UNIVERSITY OF SOUTHAMPTON

BEHAVIOURAL ASPECTS OF THE POPULATION GENETICS OF THE DOMESTIC CAT

GILES FREDERICK HORSFIELD

Thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy

Faculty of Science

Division of Biodiversity and Ecology

September 1998

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

DIVISION OF BIODIVERSITY AND ECOLOGY

Doctor of Philosophy

BEHAVIOURAL ASPECTS OF THE POPULATION GENETICS OF THE DOMESTIC CAT

by Giles Frederick Horsfield

Although the total population of owned cats *Felis silvestris catus* has been rising in recent years, the number capable of reproduction may be static or even reducing, due to the increasingly widespread adoption of neutering as a method of population control. This project investigated the effects of high levels of neutering on population dynamics and the population genetics of cat temperament. Male ranging behaviour, and the mating system of cats living in the urban environment of Southampton at the time of the study were also explored.

A population dynamics study was carried out by means of door to door surveys in and around the Southampton area. These revealed that neutering rates amongst adult cats were as high as 98% (females) and 97% (males) in the Shirley area of the city, but this figure varied between regions in the city. Overall, the city's population appeared to be stable, but the effective population had been reduced over the last 15 years, with a small number of females producing a high proportion of the population's kittens. Human mediated migration was responsible for maintaining the population in the Shirley area.

A radio tracking study of entire males revealed home ranges of up to 14 ha, with core areas of 2-6 ha. These are larger than previously documented, and demonstrate that there is the potential for overlapping home ranges and competition for mating opportunities between entire owned males even in areas where high neutering reduced their density. Microsatellite analysis of kinship relationships between cats showed that there were more males siring kittens within the Upper-Shirley area than the population dynamics survey predicted would be present, even allowing for extensive ranging behaviour shown by pet toms. I suggest that some of the sires in this area are feral cats.

Paternal genetics are known to have an important influence on cat temperament. Given the different selection pressures applied to owned and feral cats, it seems likely that feral cats tend to show traits such as lack of sociability to humans, that make them less well suited to being pets. The possible effects of an increase in owned kittens sired by feral males, promoted by neutering of owned males, was investigated by a temperament testing study. Temperament of litters born in areas of high and lower neutering were compared. This revealed a non significant trend at 6 months of age for kittens born in areas of high neutering to be less sociable to humans, as predicted by the hypothesis. These differences were not apparent when the kittens were re-tested at 18 months.

Table of Contents

Appendix 2

List of Figures

Chapter 2

Chapter 3

List of Tables

Chapter 6

Acknowledgements

I would like to thank my supervisors John Bradshaw and John Allen for their help, support and patience throughout the course of this study.

I am grateful to the BBSRC and WALTHAM for funding the project.

Many thanks are due to David Shepherd, for letting me use his laboratory to do the microsatellite work, and to Tony Long, for his help while working there. I am very grateful to Gugs Lushai for help with technical aspects of the microsatellite work, and also for help with some of the figures. Arati Iyengar also contributed help with lab work. Toby Carter has been a constant source of advice and encouragement while writing up, and is warmly thanked, as is Keith Anderson for various aid he has provided over the course of the project. The members of the AzI are all thanked, especially Debbie Goodwin for her help early on and Sarah Cook for advice on temperament testing in homed cats. Sandra McCune also taught me temperament testing techniques. Dr Menotti-Raymond is owed a debt of gratitude for proving us with microsatellite sequences ahead of their publication.

This study would not have been possible without volunteers who allowed me to use their cats as subjects in this study, thank you all. Barbara Dollery and Helen Farrier from Cat and Kitten rescue and Steve Broomfield from the Blue Cross were kind to let me examine their records. I would also like to thank the inhabitants of Southampton, both feline and human, who have been admirably tolerant of my eccentric activities over the last few years.

Darren Williams has been a source of moral support while writing up, as have Richard Hankins and Simon Griffiths at a longer distance. Life in Southampton would not have been the same without the SUMC, and I would like to thank everyone there for the laughs. Noodles and Twizzle reminded me what I was supposed to be writing about, and are offered a miaow of gratitude.

Thank you to Mum, Dad and Al for their love and support over the years. And, of course, to Gill McDonald, who has done more than anyone to preserve my sanity during the writing of this thesis.

Definitions of terms used in this thesis

Entire cat: A cat that has not been neutered

Tom: An adult male cat that has not been neutered.

Behavioural Aspects of the Population Genetics of the Domestic Cat

1 Introduction

1.1. General Introduction

The domestic cat is an animal that everyone is familiar with, yet it retains much of the behaviour of a wild mammal. Cats frequently live a feral lifestyle with little or no contact with humans. With the exception of pedigree breeds, cat breeding is generally not under human control, and cats are free to compete for mating opportunities and exert mate choice. Yet human activity does influence cat behaviour. By keeping cats or providing areas where they can live on their own, humans have promoted a species which now lives in wide range of habitats at a wide ranges of population densities. This could not have happened without the behavioural flexibility exhibited by the cat. These factors make the domestic cat a valuable model species for a zoologist.

This study examines the effects that a regime of high neutering has had on the cat population of Southampton, with respect to population dynamics and the population genetics of cat temperament. It also investigates the mating system of cats in the city, and the structure of the population.

1.2. Literature Review

1.2.1. Domestication

The domestic cat (*Felis catus*) belongs to the *Felis silvestris* species complex, a group that includes a number of races of small cats whose range covers most of Europe and much of Asia and North America (Robinson, 1984). Studies on the ancestry of the domestic cat have focused on two subspecies: the European wildcat (*Felis silvestris silvestris*), and the African wildcat (*Felis silvestris libyca*). The latter is now widely accepted as the domestic cat's ancestor due to evidence from archaeological remains (Zeuner, 1963), its relatively docile behaviour (eg. Smithers, 1968), comparisons of

skull structure (Ragni & Randi, 1986), and genetic similarity (Randi & Ragni 1991).

The association between humans and cats probably arose in the Middle East, and has been in existence since at least 2000 B.C. and possibly since 7000 B.C. (Clutton-Brock, 1994). At first, cats probably had a commensal relationship with humans (Serpell 1988) foraging cats being tolerated, and eventually encouraged, in agricultural settlements, because of their vermin controlling qualities. It seems likely that the movement of cats west into Europe followed the spread of agriculture from the Middle East. The association could then have become increasingly close, leading eventually to full domestication (Baldwin 1975).

There is little evidence that humans have ever actively sought to influence the nature of the domestic cat (Todd, 1977), except in the case of pedigree breeds. However, cats do exhibit many of the changes in physical characteristics that typically accompany domestication (Clutton-Brock, 1994). These include reduction in total body size, shortening of the jaw, and a reduction in brain volume of around 10-15% (Robinson 1984). Neoteny, the retention of juvenile characteristics into adulthood, is probably an important mechanism for such changes. The rapid phenotypic changes that occur during domestication may under certain circumstances be reversed: there may be selection in feral cats for traits that are not selected for in domestic cats, such as ability to hunt. Price (1984) views this process of feralisation as being "domestication in reverse".

1.2.2. Population trends in domestic cats

1.2.2.1. Population numbers

The popularity of cats as pets has been rising in Europe, and the number of households with cats has recently overtaken the number with dogs in the USA (Anon., 1995). One of the reasons for this trend may be that the cat, needing relatively little attention on a day to day basis due to its independent lifestyle, is well suited to life as a pet in modern households where frequently both partners go out to work (McCune,

1992; Anon., 1998).

Cat ownership was estimated at 0.51 cats/ household in Manhattan, New York, USA (Nassar and Mosier, 1982). In Australia 25.2% of households owned at least one cat, with an overall frequency of 0.36 cats/household (Anon., 1994). In Britain, an overall cat: human ratio of 1:12.5; ie. around 5 million cats was estimated (Carding, 1975, cited in Blumenburg and Lloyd, 1980). The frequency of cat owning households was estimated at 21.5%, with a mean of 0.32 cats/household (Tabor, 1983; cited in Chipman, 1990). Door to door surveys revealed 0.18 cats/household in a suburb of Manchester (Chipman, 1990). A national UK survey of social trends showed that the number of pet cats had increased from 4.7 million in 1981 to nearly 8 million in 1996 (Anon., 1998). The report also shows that the number of cats exceeded the number of dogs in 1993, and was still slightly higher than the number of dogs in 1996.

In addition to owned cats, there are feral cats. Feral cats sometimes live in colonies in cities, for example in the grounds of hospitals (Rees, 1981), parks (Neville and Remfry, 1984), urban historical ruins (Natoli and De Vito, 1988) and dockyards (Dards, 1978). Seven hundred and four colonies were located in the UK by a nationwide survey, of which only 7% were thought to consist of more than 50 cats (Rees, 1981). Two residential districts of Brooklyn, New York, which were rich in abandoned houses and refuse, supported 4.8 and 2.0 cats ha^{-1} respectively (Calhoon and Haspell, 1989). There are also feral cats that do not live in groups (e.g. Corbett, 1978). The division between pet and feral cats is not always clear; there is a continuum onto which fall individuals who are fed but are not attached to a household, and cats which visit one or more households but are not owned by them (Liberg and Sandell, 1988). The surreptitious nature of truly feral cats, the problems of definition, and the problems involved in identifying a cat as feral, make the population of solitary feral cats almost impossible to census accurately.

1.2.2.2. Neutering

Mate choice, given the independence enjoyed by most pet cats, is rarely under direct

human control, with the exception of pedigree cats. Increasingly, however, cat owners have sought to limit the reproduction of their pets by neutering. There are advantages to neutering besides litter prevention; unwanted toms are not attracted to oestrus females, and odorous spraying by tom cats is reduced (Bradshaw, 1992). Neutering also gives rise to improved health for individual cats, fighting is reduced (Hart and Barrett, 1973) and life expectancy has been found to be significantly longer (Hamilton, 1969).

There has been concern about feral cats; for their welfare and health, their potential to infect house cats with disease (Kristensen, 1981), and in some cases for the threat they pose to human hygiene, such as in the hospital colony described by Rees (1981). Euthanasia is unsatisfactory for a number of reasons; cat lovers may shield their favourite animals by hiding them (Robinson, 1992), and the vacuum left by their absence may encourage other cats to simply fill the gap (Kristensen, 1981). A policy of neutering largely solves these problems; the antisocial (from a human perspective) behaviour of the cats is greatly reduced (Hart and Barrett, 1973) and no litters are produced. Furthermore, strange cats are not simply drawn into the area to fill the vacant niche. This latter point has attracted controversy, because it was feared that neutered cats would be displaced by the more aggressive toms. However, observation of neutered cats has shown that although they are more tolerant of other cats, they are not driven away by them, although they may be less territorial than entire cats (Bradshaw and Brown, 1996). Neutered colonies have been successfully re-established with only gradual subsequent immigration by entire cats (Neville and Remfry, 1984; Tabor, 1989). Chipman (1990) found that male home ranges, which generally become larger with age, remain frozen following neutering. Neutering thus appears to be a satisfactory method of keeping feral and house cat populations in check, and it is now the standard policy of rescue organisations such as the RSPCA and Blue Cross to neuter any cats that pass through their care.

Fifty nine percent of females were found to be neutered in Manhatten New York, the authors estimated that 88% would need to be neutered to ensure a stable population, however it should be noted that mortality rates were high (Nassar and Mossier, 1982).

A telephone survey in St. Josephs county in the USA showed overall neutering rates at 79.8% (Patronek *et al*, 1997). Very high rates of neutering across Australia (93.6% of adult cats) gave rise to a 10% fall in the owned population between 1993 and 1994 (Anon, 1994). Chipman's (1990) sample of cats in Manchester revealed 59/78 males to be neutered, and 72/74 females, although some owners were prepared to allow their cats to have one litter before carrying out neutering. There are few recent data on neutering rates in the UK available.

1.2.3. Population genetics and coat colours.

Domestic cats are unusual, possibly unique among animals with such great freedom of movement and mate choice, in that they have at least 6 genes with easily identifiable mutant alleles affecting coat colour (Clark 1975); sex linked non-orange ($\mathbf{o^+}$, **O**), and autosomal loci controlling, agouti, non agouti ($\mathbf{a^+}$, **a**), striped and blotched tabby ($\mathbf{t^+}$, $\mathbf{t^b}$), non dilute, dilute ($\mathbf{d^+}$, **d**), short hair, long hair ($\mathbf{l^+}$, **l**), dominant piebald spotting, non spotted (**S**, $\mathbf{s^+}$), dominant white, pigmented (**W**, $\mathbf{w^+}$). This has made them a popular study species for population genetics. Although this study is not directly concerned with coat colour genetics, the results of such studies give an insight into the way that cat populations are structured with respect to gene flow. Also, they give an indication of the ways in which artificial selection, in the form of human preferences, and natural selection, may both influence cat evolution.

Todd (1977) showed that the worldwide distribution of cat coat colour phenotypes can be linked to human dispersal. For example, the blotched tabby genotype, which has been rising in frequency in Britain since its origin around 300 years ago, is present in localities colonised by Britain (eg. Australia, Canada), at frequencies corresponding to the frequency of the genotype in Britain at the time of colonisation. The same effect may have caused the general heterogeneity of coat genotypes in the U.S.S.R. (Robinson and Manchencko, 1981), and Scotland (Clark, 1976), where the cat populations of isolated areas still represent the coat colours of Viking preference. A study conducted on mainland Spain, and on adjacent islands (Ruiz-Garcia, 1993),

found some heterogeneity, possibly as a result of the introduction of mutant alleles by humans. However, they concluded that gene flow between geographic areas was very high, resulting in very large effective populations operating close to panmixia.

Factors other than historical legacy also influence the distribution of cat colour types. Identifying the selective pressures involved has proved to be difficult; coat type may prejudice human preference or affect survival ability *per se,* (eg. the camouflage offered by darker phenotypes), or colour type may be linked to other characteristics. A well documented example of the latter is the dominant white allele, which confers the disadvantages of reduced parental care, deafness, and increased susceptibility to disease (Todd, 1977). In this case, the genotype is clearly preserved by means of human preference. In one survey (Clark, 1975), 35% of those questioned expressed a preference for dominant white cats, while the actual frequency was very low. Cats with the dark **a,t,b** and $\mathbf{s^+}$ alleles have been found to have low body weight and small adrenal glands (Van Aarde and Blumenburg, 1980). Other phenotypes have been tentatively linked with distinctive behavioural styles; from a survey of road deaths, Reichholf (1983), reported that black, and black and white cats tend to roam further from their homes than other cats. Also, there is extensive anecdotal evidence concerning the extrovert and vocal nature of Siamese cats (Robinson, 1992).

The importance of human preference in preserving the detrimental W allele is unequivocal, the situation is less clear, however, for other coat type alleles. This is well illustrated by the comparison of coat preferences and their actual frequencies among cats in Glasgow (Clark, 1975). A consistent preference for lighter phenotypes was found, well in excess of their actual frequency. Moreover, for all loci studied, the darker alleles were significantly more frequent in the poorer areas than in the more affluent areas. The interpretation of this situation was confused by the paradox that in the more affluent areas, where residents exercised more control over the cat population, the incidence of neutering was much higher, meaning that human selection may not be directly translated into allele frequencies, and may even have the reverse effect. Clarke concluded that the difference in cat genotypes between the areas was largely explicable in terms of the adaptive value of being inconspicuous in an urban feral situation,

together with some artificial selection for lighter phenotypes.

In general, Clark's findings have been supported by further research. Unwanted cats in Austria (Hoger, 1994), have a lower incidence of **O**, $\mathbf{T^b}$, **S** and **I** alleles, indicating a similar set of preferences to those in Glasgow. The increase of the **O** allele in Southern England in recent decades can be linked to artificial selection for orange and tortoiseshell cats (Robinson and Silson, 1969; Searle, 1949). Conversely, densely populated city areas in the USSR, similar to the "urban feral" situation in Glasgow, showed a high frequency of the dark **a** (non agouti) allele (Robinson and Manchenko, 1981). When 28 Scottish locations were sampled (Clarke, 1976), a highly significant positive correlation was found between an overall index of phenotypic darkness and the log of human population density. In this case there was clear heterogeneity between cities and rural areas possibly due in part to differential migration and the isolation of parts of Scotland.

Vinogradov (1994) performed principal components analysis on the results of 204 surveys of cat coat allele frequencies, and found that overall the alleles **d**, **l** and **W** are promoted by artificial selection, while the **a** and **tb** alleles can be traced to heavily urbanised areas. Blumenburg and Lloyd (1980) analysed data from surveys from worldwide locations, with emphasis on UK and Eire populations, and also found a correlation between darkness and population density. However their favoured explanation of the selective pressures involved differed from Clark's, citing evidence of pleiotropy linking the **a**, $\mathbf{t^b}$ and $\mathbf{s^+}$ alleles to lower body and adrenal weight (Van Aard and Blumenburg, 1980), they speculate that these characteristics should be favourable in an urban habitat requiring a high degree of tolerance.

It is possible, therefore, to pick out trends in cat population genetics with respect to coat alleles. How easy this will prove to be when considering cat "personalities" remains to be seen. One advantage of my study is that the effect of neutering will be a central consideration, rather than merely a confounding variable.

1.2.4. Individuality in the domestic cat

Those who come into contact with cats have long been of the opinion that individual animals exhibit distinctive "personalities" or temperaments. This view conflicts with the traditional ethological standpoint which considers intraspecific differences in behaviour to be trivial, or "noise" in the data. More recently, however, it has been accepted that there may be sound evolutionary reasons for individuals within a population to pursue different behavioural strategies (Mendl and Harcourt, 1988; Wilson *et al,* 1994). Empirical evidence for the existence of genuinely different behavioural styles in individual cats has accumulated. Cat "personalities", and the influences that shape them, have important implications for the future of the human-cat relationship.

Field studies have revealed that individuals living in the same area often follow different strategies, especially when spacing and mating behaviour are concerned (see below). In some cases these differences can be linked to observable differences in social status (e.g. Liberg 1980), but sometimes seem to represent alternative strategies amongst individuals of comparative social status (Natoli and DeVito, 1991). Free ranging cats were approached in a housing area (Meier and Turner, 1985), and the distance to which the experimenters were allowed to approach, and the cats subsequent reactions to attempts at petting, were recorded. The cats appeared to fall into two distinct personality types; trusting and shy, rather than along the expected continuum. Also the cats' reactions showed little change over time, indicating that their behaviour was guided by underlying differences in temperament, rather than temporary differences in motivation.

Methods of assessing the individuality of cats under controlled conditions were developed by Feaver *et al* (1986). Observers, who had been able to interact with the cats over a period of 3 months, rated 18 aspects of the cats' behavioural style. Significant inter-observer correlations were obtained for 15 of these. When only the 7 aspects that showed inter observer correlations of >0.7 were considered, three reasonably independent dimensions of behavioural style could be defined by grouping

the categories that showed either strong negative or positive correlations. The behavioural dimensions defined were; alert (consisting of ratings for active and curious), sociable (sociable with people, fearful of people, hostile to people, and tense), and equable with cats. It is interesting to note that friendliness to humans appeared to be unrelated to friendliness to cats. The second part of this experiment involved the direct recording of strictly defined categories of behaviour in the same cats, which gave good agreement with, and thereby validated, the equivalent subjective ratings given to individual cats.

Mertens and Turner (1988) used 19 cats and 240 volunteers in a study of interactions during first encounters between cats and people. Latencies for interaction and occurrences of sociable behaviour were recorded. It was found that each cat performed consistently throughout the experiments, even after taking the differences of test person into account.

1.2.4.1. Sources of variation in temperament

There is no doubting the profound effects of experience, particularly at early age, on the reactions of cats towards humans. Collard (1967) found that kittens that were exposed to 5 people between 5 and 9 weeks of age, showed significantly less fear of strangers than cats exposed to one or no people, although some individuals appeared to more susceptible than others to the risks of exposure. Karsh (1983) devised a series of tests to measure the friendliness of cats towards people, using two groups of kittens; one of which had been handled regularly and another which had not. Her tests included; the preference of the 4 month old kittens for a person or another cat when placed in a 1.8mx1.8m room, and the latency of the kittens to approach a person. In both cases the handled cats were significantly friendlier than the non handled kittens. When the cats were held, but not restrained, the handled kittens were significantly less likely to make escape attempts. Friendliness, at least on some measures, and the optimum time for it to occur is known as the sensitive period, and improves with handling up to about 40 minutes/day.

This process bears some superficial similarity to imprinting by waterfowl, although some authors, (e.g. Chalmers, 1983) regard the two as related but distinct. One crucial difference is that imprinting results in attachment to a single maternal (or pseudo maternal) figure, whereas exposure of kittens to people produces a more generalised effect that inhibits fear towards all similar people. This process is usually distinguished by the term socialisation, as opposed to the term "critical period", which is generally not used for mammals (Lorenz, 1937; cited in Karsh and Turner, 1988). The sensitive period for the domestic cat was identified by Karsh (1983) by handling different groups of kittens for 40 minutes daily for 4 weeks commencing in week 1, 2, 3, or 4 of the kitten's life. The kittens then underwent the holding test, as above. The weeks 2-7 appeared to be the most important for attachment to humans. Interestingly, some of the cats seemed to be intrinsically shy, and the data analysis had to be adjusted accordingly. A study of 100 kittens, born in both feral and domestic environments, revealed that feral born kittens generally make satisfactory pets if socialisation begins before they reach 7 weeks old (Bradshaw and Cook, 1997).

Further research has focused on the genetic and environmental aspects of cat individuality. Overall observer rankings of friendliness were used by Turner *et al* (1986), who provided evidence for the influence of genetics on cat personality. Cats from two separate colonies were used; one based in Cambridge, and the other in Zurich. The cats were sired by one of two males in both colonies, in both cases the kittens that were rated "friendly" were unevenly distributed between the two parent males. Strong inter-observer correlations were obtained. As the fathers had no social contact with their offspring it would seem that this effect is, at least partially, directly genetically mediated.

Reisner *et al* (1994) used kittens sired by five males, which were exposed to different levels of handling between 4 and 8 weeks of age. No differences due to treatment were found, though this may have been due to the low levels of handling to which the "socialised" kittens were exposed; 15 minutes, three times per week. However, there were significant paternal effects on the kittens' friendliness and defensive aggression.

McCune (1992) found the effects of socialisation and paternity to be additive. Cats that had friendly fathers and were also socialised were the most friendly. Unsocialised cats with friendly fathers and socialised cats with unfriendly fathers were generally similar in their responses to familiar people and strangers. When exposed to a novel object, however, paternity was the most important factor in determining the cats' response. Ultimately, friendly father influences proved to be the more important factor determining the cats personalities (McCune, 1995). McCune proposes that one of the genetic components of friendliness can be viewed as a "boldness" characteristic, generalisable to both people and other novel situations, which therefore promotes the socialisation process.

There have been other indications of genetic influences on behaviour; cat show judges showed consistent agreement when asked to describe the temperaments of pedigree cats (Hart and Hart, 1984). Certain lines of cats have been found to be consistently timid (Beaver, 1976), and out-breeding can reduce timidity in a cat pedigree (Hurni and Rossbach, 1987, cited in McCune, 1992).

1.2.4.2. Identifying behavioural styles in cats

It is worth considering the categories of behavioural style that have been labelled for cats. Already mentioned are the bold/shy trait identified by Meier and Turner (1985), and sociable/alert/equable to cats (Feaver *et al,* 1986). Active, timid and confident were identified by Karsh (cited in McCune, 1995), while sociable to humans and generally active were the most important components extracted by Bradshaw and Cook (1996). McCune (1995) separated a friendliness trait into sociability to humans, promoted by socialisation, and boldness in a novel situation, mediated by genetic influences (demonstrated through paternity).

1.2.4.3. Consistency of temperament between ages

Kittens raised in a home environment were tested at intervals between 2 months and 36 months of age (Cook and Bradshaw, 1996; submitted). At 12 months old there was

a peak in the frequency of distress being exhibited. Otherwise there were positive correlations between age groups for distress while being handled and escape attempts while being handled, but not incidence of purring. Despite these similarities, there was evidence of changes in behavioural style over time, with some friendly cats at 4 months later becoming unfriendly and vice versa.

1.2.4.4. Cat temperament and the human-cat relationship

Different people demand a wide range of different levels of companionship and affection from their cats, and it is a tribute to the cat's flexibility that it is so often able to meet these needs. When Turner and Stambach-Geering (1990) asked cat owners to compare their ideal cats with the ones they owned, 75% of owners considered their present cats to be close to ideal. A symmetry appears to exist between the wishes of cat and human (Turner, 1991); high compliance to interact by the human was associated with high compliance on the part of the cat. However, there were some grounds for dissatisfaction; where humans frequently tried to initiate interaction, total interaction time was reduced. Playfulness and willingness to be petted are two characteristics that are both frequently desired by cat owners, yet, contrary to expectation, these traits are uncorrelated.

In general people require friendly, interactive cats. A questionnaire distributed to cat owners revealed that they tended to be less attached to less interactive cats Bradshaw and Cook (1994). Any trend in the cat population away from these traits is therefore likely to be damaging to the human/cat relationship.

1.2.5. Social organisation

There has been considerable interest focused on the spatial use and social behaviour of domestic cats. What has emerged is a picture of an animal that is highly adaptable in terms of social systems, which vary greatly in different ecological conditions and population density. Studies of mating behaviour have sometimes given conflicting accounts (Liberg, 1981; Natoli and DeVito 1988 and Natoli and DeVito, 1991), leading

to speculation over the possible causes of such differences. Estimation of reproductive success has so far been based on observation of copulatory success (Natoli and DeVito, 1988), or an index using territory and coat colour inheritance (Liberg, 1981; Pontier and Natoli, 1996). Reliable measures of paternity, using recently available molecular techniques, would give unequivocal measures of male reproductive success. The information gained from such a study would be of great theoretical interest in its own right, and would also be vital in assessing any changes in cat population genetics as a result of neutering programs.

1.2.5.1. Population structure

There is great variation in the population densities at which domestic cats can be found; ranging from 0.9 for a remote feral population to 2350 per km^2 in a dense urban situation (Langeveld & Niewold, 1985; Izawa *et al,* 1982), in the studies reviewed by Kerby and Macdonald (1988). In general, home range size can be shown to be negatively correlated to population density (Tabor, 1983), for both males and females (Liberg & Sandell 1988). In a suburban area of Manchester median home range size was measured at 1.2ha (Chipman, 1990), with a density of 0.18 cats/ household; in rural Sweden home ranges were measured up to 50ha (Liberg, 1980), and on farmsteads in rural Illinois mean male home ranges were 228 ha (Warner, 1985).

The spacing of cats within an area is primarily determined by the quantity and distribution of food. Liberg and Sandell (1988) propose that cat populations can be broadly categorised between those where females form groups, and those where they do not. Where food resources are evenly distributed in time and space, and the cat population is low, one would be likely to find a fairly even distribution of cats, with less likelihood of overlapping home ranges, such as is the case for rural feral cats (Corbett 1978). Conversely, clumped food resources would lead to a similarly clumped distribution of cats, such as in the dockyard population studied by Dards (1978), and the population maintained by handouts from cat lovers in a park in Rome (Natoli, 1985), while an intermediate situation can be found with farm cats (Macdonald *et al*, 1987). However, Calhoon (1987) found that within his urban (New York) population,

availability of food did not influence home range size.

There has been a growing consensus that cats are capable of a far higher degree of social behaviour than was previously thought, and that groups consist of more than simple aggregations around food. Farm cats often share a communal sleeping area, and sometimes interact amicably when hunting or travelling Panaman (1981). Home ranges of cats from the same group tend to overlap substantially more than they do with cats from adjacent groups, suggesting a degree of within group tolerance (Liberg, 1981; Turner and Mertens, 1985). In one study, most aggressive behaviour observed on a farm was directed towards "outsiders" (Macdonald and Apps, 1978). Perhaps the most persuasive evidence comes from the existence of nursing coalitions, possibly based on kinship, although variation in kinship has not been enough to test this hypothesis (Macdonald *et al*, 1987; Izawa & Ono, 1986). The adaptive value of such behaviour is underlined by the higher survival rates of litters born of females holding a central position in the colony (Kerby and Macdonald, 1988).

Where groups of house cats do exist, all research to date has indicated a society based upon matriarchal lineages. The general pattern, as described by Dards (1978); Liberg (1983); Turner and Mertens (1985); and Macdonald *et al* (1987), is for the females within a group to be closely related. Female kittens tend to remain within their natal group throughout their lives. Groups of females defend their homes ranges against other females, and transfer of females between groups is rare. Males usually leave their natal group between the ages of 1 and 2 years, and may be driven out by the resident male at this time, when adult, these males may succeed in joining another group (see below).

Unusually, in a population in Japan (Izawa and Ono, 1986; Izawa *et al*, 1991), young male and female cats established home ranges that overlapped with their parental home ranges, possibly as a result of the exceptionally high population density.

Male cats are more loosely associated with groups than females are, and male's home ranges are larger than those of females and may encompass more than one female

group. Male home ranges also overlap more than female home ranges (Dards, 1978; Liberg, 1980). It has been hypothesised that female home range requirement is defined by food supply, while access to females would be the major factor influencing male home ranges, at least during the breeding season (Liberg and Sandell, 1988). This hypothesis is supported by three lines of evidence; data showing that the difference between male and female home ranges exceeds their relative food requirements, even allowing for the males' greater body mass (Kerby and Macdonald, 1988); neutered males have been shown to have smaller home ranges than entire males when they were neutered prior to reaching sexual maturity (Chipman, 1990); and in one rural area male home ranges were substantially larger during the breeding season than at other times of the year (Liberg and Sandell, 1988).

1.2.5.2. Measurement of home ranges

Home ranges have been investigated for cats in a wide range of environments, using either sighting methods or radio-telemetry. These include feral cats on Illinois farmland using telemetry (Warner, 1985), farm cats in Britain using telemetry (MacDonald and Apps, 1978) and using sighting (Panaman, 1981), farm cats in Austria using sighting (Turner and Mertens, 1985), farm cats in Sweden using telemetry (Liberg, 1983), urban feral cats in Jerusalem using sighting (Mirmovitch, 1995), feral cats in an English Dockyard using sighting (Dards, 1978), feral cats in a London park using sighting. (Tabor, 1989), and owned cats in a suburban area of Manchester using sighting (Chipman, 1990).

At the beginning of this project, no radio-telemetry studies of home ranges of urban cats were found in the literature, although more recently a radio tracking study of cats based on the edge of a suburban region of Australia has been reported (Barrett, 1997). It seems unlikely that sighting methods are adequate to investigate all aspects of ranging behaviour in an urban environment. Information on the ranging behaviour of cats in the Southampton environment would be valuable in elucidating the population structure and mating system. This information cannot be extrapolated from previous research in most cases because the environments were greatly different. In the habitat

most closely comparable to Southampton only sighting methods were used (Chipman, 1990). This is in addition to the consideration that neutering rates and topographical factors might be substantially different to those in Southampton at the time of this study.

Radio tracking has successfully been used to study ranging behaviour of foxes in cities: a similar sized mammal in a similar environment, for example in Bristol (White and Harris, 1994), Oxford (Doncaster and Macdonald, 1997) and Toronto, Canada (Adkins and Stott, 1998). This suggests that it should be a useful technique for studying free range cats, despite the difficulties imposed by the urban environment, such as the reflection of radio signals off buildings (Kenward, 1987)

1.2.5.3. Mating systems in domestic cats.

Liberg and Sandell (1988) discuss the theoretical considerations of male mating tactics. Males have a choice of concentrating on trying to monopolise females within a group or trying to gain some access to as many females as possible. Observational evidence suggests that the latter strategy is the one most commonly pursued (eg. Liberg, 1981), although Dards (1983), in her study of the Portsmouth dockyard population found that, male home ranges were fairly evenly distributed between 0.7 and 4ha., and that the males with the smaller home ranges remaining with a single group of females. Liberg and Sandell (1988) suggest that a strategy of trying to maintaining exclusive access to a group of females would be maladaptive if other members of the population were pursuing a "roaming" strategy, and that a roaming strategy should therefore predominate. Males sharing home ranges are in competition for females, and would be expected to show agonistic behaviour; Dards (1983) noted that, while adult males usually managed to avoid overt aggression, their interactions were never amicable.

A roaming strategy might not be optimal where there are clumps of females living close together and the distance between clumps is great (Liberg and Sandell, 1988).

The evaluation of distance, in ecological terms between females should be considered with reference to the surrounding habitat. Home range size is known to be related to population density, but there are other parameters that may also be important. Urban features such as roads and housing may limit ranging activity. In a city where neutering is high, the density of entire females may be low relative to the home range potential of the males, making each entire female a valuable resource at low density. Under such conditions a guarding rather than a roaming strategy might be predicted.

Two long term studies on the mating systems of cats have been carried out, one on a low density rural populations, and the other on a densely populated urban colony. Their findings are summarised below.

Liberg (1980; 1981; 1983), studied cats in a rural area of Sweden, which lived mainly in households in groups of 1-7. There were, in addition, several feral cats until severe depletion of rabbits, their staple food supply, destroyed the population. Male cats were subdivided into four categories; *novices*, who were sexually immature and still lived in their natal group; once they left their natal group, usually as a result of being driven out by the resident male on reaching sexual maturity, they were defined as *outcasts*, and often dispersed and lived as ferals; when young cats had become strong enough not to avoid confrontation with adult males they were classed as *challengers*, and cats that were able to displace others from their home range and from females were termed *breeders*.

A male's ability to displace other males, i.e. maintain "central male" position, was site specific, and the variation in the areas in which breeders could hold this position was substantial, but the number of females within the ranges was much more constant (mean: 10.8, standard deviation: 3.3). Central males guarded oestrus females and were the only individuals that succeeded in copulating, despite the frequent presence of one or more subordinate males. Copulation occurred at an average frequency of 15 per 24 hours. There were, however, often changes of central male as a result of a central male leaving the area, or being temporarily absent, or the return of a previously central male to a group. This allowed some copulatory success by challenger males.

Females did not actively choose males, and apparently always copulated with the central male; however oestrus females did frequently perform "flirt walks"; moving away rapidly in an apparent attempt to shake off the central male. This could be a way of testing the vigour of the central male, or of giving the other males present a chance to challenge the central male by breaking the asymmetry caused by the central male occupying a "holder" position (Maynard-Smith and Parker, 1976; cited in Liberg, 1983). In this way females could be promoting competition between males. Another potential means of achieving this would be to avoid synchronising oestrus, however there was significant synchrony of oestrus between groups. Liberg calculated a paternity index, based on coat colours and times of exclusive access to females. This revealed that dominance was a good predictor of reproductive success, with breeders gaining twice the indices of challengers, and 4 times that of novices and outcasts.

A population of feral cats in a park in central Rome provides data on the mating behaviour of cats living in completely different ecological conditions, and makes an interesting comparison. The work was carried out by Natoli (1985), Natoli and DeVito (1988), and Natoli and DeVito (1991). There were 81 cats; 37 adult females, 32 adult males and 12 subadults, contained in an area of 2570m^2. There were usually several males in attendance of an oestrus female, however, despite there being a clear dominance hierarchy, no males monopolised any of the females, and dominance rank did not correlate with copulatory success. Interestingly two male strategies were evident; most invested allot of time courting females, but around 30% did not. These "occasional males" performed less copulations overall, but were sometimes able to copulate with a female without investing much time in courtship. It is possible that the regular males in one area may have been occasional males in another area. The reasons for such tolerant male behaviour around fertile females are unclear.

A case study of cat mating systems in rural France demonstrated that it is possible for one male to secure a high level of reproductive success within a population (Pontier and Natoli, 1996). Coat colour genetics revealed that one year a tom homozygous for the rare dominant white allele fathered 95.5% (N=66) of kittens within an area of

approximately 2.2km^2. There were estimated to have been two or three other adult males previously in the area, though the authors do not state specifically that they were entire.

The use of DNA profiling has great potential for elucidating actual reproductive success in the domestic cat, as it has done for many other species (see below). No previous studies focussing on mating in cats in an urban housed area were found in the literature, highlighting the need for such information.

1.2.5.4. Social systems in another felid species.

It is worth briefly considering the social systems of the lion, *Panthero leo*, the only other felid widely recognised as showing extensive social behaviour. Prides consist of 4-12 females consorted by two or more males (Scaller, 1972; cited in Leyhausen, 1988). The only females recruited to the pride are the offspring of pride members, while adolescent males are driven away from their natant group, a parallel situation to that found in the domestic cat. Adult males form coalitions and attempt to drive the resident males from an established pride. If successful, this is followed by the infanticide of the pride offspring, which serves to advance oestrus in the pride females and allows them to sire offspring with minimal delay. Observational evidence shows that male lions do mate with more than one female (Packer & Pusey, 1982), but one male gains temporary dominance over the others when consorting a particular female. Cooperation between males has been observed, for example in patrolling the perimeter of the territory (Packer *et al*, 1991a).

Analysis of lion social structure using DNA fingerprinting revealed that pride females are indeed closely related (Packer *et al,* 1991a). Males were found to form coalitions with non relatives, but only when the total number of males was small, i.e. 2 or 3, larger (4-9) male coalitions always consisted of related males. Interestingly, male reproductive success became increasingly skewed as the number of males in the pride increases; both males always sired some offspring in a two male pride, whereas the distribution of offspring among one 4-male pride was; 9, 8, 1 and 0. One male fathered

all the members of 23 out of 24 litters. It was concluded that the non breeding males served as helpers within the pride, a system that depends on kinship for its continuance as an evolutionary stable strategy.

1.2.6. Using molecular methods to determine kinship

Areas of biology, such as behavioural ecology, that require the accurate determination of the relatedness of individuals, have been revolutionised by the application of DNA profiling. This offers far greater variability between individuals, and therefore more accurate estimation of relatedness than previously available techniques such as electrophoresis of allozyme loci (Burke, 1989). DNA profiling was made possible by the discovery of minisatellite DNA by Jeffreys *et al* (1985). Minisatellites, also known as variable number repeat loci (VNTR) are usually less than 20,000 base pairs in length and consist of multiple copies of tandem repeat units, typically 20-65 base pairs in length (Burke *et al*, 1991). The statistical power of minisatellites is very high, sometimes the probability of unrelated individuals sharing identical profiles is less than the reciprocal of the worlds population. However, minisatellites carry disadvantages such as the requirement for around 0.5ug of purified DNA per sample and difficulty in comparing between gels. These are largely overcome by the use of a subsequently developed technique; microsatellite analysis (Queller *et al*, 1993).

The repeat units in microsatellites are less than 6 base pairs in length. Those used in kinship analysis are typically 2-4 base pairs, one common example being repeats of the CA unit (CA)n. Microsatellites are commonly 100-200 base pairs in length, the difference in size between alleles can be as little as one base pair. There are often high levels of allelelic variation at microsatellite loci and they are inherited in a simple Mendelian fashion. This combination of factors, together with their selective neutrality, makes them extremely useful genetic markers. They can be used for estimating relatedness at levels from first order relatives to population differentiation (Bruford and Wayne, 1993). The statistical power of the loci available depends on the number and frequency of alleles within the population being considered, see Chakaborty *et al* (1988), and Morin and Woodruff (1992). Mutational events have been estimated to

occur in the range of 1×10^{-3} to 10^{-5} per generation (Edwards *et al*, 1992).

The small size of the hypervariable regions mean that microsatellites need to be amplified by the polymerase chain reaction (PCR), before being separated by electrophoresis. It is necessary to identify a number of microsatellites and sequence the flanking DNA on either side of the hypervariable region. From these, complementary primers can be designed, which bind to the locus specific flanking sequences during PCR, and start the enzymatic binding of nucleotides in solution to the microsatellite region. Details can be found in Hughes and Queller (1993), and Sampson (1994).

One of the great advantages of microsatellites is that only nanogram quantities of DNA are required, and thus only very small quantities of tissue are needed, and adequate quantities of DNA can be extracted from 0.1 μl of mammalian blood. Faeces have been a useful DNA source where the study species has been difficult to approach directly, e.g. marine mammals (Tikel *et al*, 1996). Hair roots have been used on chimpanzee *Pan troglodytes* (Morin and Woodruff, 1992, Morin *et al,* 1994), brown bear *Ursus arctos* (Paetkau and Strobeck, 1994). Other possible DNA sources include nails and buccal swabs (Bruford and Wayne, 1993). In this study it was necessary to obtain permission from cat owners to collect DNA from their cats; since the success of the project was largely dependent on maintaining the goodwill of cat owners, it was therefore of great importance to employ a method of DNA extraction that caused minimal distress to the cats. If PCR can be performed on DNA extracted from hair roots, it would seem to be the ideal method for this study.

A further advantage of using microsatellites is that alleles can be sized and identified at a given locus, and the information can be stored on a database for subsequent analysis. This makes comparisons between gels relatively straight forward (Morin *et al,* 1994). This is a valuable property in a study such as this one where a large number of individuals are to be profiled, and many combinations of individuals have to be screened for allele sharing.

1.2.6.1. Microsatellites and population genetics

The versatility of microsatellites means there may be the potential to apply them to population genetics questions as well as kinship analysis in the course of this study. If cats are genotyped from different geographic regions it would be possible to test for population sub-differentiation. Microsatellites have been used in this capacity, for example, island populations of red foxes *Vulpes vulpes* (Lade *et al,* 1996) and isolated populations of Canadian polar bears *Ursus maritimus* (Paetkau *et al,* 1995).

High levels of neutering may have led to a reduction in the breeding (effective) population size of domestic cats, and could give rise to an increase in inbreeding. If these effects were severe enough, a measurable reduction in genetic variability might result eventually. A reduction in heterozygosity in microsatellite loci has been demonstrated for cheetah *Acinonyx jubatus*, a species which is thought to have undergone a bottleneck around 10,000 years ago. Also, a sample from a population of Asiatic lions *P. leo persica* , which underwent a severe bottleneck around 100 years ago, showed substantially depressed heterozygosity at all loci (Mennotti-Raymond and O'Brien, 1995). Genetic depletion has been demonstrated by enzyme electrophoresis for the Florida panther *Felis concolor corti* (Roelke *et al*, 1993) and a population of lions living in the Ngorongo crater (Packer *et al*, 1991b). A relationship between minisatellite diversity and population history was shown for lions (Gilbert *et al,* 1991) .

1.2.6.2. Primers for microsatellites in felids

It was fortunate for this project that suitable feline microsatellite primers had been developed at 10 loci (Menotti-Raymond and O'Brien 1995). The microsatellites were well conserved between feline species, and amplified in representative species across feline phylogeny. Preliminary analysis revealed a high level of variability, with an average heterozygosity of 0.77. More recently, hair was used from a domestic cat as a forensic tool in a murder case (Mennotti-Raymond *et al,* 1997a). The potential of cat microsatellites in forensic applications is explored in Mennotti-Raymond *et al* (1997b).

1.2.6.3. The application of molecular markers to mating systems in other animals.

There has been a proliferation of studies in recent years using molecular markers to tackle questions of parentage and kinship. A few examples are described here to illustrate the diverse groups of animals to which the technique has been applied.

Behavioural estimates of reproductive success were evaluated with DNA fingerprinting techniques for the red deer *Cervus elaphus*; a species where males hold harems for variable lengths of time (Pemberton *et al,* 1992). Behavioural estimates were found to be good predictors of relative reproductive success, but did not accurately predict absolute numbers of offspring. In fact some males failed to father any offspring, while others were more successful than behavioural estimates suggested; thus DNA fingerprinting revealed the greater variance in reproductive success. Indeed there is often a substantial difference between number of offspring estimated by field observations and the figures revealed by DNA fingerprinting (Pemberton *et al*, 1992).

Many aspects of mating systems in birds have been investigated: The complex systems of polygynandry used by dunnocks *Prunella modularis* (Davies *et al,* 1992), the relationship between certainty of paternity and paternal investment in reed warblers *Acrocephalus palustris* (Dixon *et al*, 1994), and rates of monogamous and polygynous mating in the great spotted cuckoo *Glamator glandarius* (Martinez *et al*, 1998).

Amos *et al* (1993) used microsatellites to investigate the social structure of long finned pilot whales *Globicephala melas*, males belonging to a group were eliminated as potential fathers of young born into a group. Whales born within a cohort were sired by a number of non-group males, rather than one male dominating mating opportunities.

In a study of free ranging chimpanzees, paternity could be ascertained or excluded in cases where the mother was known, however where the mother was not known it was not always possible to exclude all males as potential fathers (Morin *et al*, 1994).

Other species include grey seal *Halichoerus grypus* (Amos, 1992), green turtle

Chelonia mydas (Fitzsimmons, 1998) and three species of neotropical wasps (Strassmann *et al*, 1998). Zajc *et a*l (1994) isolated microsatellites from domestic dogs *Canis familiaris*, and concluded that they were variable enough to determine parentage even within an inbred pedigree population.

1.3. Possible Effects of High Levels of Neutering on The Southampton Cat Population

Rates of neutering have been rising among domestic cats in recent years. The practice has been encouraged by veterinary surgeons and rescue organisations in order to reduce the number of unwanted kittens. Householders, not wanting to deal with litters, and wanting to reduce their cats' undesirable sexual behaviour, have complied.

High rates of neutering may ultimately have the effect of reducing the size of the population. Alternatively the effects may be more subtle; the number of kittens being born could be enough to maintain the population but these kittens would be the progeny of a reduced number of females (the situation with males is harder to predict, since male reproductive success can be much more skewed than in females). This would mean a reduction in the size of the breeding (effective) population size.

Selection may be taking place if the individuals that remain entire under a regime of high neutering, i.e. those that gain a selective advantage from neutering, did not represent a genetic cross section of the population. There are a number of reasons why a cat could escape neutering. Entire cats could be "lucky" house cats, whose owners happen, for whatever reason, not to neuter them. There is no reason to suppose that these individuals should, on average, differ from the domestic population as a whole. Another category that may largely escape neutering is feral cats. Cats may become feral because:

i. Their owners desert them. In this case some individuals will be better able than others to cope with a feral lifestyle. These individuals would be more likely to survive,

and may be more likely to avoid being re-domesticated.

ii. They choose to leave their domestic environment and become feral. These individuals would be poorly adapted to a domestic lifestyle.

iii. They are born feral.

Feral cats may therefore be different from their pet counterparts; they are individuals, or descendants of individuals that prefer a "wild" lifestyle, or they are selected for the ability to survive as feral cats. Traits that may be important for survival include hunting ability and territory holding ability. In addition, in order to remain feral (and therefore entire), cats must avoid being adopted, i.e. avoid human company.

There are interactions between feral and owned cat populations. Pet cats can become feral, or *vice versa*, and feral kittens can be adopted, often *via* rescue organisations. Another, less obvious, route for gene flow between feral and owned cats is the mating of feral males with domestic females, thereby siring domestic kittens (this gene flow could also occur in the opposite direction).

In summary, it seems reasonable to postulate that feral cats require different behavioural traits to domestic cats, and behavioural traits are genetically mediated to an important extent. So it possible that this has led to some genetic differentiation between feral and owned cats, and that high neutering among the domestic population will hand a selective advantage to the "wilder", and less friendly, feral cats.

This reasoning leads to the hypothesis that increased rates of neutering amongst the owned population will lead to an increase in the ratio of entire feral males to entire owned males. This may have lead to an decrease in pet kittens being sired by domestic males which, given the heritability of temperament, could have effects on the temperament of domestic- born kittens.

1.4. Aims of this project

1. To investigate the effects of high rates of neutering on cat population dynamics and population genetics, with particular reference to the population genetics of cat temperament.

2. To investigate the mating system of the domestic cat population in Southampton.

Knowledge of the population structure and mating system is of theoretical interest in its own right. However, the aims are interlinked; knowledge of the mating system is needed for a full understanding of any shifts in population genetics that may be occurring, and to predict how these trends may be continued in the future.

Ultimately, it was hoped to establish how reproductive success was distributed amongst male cats in an urban environment. It might be possible to assign paternity to some kittens, if entire males can be located. Where paternity cannot be assigned, it might still be possible to identify litters that share the same father. If there is a measurable differential in male reproductive success, ie some males can be identified as having sired a large number of kittens, then reproductive success of males could be compared to the temperament of their offspring. Alternatively, if reproductive success is more evenly distributed among males the population level effects of neutering on temperament could be tested by comparing the temperaments of litters born in areas of high and lower neutering. In either case, it would then be possible to test the hypothesis that, in an urban environment with the present neutering regime, males with an unsociable temperament (to humans) have a selective advantage.

