J.L. and Wong, C. (1993). Mutation of human short tandem repeats. *Human Molecular Genetics*, **2**: 1123-1128.

Weir, B.S. and Cockerham, C.C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, **38**: 1358-1370.

White, T.H., Bowman, J.L., Leopold, B.D., Jacobson, H.A., Smith, W.P. and Vilella, F.J. (2000). Influence of Mississippi alluvial valley rivers on black bear movements and dispersal: implications for Louisiana black bear recovery. *Biological Conservation*, **95**(3): 323-331.

Wierdl, M., Dominska, M. and Petes, T.D. (1997). Microsatellite instability in yeast: dependence on the length of the microsatellite. *Genetics*, **146**: 769-779.

Wolff, J.O. (1993). What is the role of adults in mammalian juvenile dispersal? *Oikos*, **68**: 173-176.

Wolff, J.O. (1994). More on juvenile dispersal in mammals. *Oikos*, **71**: 349-352.

Woolpy, J.H. and Eckstrand, I. (1979). Wolf pack genetics, a computer simulation with theory. In: *The Behaviour and Ecology of Wolves*. E. Klinghammer (ed.). Garland STPM Press, New York. p.206-224.

Wright, S. (1943). Isolation by distance. *Genetics*, **28**: 114-138.

Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics*, **15**: 323-354.

Wright, S. (1969). *Evolution and the Genetics of Populations.* University of Chicago Press, Chicago, 511 pp.

Zhang, D. and Hewitt, G.M. (1996). Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution*, **11**(6): 247-251.

Zischler, H., Geisert, H. and Castresana, J. (1998). A hominoid-specific nuclear insertion of the mitochondrial D-loop: implications for reconstructing ancestral mitochondrial sequences. *Molecular Biological and Evolution*, **15**(4): 463-469.

Zuckerkandl, E. (1991). Dispensability of parts of histones and the molecular clock. *Journal of Molecular Evolution*, **32**(4): 271-273.

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