These aims were pursued through the following lines of research:

- Door to door surveys were carried out in the Southampton area to establish cat population density and population dynamics **(Chapter 2)**.

- A sample of kittens from the Southampton population was recruited for:

  i. Assessment of temperament.

  ii. Use of molecular markers to establish paternity of as many kittens as possible.

  (Chapter 3)

- Tom cat behaviour was directly recorded by radio-telemetry to establish home ranges, and the maximum range available to toms for mating behaviour (**Chapter 4**).

- Allocation of paternity and sharing of paternity between litters was tested for by use of molecular markers (microsatellites) (**Chapter 5**).

- Temperament of kittens was measured through tests carried out during house visits, and comparisons were made between areas with high and low neutering (**Chapter 6**)

- These findings are considered as a whole (**Chapter 7**)

2 Population Dynamics

2.1. Introduction

Population dynamics, population densities and levels of neutering form an important component in understanding the structure of any cat population. It was also necessary, as a background for designing the rest of this project, to obtain data on how these parameters vary between areas, and how they may have changed over time.

There is limited recent information on cat ownership and neutering rates. In Manhatten, New York, USA, mailed questionnaires were used to census a random sample of the population; this revealed that 59% of females were neutered in a population of 0.51 cats/household (Nassar and Mosier, 1982). A more recent study, using telephone surveys, in St. Josephs county in the USA (Patronek *et al*, 1997), showed neutering amongst adult cats at 79.8%. In Australia (Anon, 1994), overall neutering rates of 93.6% of adult cats led to a 10% decrease in the overall numbers of pet cats between 1993 and 1994 to 0.36 cats/household.

It as been estimated that in 1989 in the UK overall, 21.5% of households owned cats, and these households contained a mean of 1.5 cats each (Tabor, 1983; cited in Chipman, 1990). The most recent available data from a British cat population comes from a survey conducted in Manchester Chipman (1990). Here it was found that 59 of 78 males were neutered, at less than one year old in 51 of 54 known cases. Seventy two of seventy four females were spayed, although 31 of these were allowed to produce one litter first. This survey was carried out in 1989. There is a paucity of recent information on British domestic cat populations, and it is evident that figures are likely to vary between regions and over short spaces of time.

The aims of the work described in this chapter are to:

i. Obtain detailed information on the structure of the domestic cat population in and around Southampton, and how it varies from one area to another.

ii. Use retrospective analysis to identify any recent trends in neutering rates and population dynamics.

Door to door surveys were decided to be the best technique for obtaining comprehensive data within a geographic area. This would maximise the proportion of residents interviewed, and would be quicker than sending out questionnaires. Door to door questioning allows an area to be rapidly and systematically surveyed.

2.2. Shirley Cat Survey

2.2.1. Introduction

The object of this survey was to provide comprehensive information on domestic cat ownership, levels of neutering and reproduction within a defined area of Southampton.

The survey was carried out in the Shirley area of Southampton (see **Fig 2.1.**), bounded by Languard Rd, Hill Lane, Stafford Rd, Malmesbury Rd and Raymond Rd; comprising a total area of 54ha. The site contained a total of 1175 residences of which 1079 (91.83%) were houses, 89 (7.57%) were flats, and 7 (0.06%) were rest homes. The houses were a mixture of terraced, detached, and semi-detached.

2.2.2. Methods

2.2.2.1. Procedure

The survey was carried out systematically on a door to door basis. The most efficient time of day for data collection was found to be between 5pm and 7pm.
If there was no reply, the residence would be tried again approximately one week later. It was found from experience that if the door was not answered on the second attempt, there was little chance of success on subsequent visits. I therefore made a maximum of 2 visits per residence.

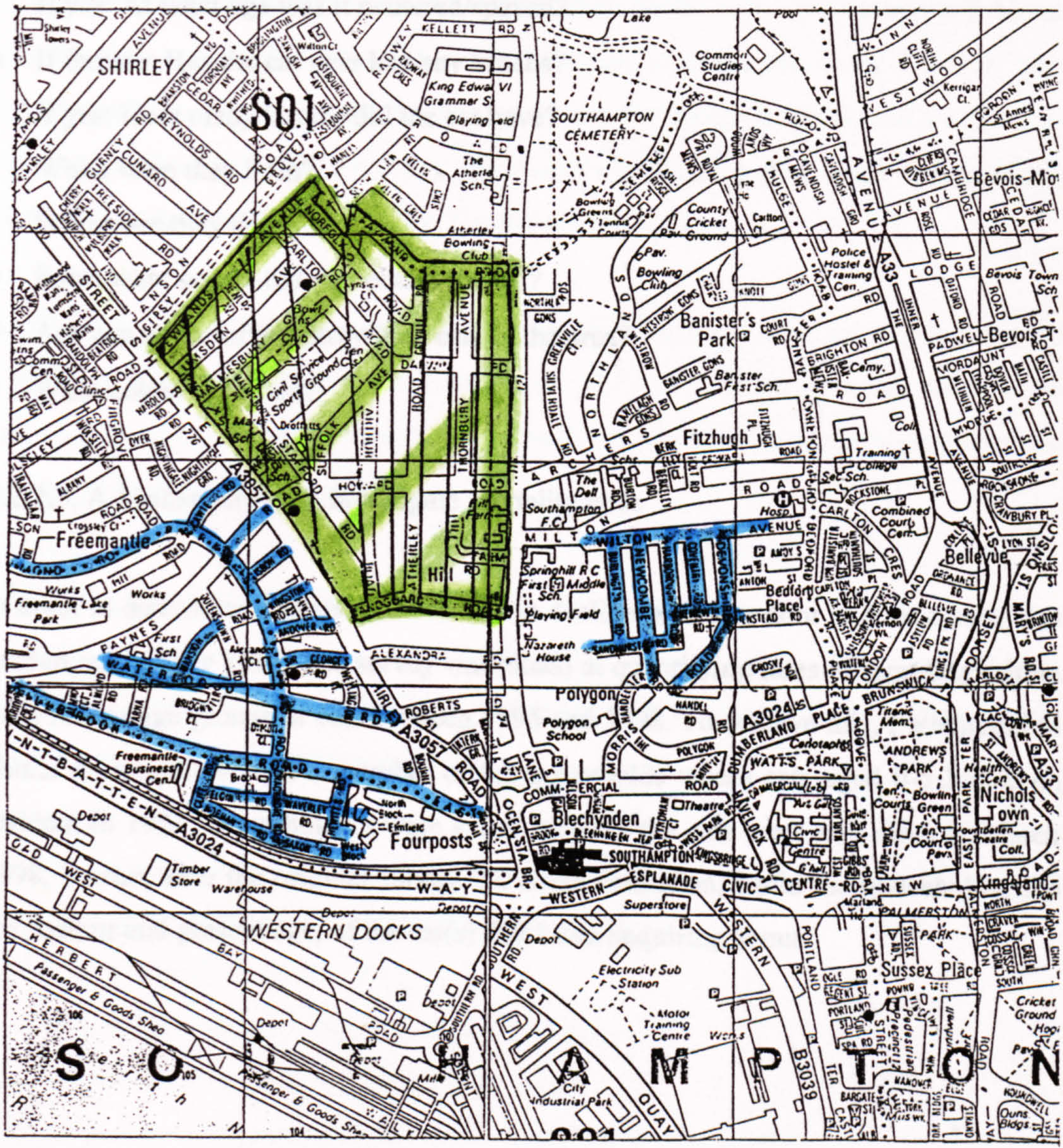
Fig. 2.1. Area covered by:

i. Main Shirley survey

ii. Freemantle/Polygon area survey

Area with relatively low neutering rates

From A to Z street atlas, scale 1: 15,840, 4 inches to 1 mile.



2.2.2.2. Questions

Each householder was first asked if they owned any cats. If they did they were then asked the following questions about each of their cats:

- What sex is the cat?
- What age is the cat?
- Has it been neutered/spayed?

  If yes: At what age was it neutered/spayed?
- If female: Has the cat ever had any kittens?

  If yes: How many kittens did the cat have?

  When were they born?

  Where are they living now?
- From what source did you obtain the cat?
- Are you aware of any stray/feral cats in the area?
- What colour is the cat?

2.2.2.3. A further survey to investigate mortality rates

In order to determine if the population was in decline, a further door to door survey was carried out in May 1998. The survey was aimed at quantifying rates of mortality within the younger age groups of cats between 1995 and 1998. To achieve this, I attempted to obtain an interview with a member of the households where cats aged 0-5 had been resident in 1995. Householders were asked whether the cats were still alive in March 1998, 3 years after the original survey. To avoid confusion, I told the householder the sex, colour and present age, of the cat(s) that I was enquiring about.

2.2.2.4. Analysis

Calculation of smoothed age distributions and life tables

A life table was calculated using the age distribution of the cats in the survey. Life tables give the expected numbers of survivors, at each age category, for a hypothetical cohort of individuals born at the same time within a population. Logically, the frequencies of individuals within a cohort of age X must always be greater than those of age X+1 to avoid the impossibility of negative death rates. Ecological data do not always give age distributions that conform to this requirement. Here the data were transformed in order to meet the preconditions for calculating life tables: the age distribution was smoothed using log polynomials to the power x^2 prior to calculation of the life table, as recommended by Caughley (1977).

A life table constructed from a standing age distribution makes the assumption that the population structure is stable, and is therefore of limited value. However some crude information about the population can be gained, and comparisons between populations can be made (see Caughley, 1977).

Assigning an area of origin to cats

The areas that the cats originated from, or the places their owners obtained them from, were divided into the following categories;

1. Cats obtained directly from litters in private houses (including own house). This category was subdivided into geographic areas as follows:

i. Cats born within the Shirley area.

ii. Cats born in Southampton, but outside of Shirley.

iii. Cats born in rural areas (including farm cats).

iv. Cats born in urban and suburban areas outside of Southampton

2. Strays adopted directly by the householder

3. Cats bought from shops.

4. Pedigree cats.

5. Cats obtained from rescue centres

2.2.3. Results

Interviews were eventually obtained for 949 (80.8%) of the 1175 residences in the survey area. A total of 315 cats (0.331 cats per residence) were owned in 218 (22.97%) of the residences. Twenty one of the cats in the survey had a pedigree; it was decided to consider these separately because their breeding is kept under strict control, and they are largely prevented from breeding with the cross-breed population by their owners. Of the 295 cross bred cats, 134 were male and 160 female, and one was of unknown gender.

2.2.3.1. Age distribution and life table of cats from the Shirley survey

The age distribution of cats from the survey and the life table derived from it (**Table 2.1.**) gave no indication that the population was in decline. The age distribution obtained here (**Fig. 2.2.**) was of a shape frequently associated with stable mammalian populations (Caughley, 1977). The only available mortality figures for a cat population come from work in Manhatten, New York (Nasser and Mossier, 1982), which showed much higher mortality amongst young cats than was calculated here. The survival rates from 0-5 years were 56% (Manhatten) compared to 80.5% (Southampton). If the data from the static age distribution was misleading, and mortality rates in Southampton were comparable to those experienced in Manhatten, the age distribution obtained here would indicate that the population was declining.

Table 2.1. Age Distribution of Shirley domestic cat population, calculated using smoothed age distribution calculated from static age distribution

Age	Sampled Frequency	Smoothed Frequency	Survival lx	Mortality dx	Mortality Rate qx	Survival Rate
0	31	26.2	1.000	0.031	0.031	0.969
1	19	25.4	0.969	0.038	0.039	0.961
2	32	24.4	0.931	0.038	0.041	0.959
3	21	23.4	0.893	0.042	0.047	0.953
4	21	22.3	0.851	0.046	0.054	0.946
5	14	21.1	0.805	0.038	0.047	0.953
6	18	20.1	0.767	0.046	0.060	0.940
7	15	18.9	0.721	0.046	0.063	0.937
8	23	17.7	0.676	0.053	0.079	0.921
9	17	16.3	0.622	0.053	0.086	0.914
10	25	14.9	0.569	0.065	0.114	0.886
11	10	13.2	0.504	0.046	0.091	0.909
12	13	12.0	0.458	0.053	0.117	0.883
13	11	10.6	0.405	0.073	0.179	0.821
14	8	8.7	0.332	0.062	0.187	0.813
15	5	7.1	0.270	0.056	0.208	0.792
16	7	5.6	0.214	0.073	0.339	0.661
17	1	3.7	0.141	0.065	0.459	0.541
18	3	2.0	0.076			

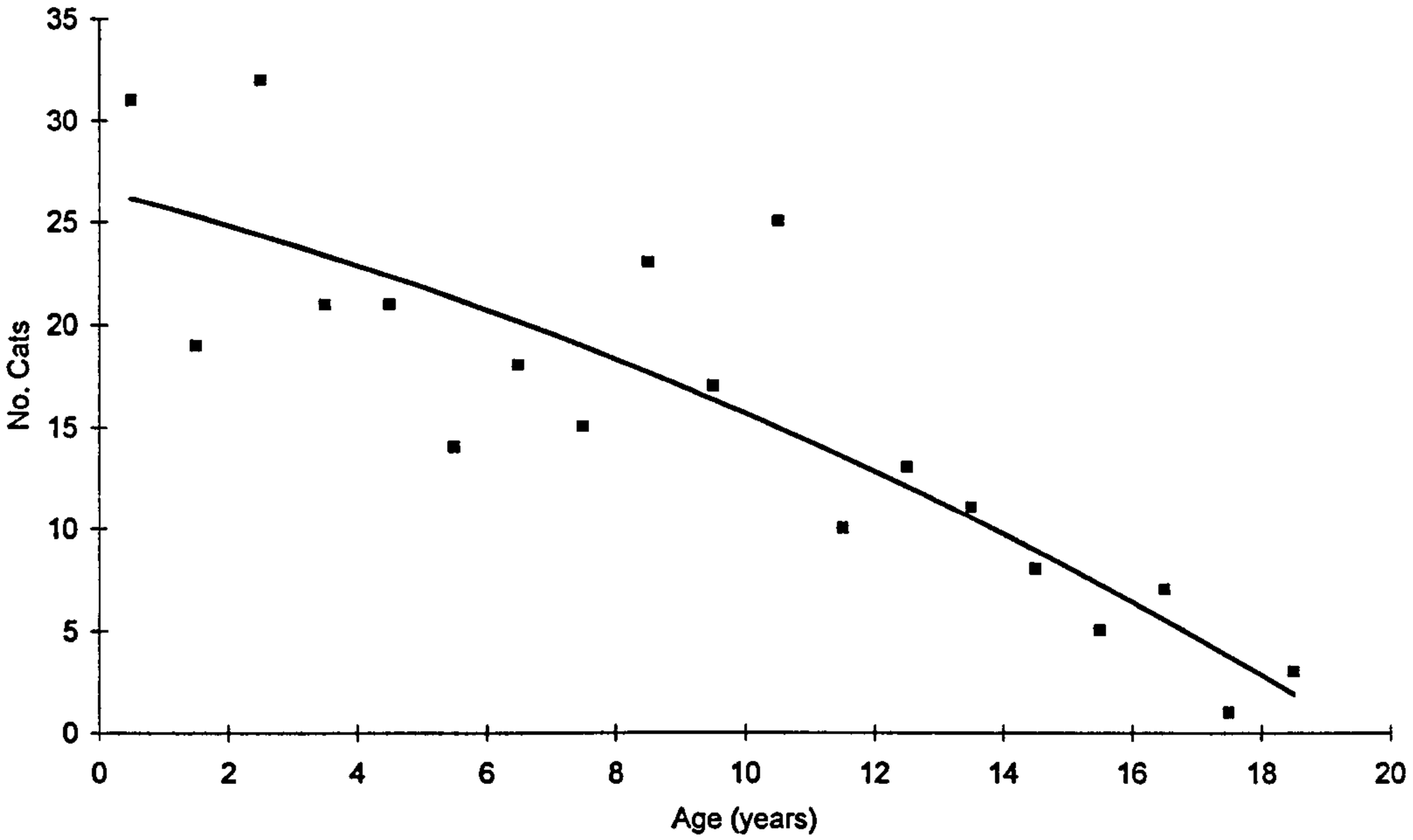


Fig. 2.2. Smoothed age distribution of cats from Shirley survey, calculated using log polynomials to the power x^2

2.2.3.2. Survival rates and trends in population size

In the further surveys carried out in May 1998 to assess mortality rates I attempted to interview a householder from all the 86 residences that had homed the 138 cats aged 0-5 from the original survey. Interviews were obtained for 69 (80.2%) of the households. Of these, the householders were the same people as in the original survey in 62 (89.9%) of the residences, while the remaining 7 householders (10.1%) had moved house. The 62 households (72.1%), for which repeat interviews were obtained, accounted for 92/138 (63.8%) of the cats aged 0-5 years in the original survey. Of these 4 (4.3%) were reported to have disappeared, these were excluded from the analysis because it was not known whether they had died, moved to a different household or even become feral. The mortality data was therefore calculated from a sample of 88/134 (63.7%) of the original sampled population.

<u>Predicting the numbers of survivors in each age group</u>

The numbers of cats in each age class in 1995 were converted into the predicted number of survivors in 1998 using the survival rates derived from the life table (**Table 2.1.**). The survival rates (calculated from the static age distribution) for the next three yearly age increments were applied to the 1995 figures, hence:

$$F(x+3)_{1998\ predicted} = F(x)_{1995} * P(x) * P(x+1) * P(x+2)$$

Where F(x) is the number of cats at age x, and P(x) is the survival rate from age x to age x+1.

The total numbers of predicted survivors were converted to the predicted numbers of sampled survivors by multiplying by the fraction of cats in each age group for which 1998 data was obtained.

Testing the stability of the population

The predicted (column 8) and actual (column 6) numbers of surviving cats are compared in **Table 2.2.** The close statistical similarity of the two distributions is strong evidence that the age distribution of 4-9 year old cats in March 1998 was similar to the distribution of these age groups in 1995 (see **Fig. 2.3**). Assuming that age dependent mortality has remained constant, this implies that recruitment in the years 1990-1994 was equivalent to recruitment in the years 1987-1990. The population has therefore remained stable over between the late 1980's and early 1990's despite the recent increases in neutering (see below). It is possible that the population may have declined prior to 1987, but has since stabilised.

This analysis also shows that mortality rates amongst the younger cats in the Shirley population are much lower than in the Manhatten, New York population (Nasser and Mossier, 1982).

Table 2.2. Proportion of cats from year groups 0-5 in 1995 survey that were re-surveyed in 1998, and survivorship rates between 1995 and 1998 for this sample.

Age in March 1995 (years)	Age in March 1998	Number of cats (1995)	Cats accounted for (1998)	% resampled (1998)	No. cats alive in 1998	Survival rate (1995-1998) %	Predicted number of sampled survivors	Predicted survival rate (%)
0-1	3-4	31	21	67.7	17	81.0	18.75	89.3
1-2	4-5	19	16	84.2	14	87.5	14.05	87.8
2-3	5-6	32	17	53.1	14	82.4	14.69	86.5
3-4	6-7	21	17	81.0	17	100	14.61	85.9
4-5	7-8	21	7	33.3	6	85.7	5.93	84.8
5-6	8-9	14	10	71.4	9	90	8.39	83.9

Fig. 2.3. Predicted number of surviving cats based on 1995 survival rates (white bars) and actual number of surviving cats (dark bars) resurveyed in 1998. There were no significant differences between the two distributions ($\chi^2 = 1.55$, d.f. = 5, $p > 0.05$)

2.2.3.3. Density

The 294 cross bred-cats located in the 80.8% of residences where interviews were obtained gives an overall estimate of 364 cross bred cats in an area of 54 hectares. Giving an overall density of 6.74 cats per hectare.

There were a minimum of 4 entire males in the survey site (see below). The entire males were therefore living at a minimum density of 1 per 13.5 hectares. The two identified entire females lived at a density of 1 per 27 hectares.

2.2.3.4. Neutering

Cats are not usually neutered until they are 5 or 6 months old, and are not sexually active until about 9 months old. Of the 124 males over 9 months old, 120 (96.8%) had been neutered. Of the 148 females over 9 months old, 146 (98.7%) had been neutered (or were kept permanently indoors, in the case of two females).

2.2.3.5. Reproduction

An overwhelming proportion of the cats was neutered. However, many cats had the opportunity to reproduce before being neutered. It was possible to quantify lifetime reproduction of females from this survey. For males the information was much less comprehensive, it only being possible to quantify for how long a male had been entire while also potentially sexually active.

<u>Females</u>

Trends in lifetime female reproduction were investigated by calculating the number of litters produced per female for each age group (year of birth) retrospectively for 18 years, the age of the oldest female in the survey. The number of females in each year group declined with age, so for females born before 1987, year groups were combined to give comparable numbers of cats in each age group.

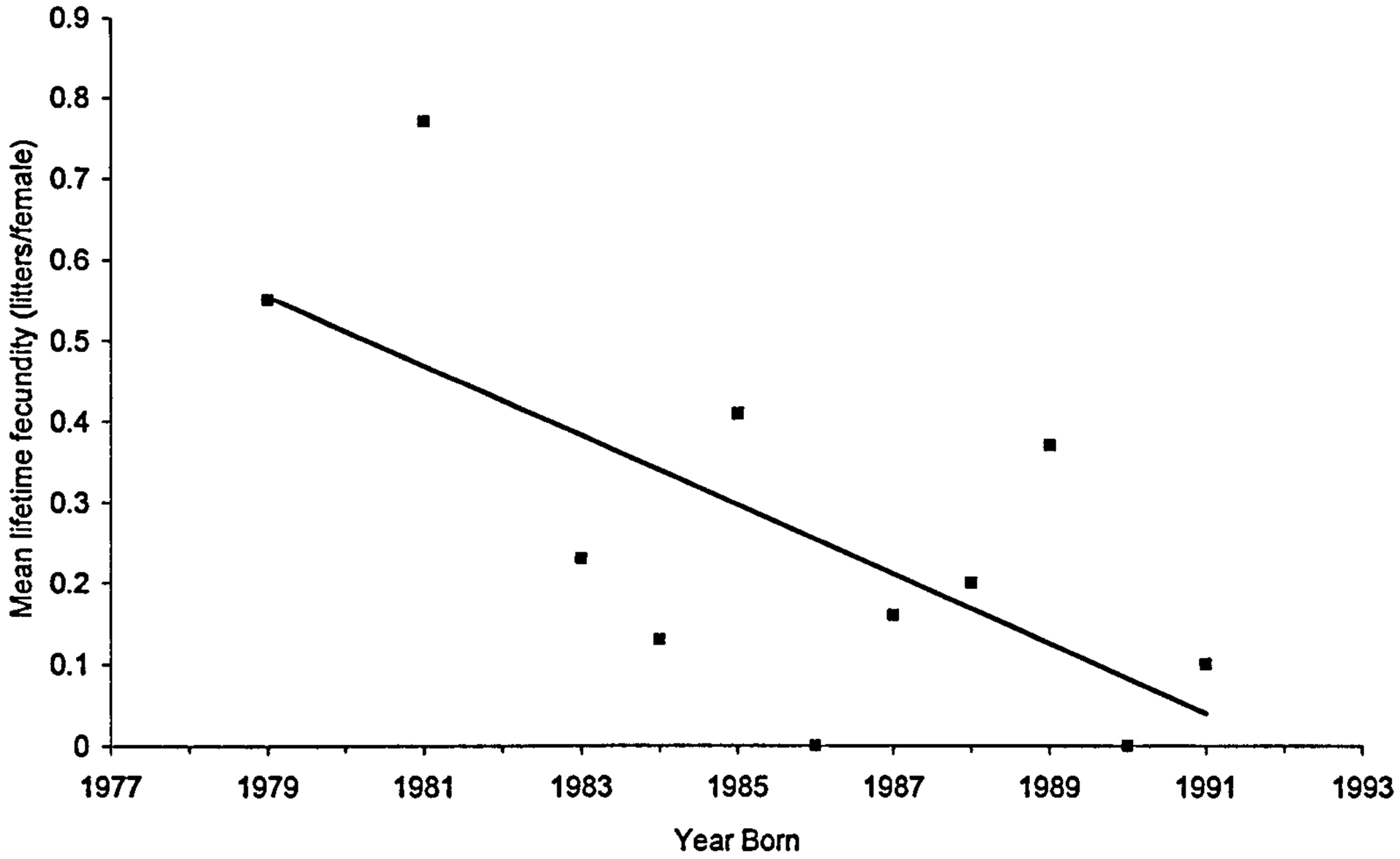
It was not possible to state lifetime reproduction for females that were still entire, and potentially able to have more litters. No entire females over 3 years old were found in the survey, so only females of 3 years upwards were considered in this analysis. Many cat owners were not able to remember the exact numbers of kittens in each litter, but almost all were confident of the number of litters that their cats had produced. Number of litters per lifetime, rather than estimated number of kittens, was therefore used as the measure of fecundity.

Linear regression analysis was performed to investigate the effect of year of birth on the mean lifetime number of litters produced per female (**Fig. 2.4.**). This revealed a significant decline in fecundity ($p=0.021$) from 0.58 litters/female in 1979 to 0.07 in 1991.

The mean number of kittens/litter, where the information was available, was 4.09. Assuming equal sex ratios, the population would require about 0.5 litters/female to be self-replicating; this would give lifetime fecundity figures of approximately one female per female. Females in the survey passed below this threshold in 1981, and by 1995 were able to produce only a fraction of the kittens needed to keep the population stable.

This method of analysis made the assumption that having kittens did not affect the life expectancy of the mother. However, it seems likely that, if there is any effect, females that had kittens would have a lower life expectancy (Tabor, 1989), and would therefore be under-represented in the older age groups.

One householder was aware of one feral female (not included in the analysis), who had produced 2 litters per year for at least the last 5 years, before finally being neutered. The kittens were all adopted via rescue organisations. This example illustrates how much the odd female can contribute to the cat population when reproduction has been prevented in the majority of females.



Year(s) born	1977-1979	1980-1981	1982-1883	1984	1985	1986	1987	1988	1989	1990	1991
Number of females	9	9	13	15	12	12	6	10	8	11	16

Fig. 2.4. Lifetime fecundity of female cats in Shirley survey, by year of birth. Linear regression revealed a significant decline in fecundity ($F = 7.74$, d.f. = 1,9, $p = 0.021$)

Males

There were a total of 11 males (8.9%) that had been entire over the age of 9 months, and 7 of these had subsequently been neutered. This was too few to analyse for trends in neutering of males, particularly as entire males are probably the most vulnerable to deaths due to road accidents etc. because of their greater tendencies to wander.

It is interesting to note that 7 of these were males that had previously been stray or feral, including one still semi-feral that was fed regularly by a household. A few householders thought that there might be feral males in the area.

2.2.3.6. Origins of cats from Shirley survey

The survey results were analysed to find out where the cats in the population had originated, and investigate any trends that may have occurred alongside the decline in kittens being born in the area. A region of origin was categorised for 259 (82.2%) of the cats in the survey (**Table 2.3.**), the owners of the remaining cats were unable to tell me where their cats had been obtained.

There has been some change in the origins of the cats in the survey; a simple regression analysis revealed a significant increase in the proportion of cats from shelters ($p<0.05$). There has also been an increase in the number of cats from other areas of Southampton when the 0-4 year age group is compared to the 10+ age group ($\chi^2=3.86$, $p<0.05$, 1df). However the proportion of "immigrant" cats (those not born in the Shirley area) has been consistently high (around 75%).

Table 2.3. Origins of cats from Shirley survey, divided into three age groups.

Age	0-4	5-9	10-	Total
Shirley area	29 (25.2%)	21 (26.9%)	17 (26.2%)	67 (25.8%)
Southampton	14 (12.2%)	7 (9.0%)	2 (3.1%)	23 (8.9%)
Rural Areas	10 (8.7%)	9 (11.5%)	12 (18.5%)	31 (12.0%)
Urban Areas	8 (7.0%)	9 (11.5%)	3 (4.6%)	20 (7.7%)
Stray	12 (10.4%)	11 (14.1%)	7 (10.8%)	30 (11.6%)
Shop	7 (6.1%)	6 (7.7%)	9 (13.9%)	22 (8.5%)
Pedigree	12 (10.4%)	2 (2.7%)	7 (10.8%)	21 (8.1%)
Shelter	23 (20.0%)	14 (18.0%)	8 (12.3%)	45 (17.4%)
Total	115 (40.4%)	79 (30.5%)	65 (25.1%)	259

2.2.4. Discussion of Shirley survey

The overall picture is of a population with high levels of neutering, and therefore little reproductive activity. Female reproduction, controlled by neutering, has declined over the last 16-18 years to extent that the population in 1995 no longer contained enough entire females to sustain itself at its present levels. However, the life table and mortality data indicate that the population is stable.

The key to sustaining the population at its present level is immigration. Over the time covered by the survey, around 65% of the area's cats have been actively introduced to the area by humans. Immigrant cats, as a proportion of the population, have remained constant while neutering levels have increased. It would be interesting to examine trends in human-mediated emigration of cats over the same time period, to investigate

whether this has declined as the surplus of cats in the area has diminished. Shelters have become increasingly important as a source of cats to the area, providing 20% of the population in the 0-4 year age group.

The maximum male home range recorded for a similar urban environment was about 6 hectares (Chipman, 1990), well under the 13.5 hectares (total area = 54 hectares/4 entire males found) available to each entire male identified in this survey. The number of feral cats in the area is unknown. On this evidence, the level of competition between males for mating opportunities with the few remaining breeding females would be expected to be low. However, there is the possibility that males may be able to utilise much larger areas of space in urban environments than has so far been shown (see **Chapter 4**).

2.3. Door to door surveys in other areas

As explained, the maintenance of the cat population in the Shirley area is heavily dependent on immigrants. This raises the question of what is the status of the cat population in other areas; where, if anywhere, are enough kittens being born to supply areas with very high rates of neutering? Thus further door to door surveys were carried out in other areas. These were not as comprehensive as the main Shirley survey, and no attempt was made to do any further blanket surveys. However, samples were taken in a number of areas to gain a general idea of the state of their cat populations.

Three demographically distinct areas were chosen for these surveys to represent the range of housing types in and around Southampton:

i. A suburban area, just north of Eastleigh.

ii. Villages in the Romsey area, about 12 miles away from Southampton.

iii. Southampton, within and close to the area of Freemantle.

In all three areas the same questions were asked as in the main survey area (see methods).

2.3.1. Eastleigh Suburbs Survey

This survey took place in a suburban area outside Southampton, just north of Eastleigh. An affluent area, containing mainly quite large houses with sizeable gardens. To the south of the survey area there was more housing, farmland lay less than a kilometre to the north.

2.3.1.1. Results

153 householders were interviewed, reporting a total of 56 cats (0.37 cats per household) in 47 households (30.7% of households). There were 32 males and 24 females and all were neutered.

Reproduction

Only two of the cats in this survey had reproduced; one 10 year old female had 2 litters, totalling 9 kittens, 8 and 9 years previously, one 14 year old female had two litters, 12 years previously.

Age distribution

The age distribution for this population is shown in **Fig 2.5.** An age distribution of this type, where there are fewer individuals in young age groups than older age groups, is strongly indicative of a population in decline (Caughly 1977), although it should be remembered that the sample size was small (N= 56).

Cat Origins

Origins were identified for 52 (92.9%) of the 56 cats in the survey (**Table 2.4.**). The proportion of cats obtained directly from pet litters (rather than through shelters, shops or adopted strays) was significantly lower in the 0-4 age group than in cats from the other two age categories combined ($p<0.05$, $\chi^2=4.77$, 1df). In the same time span, the proportion of shelter cats had significantly increased ($p<0.01$, $\chi^2=7.99$, 1df).

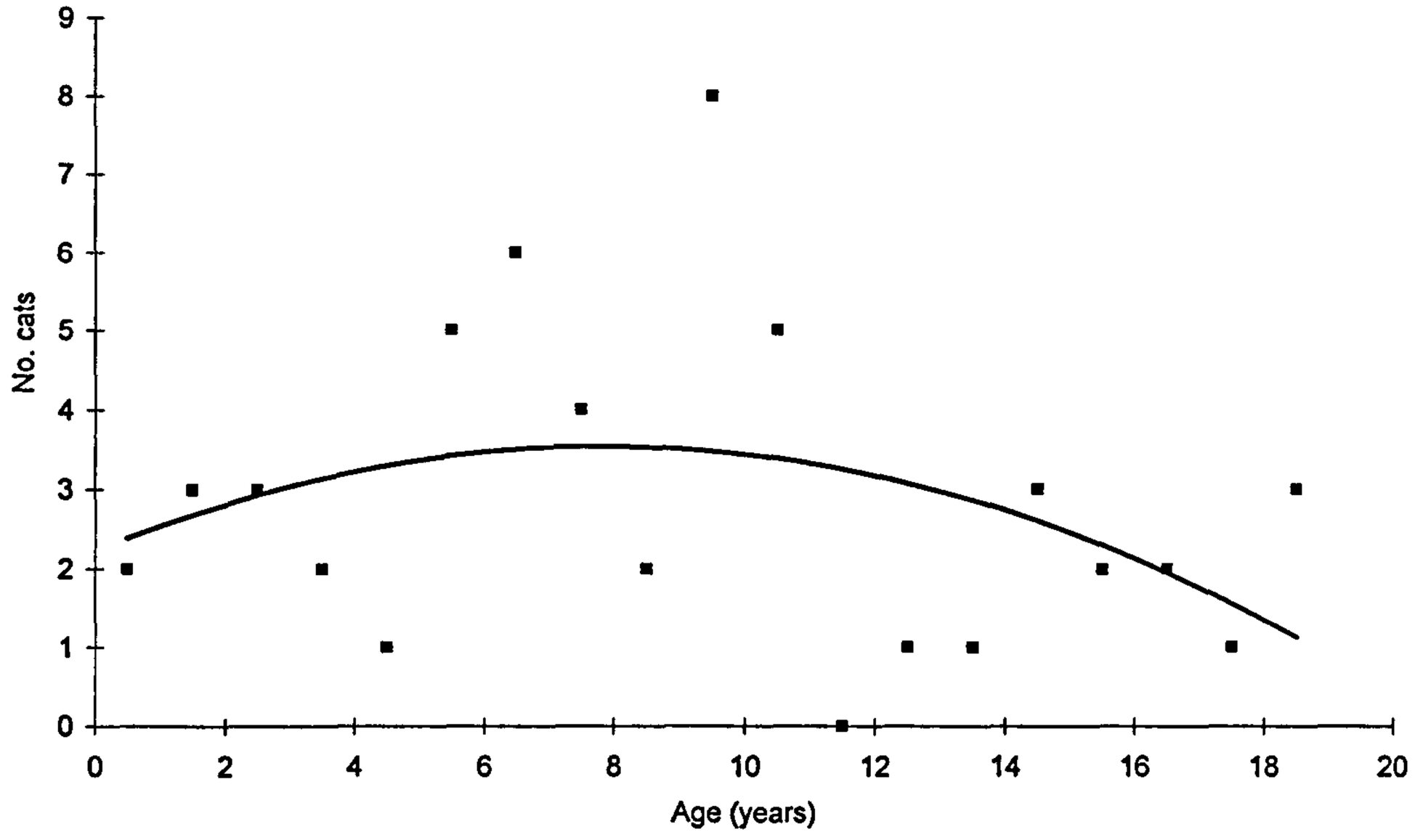


Fig. 2.5. Smoothed age distribution of the Eastleigh cat population, calculated using log polynomials to the power x^2.

Table 2.4. Summary of cats origins from Eastleigh- Suburbs population

Age	0-4	5-9	10-	Total
Local area	1 (9.1%)	10 (41.7%)	2 (11.1%)	13 (25.0%)
Southampton	0 (0.0%)	4 (16.7%)	1 (5.6%)	5 (9.6%)
Rural Areas	0 (0.0%)	2 (8.3%)	3 (16.7%)	5 (9.6%)
Urban Areas	1 (9.1%)	2 (8.3%)	3 (16.7%)	6 (11.5%)
Stray	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0%)
Shop	0 (0.0%)	1 (4.2%)	3 (16.7%)	4 (7.7%)
Pedigree	1 (9.1%)	2 (8.3%)	0 (0.0%)	3 (5.8%)
Shelter	8 (72.7%)	4 (16.7%)	4 (22.2%)	16 (30.8%)
Total	11 (21.2%)	25 (48.1%)	16 (30.8%)	52

2.3.1.2. Summary

These results indicate a cat population that is declining. No cat in the survey under 10 years old had ever bred, and only 1 cat of the 11 under 5 years old had been born locally. Residents who want cats now appear primarily to turn to rescue organisations.

2.3.2. Village Survey

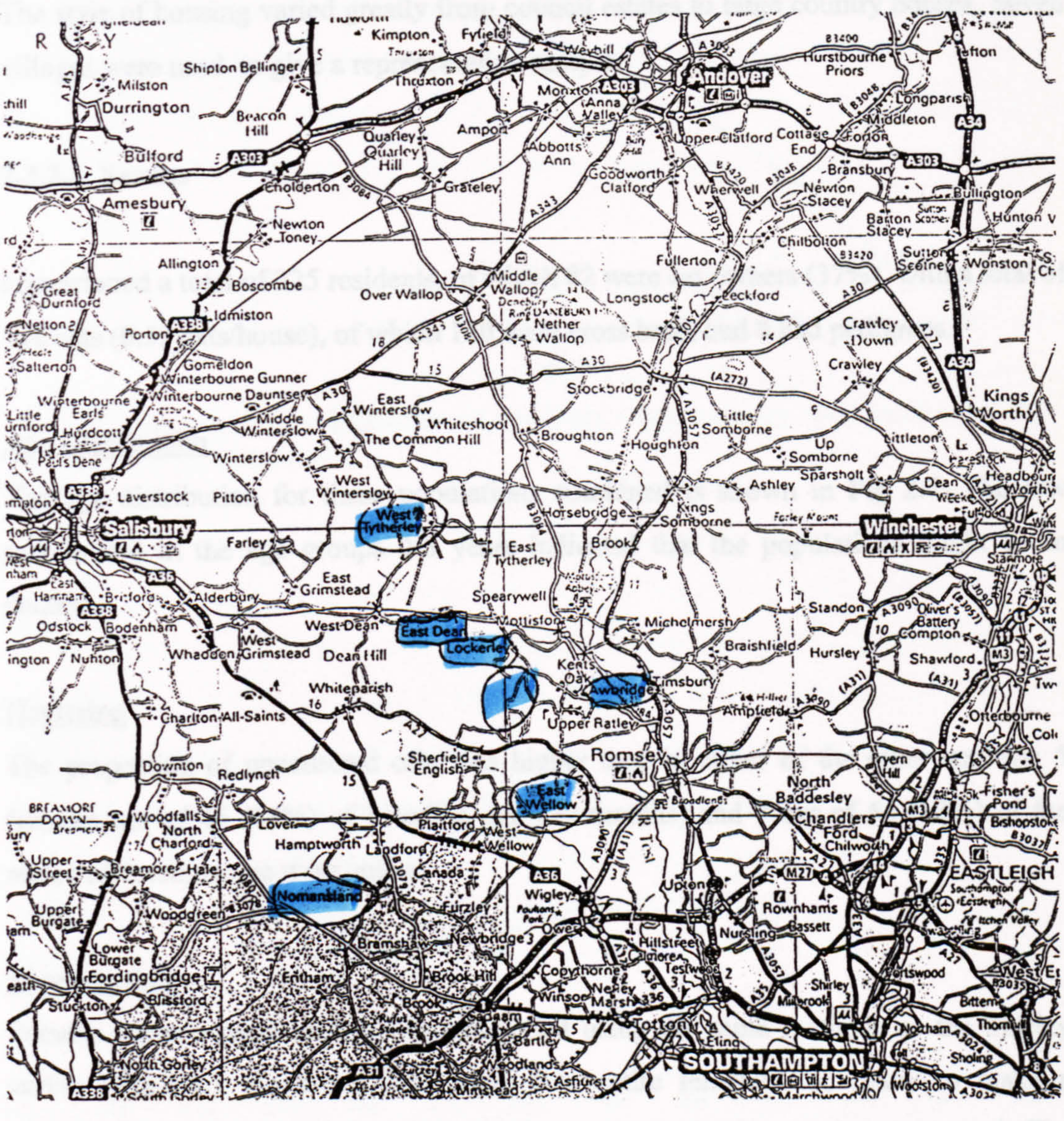
Having sampled the cat population in the leafy suburbs close to Southampton, and found it to be very non-productive, I decided to investigate the more traditional rural areas, outside the commuter belt. There are a number of villages in the countryside West of Romsey see fig.2, about 12 miles outside Southampton (see **Fig. 2.6**). Seven of these were each the subject of this survey:

Awbridge

East Wellow

Fig. 2.6. Locations of surveyed villages

From AA road atlas of Great Britain
Scale 1: 250 000



East Deane

Nomansland

Dunbridge

West Titherly

The style of housing varied greatly from council estates to large country houses. Seven villages were used to give a representative sample.

2.3.2.1. Results

I questioned a total of 225 residents, of which 82 were cat owners (37%), with a total of 118 cats (0.52 cats/house), of which 110 were cross bred, and 8 had pedigrees.

Age Distribution

The age distribution for these populations combined is shown in **Fig 2.7.** The low recruitment in the age groups 0-3 years indicated that the populations might be in decline.

Neutering

The proportion of unneutered cats was higher than in either of the other surveys; 5 females out of 46 (11%) of breeding age (>9 months) and 7 out of 54 (13%) of the males of breeding age were entire.

Reproduction

Female: Female reproduction was quantified using the same methods as the Shirley survey (**Fig.2.8.**). However, there were five entire females: too many to make a reasonable estimate of lifetime reproduction, so reproduction to date was used. The fecundity figures are therefore likely to be underestimates for the younger age groups of cats. If one considers lifetime reproduction to date, there has been no significant change over the last 13 years.

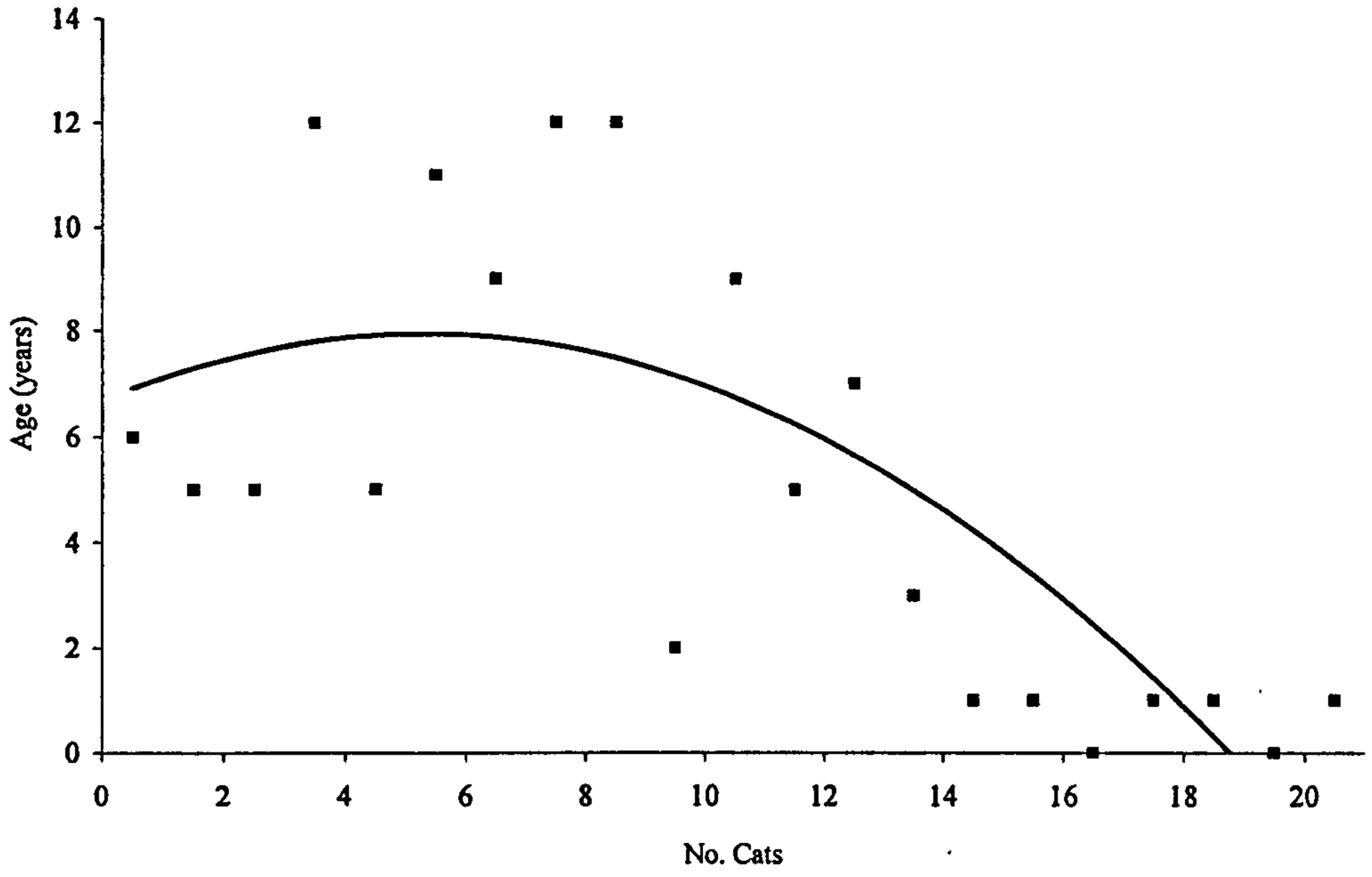
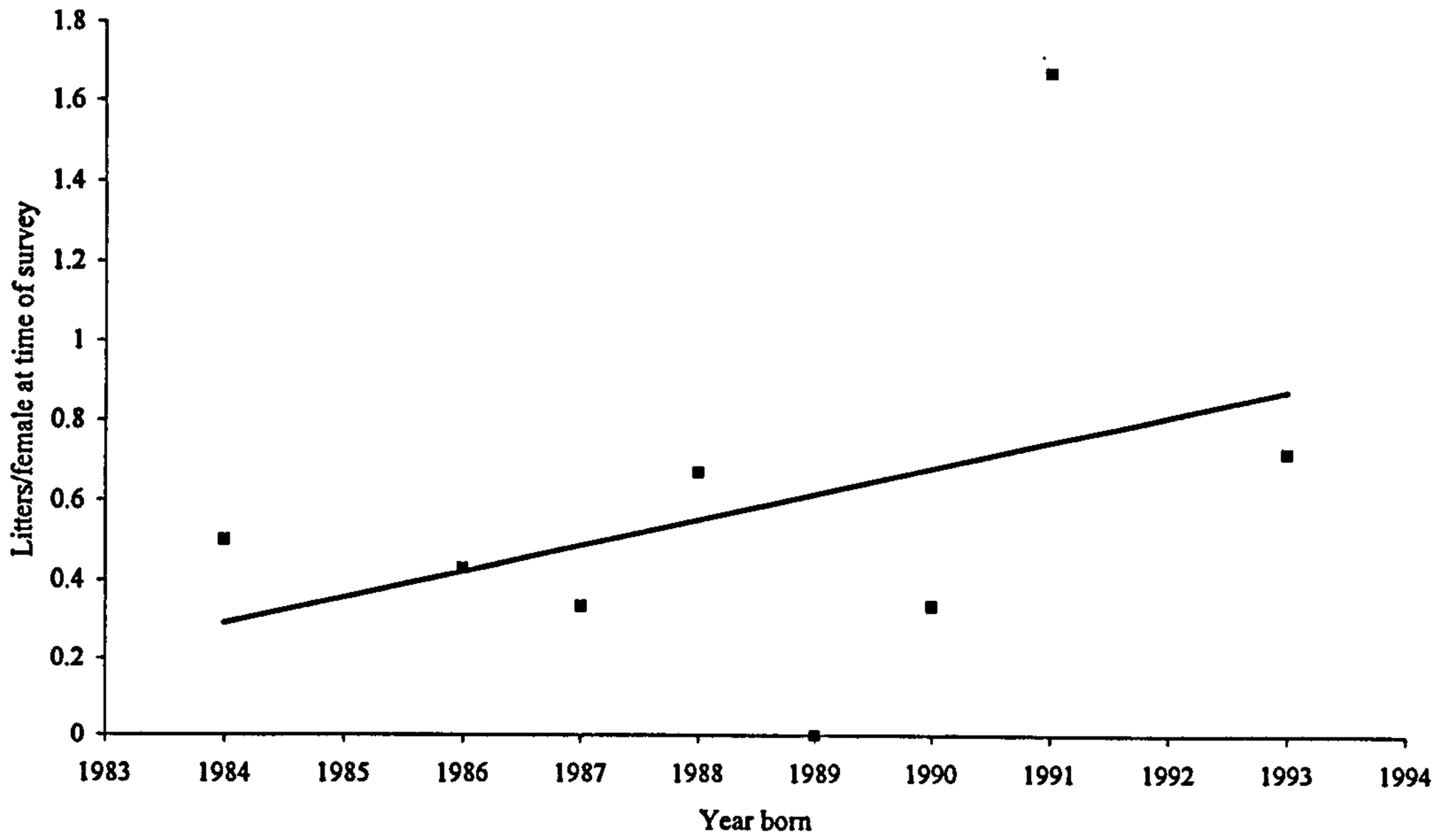


Fig. 2.7. Smoothed age distribution of cats from villages survey, calculated using log polynomials to the power x^2.



Year(s) born	1983-1984	1984-1985	1986	1987	1988	1989	1990	1991-1992
Number of females	4	6	6	6	5	6	3	7

Fig. 2.8. Fecundity to date of females in Villages survey, by year of birth. Linear regression: ($F = 0.91$, d.f .= 1, 6, $p > 0.05$.)

The overall mean number of litters per female was 0.459 and the average litter size was 4.0. Assuming equal sex ratios, these data indicate that the village cat populations are, overall, bordering on being self-replacing. The number of female births per female was at least 0.459x4/2 =0.918. The figure could be higher than this if the remaining entire females do breed.

Female reproduction is heavily skewed towards a few individuals; 15 out of 50 females (30%) have had, or are likely to have in the future, the chance to reproduce. Of 23 litters born, 9 were the offspring of just two females (one of which was still entire at the time of the survey).

Seven adult males (13%) were entire. In addition, a further 3 cats had been entire during their adult lives, so that 18.5% of the males were potentially reproductive at some stage.

Density

A complete census of houses was not taken during this survey. However, some information was obtained from the Hampshire Census (1991). This revealed that Dun Valley ward; which overlapped with the North of the survey site, contains 777 households within an area of 49 km^2. If one applies the survey's finding of 0.5244 cats/household, this gives a total of 408 domestic cats in the area. Assuming equal sex ratios, and applying the proportion of entire cats found in the survey (11% female, 13% male), gives an estimated 22.5 entire females, and 26.5 entire males; an average of almost one entire domestic male per 2km^2.

Origins

No significant changes in cat origin with age were found (**Table 2.5**).

Table 2.5. Summary of origins of cats from villages survey, where known, divided into three age groups.

Age	0-4	5-9	10-	Total
Local area	11 (32.3%)	17 (46.8%)	10 (40%)	38 (40.4%)
Southampton	2 (5.9%)	1 (2.9%)	0 (0.0%)	3 (3.2%)
Rural Areas	5 (14.7%)	5 (14.3%)	2 (8.0%)	12 (12.8%)
Urban Areas	4 (11.8%)	5 (14.3%)	5 (20.0%)	14 (14.9%)
Stray	2 (5.9%)	1 (2.9%)	1 (4%)	4 (4.3%)
Shop	1 (2.9%)	1 (2.9%)	0 (0.0%)	2 (2.1%)
Pedigree	5 (14.7%)	1 (2.9%)	1 (4%)	7 (7.4%)
Shelter	4 (11.8%)	4 (11.4%)	6 (24.0%)	14 (14.8%)
Total	34 (36.2%)	25 (37.2%)	25 (26.6%)	94

Other Observations

The houses in the villages were usually close to farmland, and some residents were of the opinion that farm cats, or feral cats were in the neighbourhood.

2.3.2.2. Summary

The village cat populations may be in slight decline; as indicated by the age distribution, however the trend was too slight and the sample size too small to draw firm conclusions. There were enough breeding females in the survey to perpetuate the population, although this was heavily reliant on a small number of females who had produced several litters each.

This rural area is in some ways promising as a potential site for studying mating systems in cats, the main problem being the low overall density of domestic cats. The breeding and entire cats appear to be found in widely separated "pockets", and may

therefore represent isolated mini-populations, with little gene flow between them, except by means of human mediated migration. The entire domestic males in this area live at a calculated density of one per 200 hectares, which may be a considerable underestimate because farm and feral cats were not taken into account. The maximum figure for male home range cited in Liberg and Sandell (1988) is 620 hectares (Jones and Coman, 1982). Although this figure was calculated for Australian grassland, it does illustrate the ability of cats to utilise large areas of land.

2.3.3. The Freemantle/Polygon survey

This area is situated very close to the Shirley area of the main survey (see **Fig. 2.1.**). However the area was socio-economically distinct from Shirley, consisting of smaller terraced houses. It was hoped that these more "traditional" areas; with less flats, fewer student residents, and possibly fewer short term residents, might show lower rates of neutering. However, it should be noted that this survey covered a variety of housing types and therefore cannot be regarded as demographically homogenous.

2.3.3.1. Results

A total of 360 householders were questioned, reporting a total of 161 cross- bred cats and 10 pedigree cats (0.45 cats per household). Of the cross- bred cats of known gender, 74 were female and 91 were male. Of the cats of breeding age (over 9 months) there were 6/70 (8.57%) entire females and 8/90 (8.89%) entire males. In addition, there were two 18 month old entire cats of unknown gender.

<u>Age Distribution</u>

The age distribution of the population in this survey area (**Fig. 2.9.**) was indicative of a stable population.

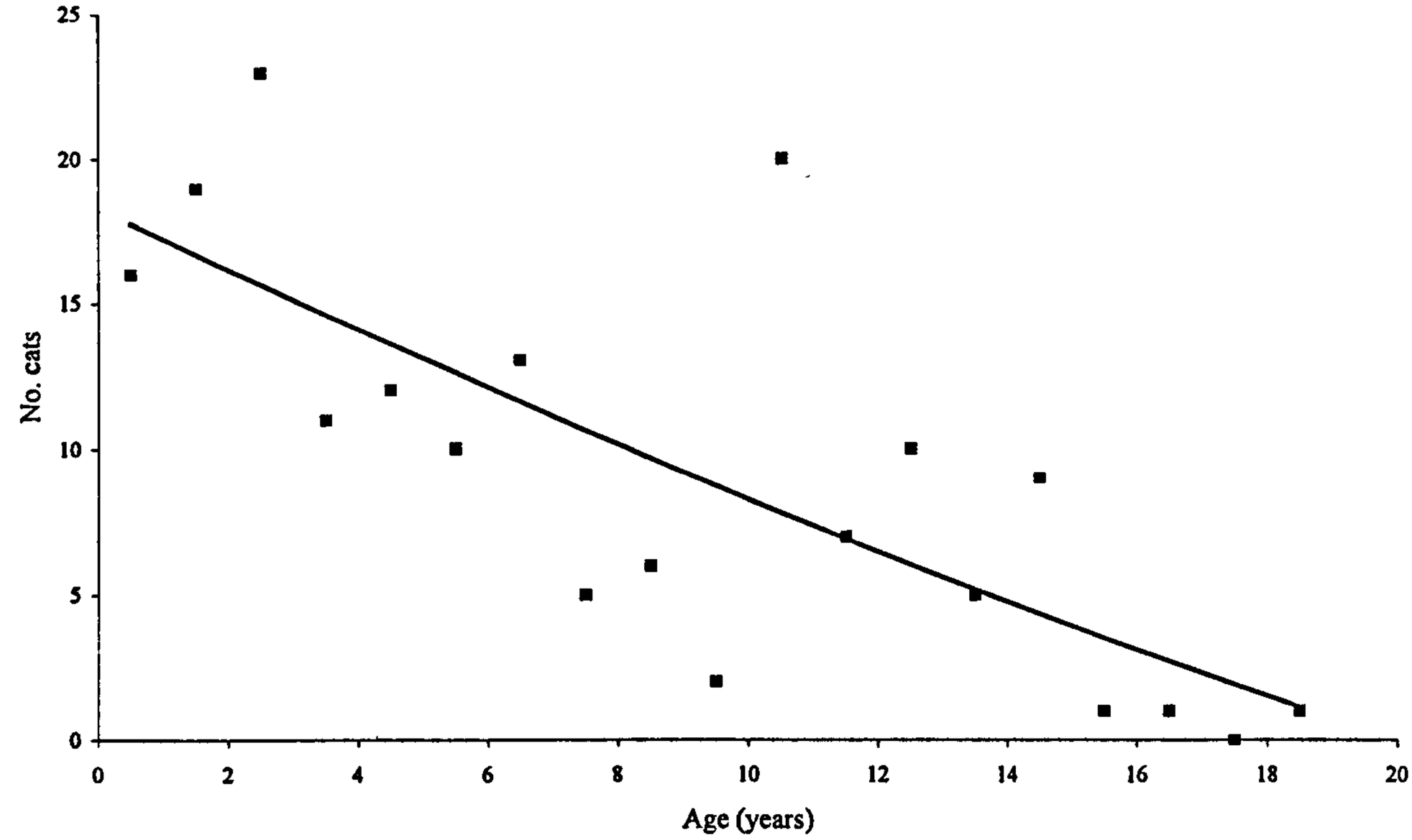
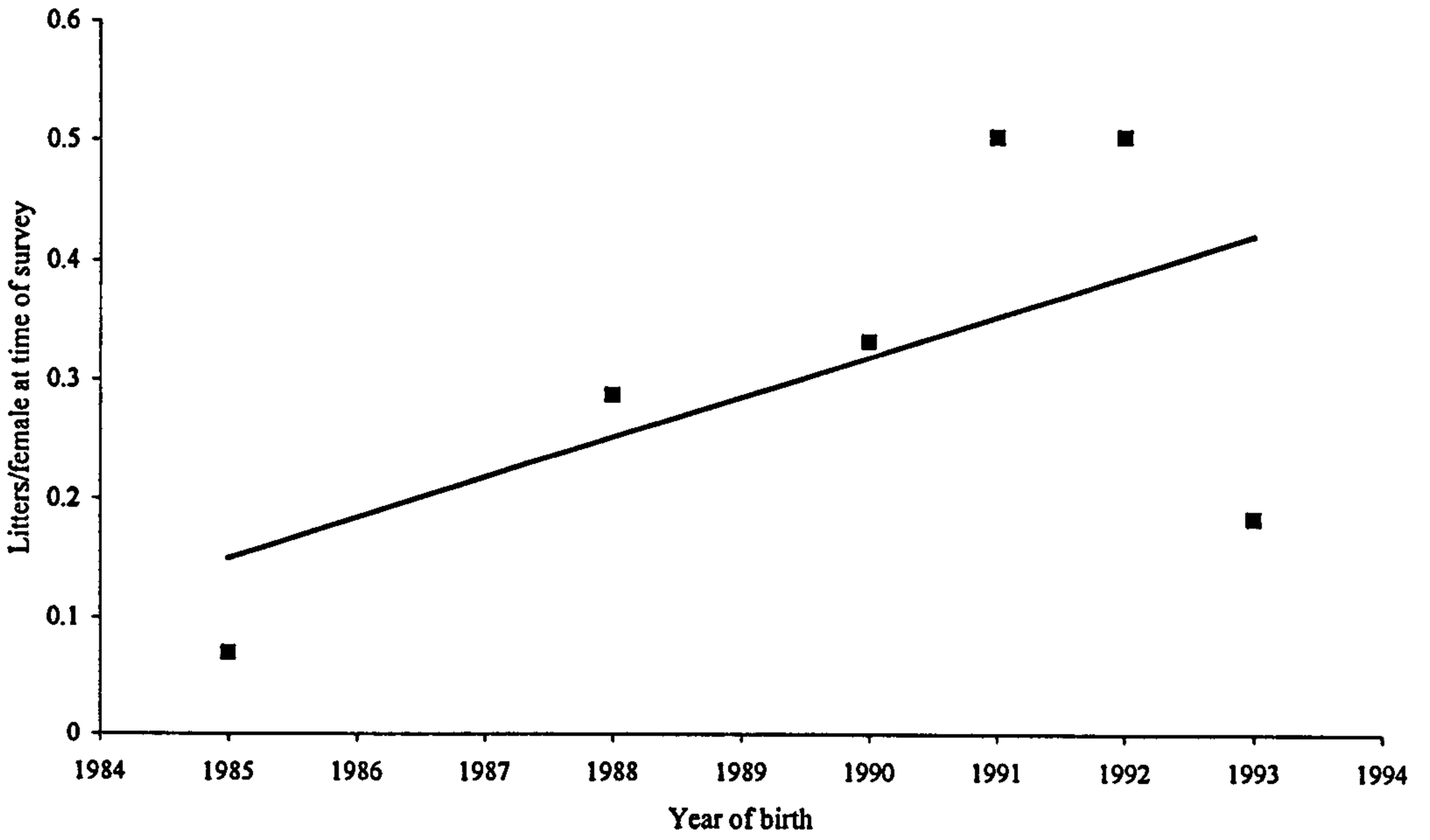


Fig. 2.9. Smoothed age distribution of cats in Freemantle/Polygon area survey, calculated using log polynomials to the power x^2.



Year(s) of birth	1983-1985	1986-1988	1989-1990	1991	1992	1993
Number of females	15	7	9	8	6	11

Fig.2.10. Fecundity to date of cats in Freemantle/Polygon area survey, by age group. Linear regression ($F = 2.0$, d.f. = 1,4, $p > 0.05$).

Reproduction

Reproduction for the females in this survey (**Fig. 2.10.**) was measured as lifetime reproduction to date, as with the village survey. There was no significant change in fecundity over the last 13 years, though most of the entire females were young (1-4 years old), so the fecundity values are a minimum value for cats of this age group. Overall, the mean number of litters per female was 0.23 (16/70), and the mean litter size was about 3.5. If these figure are representative, the Freemantle/Polygon population is not fecund enough to be self perpetuating at 3.5/2 x 16/70= 0.4 female births per female.

Female reproduction was not as skewed as in the village survey, with no female having more than 9 kittens. However, the area was not geographically homogenous, the reproductive females were largely concentrated in one area (see **Fig. 2.1.**). Conversely, some streets contained no reproductive cats at all. There were no obvious demographic differences to explain this diversity.

The 8/90 (8.9%) entire males, and 11 (12.2%) males who were potential reproducers, were also mainly concentrated into the same geographic area as the entire females.

The proportion of households (12/184) owning entire cats was higher in the subsection of the survey shown on **Fig 2.1.** than for the rest of the survey area (2/176); χ^2= 6.71, P<0.05, 1df.

Origins

The only significant trend that could be identified in cat origins (**Table 2.6.**) was an increase in locally born cats among the 0-4 age group (p<0.05, χ^2= 6.21, 1df). This may reflect the relative fecundity of the Freemantle/Polygon cats, compared to those in the neighbouring Shirley area.

Table 2.6. Summary of origins of cats, where known, from the Freemantle/Polygon survey

Age	0-4	5-9	10-	Total
Local area	31 (47.7%)	5 (15.6%)	10 (22.7%)	46 (32.6%)
Southampton	12 (18.5%)	6 (18.8%)	8 (18.2%)	26 (18.4%)
Rural Areas	4 (6.2%)	2 (6.3%)	3 (6.8%)	9 (6.4%)
Urban Areas	2 (3.1%)	0 (0.0%)	5 (11.4%)	7 (5.0%)
Stray	1 (16.7%)	1 (3.13%)	6 (13.6%)	8 (5.7%)
Shop	0 (0.0%)	4 (12.5%)	6 (13.6%)	10 (7.1%)
Pedigree	5 (7.7%)	2 (6.3%)	1 (2.3%)	8 (5.7%)
Shelter	10 (15.4%)	12 (37.5%)	5 (11.4%)	27 (19.1%)
Total	65 (46.1%)	32 (22.7%)	44 (31.2%)	141

2.3.3.2. Freemantle Area Survey: Discussion

The overall picture given by this survey is of an urban population with lower rates of neutering and higher reproduction than the Shirley area. The proportion of entire cats was higher (χ^2 = 8.79, $p<0.01$. 1d.f.), without the recent decline in reproduction witnessed in Shirley. However, the high localisation of the entire cats shows that the cats in this area cannot be treated as a homogenous population.

2.4. General Conclusions: Surveys

The main Shirley survey revealed that neutering had risen over the period 1979-1994 more than was anticipated. About three quarters of the area's cats were brought in from outside Shirley, and the age distribution gave no indication of a population in rapid decline. This indicated that the situation might be different elsewhere, and prompted surveys in other areas. These revealed that in some areas (Eastleigh suburbs) virtually

all cats were neutered, while in other areas (villages, and certain parts of Southampton) the neutering and reproduction rates were more favourable for cat reproduction, although the population is highly bottle-necked because reproduction is heavily reliant on a handful of entire females. However the low density of entire domestic males (except in the Freemantle "hotspot") seemed to give little scope for a measurable differential in male reproductive success. The number of entire feral males was hard to estimate, though some were adopted in the Shirley area. One village householder complained that it took her adult female over two years to become pregnant. In this case at least, there would appear to be few entire males around. However there are other possible explanations for this; such as the fear that virgin females often show for males, or physiological reasons affecting conception.

2.5. Cat Shelter Record Study

2.5.1. Introduction

Rescue organisations are important suppliers of domestic cats, particularly in areas where neutering rates are high. In this study 8 out of 11 cats under 5 years old in the "Eastleigh suburbs" population came from shelters, while in the main Shirley survey the proportion of shelter cats significantly increased between the early 1980's and the early 1990's, concurrent with the decrease in breeding of the resident cats. It is likely that shelters help meet the demand created by the low breeding by local cats. Interestingly, it is the policy of most shelters to ensure that all cats that pass through their hands are neutered, so there could be positive feed back; an increase in demand for shelter cats due to high local rates of neutering could lead to a further increase in the proportion of neutered cats.

The increased supply of cats from shelters in many areas raises the question: where do shelters obtain their cats? It was hoped to answer this question by examination of records kept by rescue organisations and searching for geographic trends; areas, or classes of area, where cats tend to be imported into shelters. These might be areas

where neutering rates are relatively low and consequently there is a surplus of cats. Conversely, it would be interesting to note areas where very few cats are produced, which could indicate high neutering rates.

2.5.2. Methods

2.5.2.1. Recording data from rescue organisation records.

Records of two rescue organisations were examined dating from 1993 to 1995 (up to just before the time of the study)

1. The Shirley based branch of Cat and Kitten rescue, run by Mrs Dollery.

2. The Blue Cross branch at Hedge End (situated 10 km north east of Southampton).

Both these organisations keep separate records for "cats in" and "cats out". "Cats in" records give details of where the cats originated geographically, and whether they were owned cats or strays. The term stray was applied loosely; covering both captured feral cats, and recently deserted domestic cats. The "cats out" records gave details of the location of the cats' adopters. In addition there was information on cat age, colour and gender.

From cat and kitten rescue the origins of 203 cats, and the destination of 193 cats were recorded. From the Blue Cross the origins of 92 cats and the destinations of 102 cats were recorded, giving a total of 295 origins, and 295 destinations.

2.5.2.2. Assignation of Origins and Destinations.

Cats were categorised into owned cats and strays. Geographic categories were then defined as follows:

1. The Shirley area; the region of Southampton where the main survey was sited.
2. Other areas of Southampton.
3. Urban and suburban areas outside Southampton including Chandlers Ford, Eastleigh, Hedge End, and any other urban areas.
4. Rural areas (including farms).
5. Outskirts of Southampton and areas that could not be definitely categorised.

Areas were categorised using an A to Z Street Atlas and a road map.

Table 2.7. Area of Origin of Cats Brought into The Blue Cross at Hedge End, and Cat and Kitten Rescue in Shirley

Source		Shirley	Southampton	Urban Areas	Rural Areas	Outskirts/ undefinable
Cat and Kitten Rescue	Owned	14	59	19	23	18
	Stray	0	29	16	7	16
Blue X	Owned	2	15	22	12	7
	Stray	2	12	6	7	7
Total		18 6.1%	115 38.9%	63 21.9%	49 16.6%	48 16.2%

Table 2.8. Destination Areas For Cats Adopted from The Blue Cross at Hedge End and Cat and Kitten Rescue In Shirley

Source	Shirley	Southampton	Urban Areas	Rural Areas	Outskirts/ Undefinable
Cat &Kitten Rescue	30	71	50	22	20
Blue X	2	26	41	22	11
Total	32 10.8%	97 32.8%	91 32.8%	44 14.9%	31 10.5%

2.5.3. Shelter Study Results

Over a third (36.9%; Blue Cross, 33.8% Cat and Kitten Rescue) of the cats were previously strays, including feral cats (**Table 2.7.**). It would be interesting to know if this figure had increased recently as a result of increased neutering among domestic cats. There were no significant differences found between the numbers of cats coming into and moving out from any classes of area, and no area stood out as an obvious location of cat breeding. However, the trends in the results supported the findings of the surveys; suburban and urban areas around Southampton adopted more cats than they supplied (**Table 2.8**), as did Shirley. Rural areas provided slightly more cats than they adopted. Southampton as a whole adopted and handed in very similar numbers of cats, perhaps indicating that Shirley has high rates of neutering relative to the rest of the city. If records for a larger number of cats were analysed, it might be possible to examine these trends on a finer and more statistically meaningful scale.

2.6. Population Dynamics: Discussion

Overall neutering rates were much higher than expected, although there was substantial

variation between regions in the Southampton area. The extremely high neutering figures for the Shirley survey were not reached in the other areas of Southampton where the population was sampled, in the Freemantle/Millbrook areas as many as 8% of males were entire. The distribution of breeding cats within each region was also uneven, especially with respect to females, this probably reflects the extremely low numbers of breeding females in the population.

Similar neutering rates to Shirley were found across Australia (Anon, 1994), where the neutering of 93.8% of adults has led to a decline in the population.

All the areas sampled within Southampton were found to contain too few breeding cats to maintain the population, but the population showed no signs of being in decline. The explanation appeared to be that the population was heavily reliant on immigration with cats being brought in from other areas. Also a large proportion of kittens was the progeny of a small number of entire females. These occasional breeding females are difficult to locate because of their low density, however there was the case mentioned above of the feral female residing in a Shirley garden that gave birth to 40 kittens over a 5 year period. I have found other similar situations during the time that I have been carrying out temperament testing in Southampton. I have located households whose cats have produced large numbers of kittens, which are dispersed across Southampton, and householders tell me of other similar households. Although this evidence is anecdotal, it does support the hypothesis that the effective (reproductive) population has been greatly reduced, while the total population size remains approximately stable.

Migrants make up a large proportion of the population (67% in Southampton overall), but they contribute relatively little to the breeding within the population; only 27% of potential breeders below 8 years old originated outside the local area in which their home base was situated, and only 10% from outside Southampton. In other words, although there is a lot of (human mediated) movement of cats between areas, the gene flow between areas is more limited than these figures suggest.

3 Recruiting cats to the project

The following three chapters involved the use of cats belonging to members of the Southampton public. This involved obtaining an adequate sample of cats, which meant gaining the co-operation of their owners. The methods employed to recruit volunteer cat owners are described briefly here. Throughout the study care was taken to state our commitment to animal welfare, and emphasise that none of the cats used would be subjected to experiences likely to cause them distress.

Ideally, all the cats for this study would have been recruited from an area small enough to allow comprehensive knowledge of the resident cat population. However, it became clear from the population dynamics study that the density of entire females was too low for this to be feasible. It was therefore decided to try to obtain a sample of kittens born in a wider catchment area: the whole of the Shirley, Freemantle and Millbrook areas of Southampton. Later, in a further attempt to increase the sample size, the catchment area was expanded to encompass kittens born in the whole of Southampton.. However, the Shirley/Freemantle/Millbrook area remained the core focus area of the study.

3.1. Recruiting kittens for temperament testing.

During October 1995 posters were placed in Veterinary Surgeries, advertising for the owners of suitable kittens to participate in the study. Five pounds worth of cat food was offered as an incentive, and respondents were given a simple form to complete. A stamped-addressed envelope was provided to return the form to me. In addition, notices were placed in the windows of shops, and in the small advertisements section of the Southern Evening Echo, a daily newspaper distributed in the Southampton area. Also, a number of kittens were recruited by "networking"; kitten owners putting me in touch with friends who also owned suitable kittens. In some cases these were the siblings of their own kittens.

Some respondents owned kittens that were born outside Southampton, and would therefore be of unknown relatedness to the cats from the Southampton population.

However, these kittens were still used for temperament testing in order to obtain a comprehensive data base of kitten behaviour types.

Respondents were contacted and details of the project were outlined to them. A mutually convenient time to carry out the temperament testing was then arranged.

The advertisements asked for kittens of 8 months old and younger, although all kittens should ideally be 6 months old at the time of testing. Kittens of 6 months and over were tested as soon as possible; the owners of younger kittens were re-contacted when their kittens reached 6 months of age.

Eighty five kittens from fifty nine litters were used. The temperament testing study is described in **Chapter 6**.

3.2. Recruitment of Males for Radio-Tracking

Advertisements were placed in the Daily Echo, asking for owners of tom cats who would be willing to allow them to be radio-tracked. Later, posters were placed in Veterinary Surgeries, complete with SAE for replies. Some toms were recruited through networking. The radio tracking study required a much longer term commitment from the owners than the temperament testing study. Owners were rewarded with information on their cat's night time habits rather than with cat food.

I had knowledge of the homes of a number of tom cats from the door to door surveys. The owners of these cats were approached and asked whether they would be willing to take part in a radio-tracking study. All these owners declined, except for one owner of a 10 year old tom. Unfortunately the cat died before the start of the radio tracking study. However, these cat owners were generally willing to allow me to collect DNA samples for paternity analysis.

Seven toms were use in the radio tracking study, which is described in **Chapter 4**.

3.3. Collection of hair samples for microsatellite analysis.

Hair samples were collected from cats used in the temperament testing and radio tracking studies. When the mother of the kitten(s) was present, hair samples were also taken from her. If the mother of the kitten(s) lived elsewhere I attempted to find out where her owners lived. If possible I approached them and asked to take a hair sample from the mother. Hair samples were taken from other cats living in the same households if they were thought to be of possible interest: relatives or putative relatives of the kitten(s) being temperament tested, or other locally born cats. A sample of cats which were unlikely to be related to other cats in the study, due to the geographical location of their birth, were still valuable as a means of building a database of allele frequencies for microsatellite analysis

One hundred and eight cats were used. The protocol for taking hair samples, and the results of the microsatellite analysis are described in **Chapter 5.**

The geographic locations of the kittens' birthplaces, where known, and the present home base of the adult cats is shown on **Fig 3.1.** The approximate locations of the kittens' birth, where this was not known precisely, is summarised in **Table 3.1.**

Fig. 3.1. Cats recruited to the study. The households of known location are shown, additional households for which location was not precisely known are summarised in the **Table 3.1.** The coloured circles represent the homes bases at the time of the study for the mother and toms, and households where the litters were born. The black rectangle represents the Shirley/Freemantle/Millbrook area.

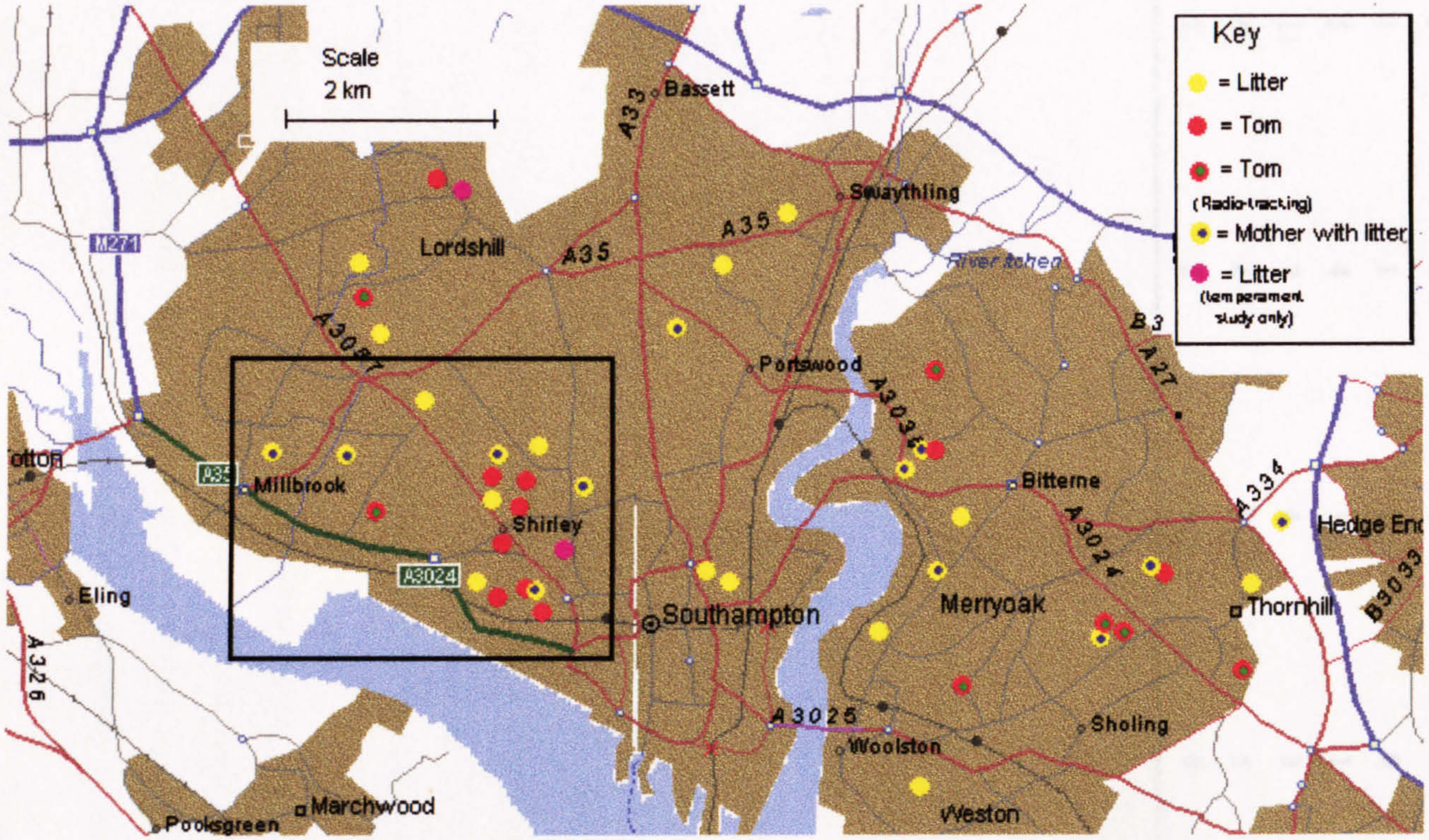


Table 3.1. Approximate locations of birth of kittens used for micrsatellite and temperament testing studies, where the exact location of birth was unknown.

	Microsatellite analysis		Temperament testing	
Area	No. Kittens	No. Litters	No. Kittens	No. Litters
Shirley	3	3	3	3
Portswood	2	1	2	1
Bitterne	1	1	1	1
Sholing	2	2	1	1
Weston	1	1	1	1
Woolston	1	1	1	1
Swaythling	1	1	0	0
Lordshill	5	4	1	1
Outskirts	11	7	4	3
Unknown	6	6	5	5
Pedigree	2	2	0	0

4 Ranging behaviour of tom cats in Southampton: a radio-tracking study

4.1. Introduction

4.1.1. Aims

The population structure of cats in Southampton is complex; there is a high density of individuals, but the great majority of these are neutered. In Shirley, it was found that 96.8% of adult males and 98.7% of adult females were neutered (see **Chapter 2**). The overall density of cats was estimated at 6.74ha^{-1}, but the density of entire males and females may be as low as 1 per 13.5 ha and 1 per 27 ha respectively. Subsequent surveys revealed that other areas of Southampton contain higher densities of entire domestic cats, which can be estimated from the neutering statistics to be in the range of approximately 1 per 4-5 ha. In addition to domestic cats, the population also contains an unknown number of feral cats.

The level of competition between males for mating opportunities depends on the ability of males to travel through an urban environment and locate females. At one end of the spectrum, cats might be able to travel long distances, and are limited by competition with other males. This would lead to a high level of competition between domestic males for mating opportunities. At the other extreme, cats might be very limited in their ability to move within an urban environment, and their ranges constrained by this. Under this scenario toms that live close to an entire female will have a great reproductive advantage, while others will be excluded from mating simply because of the location of their primary homes. This would lead to reduced competition between domestic males, and could lead to more siring of domestic kittens by feral males.

The actual situation is likely to lie somewhere between these extremes; where cats may be able to cover enough ground to allow competition between males, but their competitive ability may be site dependent. Cats have been shown to achieve more copulatory success in familiar areas, than at sites near the periphery of their range

(Liberg, 1981; Yamane *et al*, 1996). Measurement of spatial use by domestic cats in the Southampton environment, in conjunction with other lines of investigation (population surveys and molecular evidence), would be valuable in elucidating the mating system. This is interesting in its own right, and is important to understand when modelling gene flow through the city.

4.1.2. Home ranges, and other measures of spatial use.

A home range was defined by Burt (1943) as the area used by the animal in its normal activities of food gathering, mating and caring for young. There are immediate problems with this definition associated with the word "normal"; excursions beyond the normal range need to be identified and excluded (see Methods). Also, an individual's home range may change over time and season (Harris *et al*, 1990), and must therefore be considered in the context of factors such as the age and mating activities of the animal. Although the concept of a home range is flawed, measurements of home range give a good global indication of spatial use by an individual, which can easily be used for comparison between individuals and studies.

Home ranges are not the only parameter worth considering (Harris *et al*, 1990; White and Garrott, 1990; Larkin and Halkin, 1994). One useful measurement of the utilisation of space within the home range; the utilisation distribution (UD), can be provided by the identification of core areas. These are areas within the home range that receive concentrated use (Samuel *et al*, 1985). Core areas can be determined empirically (see Methods). They are often important ecologically, for example: home ranges sometimes overlap, while core areas usually remain exclusive (reviewed in Harris *et al*, 1990). Whether or not an animal uses a definable core area may in itself illuminate the ecology of that animal.

Radio-tracking data also allows other parameters of spatial use to be investigated, such as the distribution of times spent at different distances from home bases. Times spent active and inactive, and activity patterns related to position can also be explored.

4.1.3. The use of radio-telemetry

The home ranges of farm cats and rural feral cats have been measured mainly using radio telemetry, including cats in rural Sweden (Liberg, 1983), farm cats near to Zurich (Turner and Mertens, 1985) and farm cats in Oxfordshire (Kerby and Macdonald, 1988). Observational methods were used by Panaman (1981) on farm cats in Cornwall. Most studies of spatial use by urban cats have relied on sighting and observation, for example house cats in Manchester (Chipman, 1990) and feral cats in Jerusalem (Mirmovitch 1995). However, radio-tracking was used for recently published work on cats in a suburban area in Australia (Barratt, 1997).

Purely observational methods are limited because sightings are restricted to areas where cats can be seen from the road or pavement; a cat resting in a nearby garden, for example, could not usually be located. Also cats are often nocturnally active, which further reduces the power of observational methods. Observational methods may be adequate in some circumstances for measurement of home ranges (Dards 1978). However, any movement outside the normal home range, or even core area, would be extremely unlikely to be detected. This is particularly relevant when considering mating behaviour because there are indications that oestrus females can attract males from a wide area; Chipman (1990) for example, observed a number of previously unseen males aggregating around the home of an oestrus female.

4.1.4. The exclusive use of tom cats

I decided to restrict the radio-tracking study to tom cats. All previous studies have shown males to have larger home ranges than females in the same general area, and in studies where radio-tracking techniques have been used, males have travelled to seek oestrous females (e.g. Liberg, 1983), although group living females did leave their natal groups during oestrus; perhaps to avoid inbreeding. Liberg and Sandell (1988) collated data from 21 studies of cat home ranges in a variety of environments; home range size was inversely proportional to population density for both males and females, but male home ranges were on average about 3.5 larger than those of females. These results

indicate that male behaviour holds the key to unravelling the mating system of urban domestic cats.

4.2. Methods

4.2.1. Recruitment of Cats

Cats were recruited to the radio-tracking study using the methods outlined in **Chapter 3**. A total of 7 tomcats were used in the study. Two cats were rejected because they were young (under 18 months) and also lived outside the boundaries of Southampton city, which would entail extra travel.

The locations of the primary homes of the cats are shown on **Fig 3.1. (Chapter 3).**

4.2.2. Equipment

TR5 transmitters were bought from Biotrack Ltd. These weigh less than 50g, i.e. around 1% of the weight of a tom cat, well within the maximum recommended limit of 5% (S. Funk pers. comm.). Each transmitting unit is encased in waterproof plastic material and is attached to a 30cm flexible aerial. The transmitters are activated by the removal of a small magnet taped to the side of the unit, and can be de-activated when not in use by replacing the magnet. Once activated, the transmitters continuously emit a pulse on VHF frequencies.

Signals were detected using a Vega 173 receiver and a Yagi antenna. The equipment was tested at three locations in built-up areas of Southampton, and it was found that the transmitters could be detected up to a maximum distance of 700m when the location of the transmitter was previously known. When the location of the transmitters, and therefore the general direction, was not known (a more realistic situation) they could be located at distances of up to 350m.

Transmitters were attached to standard, dark coloured cat collars with electrical tape. It

was found that aerials left dangling loose, as recommended by the manufacturers, irritated the cats, which would try to remove them. To overcome this problem, aerials were taped along the length of the collars, leaving 4-6cm of aerial protruding. It was feared that this might reduce the range of transmitters, but the effect was found to be minimal. Transmitters attached in this way were discreet, and it required close inspection of the cat to allow their detection by eye.

4.2.3. Locating Cats

The volume of the high pitched "beep" noise made by the receiver increases as the antenna is pointed more directly at the transmitter, with a second lesser peak in volume when the antenna is pointing directly away from the transmitter. The volume of the noise also decreases as one moves away from the transmitter, although there is some variation caused by the number of buildings etc. obstructing the transmitter, and causing confusing reflected signals. Thus, at the start of a radio-tracking session one can rapidly ascertain the approximate position of the cat, and approach to a position close enough to obtain an accurate fix by triangulation.

The nature of urban housing means that in most cases one can approach close to the cat without disturbing it (see White and Harris, 1994), for example; the street beside the garden containing the cat. From such short distances one can almost always locate the cat to a 25m square.

4.2.4. Preliminary radio-tracking

Preliminary radio-tracking was carried out in June 1996, when several regimes of data collection were tried out. The objective was to gain background knowledge of the activity patterns of the cats, and devise a strategy for collecting data efficiently in the time available. This required identifying:

i. The times of day when the cats were likely to be active; so that tracking could be concentrated at these times.
ii. Any other factors that might influence tracking. For example, radio-tracking became

very difficult around "rush hour" times due to traffic noise and increased numbers of pedestrians.

It was found that all of the cats in the study were most active at night. Most of the radio-tracking was therefore carried out during darkness. I discovered that if the cats' owners saw me attempting radio-tracking around the time they went to bed it would influence their decision on whether to keep the cat in or let it out, despite my efforts to persuade them to ignore my presence. The outcome was that sometimes an unwilling cat might be forced to spend the night outside! This would be likely to effect its behaviour. Only two of the cats lived in houses with cat flaps. The solution was to start radio-tracking between 11.30pm and midnight, after the owners had gone to bed, and the decision on whether or not to keep the cat in for the night had already been taken.

If a cat was found to be confined indoors overnight the radio-tracking session was abandoned.

4.2.5. Data Collection

4.2.5.1. Recording locational fixes

Continuous and discontinuous tracking data were obtained for each cat. During continuous tracking, the cat's position was recorded every 5 minutes. Discontinuous recording involved recording the cat's position once before moving on.

Continuous recording is vital to gain detailed data on the activity of a cat over a given time period, however this is a highly intensive way of getting data on only one cat. It is worthwhile, in addition, to record the positions of all the cats being studied at random intervals. This provides independent data points (see below) that can be used to calculate the home range of the cat, even if continuous activity cannot be recorded

4.2.5.2. Casual observations

Any sightings of the cats were recorded, as were details of their activity, and any interactions with other cats.

4.2.6. Calculation of Home Ranges

Methods of home range calculation have been reviewed comprehensively by, for example Harris *et al* (1990), Worton (1987) and Kenward (1987) etc. A brief resume is given here:

The minimum convex polygon (MCP) method is the oldest and most popular way of calculating home range (White and Garrott, 1990). The area is constructed by connecting the outer points to form a convex polygon, i.e. the maximum area formed by joining the outer locational points. The lack of ambiguity in calculation allows easy comparison between studies (Harris *et al.* 1990). Indeed almost all previous studies of cat home ranges found in the literature have used MCP methods (Chipman, 1990; Mirmovitch, 1995; Liberg, 1980; Barratt, 1997). One exception is Turner and Mertens (1985), where a grid cell approach was used. The MCP has also been used in studies of urban foxes; a similar sized mammal in a similar environment e.g. in Bristol, (White and Harris, 1994); and Toronto, (Adkins and Stott, 1998). But a grid cell approach was preferred by Doncaster and Macdonald (1997) in Oxford.

A weakness of the MCP method is that all data points carry equal weight (Harris *et al*, 1990), meaning that a single reading outside the area normally used could lead to a considerable increase in the calculated home range. A common solution (White and Garrott, 1990) is to exclude outlying points by including only the 95% of fixes that result in the smallest overall range. The choice of a 95% parameter is arbitrary, but is at least objective and allows comparisons between studies. MCP methods are also crude with respect to internal utilisation of home ranges (Doncaster and Macdonald, 1996; Worton, 1987).

No method of calculating home ranges is perfect. The almost exclusive use of MCP in previous cat studies makes its application here useful. However the additional use of a further, probabilistic technique would be valuable for comparison.

Parametric techniques (e.g. Jennrich and Turner, 1969) require animals to conform to unwarranted assumptions (White and Garrott, 1990). The geometric centre of the range is expected to be the centre of activity, and animals are expected to move randomly around their home range.

Non-parametric probabilistic approaches have been developed. The harmonic mean method (Dixon and Chapman, 1980) has been shown to be give misleading results Worton (1987), and is not recommended by the authors of the Calhome package for analysing home-range data (Kie *et al*, 1994). Kernel methods smooth locational fixes to provide a probability density function (Worton, 1989). They are more flexible and give more representative results than previous methods (Wray *et al,* 1992, cited in White and Harris, 1994), and allow detailed analysis of the utilisation distribution within a range. However kernel methods may require a large data set (Worton, 1987), and must be applied with caution. The Calhome program was used to calculate MCP and kernel values. The program uses adaptive kernels see (Worton, 1989).

Core areas: When a grid cell method of home range calculation is used, core ranges can be defined by identifying cells which are occupied by the animal significantly more than would be predicted by assuming a uniform distribution (Samuel *et al* , 1985). An alternative method, which is appropriate to this study and therefore used here, is to plot range area against adaptive kernel isopleth value (White and Harris, 1994), or against MCP percentage point inclusion. The major point of inflection was identified, and the isopleth or percentage point inclusion value at that point was use to define the core area.

4.2.7. Independence of Observations

Almost all methods for estimating home ranges require that each locational fix be

independent from any other, i.e. sufficient time should elapse between fixes for the location of the cat at one fix not to influence its position at the next fix. There is controversy over methods of ensuring independence. However, a reasonable rule of thumb is that the time between fixes should be adequate for the animal to traverse its home range (White and Garrott, 1990). A conservative estimate of the length of time necessary for any of the cats studied to traverse their range is one hour. Therefore a positional fix was taken at the start of each recording session, and at one hour intervals thereafter. In addition, when discontinuous recording was carried out, the single fixes taken were used as independent data points for the calculation of home ranges.

4.3. Results

4.2.8. Data

The quantity of radio-tracking data collected varied between cats **(Table 4.1.)**

Table 4.1. Summary of radio-tracking data.

a. Radio tracking data collected in 1996

Cat	Age in June 1996	Location of home base	No. locational fixes	Total hours of continuous recording
Whit	14 months	Freemantle	35	3
Ziggy	14 months	Sholing	14	0
Zoe	15 months	Sholing	30	9
Ebony	2 years	Sholing	54	37
Jake*	7 years	Bitterne	11	6

*Jake was removed from the study after one week because he was suffering adverse effects from wearing the transmitter/collar: He lost hair in the neck area, became sore, and started to bleed. The exact cause of this is unclear; it may have been simply abrasion or, as the owners believed, because he developed an allergy to the collar. This was very unfortunate, both because of the discomfort caused to the cat, and because of the loss of a mature cat from the project

b. Radio tracking data collected in 1997

Cat .	Age In March 1997	Location of home base	No. locational fixes	Total hours of continuous recording
Whit	2 years	Freemantle	27	15
Ziggy	2 years	Sholing	14	6.5
Ebony	3 years	Sholing	33	27.5
Sam	3 years	Sholing	42	30.75
Marmalade	3 years	Lordshill	24	16.7

Of the cats that were available for the full 1996 radio-tracking period, only Ebony showed extensive ranging behaviour. For this reason more data was collected for Ebony than for the other cats

Ziggy and Sam lived in adjacent terraced houses. Sam was recruited to the radio-tracking study in 1997 when he was three years old; approximately one year older than Ziggy. Ziggy's mother, Suzie, was resident in the same house as Sam, to whom she was reported to be unrelated. By May 1997, Suzie had given birth to 2 further litters; one in March 1996, and one in 1997. The owners suspected that Sam was the father of all 3 litters. DNA samples were collected from Ziggy (first litter), and from 2 kittens from the second litter; which were included in the temperament testing study (see **Chapter 6**). Contrary to expectations, Sam proved not to be the father of any of these cats (see **Chapter 5**). Unfortunately, no DNA sample was obtained from any kittens from the most recent litter, so it was not possible to investigate their paternity.

4.3.1. Home ranges

Home range areas were calculated for all cats using 100% minimum convex polygons (MCP). MCP 95% point inclusion and 95% adaptive kernel isopleths were also applied to the mature cats; Ebony, Sam and Marmalade, for which sufficient data had been collected to use these methods (**Table 4.2.**). Enough data was collected for Jake to construct a minimum value for MCP 100%.

Table 4.2. Calculated home ranges of radio-tracked cats. Insufficient data was collected from the first four cats in the table to use MCP 95% point inclusion and ADK 95% isopleths.

Cat	Year	MCP 100 (ha)	MCP 95 (ha)	ADK 95 (ha)
Zo	1996	0.5	NA	NA
Ziggy	1996	0.1	NA	NA
	1997	1.44	NA	NA
Whit	1996	0.2	NA	NA
	1997	1.67	NA	NA
Jake*	1996	4.1	NA	NA
Sam	1997	7.38	5.14	12.45
Marmalade	1997	7.25	4.56	14.30
Ebony	1996/1997	14.28	6.28	12.70

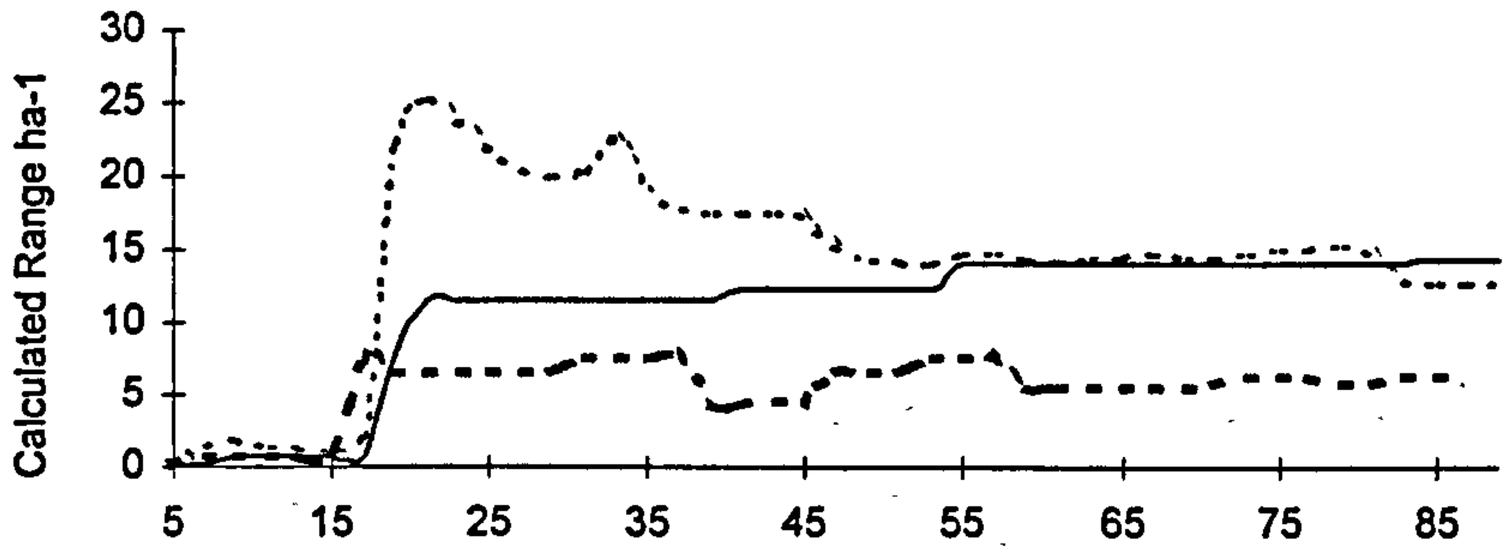
*Removed from the study due to discomfort caused by wearing collar.

Visual inspection showed little difference in the area used by Ebony in 1996 and 1997, so the data was pooled for this cat between the two radio-tracking periods. Ziggy and Whit showed considerable differences between 1996 and 1997, and the data is considered separately. Zo, Marmalade, Sam and Jake were only used for one of the tracking periods.

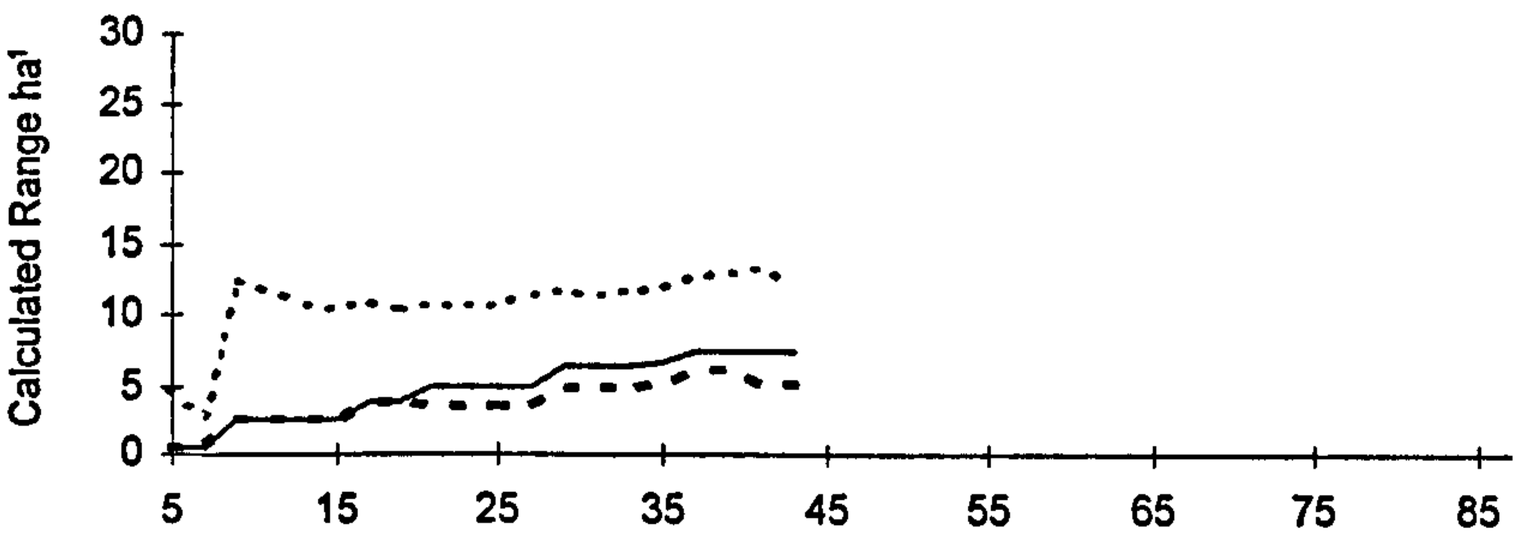
In order to ascertain whether enough locational fixes had been obtained to provide reliable home ranges, fixes were removed one at a time sequentially, and calculated range was plotted against number of fixes for each of the mature cats (**Fig 4.1.**). An asymptote in range size is indicative that the full range has been identified (Morris, 1988; Harris *et al*, 1990). This method is not infallible, (Gautestad and Mysterud, 1995), because sometimes an asymptote is never reached, usually when the animal exhibits drifting territoriality (e.g. in foxes; Doncaster and Macdonald, 1997). However, the cats in this study all have easily defined primary homes, which limits the extent to which their ranges can drift.

Fig. 4.1. Home range size related to number of locational fixes. Continuous line; 100% MCP: heavy broken line; 95% MCP: light broken line; 95% adaptive kernel.

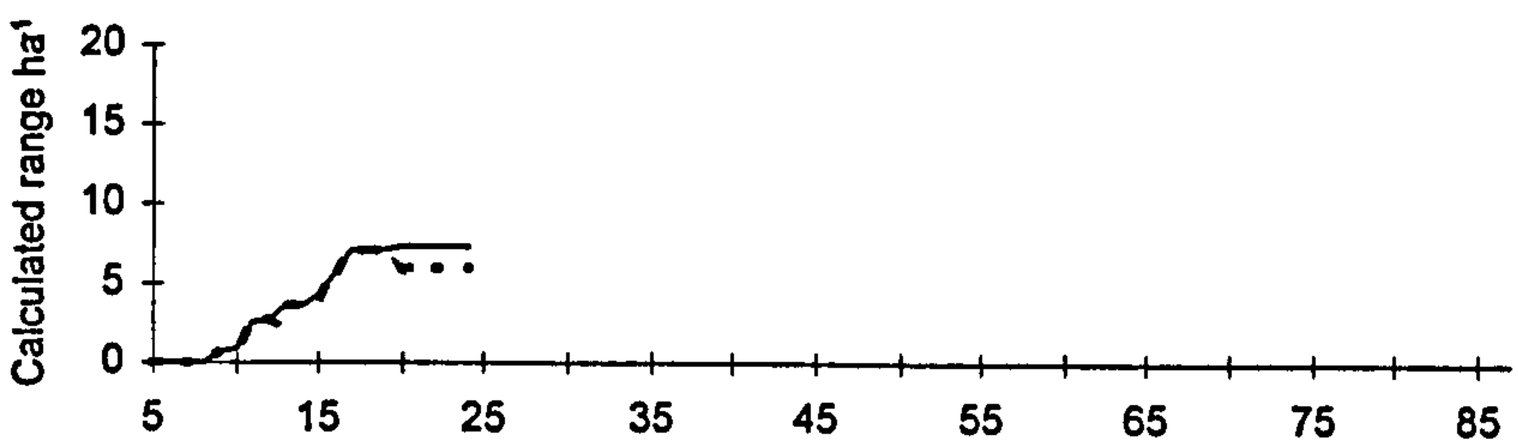
a. Ebony



b. Sam



c. Marmalade



Number of locational fixes

Ebony's calculated home range reached an asymptote after around 60 fixes for both AK and MCP methods of home range calculation, (**Fig. 4.1.a.**), although there are discrepancies between the two methods in the distribution of the ranges (**Fig 4.2.1.a.**)

The data for Sam indicate that an asymptote may have been reached (**Fig.4.1.b.**); more data are needed to confirm this. The AK 95% method gave a much larger home range (12.45 ha) than the MCP 100% (7.38 ha), and covers areas well beyond the boundaries of the locational fixes (**Fig 4.2.2.a.**). The data for Marmalade follow a similar trend (**Fig.4.1.c.**): 14.30 ha (AK 95%) and 7.25 ha (MCP 100%), range distribution is shown in **Fig 4.2.3.a.**

These results imply that around 60 locational fixes are necessary to obtain reliable measures of home range for urban cats. With smaller data sets MCP methods appear more consistent with visual inspection of the areas visited by the cats.

The three young cats (under 18 months), were never observed to move more than 50m. from their home bases. Two of these cats, Ziggy and Whit, were tracked again in 1997 when they were 2 years old. In 1997 they were observed to make occasional excursions: 3 (Whit), 1 (Ziggy). This did not allow detailed analysis of home range use, but was enough to construct a 100% MCP for comparison with the other cats.

The cats were divided into 3 age groups: young (Ziggy and Whit in 1996, Zo, immature (Ziggy and Whit in 1997) and mature (Ebony, Sam, Jake and Marmalade). There was a significant effect of age on home range size between the groups (Kruskal-Wallis test; $h = 4.5$, $df=1$, $p < 0.05$), although with such a small sample size this result must be treated with caution.

The maximum range sizes recorded for the younger cats (Zo, Ziggy in 1997 and Whit in 1997) were all smaller than the ranges of the 4 mature cats. Despite the small sample size, this difference was nearly significant (Mann -Whitney U test, $u = 0$, $p = 0.057$).

Range displacement: There was only one example of overlapping ranges: Sam and Ziggy, who lived in adjacent houses. Ziggy was starting to expand his range away from his home base in 1997, while Sam had a substantial range at this time (see **Table 4.2.**). It is interesting to note that, although Ziggy's range was contained almost entirely within Sam's range (100 MCP), there was minimal overlap between Ziggy's range and Sam's core range see (**Fig 4.2.b.**) Giving some evidence of exclusive core areas.

The home ranges of both Ziggy and Sam extended North to the same point (marked on **Fig.4.1.b.**). Ziggy lost his collar, including the radio-transmitter, towards the end of the study. I found it at the northern limit of his range. It appeared to have been torn off in a fight, giving anecdotal evidence that this site marked the edge of an area defended by another cat.

4.3.2. Core areas

Core areas (see methods) were identified by plotting percentage isopleth (Adaptive Kernel) and percent point inclusion (MCP) against calculated range and identifying the major point of inflection (**Fig 4.3.**). This could be done without ambiguity for Ebony and Sam for MCP percentage point inclusion. However the core areas identified for Sam and Ebony using AK isopleths, and for Marmalaide using both methods of calculation, must be regarded as tentative.

Table 4.3. Core areas of mature cats

Cat	MCP (ha)	% point inclusion	ADK (ha)	% Contour
Sam	2.56	88	2.43	75
Marmalade	3.50	77	4.50	75
Ebony	6.28	95	6.21	85

The distribution of core areas is shown in **Fig 4.2.** The size of the core areas is comparable to the estimated density of toms in areas of the city where neutering is relatively low (**Table 4.3.**). The data suggest that core areas can be identified for urban cats with 40-60 locations. More research is needed to quantify the importance of core areas for territorial behaviour. The association between core areas and cat activity is examined below (**4.3.3.1.** and **4.3.4.2.**)

Figs 4.2.1.-4.2.3. Home ranges and core areas calculated by MCP (fine line) and adaptive kernel (thick line). In all figures the primary home is located at (0,0), the y axis runs North-South and the X axis West-East. The scale is in meters.

Fig. 4.2.1.a. Ebony: home range

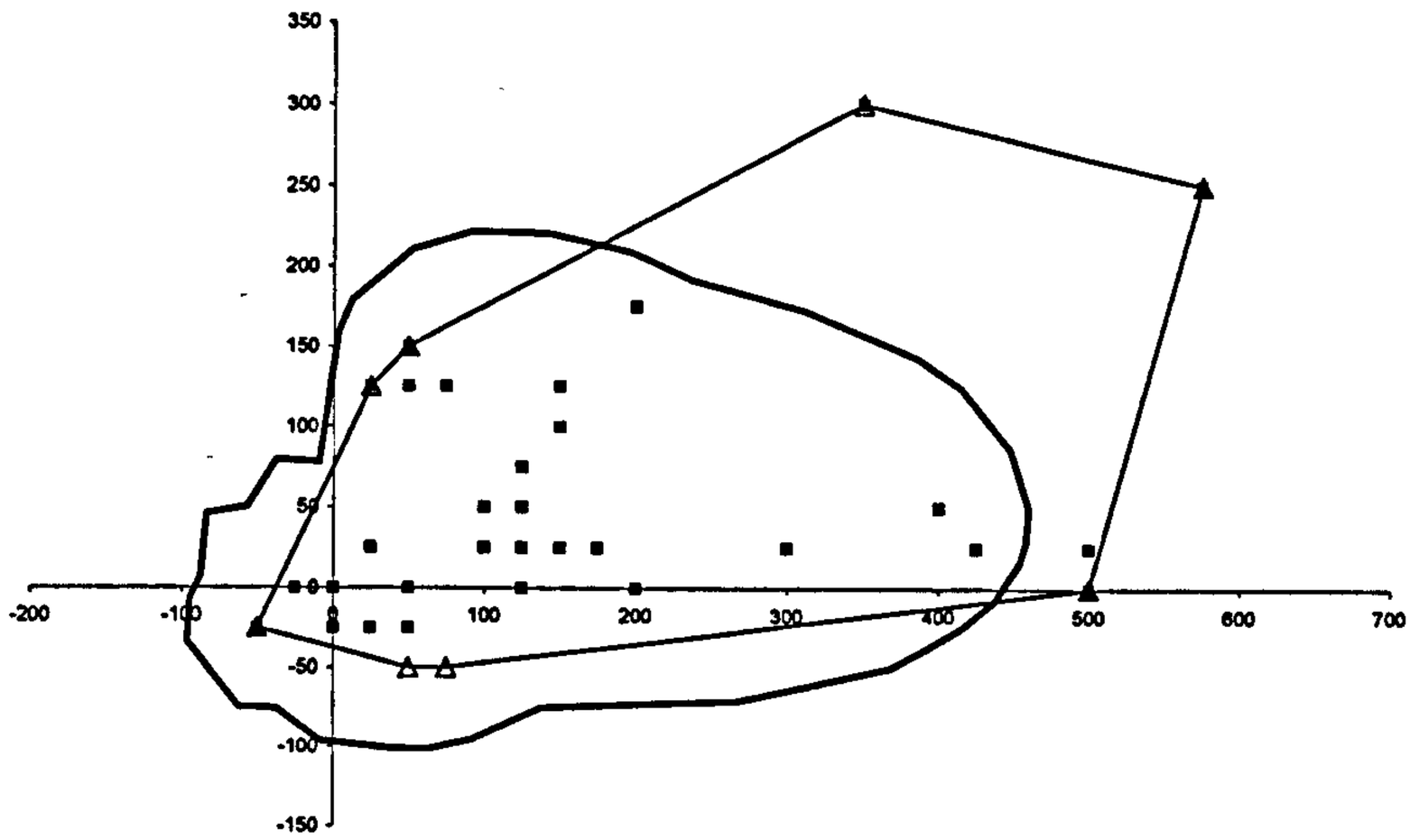


Fig. 4.2.1.b. Ebony: core area.

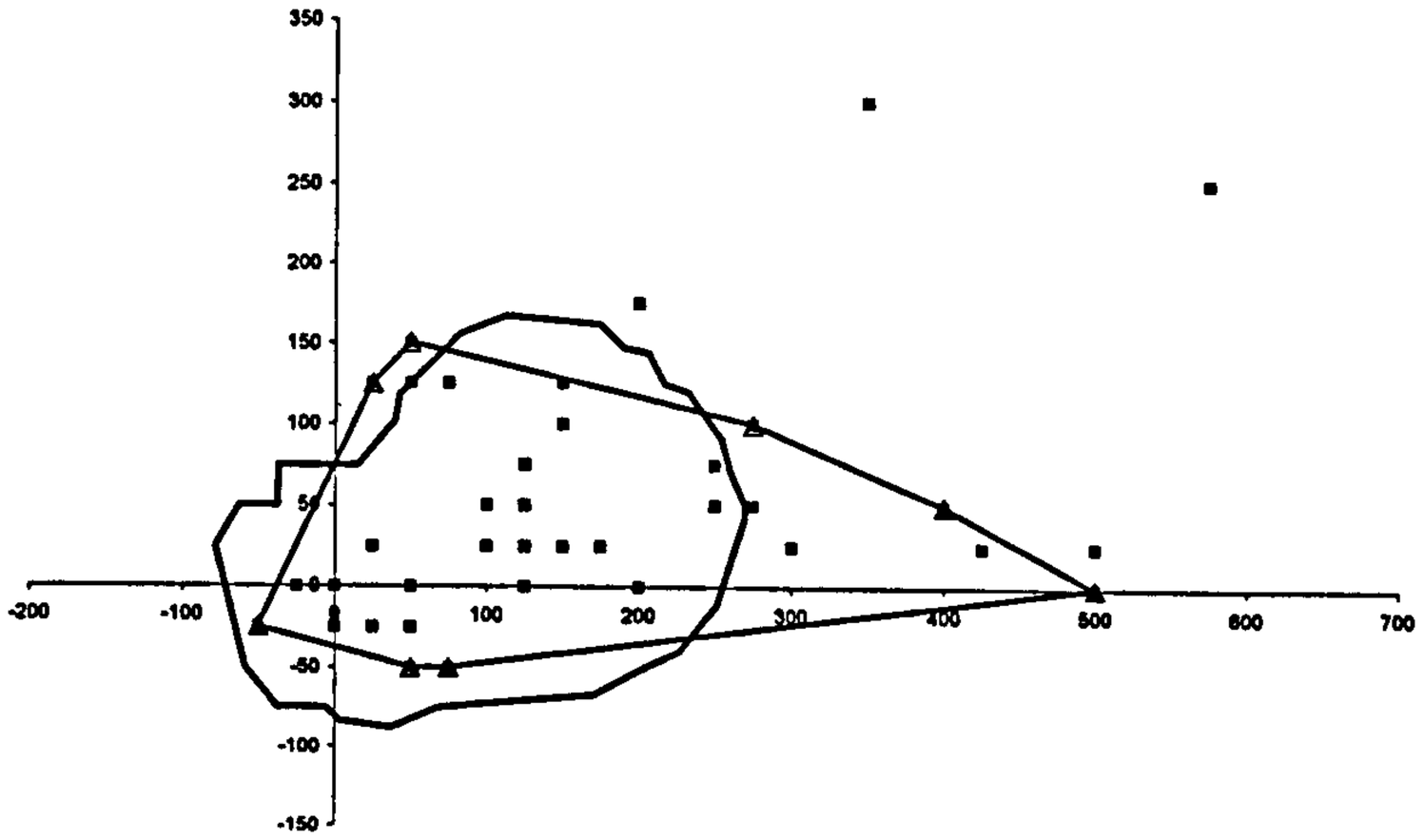


Fig. 4.2.2.a. Sam's home range.

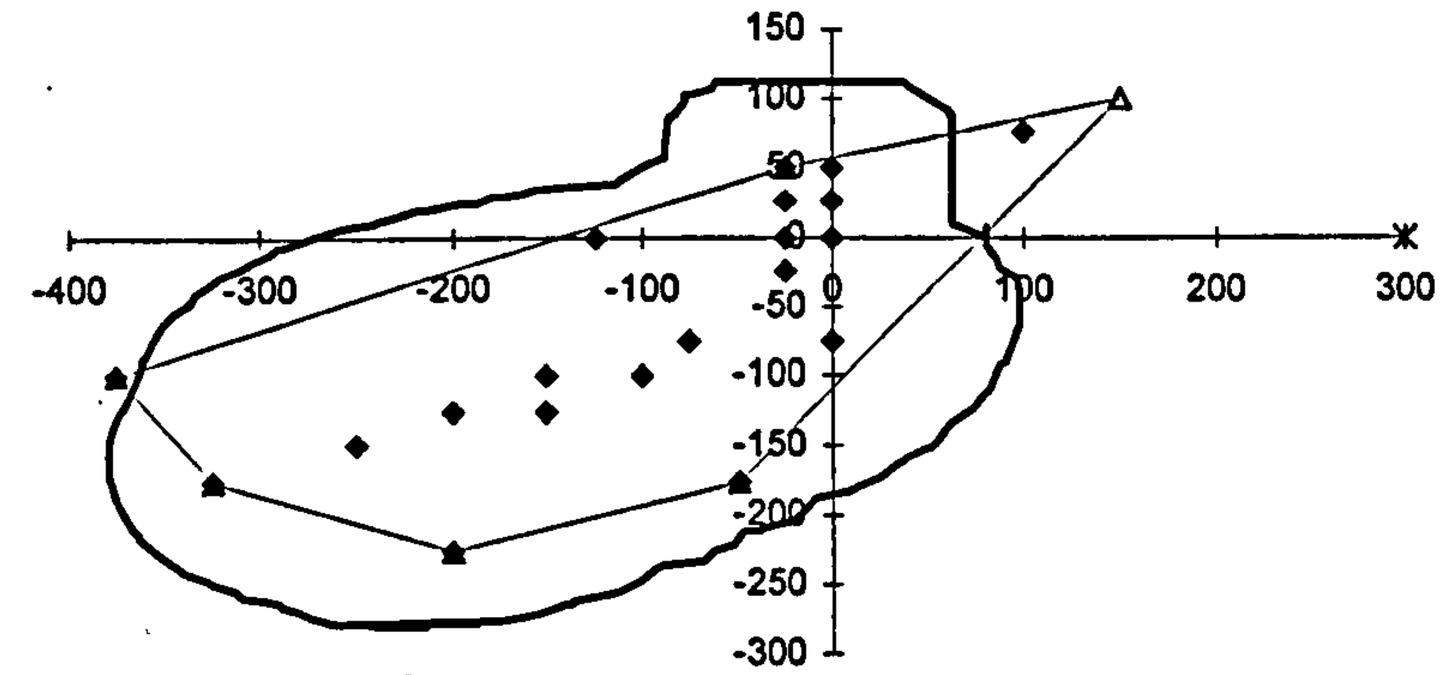


Fig 4.2.2.b. Sam's core area. Broken line indicates Ziggy's home range (100% MCP)

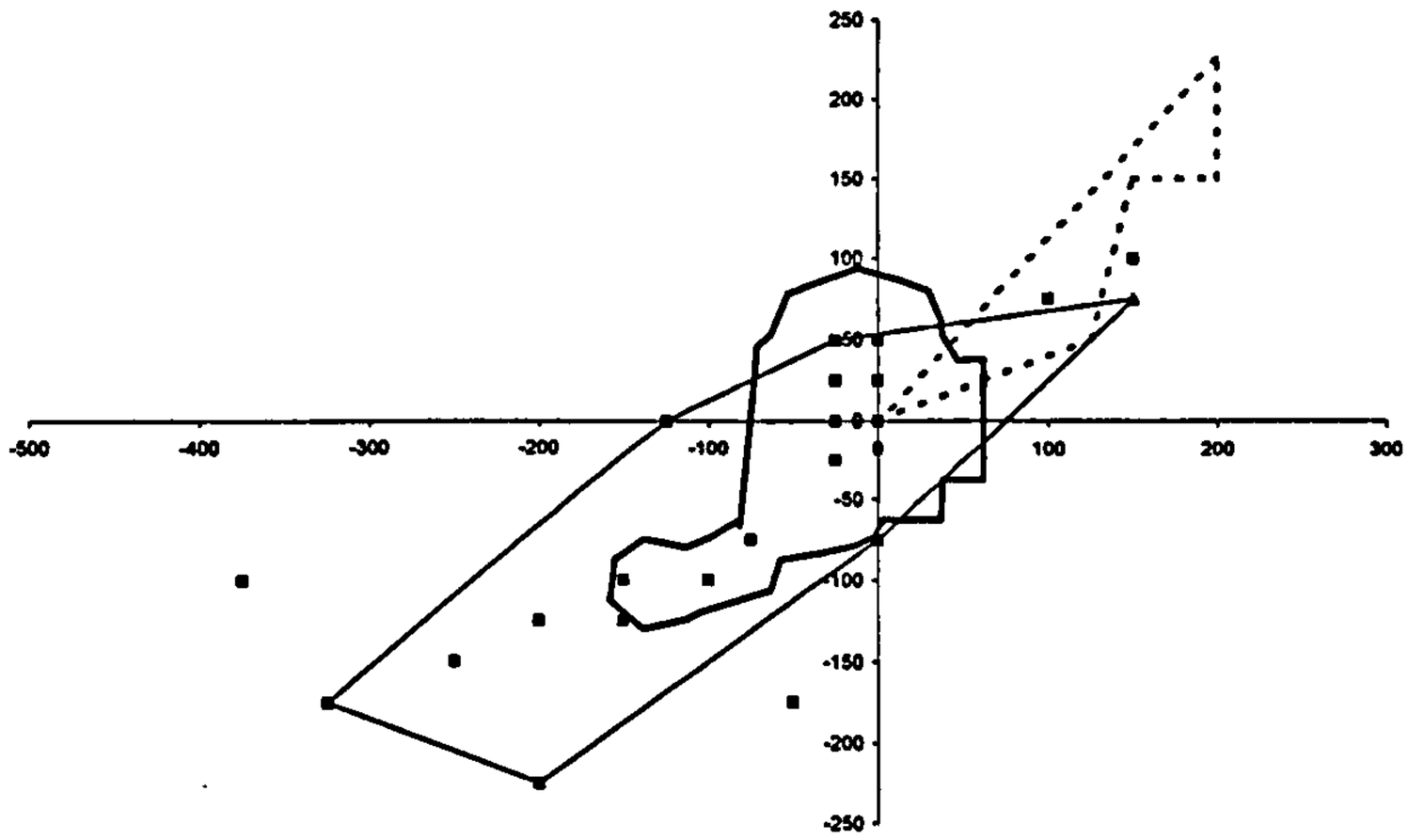


Fig.4.2.3.a. Marmalade's home range

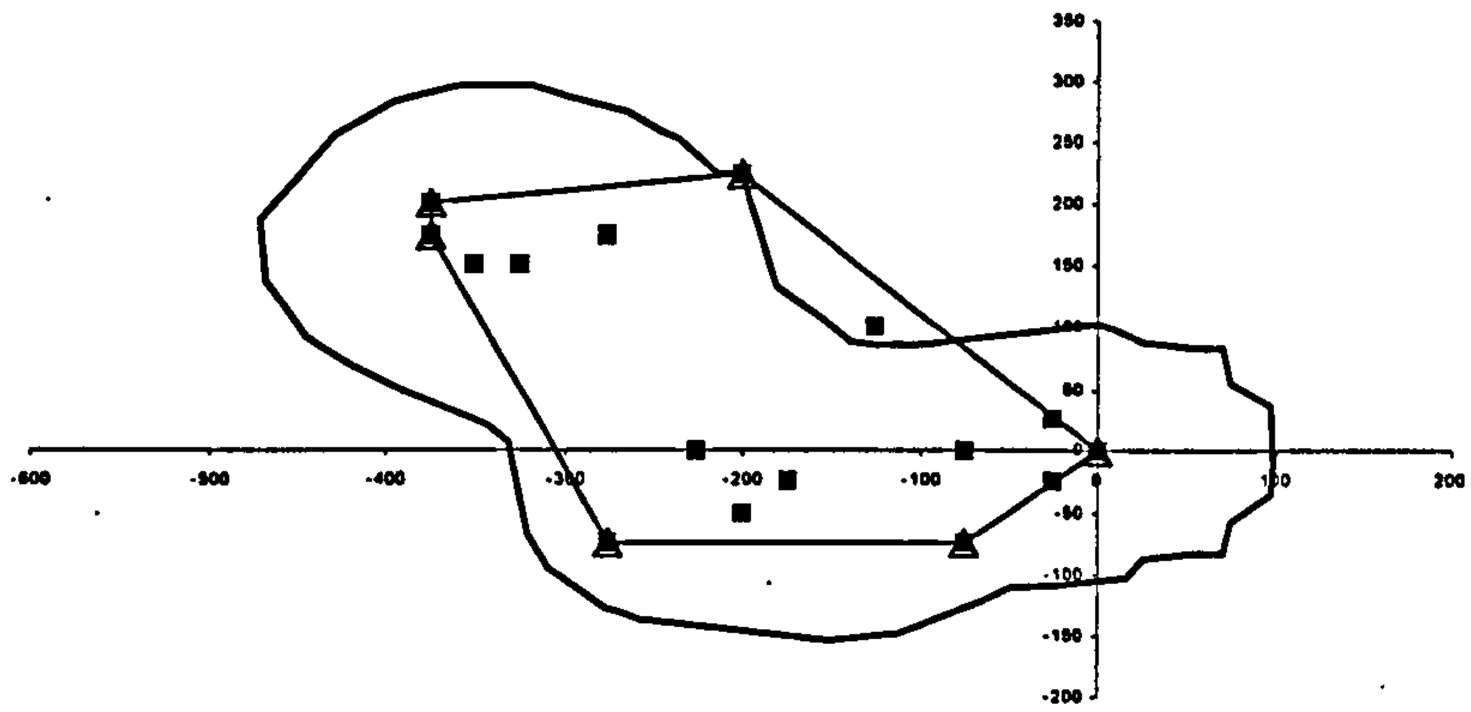


Fig.4.2.3.b. Marmalade's core areas

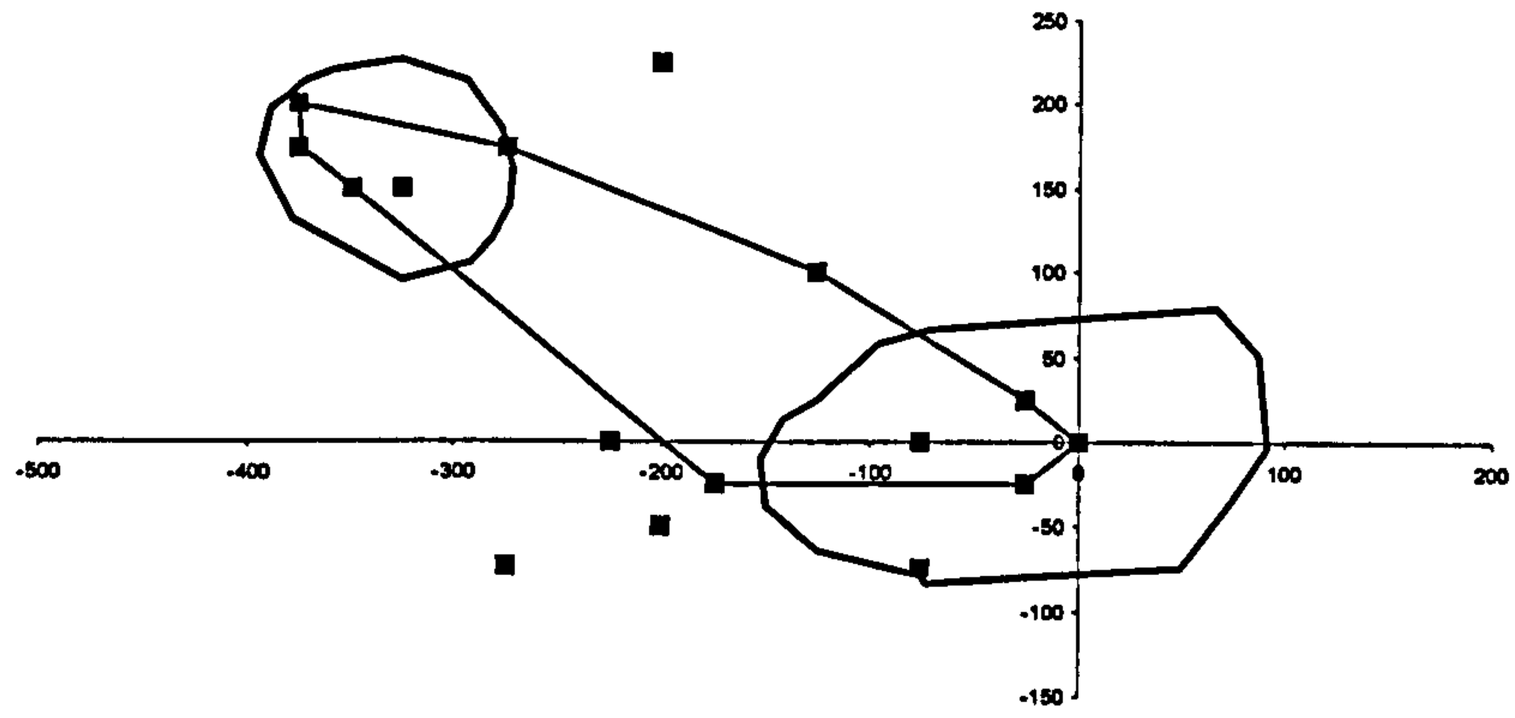
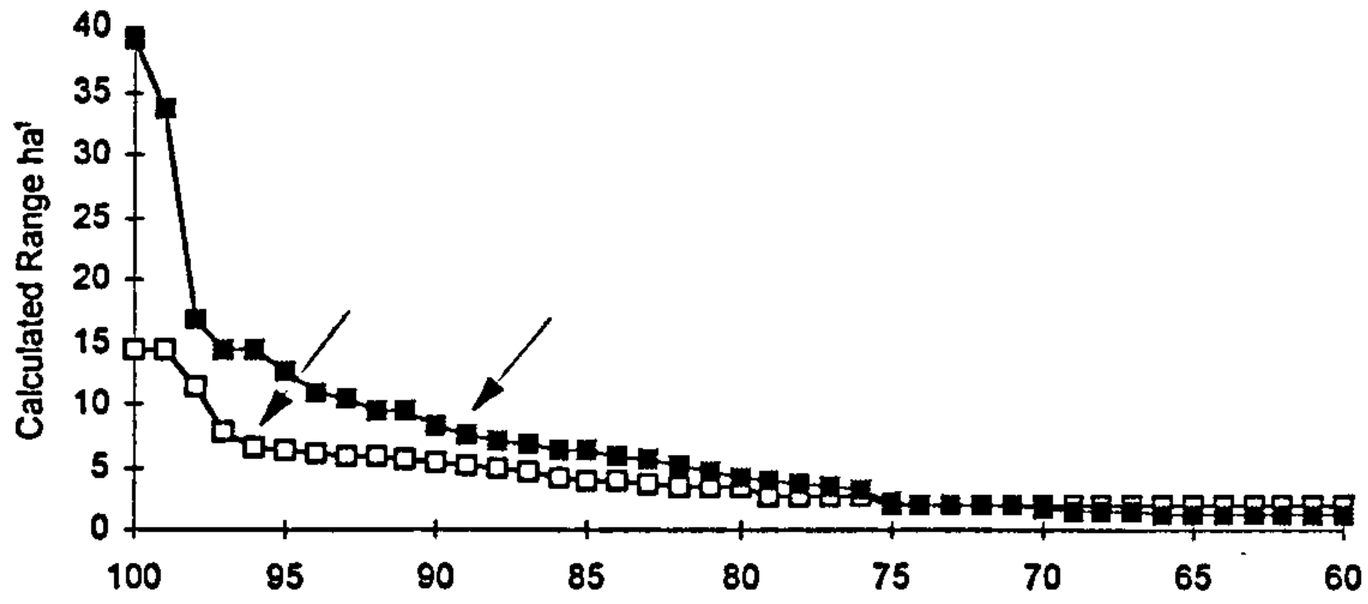
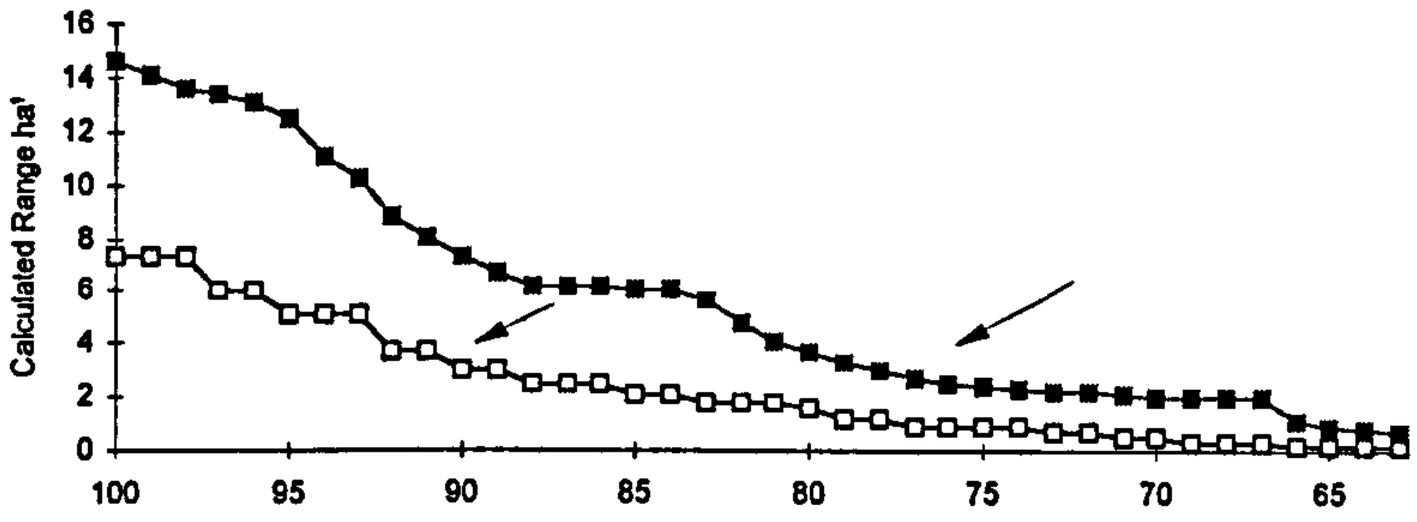


Fig 4.3. Core ranges identified by plotting calculated home range against *i.* MCP point inclusion (hollow squares), *ii.* Adaptive kernal isopleth (filled squares). Arrows indicate the chosen points of inflection.

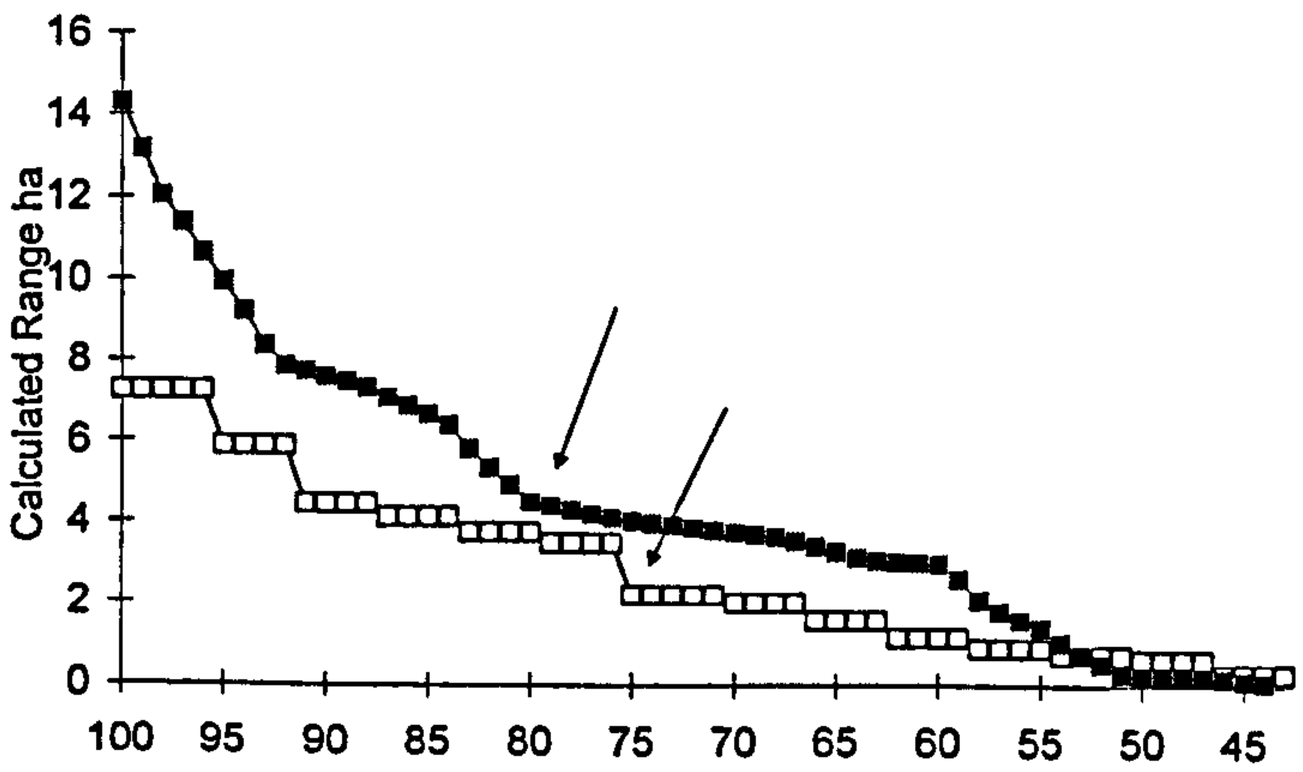
a. Ebony



b. Sam



b. Marmalade



4.3.3. Use of space over time

The positions of cats were recorded every 5 minutes throughout continuous recording sessions. For the purpose of analysing the cats' use of space over time, the cats' position was considered to be constant within each 5 minute increment in time. The data were analysed for the three mature cats that were tracked over at least one full tracking period.

4.3.3.1. Proportion of time spent outside of core ranges

The times during continuous tracking that were spent outside the calculated core ranges, and outside the 100% MCP range, were calculated as the number of 5 minute fixes and compared among the three main cats (**Fig. 4.4.**).

There was little difference in time spent in core areas between Ebony and Sam, either when calculated using adaptive kernel isopleths ($\chi^2 = 0.31$, d.f.= 1, $p>0.05$), or MCP percentage point inclusion ($\chi^2=1.23$, d.f.=1, $p>0.05$). However, Marmalade spent a significantly higher proportion of his time outside his core area than the other two cats when their data is pooled; AK: ($\chi^2= 17.03$, d.f.=1, $p<0.01$), MCP: ($\chi^2=40.03$, d.f.=1, $p<0.01$).

When time spent outside the 100% MCP range is considered, the difference between Marmalade and Sam was not significant ($\chi^2=2.41$, d.f.=1, $p>0.05$). However, both Sam ($\chi^2=5.41$, d.f.=1, $p<0.05$) and Marmalade ($\chi^2=15.77$,d.f.=1, $p<0.01$) spent a higher proportion of their time in excursions beyond their full MCP range than Ebony.

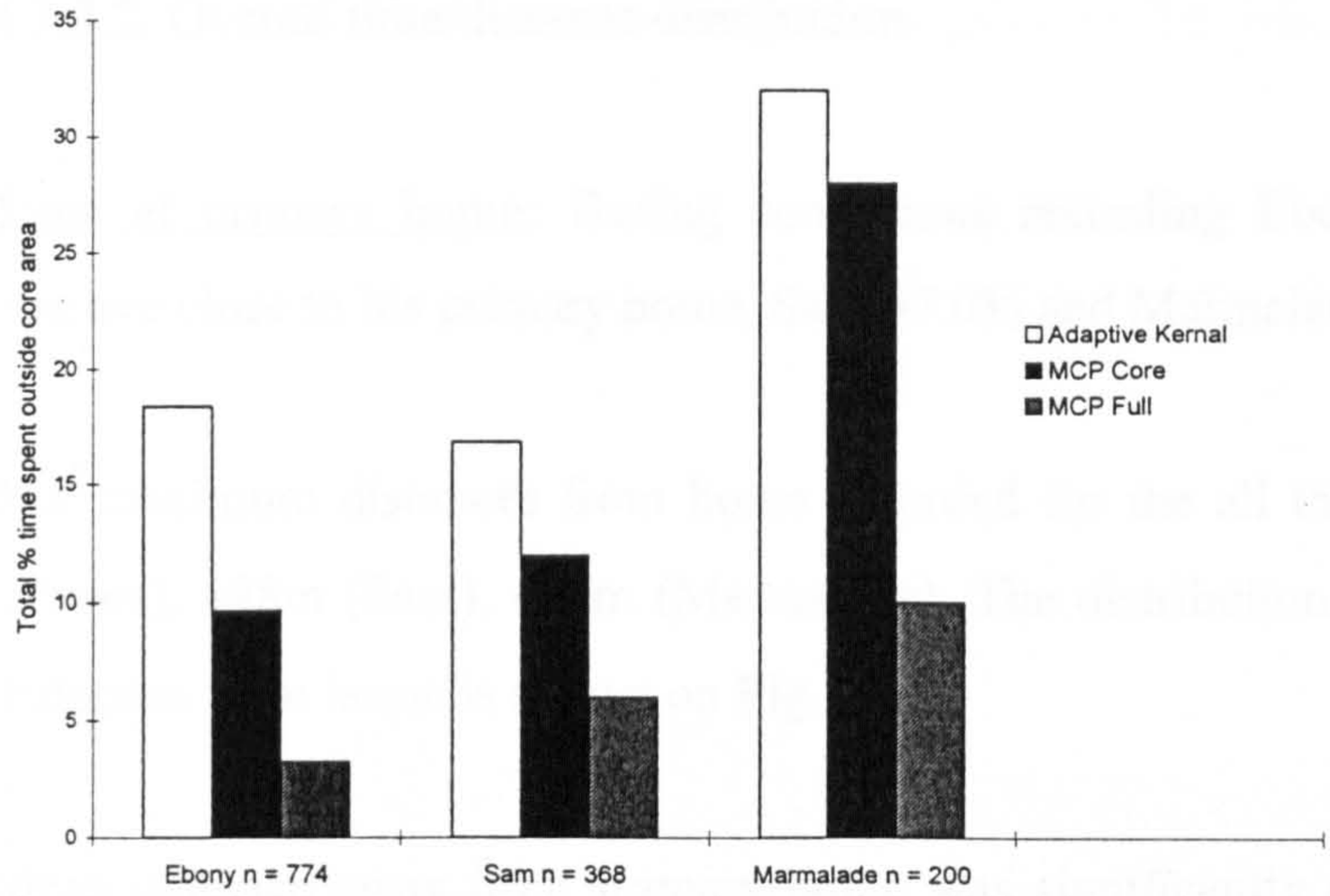


Fig. 4.4. Total percentage time spent outside core area calculated by adaptive kernal isopleths (white bars), MCP percentage point inclusion (black bars). Striped bars show percentage time spent outside home range (100% MCP).

4.3.3.2. Overall time/distance distribution

Time at primary home: During continuous recording Ebony spent 40.4% of time inactive close to his primary home, Sam 47.0% and Marmalade 30.0%.

The maximum distances from home recorded for the all three main cats were 760m (Ebony), 498m (Sam), 436m (Marmalade). The distribution of time spent at different distances from home is shown on **Fig. 4.5.**

Mean distance away from primary home was significantly different between the cats **(Table 4.4.)**. However these data give a good overall indication of the typical distances from home that tom cats might be expected to be found in areas and population densities of the type studied.

Table 4.4. Mean distances from primary home of 3 main cats, while away from primary home. Differences between mean distances: (Kruskal Wallis test: $H = 63.37$, $p<0.001$).

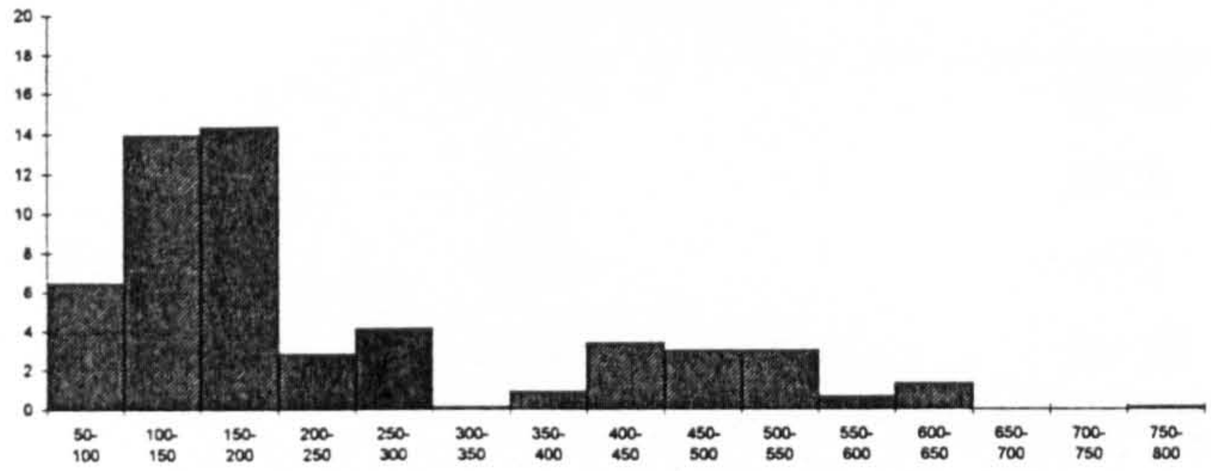
	Cat		
	Ebony	Sam	Marmalade
Mean (± s.e.) distance (m)	205.8 ± 7.2	128 ± 8.44	225 ± 10.06
Modal distance range (m)	150-200	150-200	300-350
n	464	197	142

4.3.3.3. Patterns of activity

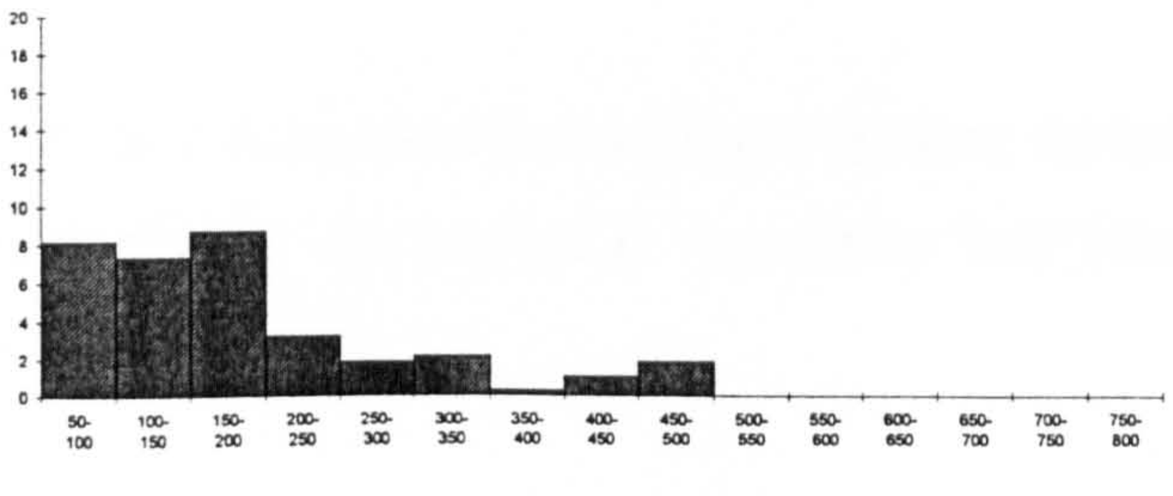
The complete data for distances away from home over continuous tracking sessions are shown in **Appendix 1.** No regular cycles in active/resting cycles were identified statistically. However the summary statistics are presented here **(Table 4.5).** Tracking sessions often finished before the cat had returned to its base: excursion times are therefore minimum values. Only occasions when the entire resting period had been recorded are included as values for resting at the primary home.

Fig. 4.5. Total time (converted to percentage) spent by cats at different distances from primary home.

a. Ebony



b. Sam



c. Marmalade

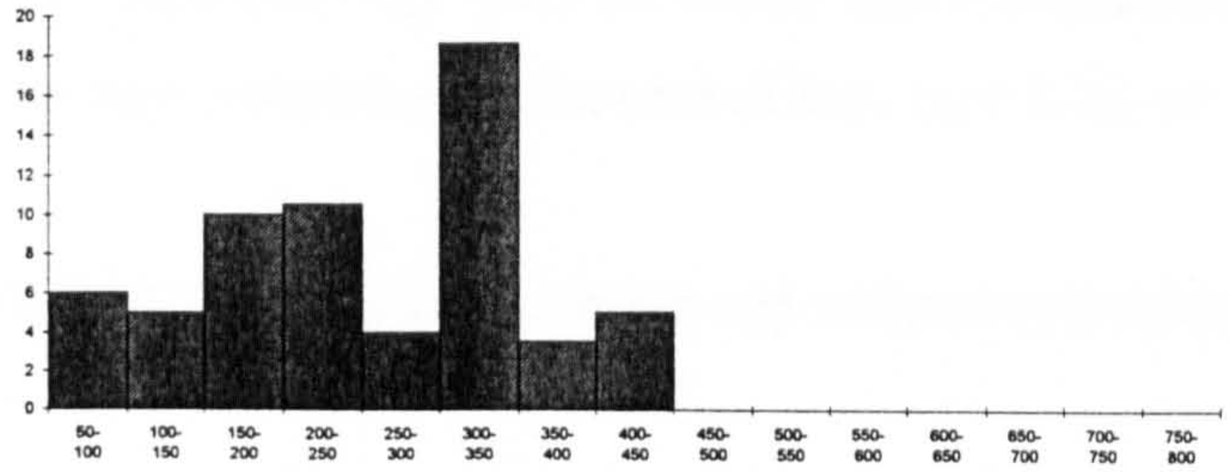


Table 4.5: Time spent at primary home and time spent active for the three main cats during continuous radio-tracking.

Cat		Mean minimum activity times (minutes)*		Mean resting times (minutes) ± S.E
Ebony	n = 15	320.0	n= 11	147.2 ± 35.3
Sam	n = 13	152.3	n= 9	101.3 ± 23.3
Marmalade	n = 4	357.0	n= 2	312.0 ± 166.2
Total	n = 32	256.6	n= 22	143.4 ± 31.3

* Standard errors were not calculated for activity times because minimum values were recorded, due to many tracking sessions finishing before the toms returned to their home bases

4.3.4. Activity

Activity was measured as the linear distance moved between 5 minute fixes. Occasions when the cat was located at its primary base (distance = 0) were excluded from the analysis.

4.3.4.1. Activity related to distance

There were no trends for activity to increase as distance away from primary home increased (see **Fig. 4.6.**) for Ebony and Marmalaide. However, there was a significant positive correlation in the case of Sam ($r_s = 3.76$, n= 197, $p<0.01$).

4.3.4.2. Activity levels within and without core areas

Levels of activity were clearly higher outside the core areas for Ebony and Sam (**Table 4.6.**). Marmalade did not exhibit this trend, though the significance of this is unclear considering the poor definition Marmalade's core area.

The results for Ebony and Sam give some empirical evidence that cats exhibit different behaviour patterns inside and outside their core areas. This supports the hypothesis that

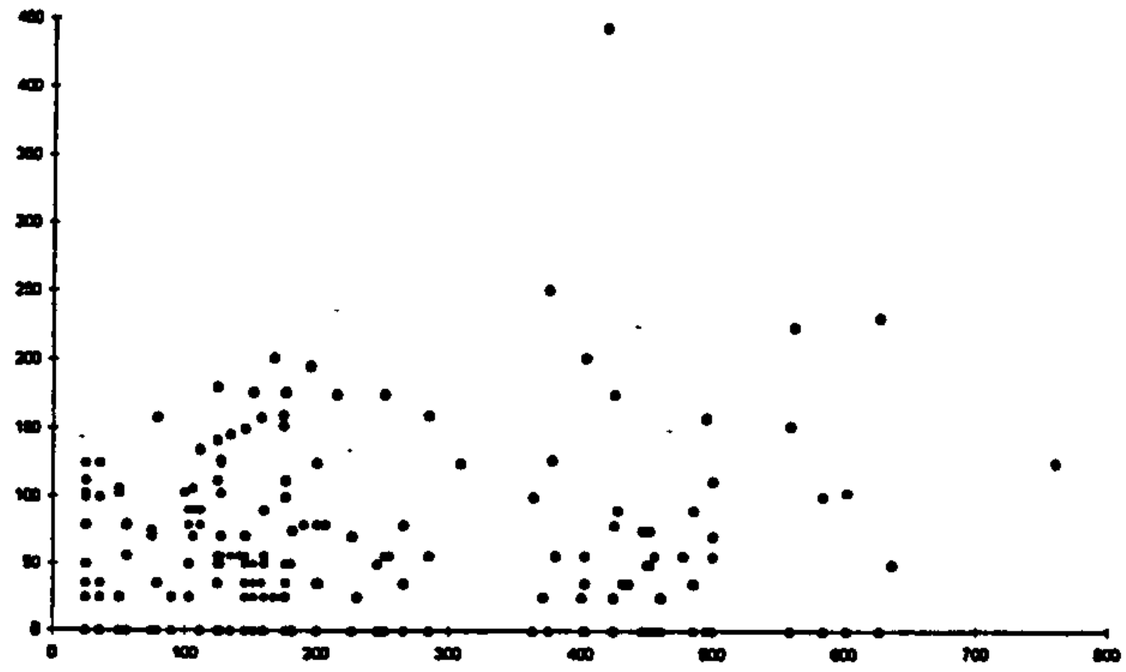
core areas are important ecologically.

Table 4.6. Activity levels inside and outside core areas calculated by MCP percentage point inclusion

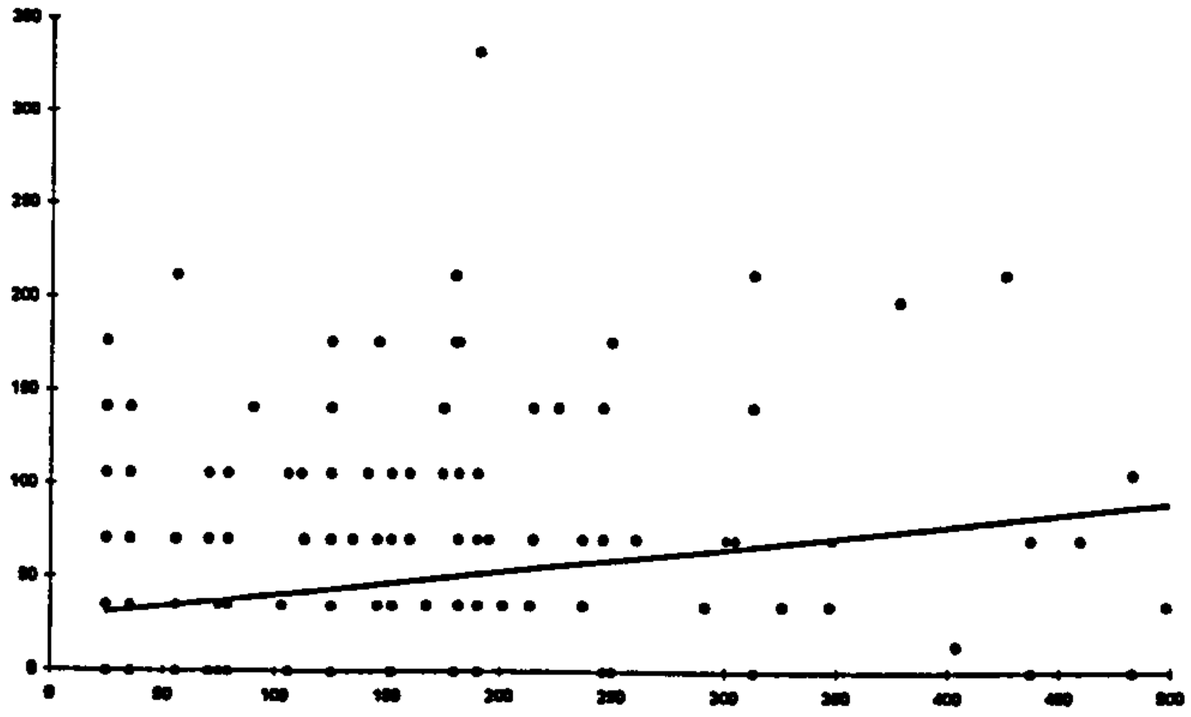
	Ebony		Sam		Marmalade	
	In core	Out of core	In core	Out of core	In core	Out of core
n	389	75	147	45	100	37
Mean activity (meters /5minutes) ± s.e.	26.45 ± 2.44	59.69 ± 10.97	37.38 ± 4.79	66.78 ± 8.48	42.71 ± 6.11	28.48 ± 8.19
Mann-Whitney U test	U = 11678 P < 0.01		U = 2024.5 P < 0.001		U = 2078.5 P > 0.05	

Fig 4.6. Scatter plot showing activity (meters moved/ 5minutes) against distance from primary home (m): Y axis.

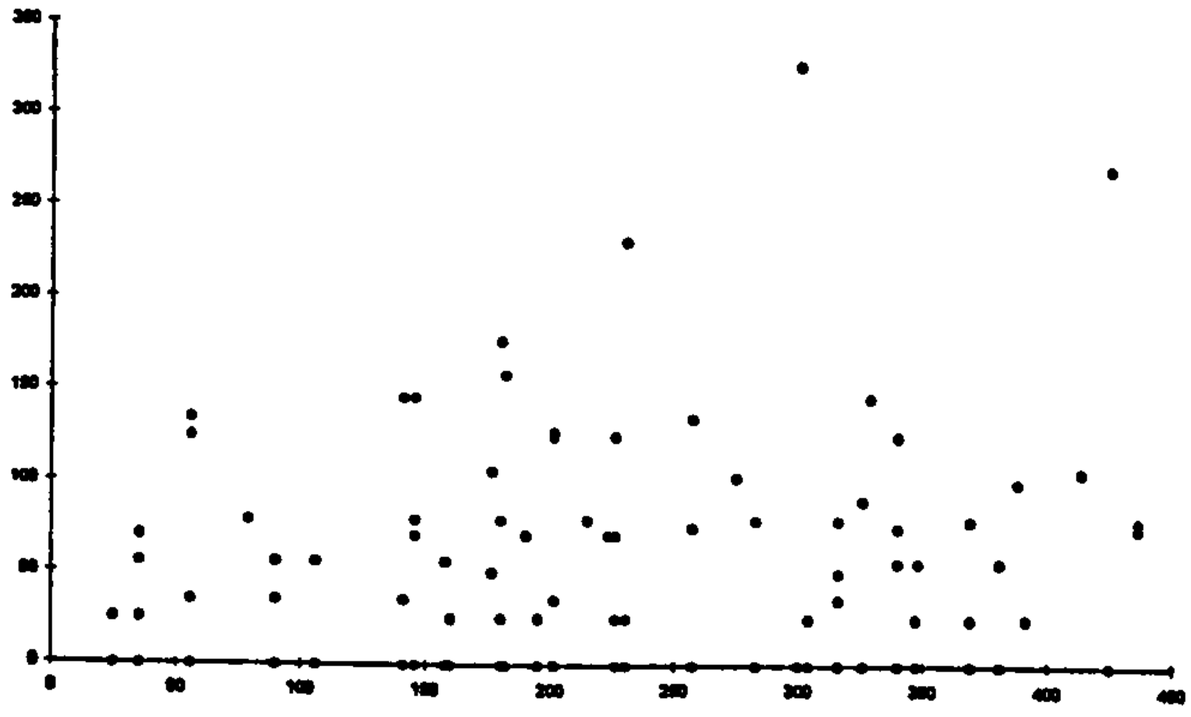
a. Ebony: (Rs = -0.01, n = 456, p > 0.05)



b. Sam: (Rs = 0.378, n = 378, P< 0.05)



c. Marmalade (Rs = -0.01, n = 137, p < 0.05)



4.4. Discussion

The home range analysis described here suggests that while an approximation to the range of an urban tom cat can be obtained with around 25 independent fixes using minimum convex polygon methods, at least 40-60 independent fixes are desirable for a robust measure of home range and core areas. MCP methods defined range areas that agreed well with those predicted by visual inspection. Adaptive kernels covered areas that were never visited by the cats, indicating that insufficient data were collected for the use of this method.

As predicted, the results from this study have revealed larger home ranges than the previous urban studies based on sighting methods. The smallest MCP 100% of an adult cat was 7.38 ha, compared to Chipman (1990) where only one male range exceeded 1.5ha (6ha), and Mirmovitch (1995) where all the home ranges were under 1 ha. These differences may partly be attributed to ecological parameters: the density of tom cats was higher in the previous work cited. However, it is difficult to compare the results of this radio-tracking based study with one based on sightings. Home ranges, calculated from radio-tracking, were larger still in suburban Australia (Barratt, 1997); mean 7.89 ± 10.57, including females and neutered males. However, the majority of the larger ranges were situated in open land beyond the edge of the suburbs and hence were not truly urban.

There was an overall positive relationship between age of cat and size of range. This supports previous work carried out on owned cats in both rural and urban environments (Chipman, 1990; Liberg, 1981; Liberg and Sandell, 1988). The general form of the change in spatial use over time conformed to the following pattern:

i. Young toms rarely move more than 50m from their home base.

ii. Toms of 18 months to 2 years make occasional forays further from home.

iii. Toms cats over 2 years old exhibit extensive ranges.

Core areas were identified, and behavioural differences inside and outside the core areas were demonstrated. Cats were more active, as defined by distance covered in five

minute intervals, outside their core areas. This could reflect exploratory behaviour away from familiar territory outside their core areas, and guarding behaviour inside their core areas (see **Chapter 5**). Further work, using more cats with potentially overlapping ranges than were available here, could investigate whether or not core areas are maintained exclusively.

The interpretation of the results presented here has to remain limited because of the small sample size and the lack of extensive data on any tom over three years old. These constraints were imposed partly by the time available, but also by the difficulties involved in finding tom cat owners who were willing to participate, in a city with very high levels of neutering. Apart from the 7 year old tom that had to be removed from the study, a 10 year old was recruited, but died before the start of radio-tracking.

More work is needed to fully elucidate the use of space by urban cats. However, the primary aim of this chapter was to investigate the range areas that toms regularly cover, and the maximum ranges that they can be expected to cover within the environment of Southampton. The home range values and maximum range values obtained here, in conjunction with the population dynamics data, indicate that competition for mating opportunities between owned males is likely to be widespread despite the low population densities of entire cats. Exploratory behaviour beyond the limits of the home range points to the "wanderer" strategy predicted by Liberg and Sandell (1988). However, if core areas prove to be areas of the range that toms attempt to maintain exclusively, and succeed in siring a high proportion of kittens within, this would indicate that a guarding strategy may predominate in an urban environment with a low density of entire females, such as the one studied here.

The actual distribution of reproductive success between males will be examined using molecular evidence in **Chapter 5.**

5 Assessing kinship using Microsatellite Analysis

5.1. Introduction

5.1.1. Aims

In order to elucidate the structure and mating system of the Southampton cat population, microsatellite analysis was used to evaluate pedigree relationships between individuals. This data could be used in conjunction with population dynamics data (**Chapter 2**) and data on male home ranges (**Chapter 4**) to relate population density and male ranging behaviour with distribution of reproductive success. The aim was to establish whether litters, born at known locations, shared a father, and also, assess the reproductive success of genotyped males. This would give an indication of the density of reproductively active toms, and how reproductive success is shared between toms. It was also hoped to relate inferred paternity with temperament of kittens (**Chapter 6**).

5.1.2. The use of microsatellites

Microsatellites consist of tandem repeats of short sequences of DNA base pairs, up to 6 bp in length. Their short length; typically 100-200 bp, allows microsatellites to be amplified using PCR techniques once primers from the flanking sequences have been identified and synthesised. Microsatellite loci often exhibit high levels of variation within a population because the repeat chains mutate quickly, often due to errors caused by slippage in replication of the DNA strands. They are inherited in simple Mendilian fashion, and are though to be selectively neutral. They have therefore been successfully applied to a wide range of problems, notably kinship analysis (Bruford and Wayne, 1993; Queller *et al*, 1993; Rico *et al,* 1996) .

Microsatellite analysis of a single locus requires minimal quantities of DNA, and can be successfully performed using *circa* one nanogram per reaction, compared to 5ug of DNA needed for minisatellite analysis (Bruford and Wayne, 1993). Hair follicles were

the DNA source in this study (for reasons discussed below), and only nanogram quantities of DNA were expected to be obtained; Morin *et al,* (1994) found that freshly plucked hairs from chimpanzee contained up to 200 ng each. Hair follicles have also been used successfully as a DNA source for microsatellite analysis in humans (Thompson *et al,* 1992), brown bear (Paetkau and Strobeck, 1994) and domestic cats; as a forensic tool in a recently reported murder case (Menotti-Raymond *et al*, 1997b).

A further advantage of using microsatellites is that allele sizes can be measured precisely, to the exact base pair, allowing standardised comparison of data between gels (Bruford and Wayne, 1993). This greatly facilitates screening a large number of individuals for paternity and half-sibling relationships. Microsatellites were therefore considered the most suitable molecular marker for this study.

5.1.3. Microsatellites used in this study

Molecular markers were made available to the project by Marilyn Menotti-Raymond and Stephen O'Brien, from the National Cancer Institute, Maryland, USA. They sent details of primer sequences for ten microsatellite loci (later published: Menotti-Raymond and O'Brien, 1995).

5.2. Materials and methods

5.2.1. Sample Collection

The method of DNA collection needed to be non-invasive for the following three reasons:

i. It was important maintain the goodwill of the cat owners involved in the project, who might not have consented to any method, e.g. collecting blood samples, that would be likely to cause distress to their cats.

ii. The UK law restricts taking of blood samples in domestic animals to veterinarians and holders of an appropriate home office license.

iii. Invasive methods are against the charter of the AzI.

Several non-invasive methods were considered. Taking buccal swabs was feasible for friendly cats, but caused distress to more fearful individuals. Extraction and amplification of DNA from faeces (see **Appendix 2.1.**) was found to be possible, but collection of samples was generally restricted to cats that used litter trays. Even then, when there was more than one cat in the household, it was not always possible to match the cats with the samples with any certainty. Dead skin, obtained by combing the cat, gave poor and inconsistent results.

Freshly plucked hairs were relatively easy to obtain for almost all cats. Although initial difficulties were experienced in amplification of DNA, a reliable protocol was eventually derived.

5.2.2. Collection of hairs for DNA extraction

Cats were firmly held during removal of hairs, either by their owner or by myself. Hairs (20-40) were plucked from the posterior of the cats' back, close to the tail.
The hairs were inspected by eye to ensure that some root was present. The hairs were packed in a paper envelope (Niceday), as recommended in Morin *et al,* (1994), care was taken not to handle the roots. The envelopes were labelled and, as soon as possible, stored at -20°C.

5.2.3. Extraction of DNA from hair roots

5.2.3.1. Preliminary attempts at DNA extraction

Several methods of extracting DNA from hair roots were attempted before a reliable method was developed. These methods are briefly described and evaluated here, and the full protocols are presented in **Appendix 2.2.**

Phenol/Chloroform extraction (Appendix **2.2.1.**)

This protocol was carried out using 50 hair roots per sample. The hair roots were crushed in an Eppendorf tube prior to the extraction process. The extract was visualised on a 0.8% agarose gel stained with ethidium bromide. Approximately 1ng of DNA was obtained per hair. Often, however, no measurable quantity of DNA was obtained. The inconsistency of the method ruled it out.

Salting out method (Appendix **2.2.2.**)

Satisfactory yields were obtained using this protocol, similar to phenol/chloroform extraction. However, salting out was also subject to an unacceptably high level of inconsistency.

Chelex method (Appendix **2.2.3.**)

It is thought that pigments present in hair shafts inhibit PCR. Chelex is a resin that removes inhibitory metal ions from solutions. The use of chelex in DNA extraction was first described by Walsh *et al,* (1991). The protocol described here includes a proteinase digestion with a non-ionic detergent. Variations on this protocol (from P. Morin, pers. com.) have been used successfully on studies using hair roots as a DNA source for studies of chimpanzee (Morin *et al,* 1994) and brown bear (Taberlet *et al,* 1997). However, success rates were very low in this study.

Single hair immersion method (Appendix **2.2.4.**)

This simple protocol proved effective, but was time consuming because a separate hair was required for each reaction. It was therefore abandoned for the more efficient

protocol described below.

5.2.3.2. Cell lysis method: development of the protocol used for this study. This original protocol was taken from Hoezel and Green (1992).

Materials

10 x PCR/lysis buffer: 500mM KCl; 100mM Tris-HCl pH 8.3; 25 mM $MgCl_2$; 1mg/ml gelatine; 5% NP40; 5% Tween-20; stored at -20°c.

Method

- 5µl 10x PCR/lysis buffer, 1 µl proteinase K solution (Sigma), and 37 µl H_20 were mixed in a PCR tube.
- A single hair root was immersed in the solution.
- Incubation was carried out at 65°C for 2 hours (or at 37°c overnight).
- The sample was incubated at 95°C for 10 minutes to inactivate the proteinase.
- Remaining PCR reactants were added (increasing reaction volume to 50 µl) and PCR proceeded with.

This protocol was adapted as follows:

Results were improved when Lysis buffer was substituted by the PCR buffer supplied with Taq polymerase (Promega). The PCR protocol described by Menotti-Raymond and O'Brien (1995) uses a reaction mixture of 10 µl. In order to maximise the quantity of DNA in the reaction mix, the maximum quantity of substrate (6.44 µl, see below) that could be incorporated into the 10 µl reaction mix was added from the DNA extract.

Eventually the protocol was adjusted by increasing the volume of the DNA extract to 150 µl to ensure that the process only needed to be carried out once for each individual. Also, the number of hairs used in each extraction was increased to 10-15, to ensure that the substrate contained an adequate quantity of DNA.

The following DNA extraction protocol was eventually used:

10-15 hair roots were washed with MQ H_2O and immersed in 150ul lysis mixture (120 µl MQ H_2O; 15 µl pK; 15 µl Taq buffer). The mixture was incubated at 65°c for 2 hrs, and then heated to 95°c for 10 minutes to deactivate the pK. The substrate was stored at –20°C. The substrate was thawed and centrifuged when required, and 6.44 µl was used in each 10 µl reaction mixture.

5.2.4. PCR procedures

5.2.4.1. Primer characteristics

The primer sequences are published in Menotti-Raymond and O'Brien (1995). Summary information on the 8 primer pairs that were used in this study is shown in **Table 5.1.**

Table 5.1. Summary of primer characteristics

Locus	No. of Repeats	PCR product size (bp)	Forward primer sequence 5' to 3'	Reverse primer sequence 5' to 3'
Fca 8	$(CA)_{24}$	144	ACTGTAAATTTCTGAGCTGG CC	TGACAGACTGTTCTGGGTA TGG
Fca 23	$(CA)_{17}$	148	CAGTTCCTTTTTCTCAAGATT GC	GCAACTCTTAATCAAGATT CCATT
Fca 35	$(CA)_{18}$	148	CTTGCCTCTGAAAAATGTAA AATG	AAACGTAGGTGGGGTTTA GTGG
Fca 43	$(CA)_{17}$	130	GAGCCACCCTAGCACATATA CC	AGACGGGATTGCATGAAA AG
Fca 77	$(CA)_{20}$	150	GGCACCTATAACTACCAGTG TGA	ATCTCTGGGGAAATAAATT TTGG
Fca 78	$(CA)_{19}$	199	TGAACTGAAGTCAGATGCTT AACC	CGGAATCAGCTATTTTTAC GG
Fca 90	$(CA)_{17}$	113	ATCAAAAGTCTTGAAGAGCA TGG	TGTTAGCTCATGTTCATGT GTCC
Fca 96	$(CA)_{17}$	213	CACGCCAAACTCTATGCTGA	CAATGTGCCGTCCAAGAA C

5.2.4.2. Initial testing of the System

The primers were tested using high quality feline ovarian DNA, obtained from a local veterinarian. Once the primers were shown to work, the same protocol was applied to DNA obtained by non-invasive means. Agarose gels (2%), stained with ethidium bromide, were used as a rapid and inexpensive means of ascertaining whether or not a PCR product had formed, before transferring to polyacridamide gels to optimise the system.

5.2.4.3. Optimising PCR conditions

The PCR conditions reported in Mennotti-Raymond and O'Brien (1995) were modified to reduce the high levels of non-specific priming that were initially obtained at most loci.

- The concentration of $MgCl_2$ can profoundly effect the results of PCR; low concentrations may lead to non-amplification of alleles, while high concentrations of $MgCl_2$ can increase non-specific amplification, giving spurious bands. A series of concentrations of $MgCl_2$ between 2mM and 0.1mM were used to establish the optimal concentration for each locus.
- Touchdown protocols were adopted (Mellersh and Sampson, 1993). Touchdown works on the principle that a high annealing temperature will allow primers to bind only where there is a perfect match with the flanking sequence. The method involves starting a PCR reaction with a high annealing temperature for the first cycle, and lowering the annealing temperature in subsequent cycles in increments of 1°C until the touchdown temperature; the standard annealing temperature, is reached. The reaction then continues for an appropriate number of cycles at the touchdown annealing temperature. In preliminary attempts, the maximum annealing temperature was varied between 62°C and 58°C in increments of 1°c.

Optimal conditions were found to vary between loci. The optimal starting temperature for Touchdown PCR and the optimal $MgCl_2$ concentration for each locus is presented in **Table 5.2.** Magnesium concentrations needed to be very low to produce

unambiguous results, the reasons for this are unclear. It was also found that concentrations of Taq polymerase (Sigma) could be lowered from 5 units (0.24) to 3.75 units (0.18 μl) without deleterious effects.

Table 5.2. Concentrations of $MgCl_2$ and maximum annealing temperatures during "touchdown" PCR used for each primer

Locus	$MgCl_2$ concentration used (mM)	Max annealing temperature (°c)
Fca 8	0.2	60
Fca 23	0.5	63
Fca 35	0.5	65
Fca 43	0.5	64
Fca 77	0.4	64
Fca 78	0.5	64
Fca 90	0.2	64
Fca 96	0.5	64

5.2.5. Genotyping procedure

5.2.5.1. PCR mix:

• 10x Taq buffer	0.36 µl
• $Mgcl_2$ (25mM)	0.08µl l-0.2µl (Varies with primers, see **Table 5.2.**)
• dNTP mix[2]	1.6 µl
• Primer 1 (@approx75pm/µl)	0.3µl
• Primer2 (@approx75pm/µl)	0.3µl
• Taq polymerase[3] (5U/µl)	0.18µl
• dATP (^{32}P) 10mCi/ml	0.05 µl
• H_20	0.56-0.68µl
+DNA solution	6.44µl
Total	**10 ul**

[1.] N.B. The DNA solution already contained Taq buffer at the appropriate concentration, the quantity of buffer added directly to the PCR mixture was therefore reduced accordingly.

[2.] The pre-mixed dNTP solution was formulated to compensate for the additional dATP in the solution in the form of ^{32}P (G Lushai, Pers. Comm.):

- 200 uM dGTP
- 200 um dCTP
- 200 uM dTTP
- 20 uM dATP

[3.] The quantity of Taq polymerase used was greater than in most studies, but represents a reduction from those used by Menotti- Raymond and O'Brien (1995).

- The DNA extract was transferred to labelled 0.5 ml PCR tubes. The PCR mix was then aliquoted into the tubes. Finally, 20ul mineral oil was added to each tube to prevent evaporation.
- In order to provide a "hot start", which reduces non-specific priming as the reaction mixture heats up during the initial denaturation phase, the tubes were kept

on ice until the microplate temperature reached 94°C, they were then placed on the microplate. The microplate was covered with silver foil to reduce the risk of contamination by ^{32}P.

5.2.5.2. PCR Cycle

PCR was carried out in a hybaid thermal cycler using the follwing cycles

• 94°c	4 minutes	x1 cycle	Initial denaturation

then

• 94°c	45 Seconds		Denaturation
• 60-65°c	30 Seconds	x1 cycle	Annealing
• 72°c	15 Seconds		Elongation

Followed by single cycles where the annealing temperature reduced was by 1°c increments per cycle, until the annealing temperature of 56°c was reached, followed by

• 94°c	1 minute		Denaturation
• 58°c	1 minute	x 29 cycles	Annealing
• 72°c	5 minutes		Elongation

Finally

• 72°c	10 minutes	Final elongation

PCR reactions were by the addition of 5µl of stop dye, and heating at 94oC for 2-3 minutes.

5.2.5.3. Gel preparation and running

- Both plates were cleaned with dH_2O followed by 70% ethanol. Sigma-cote was applied to the base plate after approximately every 4^{th} gel. The plates were assembled using clean spacers and held together using bulldog clips.
- Polyacridamide gels (6%) were poured using Sequagel 6 (National Diagnostics Ltd), a premixed solution which requires the addition of 1% vol of Amonium

Sulphate (0.1g/ml) to activate it. 600 ul of APS were added to 60 ml Sequagel, and the gel was poured as soon as possible. Gel Combs were inserted blunt end first into the gel space at the top of the gel to produce the well space.

- When the gel had set, the comb was removed and the space washed thoroughly using tap water. The gel was then placed in the gel tank, and the reservoirs filled with 0.6% TBE. The gel was pre-heated for 30 minutes. The gel space was washed again using a syringe, to remove any urea, and the comb was inserted gently, allowing the teeth to penetrate to *circa* 3mm below the top of the gel.
- 2µl aliquots of the samples were loaded and the gel was run at 75W (2600v) for 3-4 hours. M13 Sequencing ladders (see **Appendix 2.3.**) were run alongside the samples for use as molecular size markers.

5.2.5.4. Autoradiography

- The gel was disassembled after running, with the plates carefully prised away from each other. The gels were transferred to Whatmann paper, covered with cling film, and dried in a gel drier at 80°c.
- Once dry, the gel was checked for peaks of radioactive counts where the loci were expected to be. The gel was placed in a x –ray cassette and autoradiographed using x-ray film (GRI) placed on top of the gel in a dark room. The films were exposed for variable lengths of time, depending on the recorded number of counts per minute, Hence:

200 cpm and above	1-2 h
100-200	2-4 h
50-100	4-6 h
25-50	overnight
10-20	2-3 days
5-10	1 week
2-5	2 week

- The film was developed by gentle agitation in: Kodak D-19 developer for 1-2 minutes, followed by immersion in Kodak stop bath for 1 minute and finally

Kodak rapid fixer for 5-10 minutes. The film was then submerged in tap water for 10 minutes, rinsed with dH_2O and then allowed to dry.

5.2.5.5. Scoring genotypes

Positive autoradiographs produced a series of bands, each associated with one allele. One individual sample was run per lane (see **Fig. 5.1.** for examples of autoradiographs). Three M13 sequencing ladders were used for calibration, A-Term, T-Term and C-term. Each gives a unique recognisable pattern, which could be reliably interpreted (**Fig. 5.1.**) Allele sizes were then scored on the autoradiograph relative to the labelled bands on the M13 sequencing ladder. These data were then recorded on spreadsheets, prior to statistical analysis.

5.2.6. Statistical procedures

5.2.6.1. Testing for the presence of null alleles

Null alleles are alleles that do not amplify under PCR and are therefore undetected, The most commons reasons are mutations in the primer binding site, preventing amplification of the allele (Paetkau and Strobeck, 1994). Also, some alleles can be very sensitive to variable PCR conditions, and so may simply fail to amplify (Neuman and Whetton, 1996). Consequently, when one null allele is present in a heterozygote, the locus may be falsely scored as homozygous.

The possible presence of null alleles was examined using the limited number of known pedigrees available. The incidence of null alleles in the data set as a whole was estimated using the Hardy-Weinberg (H.W.) equilibrium (Neuman and Whetton, 1996). This describes the expected frequencies of genotypes at a locus, for given frequencies of each allele at the locus under consideration (Ridley, 1993). Conformity to H.W. equilibrium is expected when the population exhibits random mating, shows no selection, and is of (effectively) infinite size. The H.W. equilibrium has long been

Fig 5.1.a. Example of autoradiograph of locus Fca 8. Each number represents one sample. The lanes with marker used for calibration are marked **mT** and **mA.** Molecular weights of representative calibration bands are shown. Common alleles are marked with arrows.

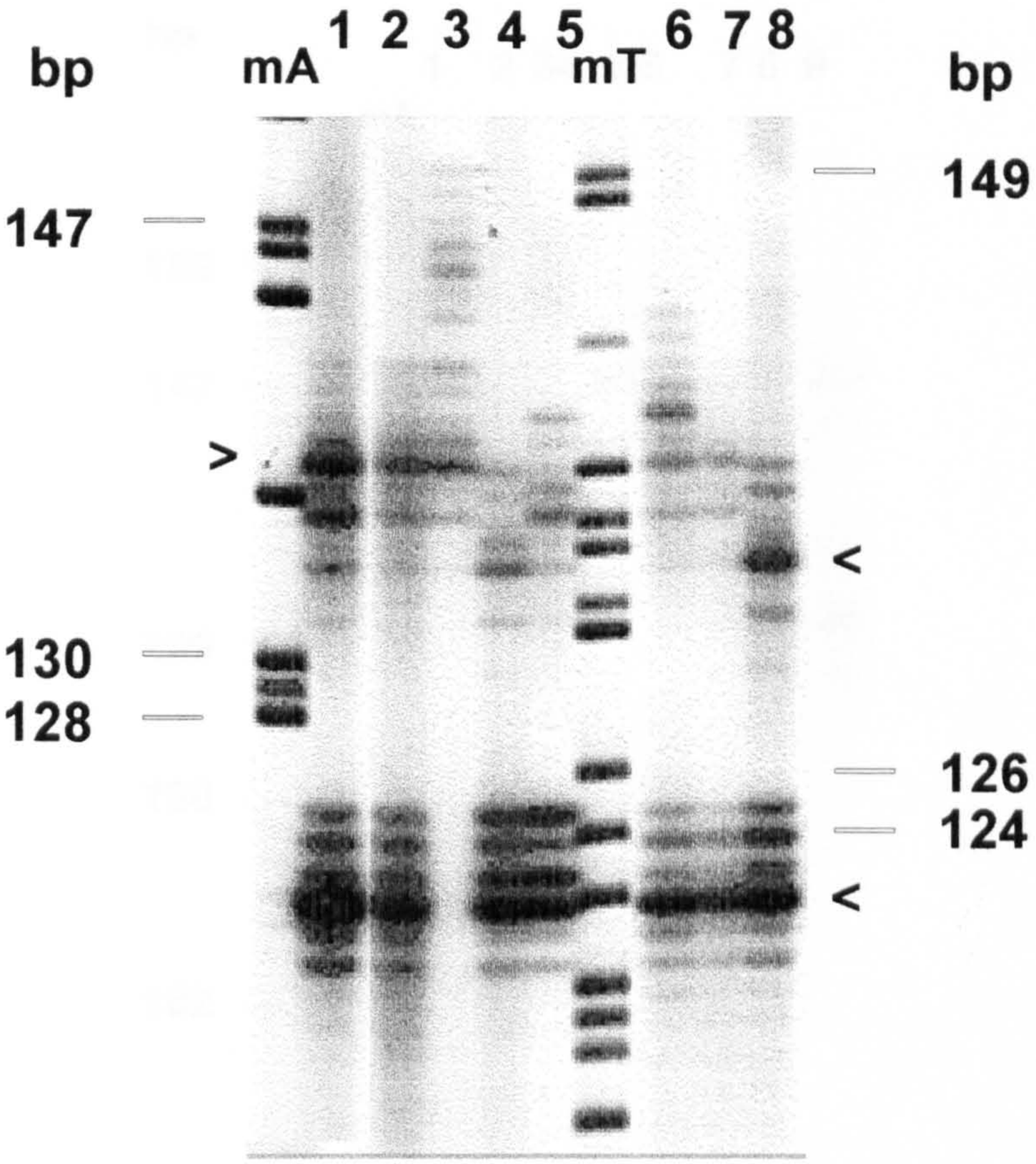


Fig 5.1.b. Example of autoradiograph of locus Fca 23. Each number represents one sample. The lane with marker used for calibration is marked **mA.** Molecular weights of representative calibration bands are shown. Common alleles are marked with arrows.

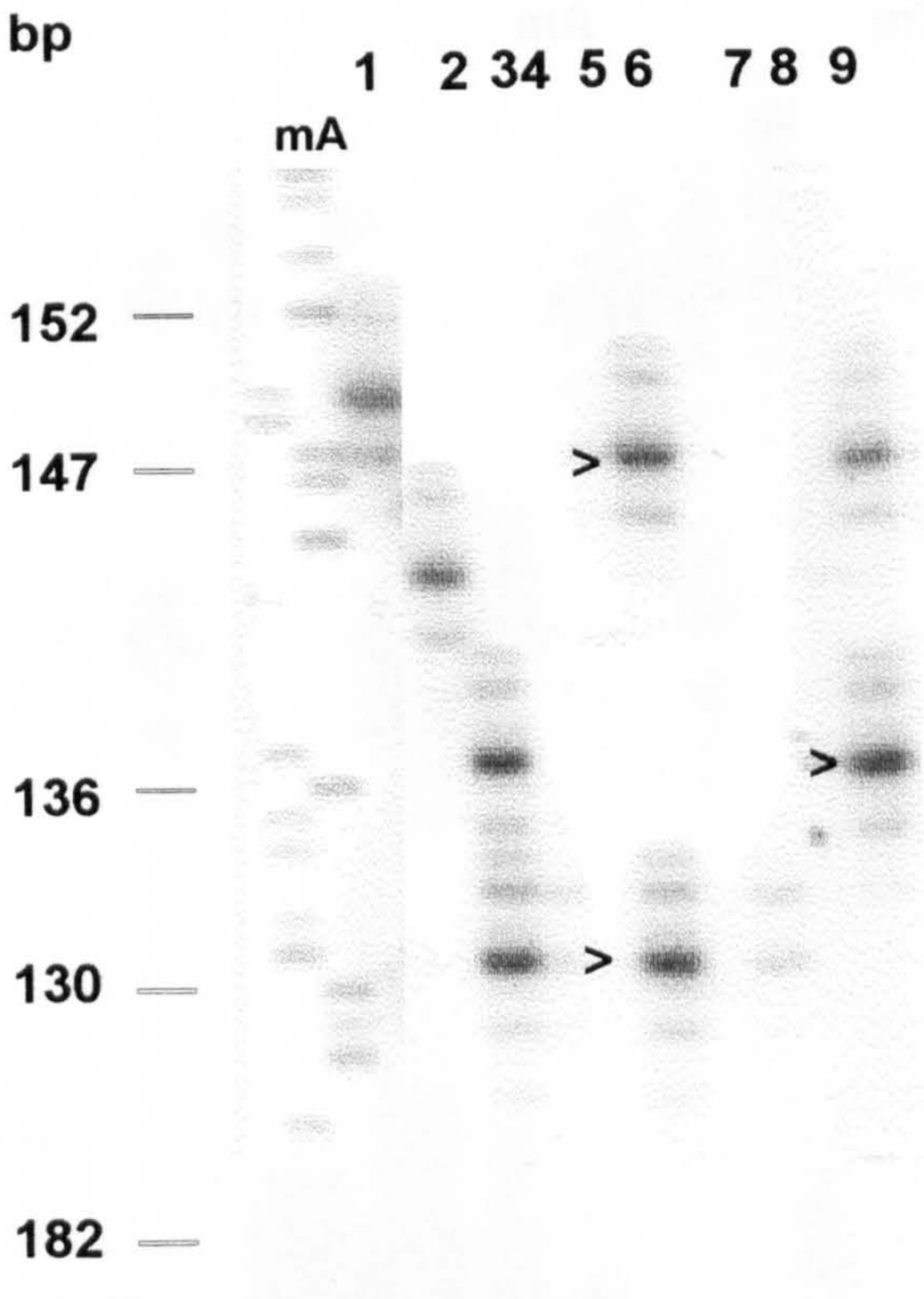


Fig 5.1.c. Example of autoradiograph of locus Fca 90. Each number represents one sample. The lanes with marker used for calibration are marked **mA and mT.** Molecular weights of representative calibration bands are shown. Common alleles are marked with arrows.

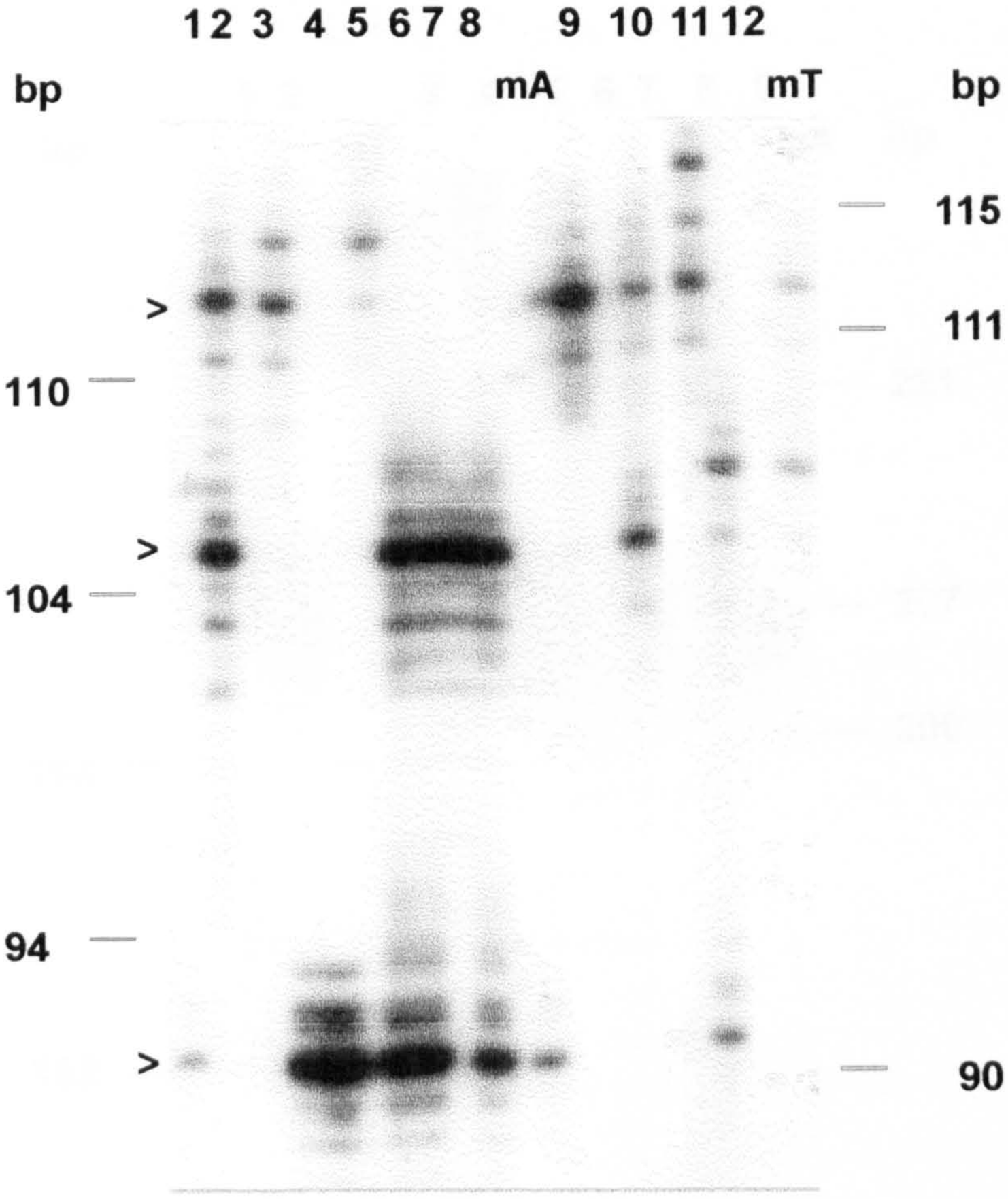
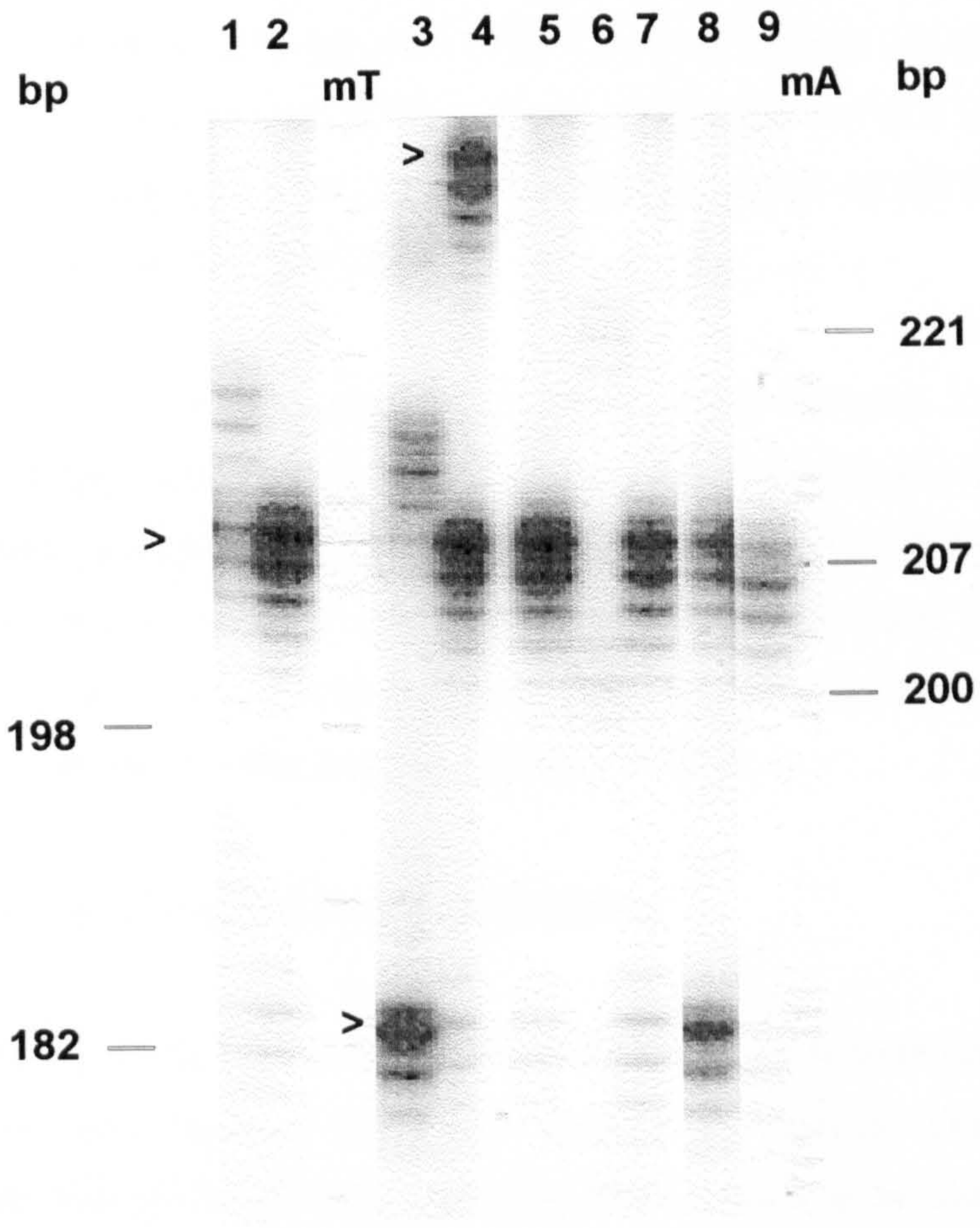


Fig 5.1.d. Example of autoradiograph of locus Fca 96. Each number represents one sample. The lanes with marker used for calibration are marked **mA and mT.** Molecular weights of representative calibration bands are shown. Common alleles are marked with arrows.



important to population genetics, often as a means of identifying a population under conditions of genetic flux (Guo and Thompson, 1992; Ridley, 1993).

The presence of a higher number of homozygotes than predicted under H.W. theory may indicate the presence of null alleles; this has frequently been used as a means of testing for the presence of null alleles in a data set (eg. Martinez *et al,* 1998). Alternatively, observed homozygous excess may reflect a genuine high frequency of homozygotes due to selection (highly unlikely at microsatellite loci) or there may be inbreeding, which is quite possible within the Southampton cat population (Ridley, 1993). The presence of null alleles does not preclude the use of a locus for parentage analysis, providing care is taken not to exclude parentage on the basis of this allele alone (Neuman and Whetton, 1996).

Assumptions of H.W. equilibrium were tested using Genepop 1.2. (Raymond and Rousset, 1995a). A Markov-Chain reaction is used, see Guo and Thompson (1992). The program calculates the error probability of rejecting the null hypothesis of H.W. equilibrium and the standard error associated with this estimate. The standard error may be reduced to an acceptable level (<0.01) by increasing the number of batches used in the calculation (Raymond and Rousset, 1995a).

5.2.6.2. Testing for population sub-differentiation

Possible levels of sub-population differentiation in allele frequencies between regions of the city were explored using two methods of analysis. First, the degree of relatedness between individuals, excluding those known to be close relatives, were compared within regions and between regions. Secondly, the random distribution of alleles at each locus between regions was tested for.

Calculating Relatedness values between individuals

The Relatedness option on Kinship 1.2. (Goodnight and Queller, 1996) calculates pairwise relatedness between individuals using the same statistics as Relatedness 4.2. (Goodnight and Queller, 1995). The program uses the allele frequencies in the data set to calculate the probability of allele sharing summed over loci and individuals, using jack-knifing. The basic equation used in the calculation is:

$$\frac{\sum\sum (Py - P^*)}{\sum\sum (Px - P^*)}$$

Where: P*: population frequency of the allele at the current locus, excluding the group of which the current individual is a member.

Px: frequency of the current allele in the current individual; 0.5 for a heterozygote,1 for a homozygote.

Py: frequency of the allele in the current individuals partners.

From Goodnight and Queller (1995).

The program assigns R values between –1 (no alleles shared at any loci) and +1 (identical genotype) to pairs of individuals, 0.5 represents first order relatives and 0 the average relatedness in a panmictic population.

The distribution of allele frequencies between regions of the city

Whether the distribution of alleles between regions conformed to random expectation, was tested using Option 3 of Genepop (Raymond and Rousett, 1995a). This uses an exact test for population differentiation based on Raymond and Rousset (1995b): The program extracts allele frequency data from individual genotypes (see **Appendix 2.4.**) for each locus, and forms RxC contingency tables. The analysis is based on a Markov-chain method, which for large data sets is much faster than the conventional exact test described by Gail and Mantel (1977), cited in Raymond and Rousset (1995b). The number of iterations and batches may be increased by the user to obtain a sufficiently precise result; preferably with a standard error under 0.01.

5.2.6.3. Testing specific hypotheses of kinship between individuals.

Kinship 1.2. either uses allele frequencies in the sampled population, or allele frequencies specified by the user, to calculate the likelihood of specified pedigree relationships (Goodnight and Queller, 1996). Pedigree relationships are specified by the user in terms of r-values for maternal (Rm) and paternal (Rp) inheritance. The relationships tested in this study and their associated r-values are presented in **Table 5.3.**

Table 5.3. Pedigree relationships tested in this study, and their associated r-values.

Relationship	r_m	r_p
Full sibling	0.5	0.5
Maternal half sibling	0.5	0.0
Paternal half sibling	0.0	0.5
Father-offspring	0.0	1.0
Unrelated	0.0	0.0

A primary and a null hypothesis were selected, and the ratio between them reported. This allows the user to establish how much more likely one outcome is than the other. For example to establish whether individuals are more likely to be full siblings than maternal half-siblings the user would enter: $r_m = 0.5$, $r_p = 0.5$, for the primary hypothesis and $r_m = 0.5$, $r_p = 0.0$, for the null hypothesis. In this way, all the potential kinship relationships of interest can be tested for.

Known groups of related individuals are specified in the input files (see **Appendix 2.5.**). An individual's relatives are expected to share alleles at a higher than chance level, which may bias background allele frequencies. The program therefore excludes an individuals relatives from background allele frequencies for likelihood calculations involving that individual.

Significance levels do not correspond to likelihood ratios in a simple way: as the size of the data set increases, the ratio needed to obtain a significant result decreases. The significance of levels of likelihood ratios are calculated empirically through simulations using the allele frequencies in the data set. The likelihood ratios required to exclude randomly generated null pairs, under the specified hypotheses, at 0.05, 0.01 and 0.001 levels of significance are calculated. Next, the proportion of randomly generated pairs that match the primary hypothesis, but are excluded at the likelihood ratios for each significance level, are reported as the type 2 error rate (false negatives). See Queller and Goodnight (1989) for a fuller description of these statistics.

5.3. Results

5.3.1. Summary of cats analysed

The cats were assigned to one of seven geographic categories: the Shirley/Freemantle area where the study was primarily focussed, five other regions of the city, and a final category for cats born in the outskirts of the city (**Table 5.4.**). The areas were assigned arbitarily, using postal districts and major roads as markers. The kittens were assigned to the area in which they were born, while the adult cats were assigned to the area in which they had been reproductively active (females) or potentially reproductively active (males). The geographical location of the cats is shown in detail for the Shirley/Freemantle region (**Fig. 5.2.**) and for the whole of Southampton (**Fig. 5.3.**)

Table. 5.4. Summary of cats genotyped in each area of Southampton, the number of kittens from each region that were included in the temperament testing study is also indicated.

Area	Potentially reproductive males	Mothers genotyped	Litters genotyped	Kittens genotyped	Kittens temperament tested	Other cats genotyped
Shirley/Freemantle	8	4	15	23	23 (83.3%)	1
Wimpson/Aldemoor	2	1	4	5	5 (100%)	0
Bitterne	2	4	5	6	5 (71.4%)	0
Highfield/Portswood	1	2	3	7	5 (72.7%)	0
Merryoak	0	1	5	7	7 (100%)	0
Sholing/Thornhill	5	3	8	11	8 (72.7%)	1
Soton other/Outskirts	0	1	14	15	15 (100%)	0
Total	**18**	**16**	**54**	**74**	**68 (91.9%)**	**2**

Two of the kittens, one from Sholing and one from Bitterne, had become mothers themselves by the time of re-testing (18 months), and are included in the table both as kittens and mothers.

5.3.2. Loci scored

A total of 107 cats from the Southampton area were genotyped. These belonged to 63 identified family groups. Not all individuals were scored for every locus (**Table 5.5.**). In some cases this was due to having obtained an insufficient quantity of DNA, for example when a member of the public collected the hair samples. In other cases, a sample repeatedly failed to amplify at a particular locus, which may have been due to poor quality DNA.

Table 5.5. Distribution of numbers of loci scored per individual.

Number of loci scored	8	7	6	5	4	3
Number of cats	80	19	5	0	3	1

5.3.3. Allele frequencies

Allele frequencies were calculated using one randomly chosen member of each family group; closely related individuals would be expected to share alleles at above chance level, and their inclusion would therefore skew the results. This subset of the data was also used to examine conformance to H.W. expectation and to explore questions of population sub-differentiation (see **Appendix 2.5.**).

Overall, the mean observed heterozygosity was 0.63, ranging from 0.373 (Fca 35) to 0.814 (Fca 23). The total number of alleles at each locus varied from 4 to 16 when considering one member for each family group, and 5 to 16 when considering all genotyped individuals. Summary data is presented in **Table 5.6.** Allele frequencies are presented, calculated using one member of each family group and also, for comparison, using all the individuals in the study (**Fig. 5.4.a-h**).

Fig. 5.2. Locations of cats from the Shirley/Freemantle area. The coloured circles show the locations of the cats used in this study. The circles represent the home bases at the time of the study for the mothers and toms, and the birthplace of the kittens. The numbers in the circles represent the number genotyped kittens born at that location. In addition to the kittens whose birthplaces are shown in the figure, there are four kittens whose precise birthplace is unknown, but which are known to have born in the area.

Scale: 1 square = 1 km^2

Birthplace of litters		1° home of toms	
Foyle (1)	War (7)	L72 (a)	C17(e)
Tell (2)	Arnold (8)	S67 (b)	ArnD (f)
Morris(3)		Ar6 (c)	W17 (G)
Pick (4)		SirG (d)	Whit (h)
Gil (5)			
Lever (6)			

Fig. 5.3. Map of Southampton showing locations of cats used in microsatellite study, where the locations were known precisely. The coloured circles represent the homes at the time of the study for the mothers and toms, and households where the kittens were born. The Shirley/Freemantle/Millbrook area is represented by the black rectangle (see **Fig. 5.2.**)

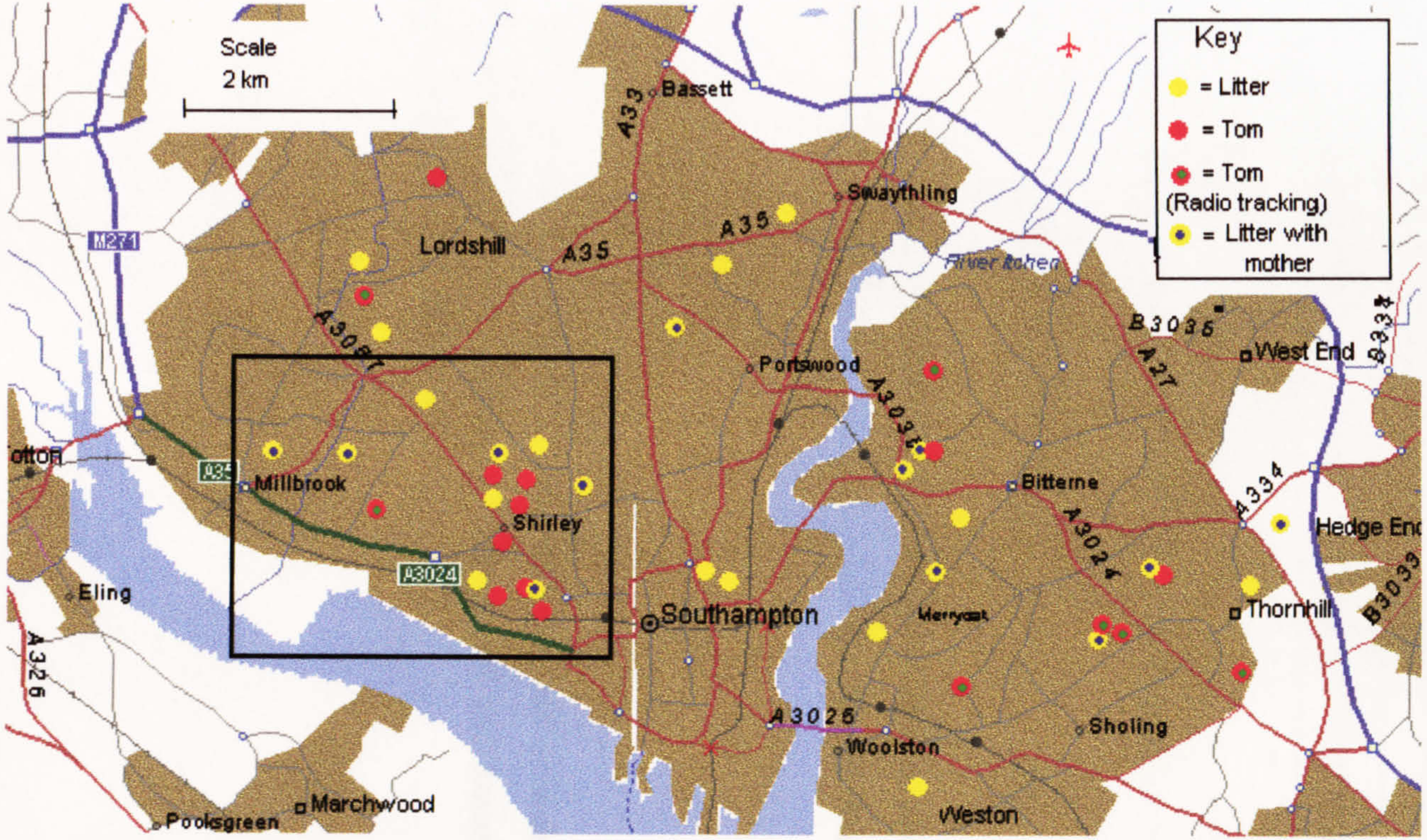


Table 5.6. Summary statistics of microsatellite data: heterozygosity, number of alleles scored per locus, and the frequency of the most common and rarest alleles

Locus	Observed heterozygosity	No. Alleles (all individuals)	No. alleles (one family member only)	Frequency of most common allele	Frequency of rarest allele
Fca 8	0.790	15	14	0.307	0.0081
Fca 23	0.814	15	14	0.322	0.0085
Fca 35	0.373	5	4	0.639	0.0082
Fca 43	0.541	8	8	0.598	0.0082
Fca 77	0.582	13	11	0.634	0.0089
Fca 78	0.760	16	16	0.186	0.0098
Fca 90	0.783	12	12	0.256	0.0083
Fca 96	0.426	11	9	0.625	0.0083
Mean	**0.634**	**11.88**	**11**	**0.446**	**0.0085**

5.3.4. Occurrence of null alleles and conformity to H.W. equilibrium

5.3.4.1. Null alleles inferred from pedigree analysis

There were 5 cases from the data set where the non-amplification of an allele could be identified or inferred. Three of these were in cats from the Arnold household (see below).

5.3.4.2. Conformity to H.W. equilibrium

Expected (H_e) and observed (H_o) numbers of heterozygotes, and probability values were calculated for each locus using Genepop 1.2. (Raymond and Rousset, 1995b). Summary statistics are presented in **Table 5.7.**

Table 5.7. Observed and expected heterozygosity at each locus.

Locus	H_o	H_e	prob	S.E.
Fca 8	0.790	0.826	0.060	0.0065
Fca 23	0.814	0.823	0.365	0.0146
Fca 35	0.373	0.477	0.11	0.0035
Fca 43	0.541	0.595	0.518	0.0093
Fca 77	0.582	0.568	0.827	0.0132
Fca 78	0.760	0.863	0.034	0.0057
Fca 90	0.783	0.829	0.346	0.0123
Fca 96	0.426	0.508	0.038	0.0054

Heterozygote deficiency There was slight, but significant, heterozygote deficiency at two of the eight loci; Fca 96 and Fca 78, suggesting that these loci may contain null alleles. One null allele was identified at each of these alleles from the data set. This indicates that caution should be used when excluding paternity at one locus only.

Figs 5.4. a-h. Allele frequencies for each of the 8 loci. Dark bars show the frequencies of each allele when one representative of each group of related individuals is considered, white bars show the allelic frequencies when all the cats are considered.

Fig. 5.4.a. Fca 8

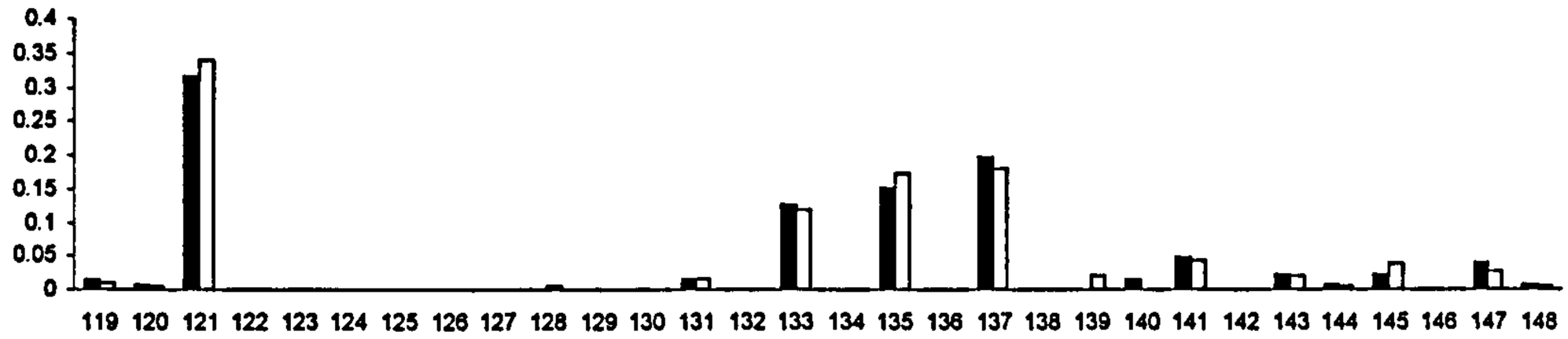


Fig. 5.4.b. Fca 23

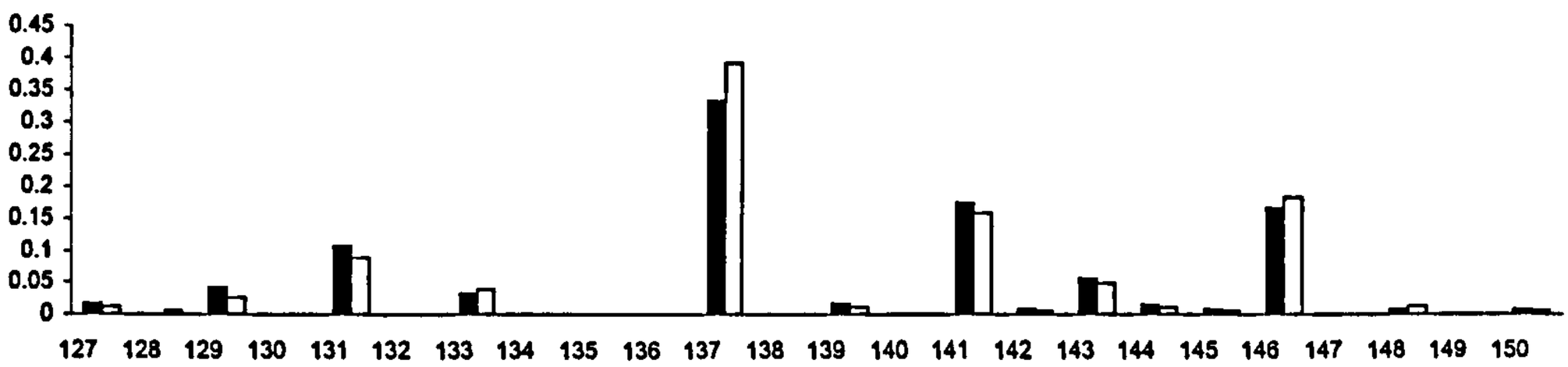


Fig. 5.4.c. Fca 35

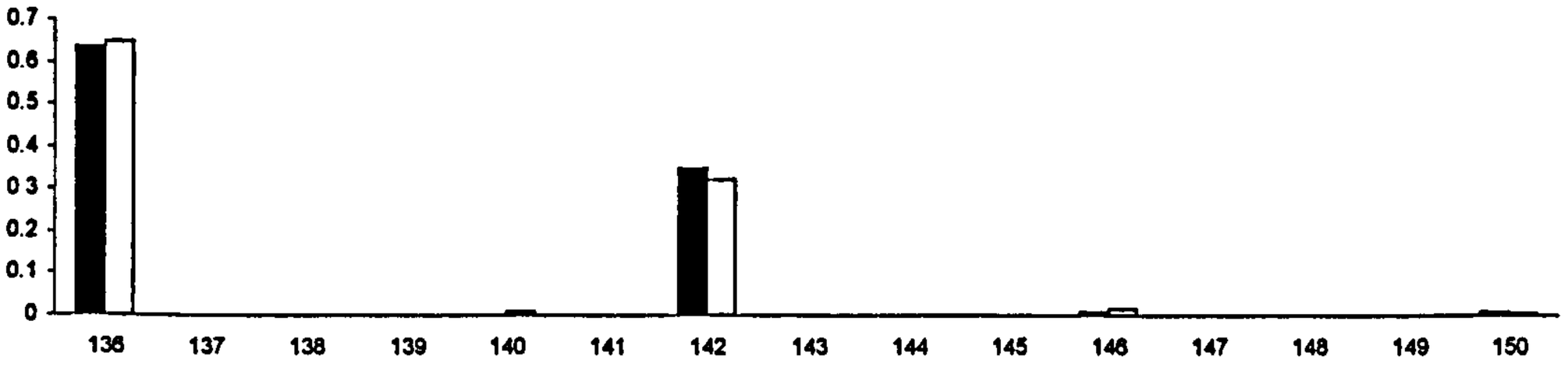


Fig 5.4.d. Fca 43

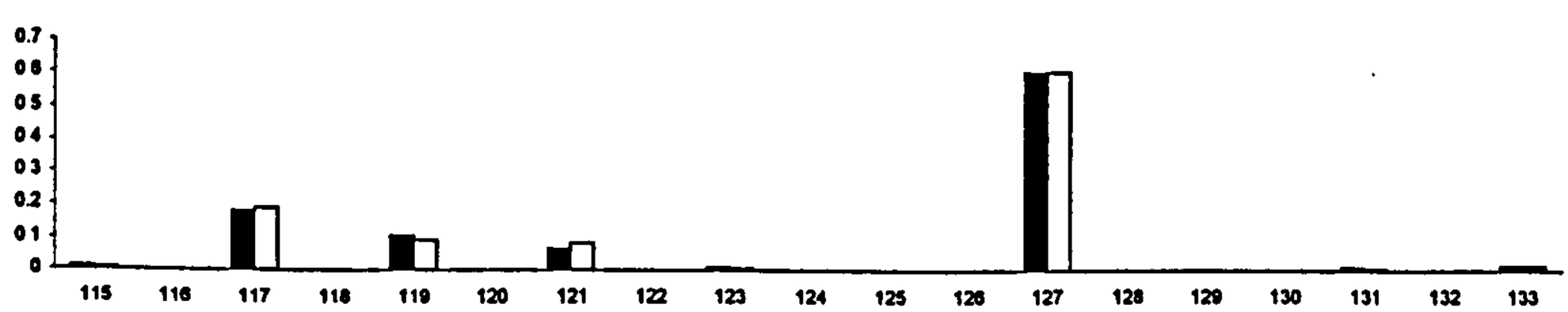


Fig. 5.4.e. Fca 77

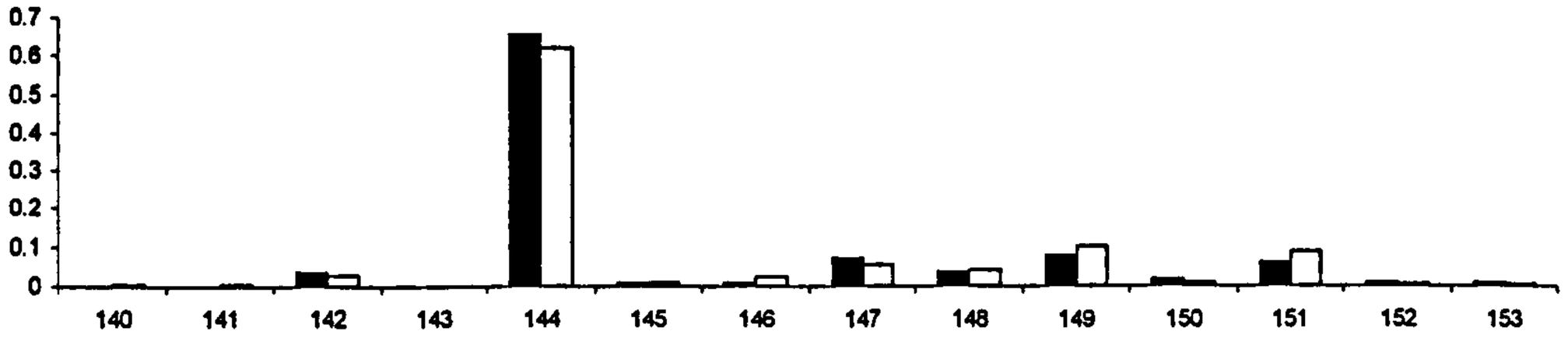


Fig. 5.4.f. Fca 78

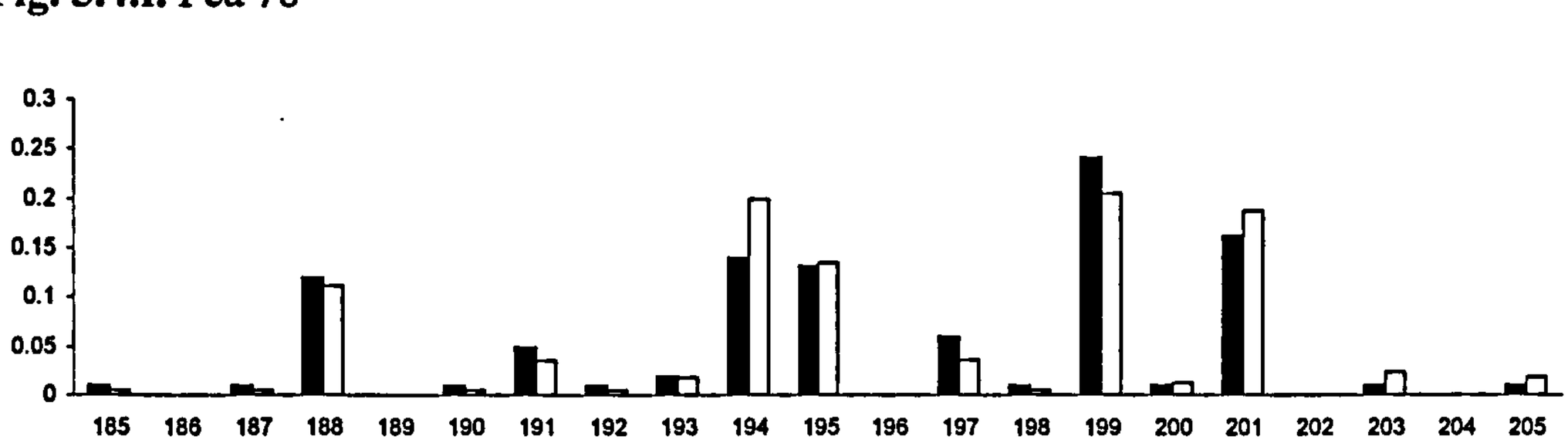


Fig. 5.4.g. Fca 90

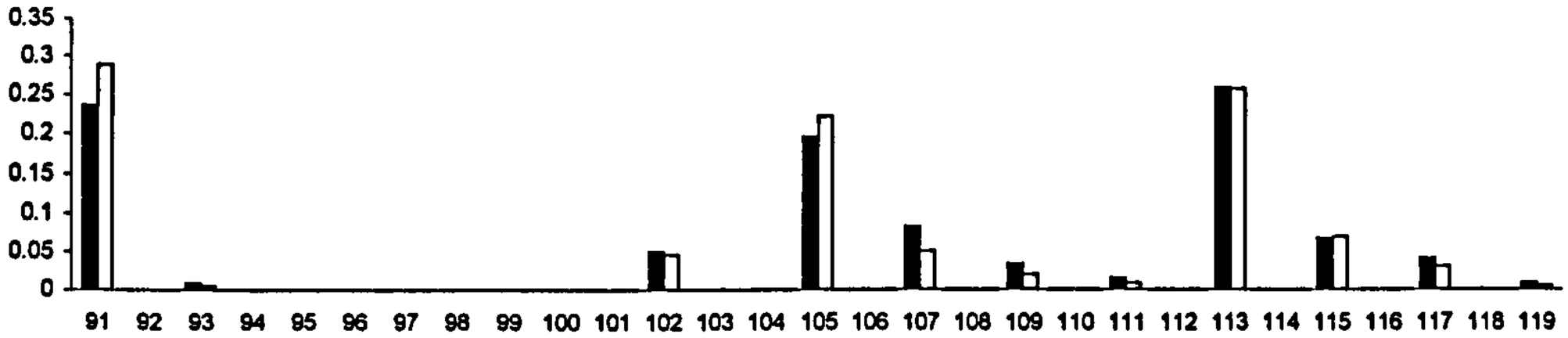
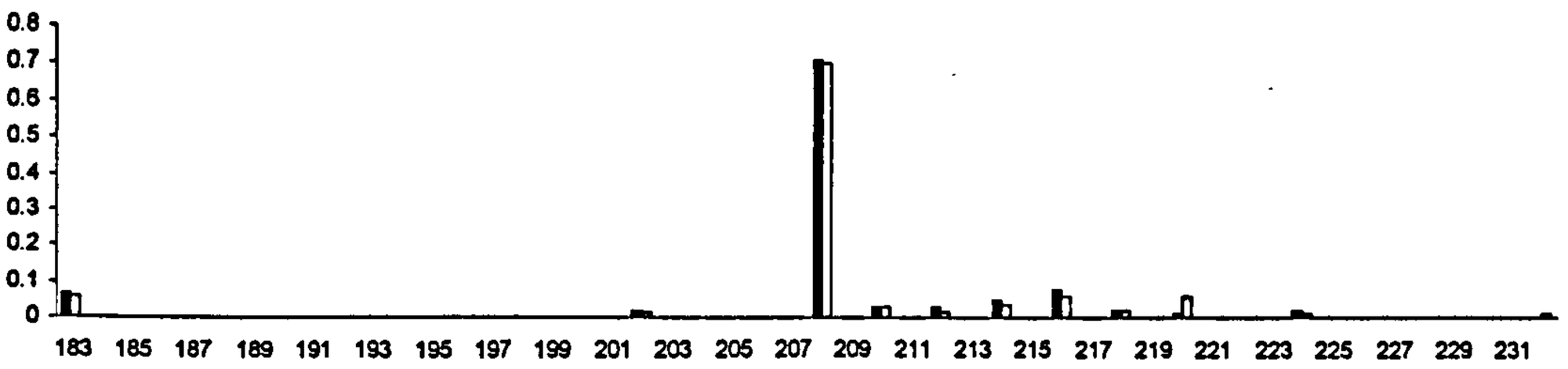


Fig. 5.4.h. Fca 96



5.3.5. Investigating variability between areas of the city

The absence of local differences in allele distribution would indicate that cats from widespread locations of the city all belong to a single panmictic population. In this case it would be legitimate to pool allele frequency data for all the cats in the study.

Before this was done, the possibility was examined that some differentiation in microsatellite allele frequencies between different areas of the city may have occurred. The high levels of human-mediated immigration and emigration would suggest that it is unlikely, even when the relatively low fecundity of immigrant cats is taken into account (**Chapter 2**). Theoretically, in the absence of selection, only one immigrant per generation is needed to preserve genetic homogeneity in a population of infinite size (Crow and Kimura, 1970). The presence of inter-regional differences of allele frequencies would demonstrate levels of effective migration well below those indicated by other sections of this study.

Two statistical approaches were used (**5.3.5.1.** and **5.3.5.2.**). First (Sunnocks *et al*, 1997) the average relatedness of individuals, calculated from overall allele sharing, was used for a comparison between:

i. Mean relatedness values between cats living in Shirley/Freemantle.

ii. Mean relatedness between Shirley cats, and cats from all other regions

The second method investigated whether allelic frequencies at each locus were distributed between regions of the city in conformance to random expectations.

5.3.5.1. Average relatedness between individuals

Pairwise relatedness was calculated between all "family representative" cats. The average within-region relatedness among cats from Shirley/Freemantle, the main focal area, was compared with the average relatedness of these cats with cats from other regions (**Table 5.8.**). Relatedness calculations were carried out using Kinship 1.2. (Goodnight and Queller, 1996).

Table 5.8. Summary statistics from Mann-Whitney-U test, comparing relatedness values within Shirley/Freemantle cats, and between these individuals and cats from other regions.

Area Category	N	Mean Rank	Sum of ranks
Shirley/Millbrook	158	463.50	73233.0
Other areas combined	756	456.25	344922.0
Total	914		

Mann-Whitney U test (U = 58776, p = 0.753)

This analysis compared the average relatedness of cats within the Shirley/Freemantle area, as calculated from sharing of microsatellite alleles, to the average relatedness of cats from this area with cats from other regions. The result indicates that a randomly chosen cat from Shirley/Freemantle is likely to be as closely related to a randomly chosen cat from elsewhere in Southampton as to another local cat.

5.3.5.2. Exact test for population differentiation

Genepop 1.2. (Raymond and Rousett, 1995b) was used to test whether the allocation of allele frequencies at each locus to local areas of Southampton was significantly different from a random distribution. This test was carried out first to investigate possible differences between the Shirley/Freemantle area and the rest of the city (**Table 5.9.**), and also to test for independent allocation of allele frequencies between all 7 regions of the city, as defined in **Methods (Table 5.10)**.

Table 5.9. Independence of allele allocation to regional location, tested using Markov-chain reaction. Shirley/ Freemantle compared to other regions combined.

Locus	Fca 8	Fca 23	Fca 35	Fca 43	Fca 77	Fca 78	Fca 90	Fca 96
Probabilty	0.777	0.752	0.542	0.459	0.998	0.079	0.11	0.505
S.E.	0.0058	0.0067	0.0049	0.0074	0.0003	0.0048	0.0049	0.0065

There were no significant differences at any loci, indicating that alleles at each locus do not differ significantly in distribution between Shirley/Freemantle and the rest of the city.

Table 5.10. Independence of allele allocation to regional location, tested using Markov- chain reaction. Compared between all regions.

Locus	Fca 8	Fca 23	Fca 35	Fca 43	Fca 77	Fca 78	Fca 90	Fca 96
Probabilty	0.514	0.126	0.718	0.037	0.321	0.392	0.446	0.761
S.E.	0.0184	0.0124	0.0090	0.0056	0.0182	0.0194	0.0164	0.0154

This was only significant for Fca 43 ($p = 0.037$). When a sequential Bonferroni adjustment is applied to the results for all 8 loci combined, the appropriate level of p for the locus showing the greatest difference in frequencies between regions is $0.05/8 = 0.00625$. This value was exceeded for Fca 43.

Therefore; No meaningful differentiation of microsatellite allele frequencies between regions of the city was detected, and the data from all areas could be pooled legitimately.

5.3.6. Testing individuals for specific pedigree relationships in the Shirley/Freemantle area.

This area was the main focus of this study. There were enough cats sampled within this defined area to draw inferences about the density of reproductively active males and their reproductive success. Twenty four kittens from this area were genotyped; these belonged to 14 litters produced by 12 females. There were 4 litters for which more than one kitten was genotyped. At one of these households one kitten from each of two subsequent litters was genotyped. A resident tom, thought to have fathered these litters, was also genotyped. Eight toms were genotyped from the whole area.

The exact location of birth of 4 kittens, belonging to separate litters, was not known but their owners believed that they were born in Shirley. These cats were included for analysis in this section because they might be identifiable as close relatives of other cats from the area. In all other cases the birthplace was known. The birth location of the kittens, where known, and the primary homes of all the toms, are shown on **Fig. 5.2.**

5.3.6.1. Paternity between the offspring of each female

As a first step towards assessing paternity between litters, it was necessary to examine paternity within litters. If kittens within a litter are known to be full siblings, this facilitates the reconstruction of paternal genotypes, which in turn aids the acceptance or rejection of paternity assignment.

There were 4 sets of kittens sharing the same mother, one of these sets comprised 3 litters (see above), the others comprised one litter each. Each multi-kitten household will be discussed separately. It was assessed whether the kittens' genotypes are compatible with single male paternity; when more than 2 paternal alleles are inferred at any locus within a litter, multiple paternity is implied, although three or more kittens need to be genotyped to identify multiple paternity in this way. In addition, the likelihood ratios, and significance levels for full sibling versus maternal half siblings, based on allele sharing, were calculated using Kinship 1.2. However, type two error rates (0.51 at $p < 0.05$) were high enough for some pairs of full siblings not to be identified as such at significant levels of probability.

Significance threshold levels and type 2 error rates are presented in **Appendix 2.7.**

<u>Foyle household</u>: Mother and all four kittens form one litter were genotyped. Paternal alleles from the kittens were consistent with single male paternity.

Table 5.11. Pairwise likelihood ratios for full sibling vs maternal half siblings for the kittens of the Foyle household.

	Kitt 1	Kitt 2	Kitt 3
Kitt 1	-		
Kitt 2	**131.03*** **	-	
Kitt 3	**96.36*** **	0.54 N.S.	-
Kitt 4	**4.00***	0.13 N.S.	**34.25** **

Not all the pairwise comparisons were significant, but each kitten was likely to be a full sibling of at least one other at minimum $p < 0.001$ (**Table 5.12.**)

Tell household: Mother and all three kittens from a litter were genotyped. Paternal alleles were consistent with all sharing the same father, favouring the acceptance of single male paternity (**Table 5.12**). However the probabilistic analysis is only significantly in favour of a full sibling relationship between kittens 1 and 3. These two kittens were used to construction the partial paternal genotype.

Table 5.12. Pairwise likelihood ratios for full sibling vs maternal half siblings for the kittens of the Tell household.

	Kitt 1	Kitt 2
Kitt 1	-	
Kitt 2	0.09 N.S.	-
Kitt 3	**10.6** **	0.61 N.S.

Arnold household

Three successive litters were born in the household, mothered by the resident female. Only one kitten was genotyped from each of the last two litters. The analysis of pedigree relations for cats at this household was hampered by the presence of null alleles (see **5.3.4.1.**). However, band sharing at the rest of the loci, assuming that the identification of null alleles was correct, allowed the resident tom to be assigned as father of all three litters (**Table 5.13.**).

Table 5.13. Likelihood ratios and significance levels of father-offspring vs. unrelated for the resident tom and kittens at the Arnold household calculated using Kinship 1.2. Significance thresholds and type 2 error rates are present in **Appendix 2.8.**

Kitten	Likelihood ratio
Litter 1, Kitten 1	**71.02****
Litter 1, Kitten 2	**54.11****
Litter 1, Kitten 3	**3.23***
Litter 2	*****
Litter 3	**142.04****

Gil household

Two kittens from a litter of unknown size were genotyped. Allele sharing indicated that they were likely to be full siblings (likelihood ratio: 43.28, $p < 0.01$).

Reconstruction of paternal genotypes

The above analysis enabled the construction of partial paternal genotypes for the Foyle, Tell and Gil litters. This was also done for the Morris kitten, for which the mother, but no siblings, were genotyped. For the Arnold household, the father was identified as the resident male, and the fathers genotype was used directly in assessment of paternity. The reconstructions of paternal genotypes are presented in **Appendix 2.6.** these were used for assessment of paternity in the following sections.

5.3.6.2. Siring of kittens by genotyped males in the Shirley/Millbrook area:

In principle, a male can be excluded as the father of a kitten if at any one locus the kitten has an allele that could not have been inherited by the male, i.e. the male must share a paternal allele at each locus with the kitten. However, there is the remote possibility of mutation events creating a parent-offspring mismatch. Mutation rates at microsatellite loci are thought to be in the order of 1×10^{3} to 10^{5} per generation (Edwards *et al,* 1992). Another, much more likely, source of error is the presence of null alleles. The presence of some null alleles has been demonstrated in this data set

(see **5.3.4.1.**). These factors indicate that exclusion of paternity based on alleles at one locus must be treated with caution (see **5.2.6.1.**)

The genotypes of each litter or kitten were tested for compatibility with each of the genotyped toms. The partly reconstructed paternal genotypes were used, when available. The number of loci at which paternity is excluded is shown for each pair-wise tom-litter comparison in **Table 5.13.** The kittens from the Arnold household (No. 8) are excluded from the table, because their father had been identified.

Table 5.14. Number of loci excluding paternity of each litter by the toms for which genotypes were obtained. For each litter, the table indicates whether the mother was genotyped, and the number of kittens genotyped from each litter. The numbers and letters in brackets identify the litter or the tom, and correspond to the locations shown in **Fig 5.4.** Bold text indicates that the litter's birthplace was known.

Kitten	Mother	No.	Birth	Genotyped males							
House-	Geno-	Kittens	location	L72	S67	Ar6	SirG	C17	ArnD	W17	Whit
hold	typed?	geno-typed	known?	9-10yrs (a)	11 yrs (b)	2 yrs (c)	(d)	(e)	(f)	10yrs (g)	1 yr (h)
Foyle (1)	**Yes**	**4**	**Yes**	**4**	**4**	**3**	**4**	**3**	**2**	**6**	**5**
Tell (2)	**Yes**	**3**	**Yes**	**2**	**4**	**5**	**4**	**6**	**3**	**5**	**5**
Morris(3)	**Yes**	**1**	**Yes**	**4**	**4**	**6**	**3**	**3**	**3**	**4**	**3**
Pick (4)	**No**	**1**	**Yes**	**4**	**1**	**4**	**1**	**0**	**2**	**3**	**2**
Gile (5)	**No**	**2**	**Yes**	**2**	**2**	**3**	**1**	**3**	**2**	**2**	**1**
Lever (6)	**No**	**1**	**Yes**	**2**	**2**	**4**	**2**	**2**	**2**	**3**	**2**
War (7)	**No**	**1**	**Yes**	**3**	**2**	**3**	**2**	**3**	**1**	**2**	**3**
Dunn (9)	**No**	**1**	**No**	**2**	**3**	**2**	**3**	**2**	**2**	**2**	**0**
How (10)	**No**	**1**	**No**	**1**	**3**	**1**	**1**	**2**	**1**	**2**	**1**
Joyse (11)	**No**	**1**	**No**	**1**	**3**	**3**	**4**	**3**	**1**	**2**	**1**
Trav (12)	**No**	**1**	**No**	**2**	**1**	**2**	**4**	**3**	**2**	**2**	**2**

Compatible father-offspring pairs

There were only two potential father-offspring pairs that were compatible at all loci. The likelihood ratios for these pairs, presented in **Table 5.15.** show that paternity in both cases is indicated at $P < 0.05$ levels of probability. C17 (Male **e**) could have sired

Pick (litter 4), although this kitten was born 1.4 km from the tom's primary home. Whit (Male h; a subject of radio-tracking) could have sired Dunn (litter 9), which was born at an unknown location.

Table 5.15. Probability ratios and significance levels (father-offspring vs unrelated, calculated using Kinship) for tom-kittens pairs where paternity was not excluded through incompatible genotypes. Probability thresholds are presented in **Appendix 2.7.**

Kitten	Tom	Likelihood ratio
Pick (4)	C17 (e)	2.71*
Dunn (9)	Whit (h)	1.36*

<u>Pairs where father-offspring relationship was excluded at one locus</u>

Given the presence of some null alleles in the data set, and the possibility of mutational events, it is worth considering pairs where a putative father-offspring relationship was excluded on the basis of alleles at one locus:

Each of the 3 kittens which were born at unknown locations, and whose paternity has not yet been considered; How (9), Joyse (10) and Travis (11), are excluded from being the offspring of between one and five of the genotyped toms based on alleles at one locus.

There are 4 potential father kitten pairs involving kittens born at known locations where paternity is excluded at only one locus:

- S67 (tom **b**)-Pick (litter **4**): ecologically possible, born 600m distant, discussed in **5.3.6.4.**
- SG40 (tom **d**)-Pick (litter **4**): Ecologically possible, born 800m distant.
- SG40 (tom **d**) –Gil (litter **5**): Ecologically unlikely, born 1200m distant.
- Arn D (tom **F**)- War (litter **7**): Ecologically very unlikely, born 1.6 km distant

These cases are considered when evaluating the implications of the results to the mating system (**5.3.6.4.**)

5.3.6.3. Paternal half siblings: Did any litters in the area share the same father?

Where there was sufficient pedigree data, it was possible to exclude shared paternity between litters on the basis of incompatible genotypes. Otherwise probabilistic analysis was carried out using Kinship 1.2. (Goodnight and Queller, 1996)

Table 5.16. Number of loci excluding shared paternity between litters (red font). Where paternity is excluded at less than two loci, likelihood ratios for unrelated vs paternal half sibling are given (calculated using Kinship 1.2.). Significant ratios are indicated: favouring unrelated (black font), favouring paternal half sibling (blue font). Threshold values for significance levels and estimated levels of type 2 errors are presented in **Appendix 2.9.**

Litter	N	Foyle (1)	Tell (2)	Morris (3)	Pick (4)	Gile (5)	Lever (6)	War (7)	Dunn (8)	How (9)	Joyse (10)
Foyle (1)	4	-									
Tell (2)	3	2	-								
Morris (3)	1	2	(1) **8.57***	-							
Pick (4)	1	(1) 2.31	(1)**23.95****	2.91	-						
Gil (5)	2	3	(1) **6.32***	2.55	0.67	-					
Lever (6)	1	3	(0)**18.46****	**13.70****	**7.68***	**6.66***	-				
War (7)	1	2	(2) **3.95***	**11.86***	**4.86***	**10.44**	**7.96***	-			
Dunn (8)	1	2	(0) 2.53	**3.25***	3.24	1.82	3.248	**10.89***	-		
How (9)	1	2	(0) **3.45***	**10.98***	1.99	0.71	**0.18***	2.91	1.37	-	
Joyse (10)	1	4	(1) **7.97***	2.02	1.54	1.11	**21.75****	**14.88****	2.9011	0.43	-
Trav (11)	1	2	(1) 1.00	**65.25*****	**12.51****	**11.47**	0.54	2.63	**10.05***	0.41	**13.42****

N = Number of kittens genotyped

<u>Comparisons between litters that were born at known locations</u>

Foyle (litter1) can be excluded from shared paternity with Pick (4); due to incompatible alleles at 1 locus in addition to likelihood ratios between the kittens favouring unrelatedness, although the mean ratio did not quite reach the significance threshold of 3.25.

It is possible but unlikely that the Morris kitten (3), shares a father with either the Pick (4) or Gil (5) kittens. There were no loci excluding shared paternity, and the likelihood ratios favouring their being unrelated did not quite reach the significance threshold (3.25) in either case. The distance between the birthplace of Morris (3) and Pick (4) is 1.6km, and for Morris (3) and Gil (5) 1.4km.

It is possible that Pick (4) shares a father with Gil (5). Statistically this is an ambiguous result. The litters were born only 600 m apart; within the realistic home range of one male. However it is worth noting that, if the litters do share a father, paternity of either litter by S67 (Male **b**), would be excluded. **(see 5.3.6.2.)**

<u>Comparisons involving litters that were born at unknown locations</u>

There are several potential half-sibling pairs that cannot be excluded. However, the only pairing where shared paternity is significantly favoured is between Lever (6), born at the south of the study area, and How (10), born at an unknown location.

5.3.6.4. Implications for the population structure and mating system of Shirley/Freemantle results.

The Shirley/Freemantle study involved 24 kittens, belonging to 14 litters produced by 12 females (see **Fig. 5.4.**), and 8 toms, with identified homes. Three of the litters (5 kittens) were all produced by one male/female pair, which lived in the same house. Four of the kittens, from separate litters, were born at unknown locations, but were believed to be within the Shirley/Freemantle area

It is not surprising that the geographically outlying kittens: Morris (3) and War (7) were not sired by any of the genotyped males, and are unlikely to share paternity with any other litters. Morris (3) was born 1.4 km distant from any of the other cats, except Whit (male **h**), which was not its father, was shown **(Chapter 4)** to have very limited ranging behaviour.

In the southern part of the Freemantle area, neutering was found to be lower than in Shirley (Chapter 2). The resident tom at the Arnold household achieved paternity of three successive litters, despite the presence of a least three toms with primary homes 50-200m away. None of the 4 genotyped males in this area was the father of Lever (6), the only other kitten genotyped from Freemantle.

The four litters from the upper-Shirley part of the core area were born at a maximum distance of 800 apart, a distance just within the limits of a tom's home range (see **Chapter 4**). The area defined by the minimum polygon covering the birthplaces of the litters was 17.7 ha, an area which could be covered by the ranges of 2 toms (Chapter 4). The litters were therefore within realistic potential ranges of 3 genotyped males (L72, S67 and Ar6), but were sired by different males. The only ambiguity is the paternity of Pick (4), were paternity by S67 (male **b**) is only excluded at one locus. The only possible sharing of paternity between litters is between Pick (4) and Gil (5) born 600m apart; if this were accepted, paternity by S67 would be ruled out.
This 17.7 ha area was therefore shown to be within the ranges of at least 6 toms, and possibly 7. Paternity of these 4 litters was shared between 3 or 4 toms. Eighty percent of the area was covered by the comprehensive Shirley survey (chapter 2), where neutering rates were amongst the highest found in Southampton.

5.3.7. Paternity of kittens born in other areas of Southampton

Cats were also genotyped from scattered locations around the city in the hope that these may also provide useful insights into the cat mating system: The number of males that sired kittens at a single household, both within and between litters, can be measured. If separate litters, born at known locations, share the same father, this will give a valuable information on the reproductive strategies of males.

<u>Paternity of successive litters</u>

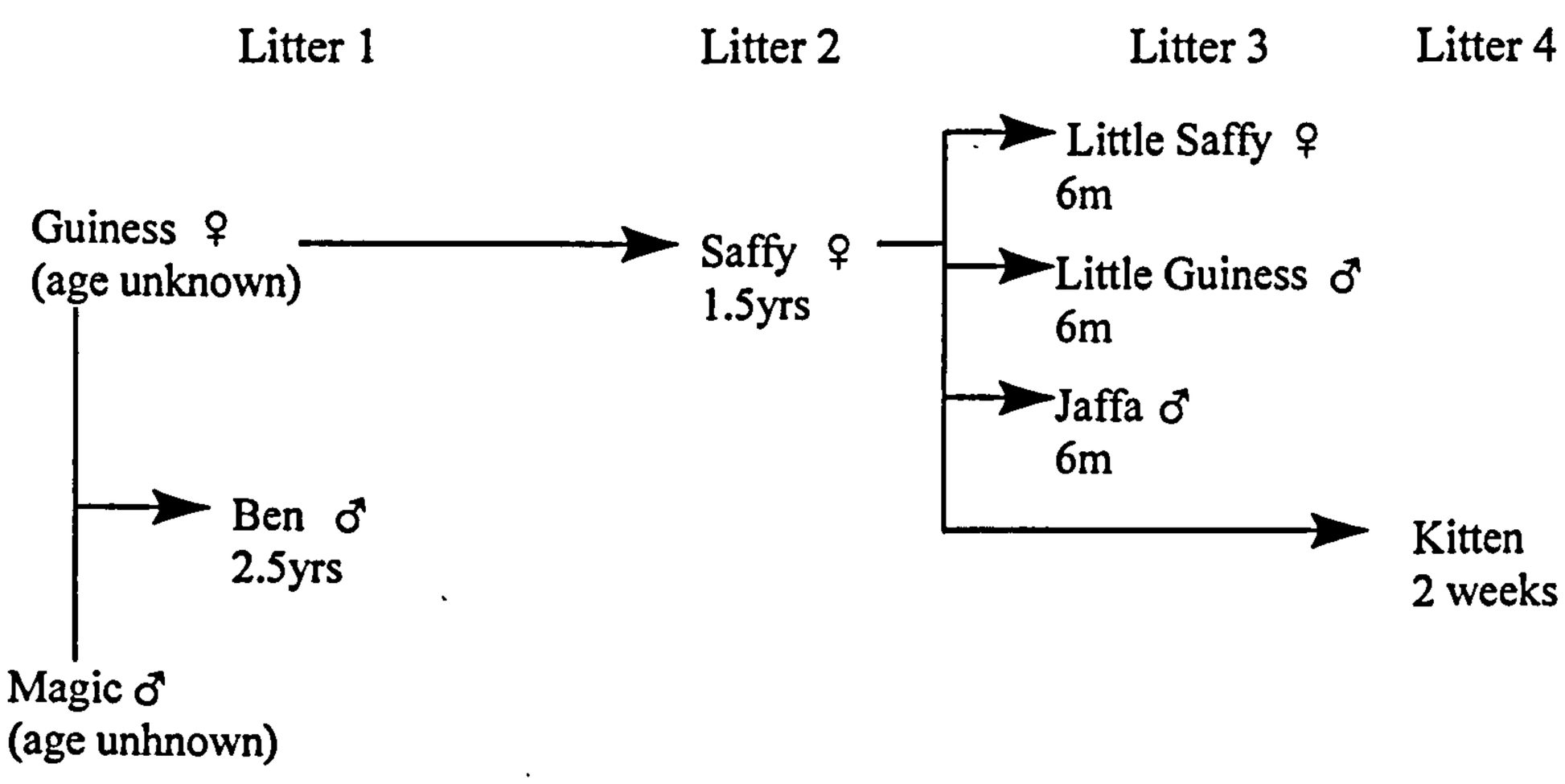
There were three households, in addition to the one in Freemantle, where kittens from successive litters were genotyped.

Hill Family (Thornhill)

A female cat of unknown age (Guinness) was an adopted stray. She had a litter which the owners believed was sired by a local stray male (Magic). One of the kittens from this litter (Litter 1; Ben) was genotyped. Ben's paternal alleles were compatible with those from Magic. Kinship 1.2. (father offspring vs unrelated) revealed a likelihood ratio of 2318.18 ($p < 0.001$) in favour accepting paternity by Magic. Magic was adopted the Hill household and neutered shortly after the litter was born.

Guinness had another litter by an unidentified tom (litter 2), including the female Saffy. Guiness was then neutered. Saffy had two litters by Summer 1996 (litters 3 and 4). All 3 kittens from the first litter were genotpyped and temperament tested, one kitten from litter 4 was genotyped. The familial relationships of the genotyped cats are shown in **Fig. 5.5.** Guinness also had two previous litters that were not genotyped.

Fig. 5.5. Familial relationships of genotpyed cats from the Hill household.



Reconstructed partial paternal genotypes for all of Saffy's kittens; those from litters 3 and 4, were compatible with them sharing the same father **(Appendix 5.5.6.)**. Likelihood ratios for full sibling vs maternal half siblings were calculated to establish the probability that they were all sired by one male **(Table 5.17.)**.

Table 5.17. Saffy's kittens: likelihood ratios and significance levels for full siblings vs. maternal half siblings, tested pairwise between all kittens using Kinship. Significance thresholds and type 2 error rates are presented in **Appendix 2.6.**

	Little Guiness (litter 3)	Jaffa (litter 3)	Little Saffy (litter 3)
Little Guiness	-		
Jaffa	72.29***	-	
Little Saffy	0.17 N.S.	0.15 N.S.	-
Kitten	0.10 N.S.	3.72*	356.37***

Not all the ratios were significant in favour of full siblings. However, the kitten from litter 4 is clearly a full sibling of Little Saffy from litter 1 ($P < 0.001$), and is significantly likely to be a full sibling of Jaffa from litter ($P < 0.05$). Jaffa and Little Guiness are clearly full siblings ($P < 001$). It can therefore be assumed that all the kittens were full siblings.

Partial paternal genotypes were constructed for litter 2, and litters 3 + 4 combined **(Appendix 2.5.)**, the paternal genotype of the father of litter 1 had already been scored (Magic). The number of loci excluding shared paternity could then be calculated between litters 1,2 and 3 + 4. **(Table 5.18)**

Table 5.18. Number of loci at which alleles exclude shared paternity; pairwise comparisons between litters 1 (father genotyped), 2 and 3+4 (fathers genotype partially reconstructed).

	Litter 1	Litter 2
Litter 1 (Magic)	_	
Litter 2	3	_
Litters 3+4	2	4

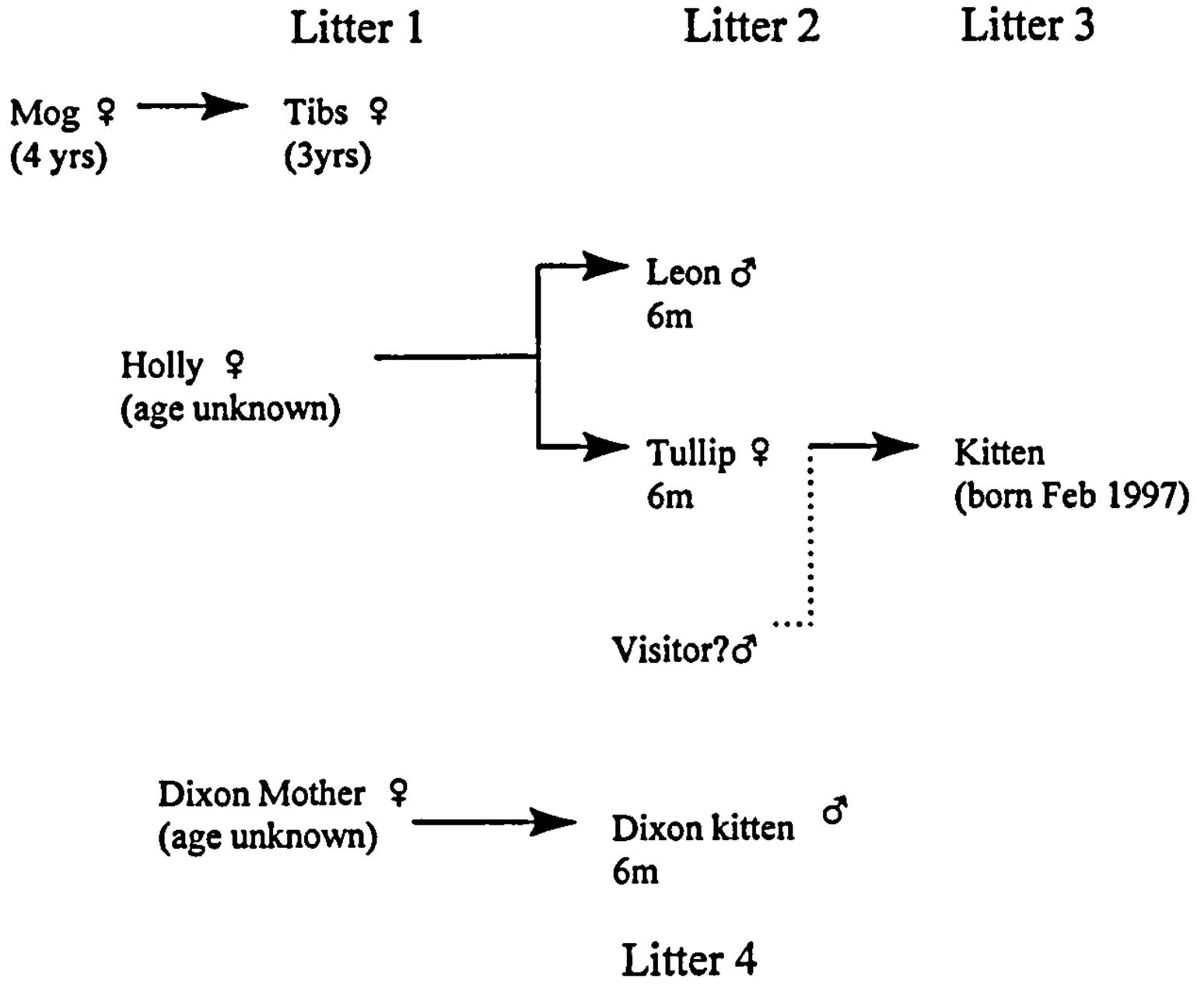
Sharing of paternity between litters 1,2 and 3+4 is excluded at least 2 loci in each case. Magic was said to have been neutered when the later litters were conceived, but the possibility of unreliable information from the owners made it worth checking his paternity of later litters.

The first litter was sired by Magic. The presence of this mature male cat, when neutered, did not prevent further litters being sired in the same house by other males. A second male sired litter 2, and a third male sired litters 3 and 4. Three separate males sired kittens at this household in successive litters. The 3rd male subsequently sired the next litter.

Norton and Dixon households (Bitterne)

These households were both situated in Bitterne, which is socio-economically similar to Shirley. The two unrelated females of the Norton household lived approximately 300m from the house where the Dixon's cat was born. Mothers and offspring were genotyped in all cases. Hair samples were collected by the owner at the Norton household from a visiting cat, believed to be a tom, when it passed through her garden. The familial relationships are summarised in **Fig. 5.6.**

Fig. 5.6. Summary of familial relationships for cats from the Norton and Dixon households. Both Mog and Tibs were neutered after the birth of the litters shown.



The kittens were tested using Kinship 1.2. for the likelihood ratios of sharing a father. In the case of Leon and Tulip the appropriate hypothesis was full sibling vs. maternal half sibling, in all the other comparisons the hypothesis was paternal half sibling vs. unrelated. The results are summarised in **Table 5.19.**

Table 5.19. Probability ratios and significance of shared paternity for kittens in the Norton and Dixon households. Details of significance thresholds and type 2 error rates are presented in **Appendices 2.6.** and **2.8.**

Kitten	Tibs (Litter 1)	Leon (Litter 2)	Tullip (Litter 2)	Tullip's Kitten (Litter 3)	Dixon Kitten Litter 4
Tibs	-				
Leon	0.014 N.S.	-			
Tullip	0.674 N.S.	16.90**	-		
Tullip's Kitten	0.27 N.S.	423.37***	258.67***	-	
Dixon Kitten	0.37 N.S.	7.96×10^{7}***	47.06***	18.99**	-

These results strongly indicate that Leon and Tullip (litter 2), Tullips kitten (litter 3) and Dixon kitten (litter 4) all share the same father, while Tibs was sired by a different male.

Partial paternal genotypes were reconstructed for all kittens (**Appendix 2.5.**). The number of loci excluding paternity the visiting tom was considered for all the kittens **(Table 5.20.)**.

Table 5.20. Number of loci where paternity is excluded between the visiting tom and kittens at the Dixon and Norton households.

Kitten	Tibs (Litter 1)	Leon (Litter 2)	Tullip (Litter 2)	Tullip's Kitten (Litter 3)	Dixon Kitten Litter 4
No.Loci excluding paternity	3	2	1	0	2

The likelihood ratio (parent-offspring vs unrelated) that the tom sired Tullip's kitten is very high: 1704.73 ($p < 0.001$) see **Appendix 2.7.** This presents a problem for the tom is excluded from being the father of litters 2 and 4. One solution is that the genotyped male is a close relation of the actual father. Alternatively, the genotyped male may have fathered litter 3, and a close relative fathered litters 2 and 4. However the probability that one male sired both litters 2 and 4, at least, is very high. This demonstrates that one male can successfully sire kittens in two households 300m apart within the space of a few weeks.

<u>Butts household (Sholing)</u>

In the Butts household, two litters were born 1 year apart. There was a resident tom in the house (Sam), which was young; 1year and 2 years, at the time the kittens were conceived. One kitten from the first litter (Ziggy), two kittens from the second litter (Molly and Max), their mother, and the resident male were all genotyped, although one

of the kittens of the second litter was scored at only 5 loci due to insufficient DNA samples.

Table 5.21. Probability ratios and significance levels for the kittens at the Butts household sharing the same father; full siblings vs. maternal half siblings (Kinship).

	Ziggy (litter 1)	Molly (Litter 2)
Ziggy	-	
Molly	73.6***	-
Max	0.184 N.S	0.112 N.S.

Six loci were scored for Max, it is unclear whether he was a full sibling of litter-mate Molly and Ziggy from the first litter (**Table 5.21.**). The level of significance between Ziggy (litter 1) and Molly (litter 2) clearly indicates that one male sired kittens in both litters. Partial paternal genotypes were constructed for all 3 kittens and compared with the genotype of the resident male (**Table 5.22.**).

Table 5.22. Number of loci excluding paternity of kittens at Butts household by resident male.

Kitten	Ziggy (litter 1)	Molly (litter 2)	Max (litter 2)
Number of loci	4	3	4

The resident male (Sam) did not sire any of these kittens. This is an example of a resident tom failing to sire the kittens born in the household in which it lives. Although it must be remembered that the tom was young (1 year and 2 years) when the kittens were conceived. Both Sam and Ziggy were subjects of the radio-tracking study (Chapter 4).

Meade household (Highfield): A hair sample by the owner was collected from a cat, believed to be a tom, which was often observed around the house. It was only scored at 4 loci, but was excluded from being the father of any of the kittens at two of these.

Summary of data from other areas of Southampton:

The individual cases in this section can be brought together: there were 6 instances of successive litters being genotyped from one household where the father was not a resident at the household. One of the fathers, Magic, was adopted and neutered before subsequent litters were born. Of the five remaining cases; successive litters within a household were sired by different toms twice, and the same tom three times (including litters 2+3 at the Norton household. One the basis of this very small sample size, this suggests that a tom that sires one litter at a household, has a good chance of siring the subsequent litter.

There were two cases in the study where a tom lived in the same residence as a reproductively active female. At the Butts household, the young tom failed to sire either of two litters. At the Arnold household, the tom sired each of three successive litters.

It is possible for a tom to sire two litters, born 300m apart, within the space of 4 weeks.

5.4. Discussion

The results of this study imply that in areas where neutering amongst pet domestic cats is extremely high; 97% for adult males 98% for adult females in the upper Shirley region (**Chapter 2**) there is potentially more competition between reproductively active males than the population dynamics data predict.

The presence of at least six or seven toms was inferred in an area of 18 ha over the time-span in which the kittens were conceived (2-3 months). The failure of one tom to sire kittens by a female living in the same house is further anecdotal evidence of the existence of competition between toms. Extrapolation from the population survey data suggests a density of approximately one pet tom per 11 ha. This could be at least partly due to pet toms being able to hold larger home ranges than previously thought (see **Chapter 4**), meaning that pet toms can locate oestrus females and sire kittens a substantial distance from their home base. Pet toms, that were not genotyped in the study, could therefore have sired the kittens.

The above scenario predicts a "wanderer" strategy (Liberg and Sandell, 1988) (see **Introduction**). A wanderer strategy in is not supported by the limited data which showed that three out of five subsequent litters were sired by the same tom that sired the previous litter, five out of seven including kittens from the Arnold household. This is more indicative of a "guarding" strategy, but more data are required to elucidate this hypothesis. However, paternity by a single male of 2 litters born 300 m apart was demonstrated.

The 4 upper Shirley litters were the progeny of 3 or 4 toms. This implies that the spacing between entire females does not allow one male to secure all the mating opportunities within his potential range size. If this was representative, it would imply that there is limited opportunity for one tom to secure high levels of reproductive success, unless a female remains entire long enough for him to sire several litters. The evidence against this is the case, already mentioned, of paternity of litters in two households by a tom in Bitterne.

The large number of fathers inferred in the upper Shirley area could also be explained by unidentified feral males siring litters. There is no direct evidence for this, but if a guarding strategy was found to be predominant, it would strengthen this hypothesis greatly. If a low density of domestic toms leads to an increase in feral males siring kittens, this may be reflected in the temperament of the kittens. The prediction of this hypothesis would be for the heritable component of temperament in Shirley-born kittens to be different to kittens born in areas with lower neutering. This hypothesis can be test using the data from **Chapter 6.**

6 Temperament Testing

6.1. Introduction

6.1.1. Aims

The aim of this study was to test the hypothesis that kittens born in an area of low neutering are more likely to exhibit unfriendly or fearful characteristics than kittens born in other areas with less neutering. This hypothesis has been formulated to provide indirect evidence to support the original hypotheses formulated in the introduction:

i. Kittens born in areas of high neutering have an increased chance of being sired by feral males.
ii. Kittens sired by feral males are likely to inherit fearful and unfriendly characteristics from their fathers.

Temperament types, or behavioural styles, have been shown to be reliably measurable in cats (Meier and Turner, 1985; Feaver *et al,* 1986; Mertens and Turner, 1988). The influence of socialisation on the reactions of cats to humans is profound (Collard, 1967; Karsh, 1983; Bradshaw and Cook, 1997). In addition, a genetic component has been identified (Turner *et al,* 1986; Reisner *et al*, 1994). This has been demonstrated through paternity, where the father had no social contact with the kittens. One genetic component of friendliness to humans has been shown to consist of a boldness characteristic, generalisable to people and to novel situations (McCune 1995).

Several dimensions of behavioural style have been identified in cats, which show some similarity between studies. A *bold/shy* trait was identified by Meier and Turner (1985), and three traits *sociable/alert/equable to cats* by Feaver *et al* (1986). *Active*, *timid* and *confident* traits were identified by Karsh (1983) (cited in McCune, 1995), while *sociable to humans* and *generally active* were the most important components extracted by Bradshaw and Cook (1996). McCune (1995) separated a friendliness trait into

sociability to humans, promoted by socialisation, and boldness in a novel situation, mediated by genetic influences (demonstrated through paternity). Some of the differences between these various classifications can be accounted for by the methods used; e.g. encounters with cats outdoors by Meier and Turner (1985), post feeding behaviour by Bradshaw and Cook (1996).

In **Chapter 2** it was shown that the density of entire pet cats in the Shirley area is low due to the prevalence of neutering. Other areas within Southampton (parts of the Freemantle area) were shown by other, less comprehensive, surveys to have lower rates of neutering than Shirley. Experience showed that recruiting kittens was relatively easy **(Chapter 3)**, in the Sholing, Thornhill and Merryoak areas. Householders in these areas stated that kittens were frequently born in the locality, giving anecdotal evidence that neutering rates were lower in these areas. In addition there are other areas of the city where no index of neutering rates was obtained (e.g. Portswood and Bitterne, Swathling). All these areas are shown on **Fig. 3.1.** in **Chapter 3**.

In **Chapter 4** it was demonstrated that pet toms have larger home ranges in an urban environment than previously thought, such that there was potential for home range overlap and inter-male competition even in an area such as Shirley. Microsatellite analysis revealed that in the Shirley area, different litters were sired by different males. This suggested that one male does not monopolise reproductive opportunities within an area the size of a male's home range in the urban environment (shown in **Chapter 4** to be 7.4ha to 14.4 ha). The pet toms that were genotyped were not successful in siring litters. Although there is no direct evidence that any of the Shirley litters were sired by feral males, the data suggest that this interpretation is feasible.

In this chapter the aim was to compare the temperament of kittens born in Shirley with those born in other parts of the Southampton area. If there is an increased rate of siring of kittens by feral males, and this is reflected in the temperament of the kittens, the trend may be revealed by this inter-area comparison.

6.1.2. Temperament testing

A protocol was developed to obtain a reliable and consistent measure of kitten temperament. There was the constraint that the tests had to be carried out in the homes of the kitten's owners. Also, there was the necessity to recruit the maximum possible number of kittens to ensure that each sample was representative of the population from which it was drawn. Therefore the tests were kept brief and simple to avoid deterring potential volunteers from taking part in the project.

Handling tests for kittens were first devised by Karsh (1984), and have become recognised as a method of obtaining a consistent measure of a kitten's temperament. The technique was used extensively by McCune (1992), who developed three tests; the Familiar Person handling test, the Unfamiliar Person handling test and the Novel Box Test. The tests were carried out under controlled conditions. Similar tests have been carried out in the homes of volunteer kitten owners in a study of the development of temperament in kittens (Bradshaw and Cook, 1994; Cook and Bradshaw, submitted), and assessing the suitability of feral born kittens as pets (Bradshaw and Cook, 1997). The tests used here were based on those used in all these studies.

6.2. Methods

6.2.1. Recruitment of kittens

Kittens were recruited to the project using the methods described in **Chapter 3.**

6.2.2. Observer reliability

I gained experience in temperament testing of kittens from my visit to WCPN in December 1994, when I practised many of the techniques used in this study under the supervision of Sandra McCune.

Before starting on this temperament testing study I practised the protocol on cats from

the AzI colony in order to attain a high level of consistency. In addition I practised on cats belonging to friends and colleagues, under house visit conditions, with help from Sarah Cook.

6.2.3. Temperament testing protocol

Two sets of tests were carried out for each kitten when it was six months old, one week apart, or as close to this time interval as it was possible to arrange. This enabled the consistency of the results between test occasions to be assessed. At the end of the second test arrangements were made to re-contact the owners in the future to re-test the kittens when they had reached 18 months old. When the kittens were approaching 18 months old, I attempted to re-contact the owners and arrange a further set of two tests for each kitten. No test was conducted at one year of age, since Bradshaw and Cook (1994) showed that holding tests carried out at this age correlate poorly with tests conducted earlier (4 months) or later (2 years).

In order to avoid the confounding effects of appetite, kittens were tested within one hour of being fed. The actual time of day varied, because of the variation in times at which people normally fed their kittens

Testing was carried out in a quiet room with which the cat was familiar. Other people and cats were excluded from the room prior to testing.

Three tests were performed on each visit: The *Familiar Person Holding Test* was carried out first, after which the owner was asked to place the kitten on the floor and leave the room. Then the *Approach Test* was carried out and then the *Unfamiliar Person Holding Test*. Finally, a hair sample was taken for DNA analysis (see **Chapter 5**). When more than one kitten from the study was resident at one household, the procedure was repeated for each kitten.

6.2.3.1. Familiar Person Holding Test

The kitten's owner was asked to hold the kitten on his/her lap, facing outwards, and

handle and stroke it for one minute. The owner was instructed to restrain the kitten gently if it tried to escape, unless it became obvious that the kitten was extremely stressed by the experience. I observed and recorded the kitten's behaviour from an unobtrusive seated position in the same room, taking care to be silent and avoiding eye contact with the kitten.

6.2.3.2. Approach test

This test lasted for the duration of one minute, during which the kitten's behaviour was observed and recorded with a pencil and note pad, using a simple coding system.

When the kitten's owner had left the room and closed the door, having placed the kitten on the floor on the other side of the room from which I was sitting, I attempted to entice the kitten to approach me. This was done using a standard cat toy; a flat/cylindrical soft toy, made of a fluffy wool material, measuring 10cm x 6cm. This was attached to a length of string, which was used to twitch and move the toy in a manner designed to attract the kitten's attention. In addition I encouraged the kitten vocally. This was continued until the kitten had moved to a position where most of its body was estimated to be within a 50cm radius of me. At this point the toy was withdrawn. Vocal encouragement was still offered to the kitten for the remainder of the test, but other than this I did not actively interact with it even if it initiated interaction or climbed up on to my lap.

6.2.3.3. Unfamiliar Person Holding Test

This test was carried out while the kitten's owner was outside the room.

I picked up the kitten, and placed it on my lap facing outwards. For a timed minute I handled the kitten, stroking it at a standard slow rate (approximately 15 strokes per minute). Escape attempts were gently restrained. Behaviour was observed during the test and recorded immediately afterwards.

6.2.3.4. Recording behaviour

Behaviours recorded during both *Owner* and *Unfamiliar Person* holding tests:

Escape Attempts: The number of times that the kitten attempted to escape from the handler's lap. Kittens that succeeded in escaping and could not be picked up immediately were awarded a maximum score of one plus the maximum score recorded from all the kittens that did not succeed in escaping, at the same age.

Purring: Presence or absence of purring during the test was recorded. Purring was recorded on a one/zero basis when the kittens were tested at 6 months. However, preliminary analysis of the data at 6 months suggested that the results would be more informative if the duration of purring was also recorded. Therefore, when the kittens were re-tested at 18 months, the duration of purring was recorded to the nearest second.

State of Activity: Three categories were devised. Kittens were allocated to the category they were nearest to for the most time during the test.

Relaxed: Kitten was still, in a relaxed sitting or lying posture, with no immediate attempt to leave the handlers lap at the end of the test.

Tolerant: Kitten made occasional attempts to change its position, but was generally still, adopting a standing or sitting upright position.

Restless: Kitten was moving constantly, either changing its position, or moving its head to look around the room, or stretching.

Tense/Untense: Tense (distressed) behaviour was scored on a 1/0 basis during the testing at 6 months. The following behaviours were taken as indicators of tenseness: distress vocalisations (hissing or growling), stiff posture, flattened posture, extended claws, staring eyes with dilated pupils, piloerection. A cat was recorded as tense if it exhibited at least a flattened posture or a stiff posture with dilated pupils or extended claws.

Preliminary analysis showed tenseness to be important in driving some components, it was therefore decided to quantify tense behaviour more precisely when testing was carried out at 18 months. Tense behaviour was then quantified using the following

scale as a guide.

0. No tense behaviour displayed.

1. Slightly tense or flattened posture, aware and alert.

2. Definitely tense and flattened posture.

3. Protracted claws, tense posture, dilated pupils.

4. Protracted claws "wild" eyes, struggling, attempting to scratch the test person

5. Constantly struggling and attempting to scratch the test person (Test aborted).

A tense score of 2 or above at 18 months would have been scored as tense using the 1/0 system employed at 6 months. Tenseness is difficult to measure and requires practice. The generally high levels of between tests repeatability which was obtained (see **Results)** indicates that the methods were consistent.

Interact With Holder: Interaction was defined as any kneading, rubbing, sniffing or licking of the holder during the test. Interactive behaviour was recorded on a one/zero basis.

The following behaviours were recorded during the *Approach Test*:

Approach to <0.5m (1/0): Whether or not the kitten approached the test person during the test, such that most of the kitten's body was estimated to be within 50cm of the test person.

Duration of Approach (seconds): Total length of time that the kitten was within 50cm of the tester.

Interaction with test person (1/0): Scored if the kitten licked or rubbed the tester, or jumped up on to the testers lap during the test.

Play with toy (1/0): Whether the kitten lunged at, pawed, or attempted to bite the toy.

Tense Behaviour: At 6 months this was scored on a 1/0 basis; the kitten was considered to be tense if hid or cowered during the test.

At 18 months tenseness was quantified on a 4 point scale. This was cruder than the 6 point scale used during the handling tests because without handling a kittens it is harder to quantify precisely the level of distress, hence:

0. No tense behaviour displayed.
1. Cowering, wide eyed.
2. Hiding immediately when placed on the ground.
3. Trying to escape from the room.

Purring: As for the handling tests, purring was recorded on a one/zero basis at 6 months and continuously to the nearest second at 18 months.

The variables recorded are summarised in **Table 6.1.**

Table 6.1. Variables measured in temperament testing study at 6 months and 18 months.

Test	Variable	Measurement recorded
Familiar person handling test	Escape attempts	Counted
	State of activity	Scored 1-3
	Interaction with test person	Scored 0/1
	Tenseness	6 months : Scored 0/1
		18 months: Scored 0-5
	Purring	6 months : Scored 0/1
		18 months: Timed (seconds)
Approach test	Approach < 0.5m	Scored 0/1
	Duration < 0.5m	Timed (seconds)
	Play with toy	Scored 0/1
	Interact with test person	Scored 0/1
	Tenseness	6 months : Scored 0/1
		18 months: Scored 0-5
Unfamiliar person handling test	Escape attempts	Counted
	State of activity	Scored 1-3
	Interaction with test person	Scored 0/1
	Tenseness	6 months : Scored 0/1
		18 months: Scored 0-5
	Purring	6 months : Scored 0/1
		18 months: Timed (seconds)

6.2.4. Statistical analysis

Consistency of results between tests was assessed using Spearman rank correlations for variables recorded on an ordinal or interval scale, and using a Kappa test for variables scored on a 1/0 basis (Martin and Bateson, 1986). Principal components analysis was used to collapse the recorded variables into a smaller number of components, with the aim of interpreting a general theme for each component. Possible relationships between extracted components at 6 and 18 months were tested using Pearson correlations since the distribution of scores between individuals appeared to be approximately normal. The factors contributing to variation in component scores were investigated using General Linear Modelling (GLM).

All statistical tests were performed using SPSS 7.5.

6.3. Results

6.3.1. Kittens recruited

A total of 81 kittens, from 59 litters, were temperament tested as close as possible to 6 months. The actual ages ranged from 5 months to 10 months. Fifty-one of the kittens were re-tested at 18 months. Summary statistics for the kittens used for temperament testing are presented in **Tables 6.2.** and **6.3.**

The number of kittens per litter that were tested ranged from one to four (**Figs 6.1.** and **6.2.**). Forty- three of the kittens tested at 6 months belonged to litters from which more than one kitten was tested. Of these kittens, 32 were housed with at least one littermate, the remaining 11 were not. At 18 months, there were 28 kittens belonged to litters from which more than one kitten was tested, and of these 19 were housed with at least one littermate, and 9 were not.

Fig. 6.1. Number of kittens tested per litter: Distribution of numbers of litters from which 1- 4 kittens were temperament tested at 6 months.

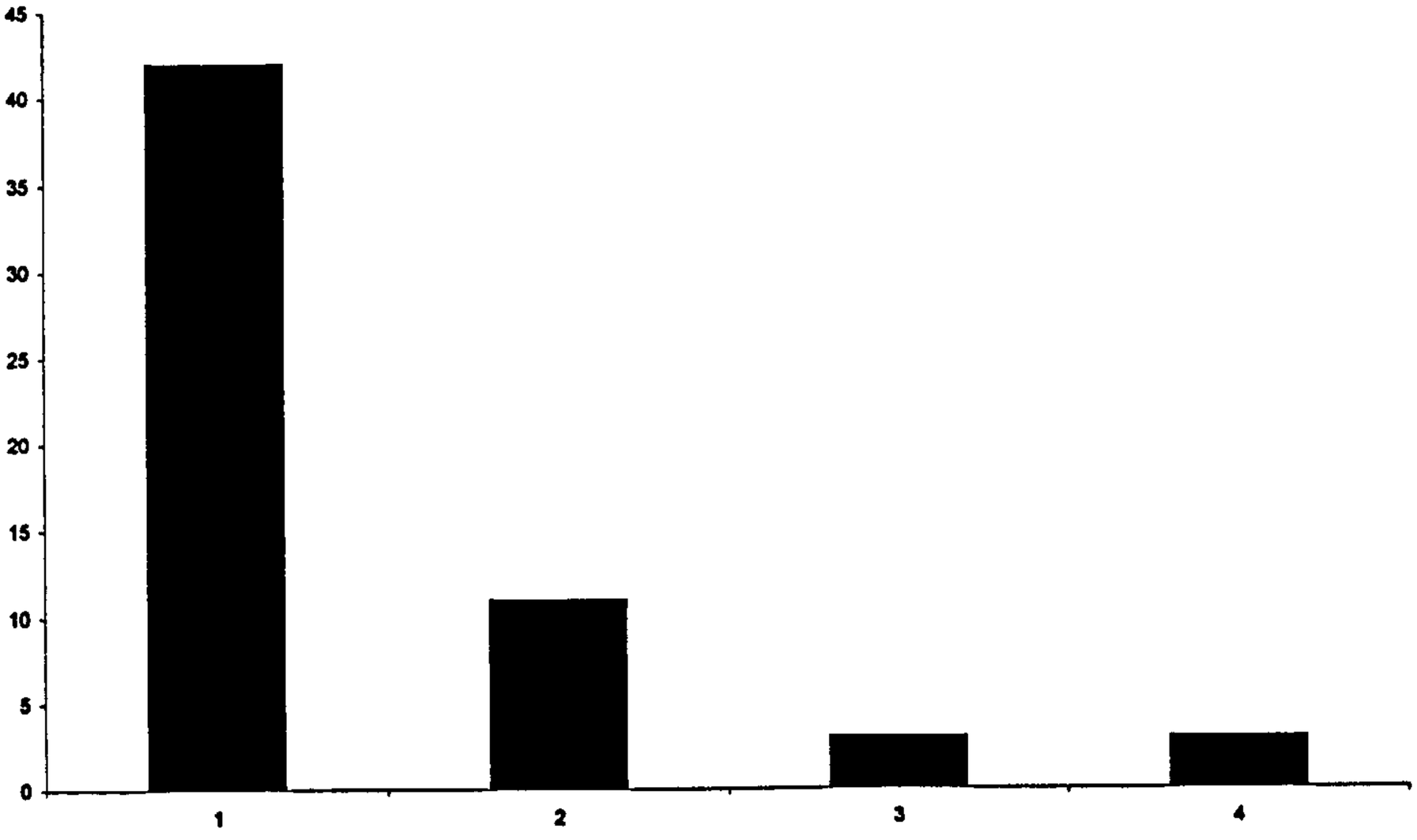


Fig. 6.2. Number of kittens tested per litter: Distribution of numbers of litters from which 1- 4 kittens were temperament tested at 18 months.

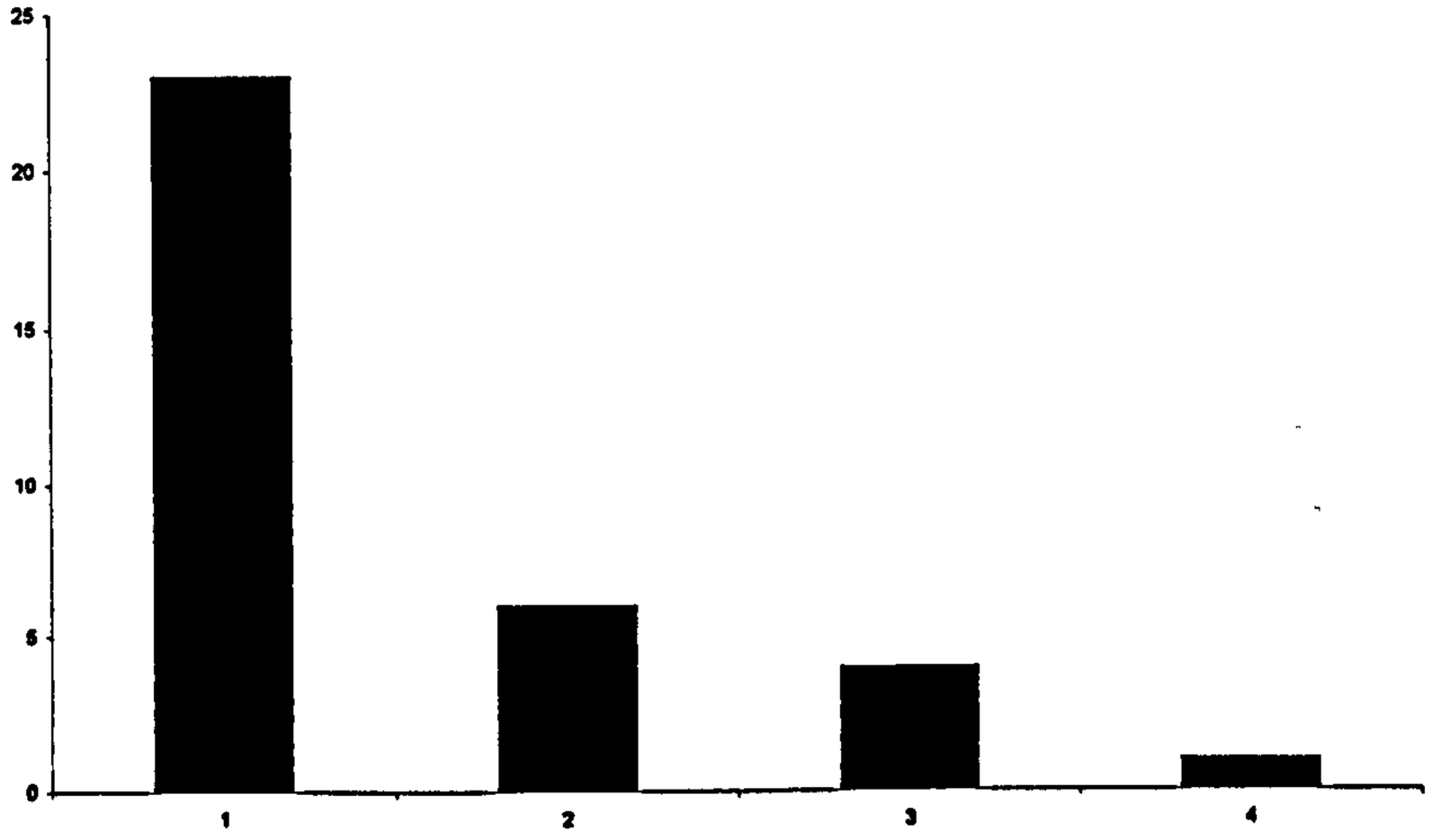


Table 6.2. Kittens used for temperament testing study at ages 5-10 months.

Age (months)		5	6	7	8	9	10	Total
Litters		5	26	10	10	5	3	59
Kittens		5	39	18	13	7	3	85
Area	Shirley	1	6	1	5	0	0	13
	Other	4	33	17	8	7	3	72
Gender	Female neutered	0	8	7	4	2	0	21
	Female entire	3	9	3	2	0	0	17
	Male neutered	0	7	7	5	2	3	24
	Male entire	2	14	1	2	1	0	20

There were two males and one female whose neutering status was unknown.

Table 6.3. Kittens used for temperament testing study, re-tested at 18 months.

Litters	Kittens	Shirley	Other	Female neutered	Female entire	Male neutered	Male entire
34	51	8	43	23	2	20	3

There were two males and one female whose neutering status was unknown.

6.3.2. Distribution of data.

The data from the temperament testing study were analysed, as described below, in order to collapse the variables recorded into a small number of components of behaviour. The aim was to interpret these, and assign values for each of these aspects of behavioural style to each kitten. The distribution of the raw data from the study is presented graphically in **Appendix 3.**

6.3.3. Reliability of measures between tests

The variables recorded needed to show consistency between tests in order to be considered biologically meaningful. Each variable was tested for a significant level of repeatability between tests one and two. Where the variable was recorded on a one/zero basis, a Kappa test was used to test for repeatability (see Martin and Bateson, 1986). In all other cases, a Spearman rank correlation was used (see **Table 6.5.** for 6 months, **Table 6.6.** for 18 months).

Martin and Bateson (1985) suggest that, as a general rule, a correlation of at least 0.7 is necessary to demonstrate adequate inter/intra observer reliability when the investigators are observing identical bouts of behaviour (eg. using video recording equipment when only one investigator is involved). Here the situation was different, since the behaviour of individual kittens was being compared between two separate occasions. A significant ($p < 0.05$) similarity was therefore taken as the cut-off point; variables where the similarity between test 1 and test 2 results was not significant were excluded.

Some kittens were only tested once; 4/81 (4.9%) at 6 months and 7/51 (11.7%) at 18 months. These kittens could not be included when evaluating reliability between tests.

6.3.3.1. Repeatability of measures at 6 months

All the variables measured in the tests at 6 months, with the exception of escape attempts when handled by the familiar person, were accepted for use in further analysis **(Table 6.4.)**.

Table 6.4. Variables measured in temperament testing at 6 months (n=81).

Test	Variable	Test used	Reliability between tests	Included/ excluded
Familiar person handling test	Escape attempts	Correlation	$r_s = 0.155$, $p > 0.05$	Excluded
	State of activity	Correlation	$r_s = 0.381$, $p < 0.001$	Included
	Interaction with test person	Kappa	$K_p = 0.336$, $p < 0.01$	Included
	Tenseness	Kappa	$K_p = 0.844$, $p < 0.001$	Included
	Purring	Kappa	$K_p = 0.482$, $p < 0.001$	Included
Approach test	Approach	Kappa	$K_p = 0.524$, $p < 0.001$	Included
	Duration < 0.5m	Correlation	$r_s = 0.495$, $p < 0.001$	Included
	Play with toy	Kappa	$K_p = 0.247$, $p < 0.05$	Included
	Interact with test person	Kappa	$Kp = 0.414$, $p < 0.001$	Included
	Purring	Kappa	$K_p = 0.575$, $p < 0.001$	Included
	Tenseness	Kappa	$Kp = 0.738$, $p < 0.001$	Included
Unfamiliar person handling test	Escape attempts	Correlation	$r_s = 0.432$, $p < 0.001$	Included
	State of activity	Correlation	$r_s = 0.315$, $p < 0.01$	Included
	Interaction with test person	Kappa	$K_p = 0.525$, $p < 0.001$	Included
	Tenseness	Kappa	$K_p = 0.550$, $p < 0.001$	Included
	Purring	Kappa	$K_p = 0.555$, $p < 0.001$	Included

6.3.3.2. Repeatability of measures at 18 months

The following variables were excluded prior to further analysis (**Table 6.5.**): interaction during the familiar person handling test, and approach, duration < 0.5m, playing with toy and purring during the approach test.

Table 6.5. Variables measured in temperament testing at 18 months (n=44).

Test	Variable	Test used	Reliability between tests	Included/ excluded
Familiar person handling test	Escape attempts	Correlation	$r_s = 0.407$, $p < 0.01$	Included
	State of activity	Correlation	$r_s = 0.534$, $p < 0.001$	Included
	Interaction with test person	Kappa	$K_p = 2.00$, $p > 0.05$	Excluded
	Tenseness	Correlation	$r_s = 0.469$, $p < 0.001$	Included
	Purring	Correlation	$r_s = 0.509$, $p < 0.001$	Included
Unfamiliar person handling test	Escape attempts	Correlation	$r_s = 0.688$, $p < 0.001$	Included
	State of activity	Correlation	$r_s = 0.591$, $p < 0.001$	Included
	Interaction with test person	Kappa	$K_p = 565$, $p < 0.001$	Included
	Tenseness	Correlation	$r_s = 0.804$, $p < 0.001$	Included
	Purring	Correlation	$r_s = 0.529$, $p < 0.001$	Included
Approach test	Approach	Kappa	$K_p = 0.182$, $p = 0.195$	Excluded
	Duration < 0.5m	Correlation	$r_s = 0.297$, $p = 0.051$	Excluded
	Play with toy	Kappa	$K_p = -0.154$, $p = 0.226$	Excluded
	Interact with test person	Kappa	$K_p = 0.283$, $p = 0.054$	Included[1]
	Purring	Correlation	$r_s = 0.129$, $p = 0.40$	Excluded
	Tenseness	Correlation	$r_s = 0.535$, $p < 0.001$	Included

[1]Interaction with test person during approach test was included, despite falling just outside significance. This was because it was felt to be important to include a measure recorded during the approach test in addition to tenseness.

For each variable that was accepted for inclusion for further analysis, a mean value was calculated for Test 1 and Test 2 for each kitten. For kittens tested only once, the single scores were used for analysis. This gave a single score for each kitten at each age. The mean scores were then used in principal components analysis (see below).

6.3.4. Principal components analysis

Principal components analysis (PCA) is a factor analysis method. It is used to reduce a data set containing a large number of variables into a smaller number of underlying components which explain a high proportion of the total variance. The technique works on the correlation coefficients between variables. Components are extracted one at a time; the first component accounts for the maximum amount of variance possible along a single axis, the second component accounts for the maximum remaining variance when the variance from the first component is removed. Third and subsequent components are calculated in a similar fashion. Each component is uncorrelated to the previous components. Each variable from the original data is assigned a weight, a positive or negative numerical value, for each component.

Ideally, the number of subjects (kittens) should exceed the number of variables by a factor of 5 (Cooley and Lohnes, 1971). This condition is met for the data set at 6 months (85 kittens; 15 variables), and is nearly met at 18 months (51 kittens; 11 variables).

All variables, other than those recorded on a 1/0 basis, were square root transformed (after adding 0.5 to avoid zero values). The SPSS program automatically transforms variables to give a mean of 0 and a standard deviation of 1, before commencing PCA.

6.3.4.1. Assessing which components to retain

In PCA each component is assigned an associated eigenvalue, which indicates the proportion of variance accounted for by the component (**Tables 6.6.** and **6.7.**). An eigenvalue of one accounts for the equivalent variance to one of the original variables. An eigenvalue of one is therefore often used as a cut-off point; components with lower eigenvalues are discarded. In practice components with eigenvalues lower than two are frequently difficult to interpret, except when only one or two variables are heavily weighted, and all others have weightings close to zero.

A further technique for evaluating components using eigenvalues is to plot a "scree" chart; component number against eigenvalue (**Figs 6.3.** and **6.4.**). An abrupt reduction in the gradient indicates the point below which components should be disregarded.

Table 6.6. Principal components analysis at six months: Eigenvalues and the percentage of variance explained by each component.

Component	Eigenvalue	% of variance	Cumulative %
1	5.240	34.931	34.931
2	2.032	13.549	48.479
3	1.678	11.188	59.668
4	1.095	7.303	66.970
5	0.997	6.646	73.616
6	0.864	5.762	79.379
7	0.669	4.458	83.837
8	0.469	3.125	86.962
9	0.415	2.767	89.729
10	0.352	2.347	92.075
11	0.340	2.270	94.345
12	0.297	1.981	96.326
13	0.234	1.563	97.889
14	0.211	1.406	99.296
15	0.106	0.704	100.000

Table 6.7. Principal components analysis at eighteen months: Eigenvalues and the percentage of variance explained by each component.

Component	Eigenvalue	% of variance	Cumulative %
1	5.026	45.689	45.689
2	1.469	13.354	59.043
3	1.208	10.985	70.029
4	0.774	7.040	77.069
5	0.678	6.168	83.237
6	0.622	5.652	88.889
7	0.498	4.523	93.412
8	0.277	2.520	95.931
9	0.245	2.223	98.155
10	0.132	1.203	99.357
11	0.071	0.643	100

Fig. 6.3. Scree chart showing eigenvalues from temperament testing at 6months

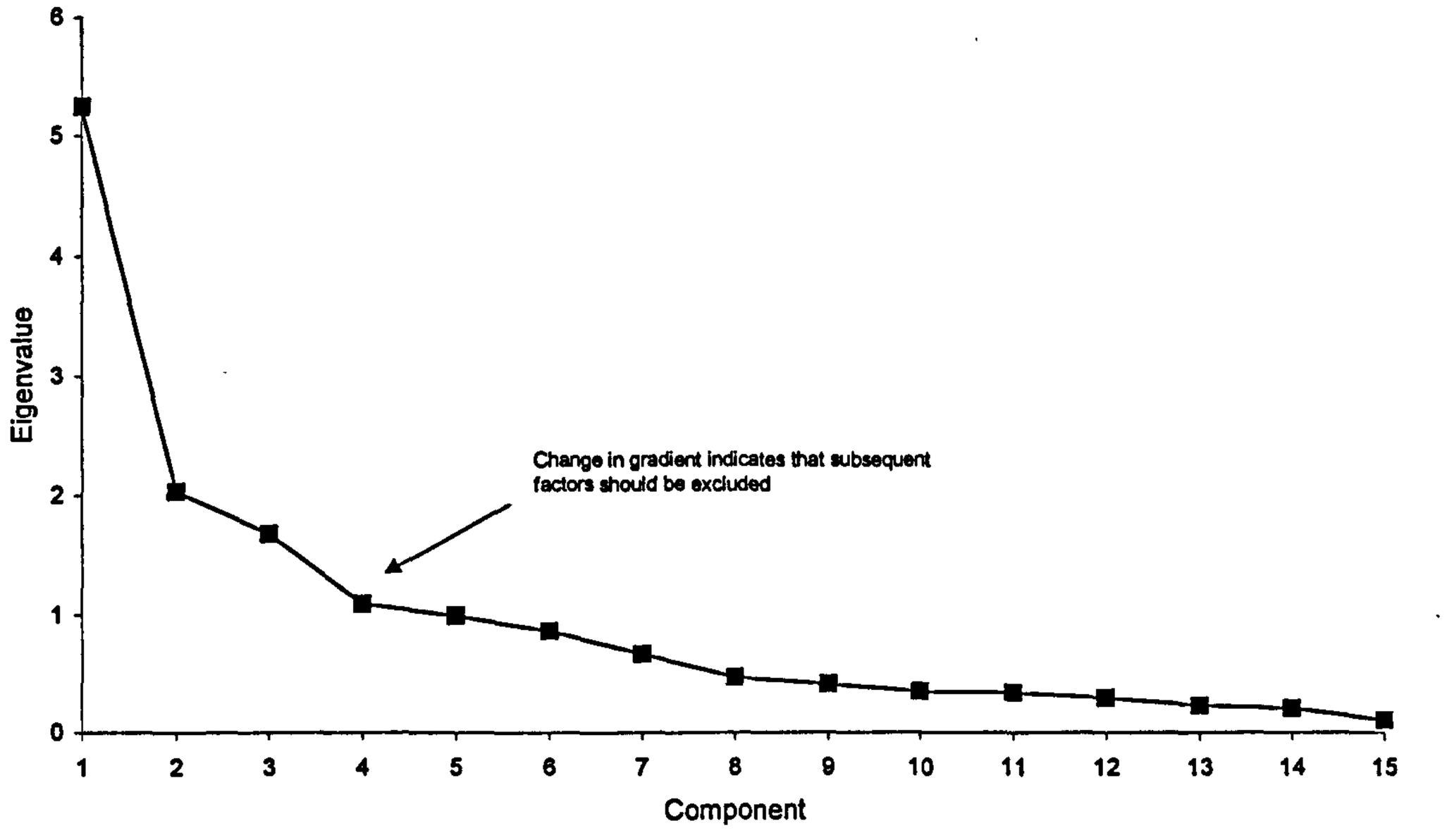
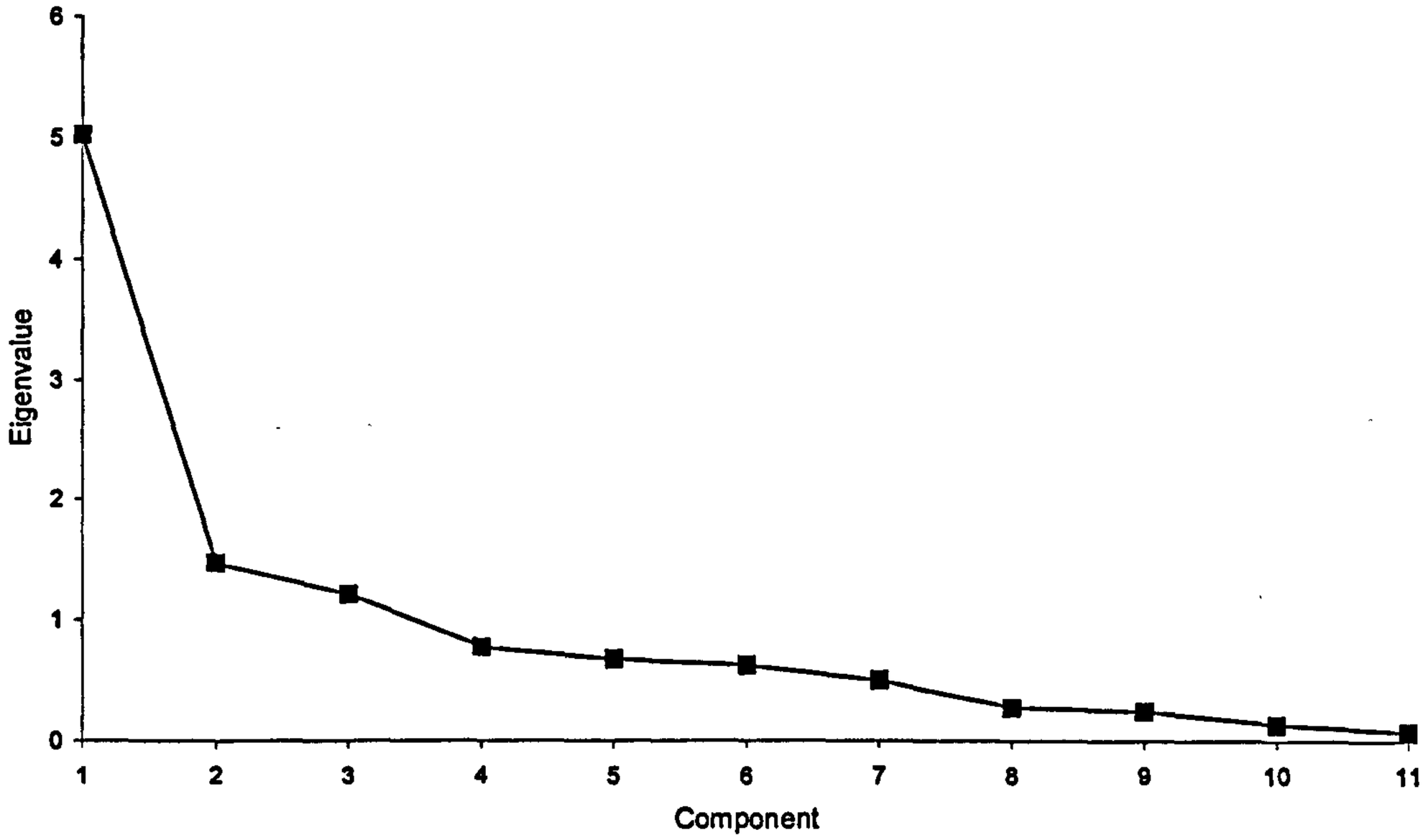


Fig. 6.4. Scree chart showing Eigenvalues from temperament testing at 18 months



Components at six months

Four components were assigned eigenvalues higher than one (**Table 6.6.**). The scree chart (**Fig 3**) indicated a cut-off point after component 3. However, it was decided to keep component 4 because it was strongly weighted for only two variables (see below), and might therefore be interpretable despite the low proportion of variance attributed to it.

Components at eighteen months

Three components were assigned eigenvalues higher than one (**Table 6.7.**). The scree chart **Fig 6.4.** also indicated that three components should be retained. The first three components from PCA at 18 months were therefore included for further analysis.

6.3.4.2. Characterising the components

The weighting of each variable in characterising each retained component was visualised by plotting component weights from different components against each other (**Figs 6.5-6.8.**). This also allows the interrelationships between components to be visualised.

The variables characterising each component were identified, first by finding the variable with the component weight showing the greatest numerical value, and then using half the numerical value of this variable, either positive or negative, as a rule-of-thumb cut-off point. Variables with a value exceeding this threshold, regardless of sign, were identified as characterising the component. However, variables falling just outside this parameter have been listed in brackets. The characteristic factors associated with each component, both positive and negative, were then listed and examined in order to identify a common theme which could be used to label the component (see below).

Key to abbreviations used in **Figs 6.5.-6.8.**

Test	Variable	Abbreviation used
Familiar person handling test	Escape attempts	OEsc
	State of activity	OState
	Interaction with test person	OInt
	Tenseness	OTense
	Purring	OPurr
Approach test	Approach < 0.5m	Approach
	Duration < 0.5m	ApDur
	Play with toy	Play
	Interact with test person	APint
	Tenseness	APTense
Unfamiliar person handling test	Escape attempts	MEsc
	State of activity	MState
	Interaction with test person	MInter
	Tenseness	MTense
	Purring	MPurr

Fig. 6.5. Scatter plot of factor component weights for component 1 against component weights for component 2, from temperament testing at 6 months.

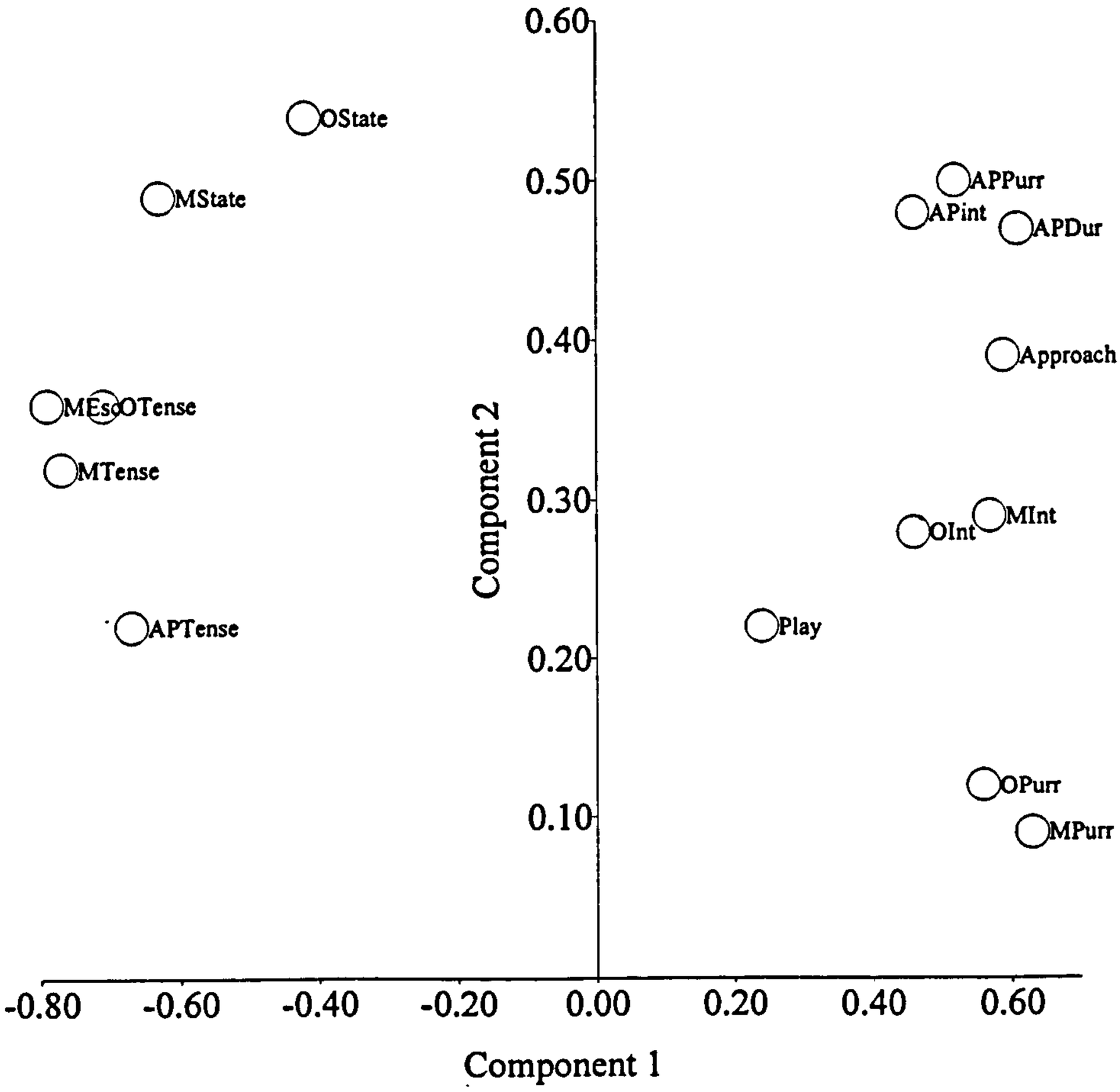


Fig. 6.6. Scatter plot of factor component weights for component 3 against component weights for component 4, from temperament testing at 6 months.

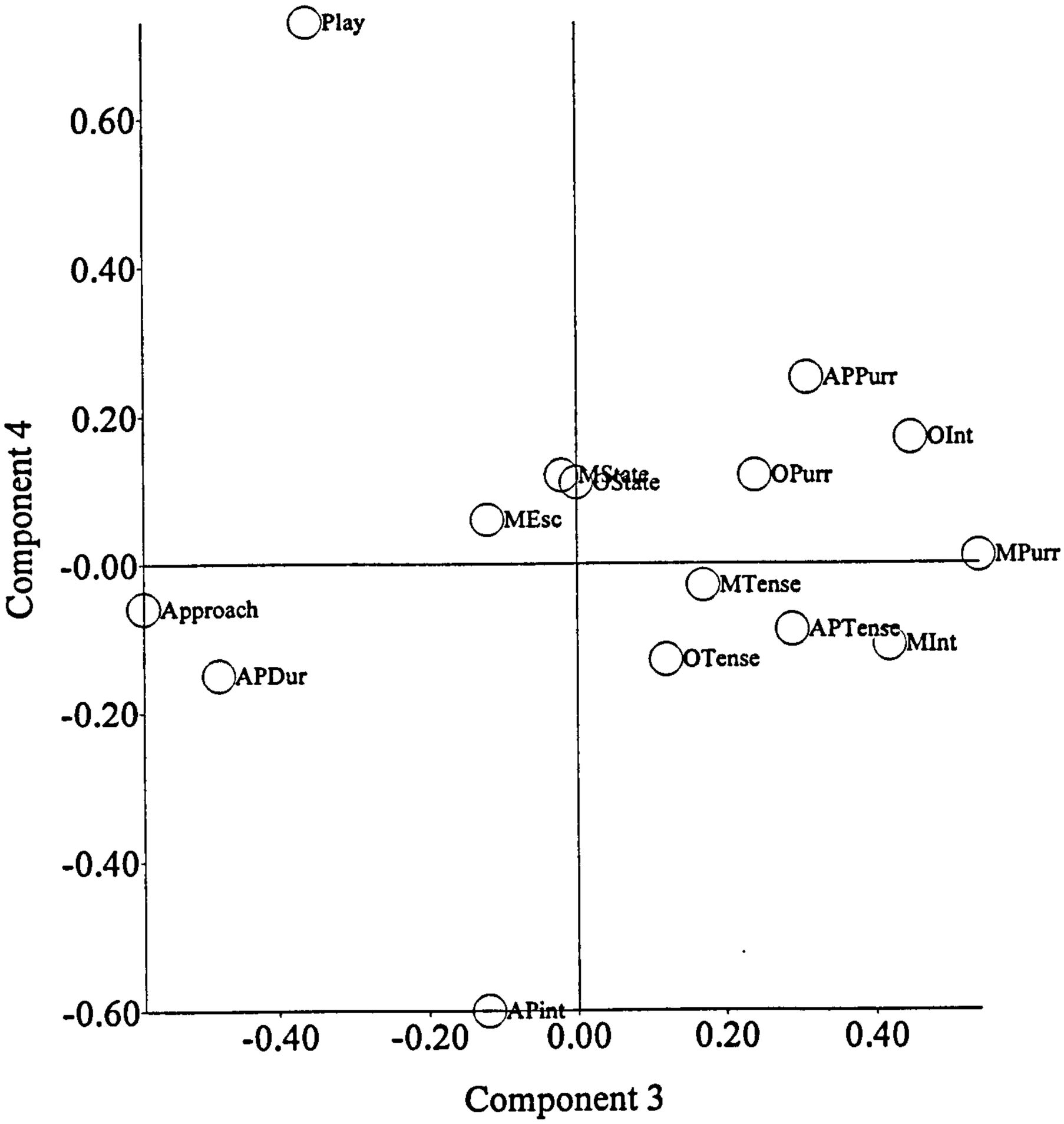


Fig. 6.7. Scatter plot of factor component weights for component 1 against component weights for component 2, from temperament testing at 18 months.

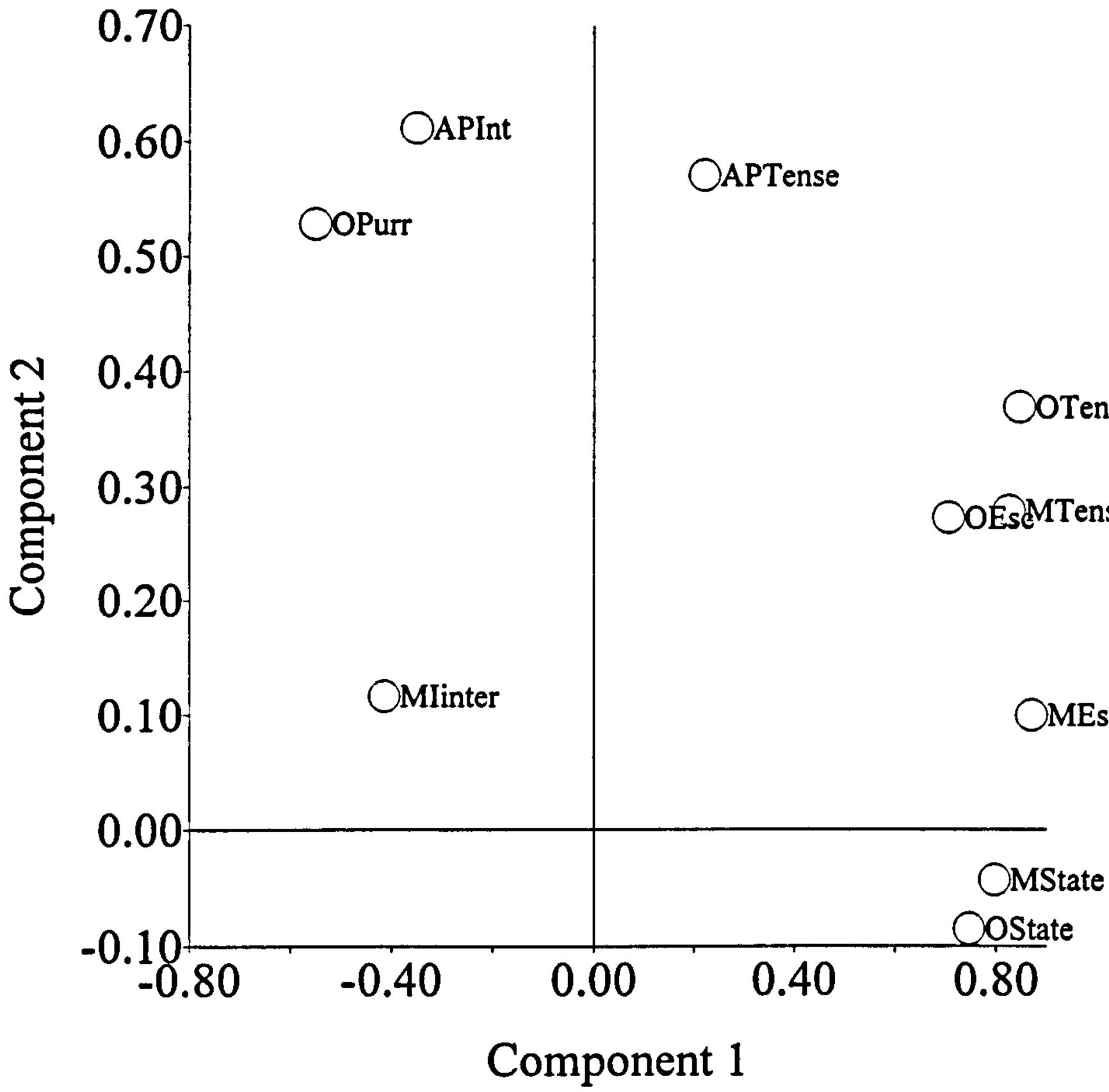
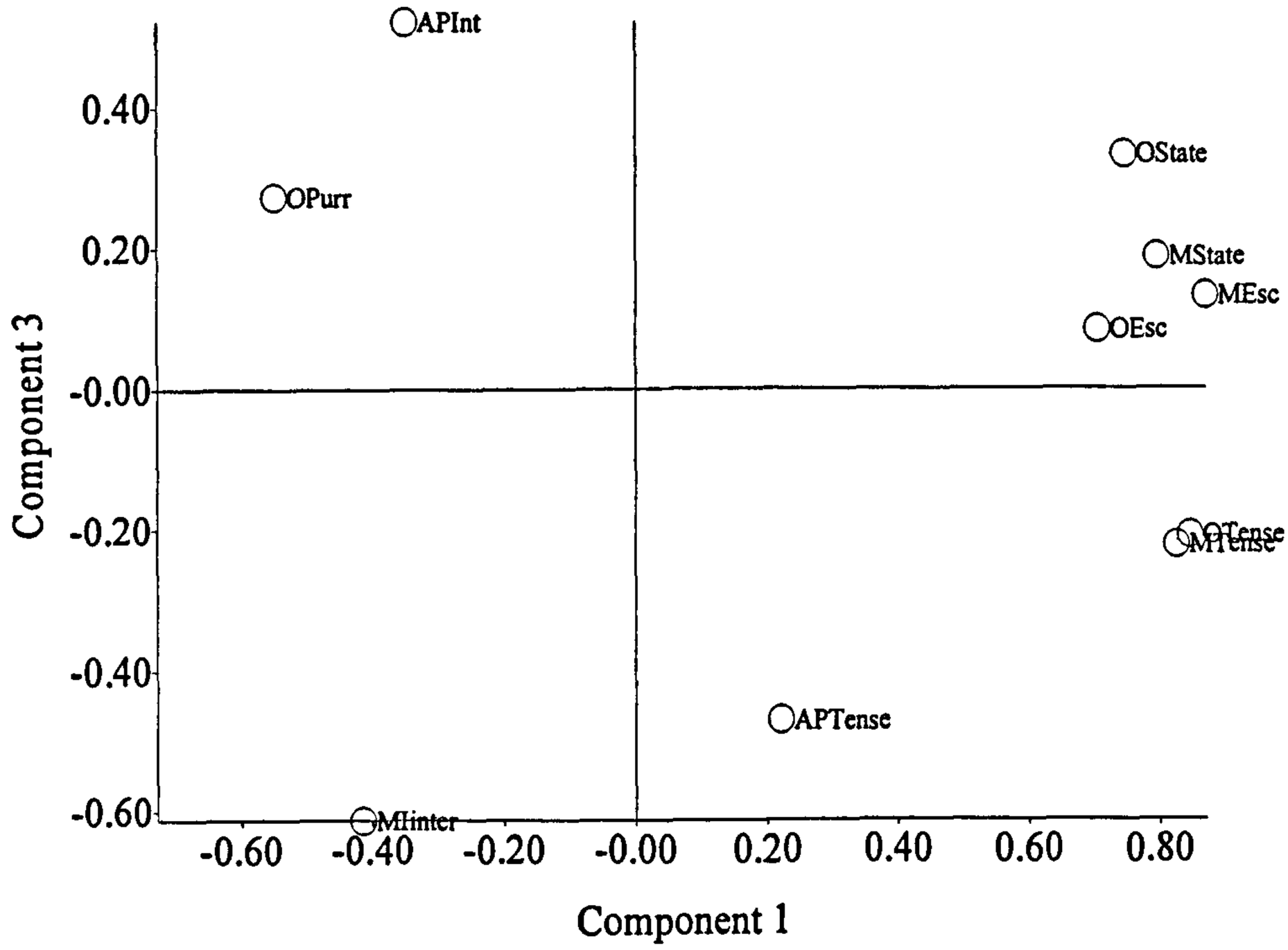


Fig. 6.8. Scatter plot of factor component weights for component 1 against component weights for component 3, from temperament testing at 18 months.



Components at six months

Component 1 (34.9% of variance)

Positively weighted:	*Interactive* in unfamiliar person handling test
	Interactive in familiar person handling test
	Purring in unfamiliar person handling test
	Purring in familiar person handling test
	Approach to <0.5m in approach test
	Duration <0.5m in approach test.
	Interact with test person in approach test.
	Purring during approach test.
Negatively weighted:	*State of activity* in unfamiliar person handling test
	State of activity in familiar person handling test
	Escape attempts during unfamiliar person handling test
	Tenseness in unfamiliar person handling test
	Tenseness in familiar person handling test
	Tenseness in approach test
Not weighted:	*Play with toy*.

Component 1 can be interpreted as characterising a *sociable-unsociable* dimension. Purring is a behaviour usually (but not always) associated with non-fearful cats. The other components with a positive weighting involved *interacting* with, *approaching*, and *remaining close to the test person*. Conversely, the components with a negative weighting indicated kittens that were *tense*, were not likely to *tolerate being handled*, and were unlikely to *approach* during the approach test.

Component 2 (13.5% of variance)

Positively weighted:	*Interactive* in unfamiliar person handling test
	Interactive in familiar person handling test
	State of activity in unfamiliar person handling test
	State of activity in familiar person handling test
	Escape attempts during unfamiliar person handling test
	Tenseness in unfamiliar person handling test
	Tenseness in familiar person handling test
	Approach to <0.5m in approach test
	Duration <0.5m in approach test.
	Interact with test person in approach test.
	(*Play with toy*)
	(*Tense* during approach test)
Negatively weighted:	None
Not weighted:	*Purring* in unfamiliar person handling test
	Purring in familiar person handling test

The interpretation of this component is not obvious because *interactive*, *approaching* and *tense* behaviours are all positively weighted. Component 2 might be characterised by kittens that are generally *active*, or are likely to show a *range of behaviour* over the course of each test. In order to test this, the number of variables for which each kitten had scored zero in both tests (kittens tested only once were excluded) was correlated (Spearman rank) against the kittens' scores for component 2. There was a negative correlation ($r_s = -0.664$, $p < 0.001$) i.e. kittens that exhibited a wide range of behaviours (scored few zeros) over the 2 tests were likely to score highly in component 2. There were no corresponding associations with component 3 ($r_s = -0.104$, $p = 0.353$), or component 4 ($r_s = -0.101$, $p = 0.368$). However there was a correlation for component 1 ($r_s = -0.497$, $p < 0.001$).

Component 3 (11.2% of the variance)

Positively weighted: *Interactive* in unfamiliar person handling test
Interactive in familiar person handling test
Purring in unfamiliar person handling test
Purring during approach test.
Tenseness in approach test
(*Purring* in familiar person handling test)

Negatively weighted: *Play with toy* during approach test
Approach to <0.5m in approach test
Duration <0.5m in approach test.

Kittens scoring highly on component 3 are likely to *purr* and *interact* when handled, but unlikely to *approach* or *play* with the toy during the approach test. This component may therefore be characterised as *timid/friendly-playful/bold*.

Component 4 (7.3% of variance)

Positively weighted: *Play with toy* during approach test

Negatively weighted: *Interact with test person* during approach test

Only two variables were weighted for this component, which separated those that *play* with the toy and those that *interact* with the test person during the approach test.

Components at eighteen months

Component 1 (45.7% of variance)

Positively weighted:	*State of activity* in unfamiliar person handling test
	State of activity in familiar person handling test
	Escape attempts during unfamiliar person handling test
	Tenseness in unfamiliar person handling test
	Tenseness in familiar person handling test
Negatively weighted:	*Interactive* in unfamiliar person handling test
	Purring in unfamiliar person handling test
	Purring in familiar person handling test
	(*Interact* with test person during approach test)
Not weighted:	*Play with toy*.

The factors weighted for the first component at 18 months were similar to the factors weighted at 6 months. Of the variables that were accepted for PCA at both 6 months and 18 months, the only difference was that *interactivity* during the approach test was just below the cut-off point at 18 months. Component 1 at 18 months therefore represents the same general *sociable-unsociable* dimension as component 1 at 6 months. Accounting for 46% of the variance, component 1 is the most important component at 18 months by a substantial margin; component 2 accounts for only 13.4% of the variance.

The analysis had arbitarily reversed the positively and negatively weighted factors. The sign was therefore reversed for component 1 scores at 18 months, for ease of interpretation, when further analysis was carried out.

Component 2 (13.4% of variance)

Positively weighted:	*Purring* in unfamiliar person handling test
	Purring in familiar person handling test
	Interact with test person in approach test.
	Tenseness during approach test.
	Tenseness in familiar person handling test
	(*Tenseness* in unfamiliar person handling test)
	(*Escape attempts* in familiar person handling test)
Negatively weighted:	None
Not weighted:	*State of activity* in familiar person handling test
	State of activity in unfamiliar person handling test
	Escape attempts in unfamiliar person handling test
	Interactive in unfamiliar person handling test

Component 2 at 18 months shows some similarity with component 2 at 6 months: *tense* behaviour in all tests, and *interactivity* during the approach test, are positively weighted. However, conversely to results at 6 months, active behaviours while being handled (*interactivity*, *state of activity*, *escape attempts*) are not weighted at 18 months, but *purring* while being handled is weighted.

This component is difficult to interpret. Cats under stress at low levels do sometimes *purr*, and the "*tense*" factors were only weakly loaded here, so the association of these factors is intuitively reasonable. *Interaction* and *tenseness* during the approach test are the two most strongly weighted factors, which is not easily explicable. There may be an element here of the "*display a wide range of behaviours*" or *active* tendency, discussed for component 2 at 6 months.

The relationship between the number of factors for which behaviours were displayed, and component 2 scores, was investigated using Spearman rank correlations, as it was at 6 months. There was a positive correlation between number of behaviours exhibited

and component 2 scores, although the relationship was not as strong as it was at 6 months (r_s = -0.485 , P < 0.001). There was no significant correlation for component 3 scores. However there was a correlation for component 1 scores (r_s = 0.500, P < 0.001), but the trend had reversed; cats which displayed a *wide range of behaviours* were more *sociable* at 6 months, but less *sociable* at 18 months.

<u>Component 3</u> (11.1% of variance)

Positively weighted:	*Interactive* during approach test
	State of activity in familiar person handling test
	(*Purring* in familiar person handling test)
Negatively weighted:	*Interactive* in unfamiliar person handling test
	Tenseness in approach test.

Component 3 clearly separated kittens that were *tense* and kittens that were *interactive* during the approach test. It also connects kittens that *interacted* during the approach test were likely with a high *state of activity* when handled by their familiar person, and *non interactivity* during the unfamiliar person handling test. The interpretation of this is not clear, but may represent a *bold–timid* towards humans axis.

6.3.5. Component scores at six months and eighteen months

In order to investigate whether there were relationships between components extracted at 6 months and 18 months, correlation analysis were performed between component scores for each component at both age categories. Pearson's correlations were used because graphical inspection of component scores revealed them to be approximately normally distributed. Only kittens tested at both 6 months and 18 months were included. Two significant correlations were found:

Component 1 at 6 months and 18 months ($r = 0.649$, $p < 0.001$)

Component 2 at 6 months and 18 months ($r = 0.427$, $p < 0.001$)

Component 1 was very similar at both age groups, the correlation found here shows a definite relationship between general friendliness at 6 months and 18 months. The correlation of the second component between the age groups gives some justification to the similarities drawn between component 2 at 6 months and 18 months.

6.3.6. Variation in temperament between litters

6.3.6.1. Factors contributing to variation between individuals

Shirley is an area where neutering rates are known to be very high (**Chapter 2**). Neutering rates in other areas are likely to be varied, and in at least some areas are known to be lower than in Shirley. Neutering rates were not estimated in all areas, but are unlikely anywhere in Southampton to be higher than in Shirley, where 98.7% of adult females in the survey were neutered. Shirley can therefore be assumed to be an area of high neutering relative to the rest of Southampton combined. In this analysis, area effects were examined by comparing Shirley (high neutering) to the other areas combined (lower neutering).

Three potential sources of inter-individual variation were examined in the principal component scores: gender, between-litter, and area of origin (Shirley - high neutering, vs. other areas combined). Graphical inspection of the principal component scores for the individual cats at both 6 and 18 months indicated that they were approximately normally distributed, and so it was appropriate to use parametric analysis of variance to test the relative importance of all three sources of variation simultaneously. The model adopted was:

Main effects:

Gender (4 levels: entire male, entire female, neutered male, neutered female)

Area of origin (2 levels: Shirley, other areas of Southampton); nested in -

Between-Litter (59 levels at 6 months, 34 levels at 18 months)

Within-litter (Residual).

No interactions were considered, since none was relevant to the hypotheses being tested.

Between-litter variation was used as the denominator for calculating the F-ratio for the area effect, since each litter came uniquely from one area or the other (nested factor). Within-litter variation was used as the denominator for calculating the F-ratio for between-litter variation, and also, by default, for variation due to gender.

The Within-litter term includes two potentially different sources of variation, differences between littermates housed separately and differences between littermates housed together. Since it might be expected *a priori* that the latter would be less than the former, two additional data sets were constructed with one member of each pair of littermates housed together excluded from each (in the case two litters of four housed together, two were excluded from each). All ANOVAs were then carried out on (a) the full data set (b) each of the reduced data sets in turn (a form of jacknifing), and the F-ratios for gender and between-litter effects compared between (a) and (b). For between litter differences, one of the F-ratios from (b) was greater than that in (a), and the other was smaller in components one and two (**Table 6.8.**), indicating that within-litter variation was unaffected by whether the kittens had been housed together or separately. For component three the F-ratios increased slightly in both jackifed data sets, while F ratios decreased slightly in both jacknifed data sets for component four. Considering gender effects, variance was slightly increased in both jacknifed data sets.

The jacknifed data sets did not reveal substantially reduced variances for any components. The F-ratios from the ANOVAs on the full data set were therefore used to estimate significance levels.

Table 6.8. Comparisons of F-ratios for between litter variation from full data set and jacknifed data sets for kittens at 6 months.

Component	F-ratios: between litter variation using full data set		F-ratios: between litter variation using jacknifed data set No.1		F-ratios: between litter variation using jacknifed data set No.2	
	Between litter	Gender	Between litter	Gender	Between litter	Gender
1	2.579	1.386	1.934 (-)	0.966(-)	3.780 (+)	4.040 (+)
2	3.226	4.109	2.756 (-)	4.516 (+)	3.916 (+)	5.148(+)
3	2.097	3.085	2.128 (+)	4.800 (+)	2.881 (+)	5.380 (+)
4	0.967	0.932	0.655 (-)	1.457 (+)	0.673 (-)	1.085 (+)

Prior to this analysis, unpaired t-tests were used to test whether kittens given their "6 month" test at 9 or 10 months old, differed in their 6-month test responses from those tested at 6-8 months, since Cook and Bradshaw (submitted) found substantial differences in responses to handling between four and twelve months of age. Significant differences were found ($p = 0.024$) and so the group tested late (N=10) was excluded from the ANOVA.

6.3.6.2. Sources of between-litter variation

General linear modelling was carried out for each retained component at both age categories **(Tables 6.9-6.15.)** .

Six months

Table 6.9. Anova table for component 1, six months.

Source	Type III S.Sq.	df	Mean Square	F	Significance
Area of origin	Hypothesis	3.832	1	3.832	3.898	0.054
Estimate of between -litter error		44.742	45.51	0.983		
Gender	Hypothesis	1.658	3	0.553	1.386	0.275
Between-litter	Hypothesis	44.200	43	1.028	2.579	0.011
Within-litter	Residual	8.371	21	0.399		

Between litter variation was significant, signifying that there are meaningful differences in this component which are attributable to genetic/early environmental effects. Gender effects were not significant. The difference between Shirley and other areas was close to significance, and was in the direction predicted by the initial hypothesis, Shirley cats (mean ±. S.D: -0.441 ±. 0.188) had lower scores for this component than cats for other areas (mean ±. S.D: 0.072 ±. 0.081), and were therefore more likely to be *unfriendly/fearful*. However, it should be noted that the sample size of Shirley litters was small (n = 10). When a similar anova was performed, comparing Shirley born litters with litters born in areas where there was some evidence of lower neutering rates (Sholing, Merryoak and Thornhill), the between area effects was not significant (p= 0.32).

Table 6.10.a. Anova table for component 2, six months.

Source		Type III S. Sq.	df	Mean Square	F	Significance.
Area of origin	Hypothesis	2.145	1	2.145	1.745	0.193
Estimate of between litter error		55.327	45.01	1.229		
Gender	Hypothesis	4.939	3	1.646	4.109	0.019
Between-litter	Hypothesis	55.580	43	1.293	3.226	0.003
Within-litter	Residual	8.414	21	0.401		

Between litter variation was highly significant for this component, but area of origin was not. Interestingly, gender effects were also significant, with entire males scoring highest and neutered males lowest **(Table 6.1.1.b.)**

Table 6.10.b. Mean gender scores for component 2, for kittens temperament tested at 6months.

Gender	N	Mean ± Std. Error
Female neutered	21	0.090 ± 0.154
Female entire	17	-0.027 ± 0.155
Male neutered	24	-0.127 ± 0.132
Male entire	20	0.231 ± 0.147

Table 6.11.a. Anova table for component 3, six months.

Source		Type III S. Sq.	df	Mean Square	F	Significance.
Area of origin	Hypothesis	0.522	1	0.522	0.432	0.514
Estimate of between –litter error		55.669	46.06	1.208		
Gender	Hypothesis	5.539	3	1.846	3.085	0.049
Between-litter	Hypothesis		43	1.255	2.097	0.035
Within-litter	Residual	12.570	21	0.599		

Gender effects, as well as litter effects, but not area effects, were significant for component 3 (**Table 6.11.a.**). Component 3 was characterised as *timid/friendly-bold/playful*. And it is interesting to note that neutered kittens and females scored more highly (more *timid/friendly*) than entire kittens and males (more *bold/playful*).

Table 6.11.b. Mean gender scores for component 2 at six months.

Gender	N	Mean ± Std. Error
Female neutered	21	0.270 ± 0.194
Female entire	17	0.142 ± 0.195
Male neutered	24	-0.009 ± 0.166
Male entire	20	-0.187 ± 0.185

Table 6.12. Anova table for component 4, six months.

Source		Type III S. Sq.	df	Mean Square	F	Significance.
Area of origin	Hypothesis	1.132	1	1.132	1.379	0.246
Estimate of between –litter error		40.596	49.44	0.821		
Gender	Hypothesis	2.370	3	0.790	0.932	0.443
Between-litter	Hypothesis	35.222	43	0.819	0.967	0.553
Within-litter	Residual	17.793	21	0.847		

For component 4 there were no significant differences attributable to any of the potential sources of variation tested here, even between-litter variation. Component 4 was therefore not considered useful for this analysis of heritable effects.

Eighteen Months

Table 6.13. Anova table for component 1, eighteen months.

Source		Type III S. Sq.	df	Mean Square	F	Significance.
Area of origin	Hypothesis	0.039	1	0.039	0.035	0.852
Estimate of between -litter error		35.373	32.00	1.105		
Gender	Hypothesis	6.661	3	2.220	2.642	0.090
Between-litter	Hypothesis	27.404	23	1.191	1.418	0.252
Within-litter	Residual	11.765	14	0.840		

Component 1 at 18 months was not affected significantly by any of the variables considered. Even between-litter affects had drifted such that the differences between litters were not significant, although a value of p = 0.252 does not rule out the existence of differences. Intuitively, one would expect between-litter differences to wane as the kittens mature and undergo different experiences. The sample size decreased between 6 months and 18 months, which effects significance levels, but the F values, which should be independent of sample size, were also correspondingly reduced.

Table 6.14. Anova table for component 2, eighteen months.

Source		Type III Sum of Squares	df	Mean Square	F	Significance.
Area of origin	Hypothesis	0.042	1	0.0417	0.032	0.859
Estimate of between -litter error		38.049	29.11	1.307		
Gender	Hypothesis	2.229	3	0.743	1.108	0.379
Between-litter	Hypothesis	34.817	23	1.514	2.257	0.0589
Within-litter	Residual	9.389	14	0.671		

Interestingly, component 2 is approaching significance between litters at 18 months, indicating that it may be more robust over time than component 1 (**Table 6.14**). However, the gender effects apparent at 6 months have disappeared.

Table 6.15. Anova table for component 3, eighteen months.

Source		Type III Sum of Squares	df	Mean Square	F	Significance.
Area of origin	Hypothesis	0.301	1	0.301	0.373	0.545
Estimate of between litter error		29.800	36.90	0.808		
Gender	Hypothesis	1.128	3	0.376	0.283	0.837
Between-litter	Hypothesis	14.688	23	0.639	0.481	0.942
Within-litter	Residual	18.571	14	1.326		

Component 3 was tentatively interpreted as representing a *timid-bold* component of behaviour. GLM revealed no between litter variation and the component is therefore not useful for this analysis **(Table 6.15)**.

6.4. Discussion

6.4.1. Components of behaviour identified at 6 months and 18 months

At both 6 months and 18 months the primary component revealed by PCA was a general *sociable-unsociable to humans* trait. This is comparable with the *sociable* characteristic identified by other workers (Feaver *et al,* 1986; Bradshaw and Cook, 1996). The interpretation of this component was straightforward at both age categories because there were no anomalous factors to consider. However, it should be remembered that component one is not identical between 6 months and 18 months, because different factors were excluded from each, due to the criteria of repeatability between tests.

Component one scores were correlated between 6 months and 18 months ($r = 0.671$), indicating that a kitten's friendliness at 18 months can be partially predicted from its friendliness at 6 months. The correlation is substantial, but not perfect, i.e. behavioural changes do occur between the age categories. GLM analysis showed that component one scores are no longer significantly different between litters at 18 months. This

supports previous research on the development of temperament in kittens; correlations were found between 4 months and 36 months in *escape attempts* and *distress* behaviour, but some cats did change from being *sociable* to being *unsociable* (Cook and Bradshaw; 1996, Cook and Bradshaw, submitted). One would intuitively expect the influence of early environment and genetic pre-disposition to diminish over time to some extent.

The relationship between the number of behaviours exhibited and component one scores, discovered when attempting to interpret component 2, is worth considering because it may shed light on the changes in behaviour between 6 months and 18 months. The correlations at 6 months revealed that cats which *display more behaviour types* during tests tend to be more *friendly*, while at 18 months this was reversed. This may reflect the fact that the factors *approaching* during the Approach Test, and *duration <0.5m* during the Approach Test, were not repeatable between tests at 18 months. At 18 months factors which involved less activity by the cat; *purring* while being handled, and *interactivity* while being handled, were more important in characterising *sociability* or *friendliness* than they were at 6 months.

Component two was harder to interpret. There were elements of a tendency to *display a wide range of behaviours* (indicating *inconsistent* or *active traits*) at both ages, as revealed by correlation analysis, although this was more robust at 6 months than at 18 months. There was a moderate correlation between component 2 scores at 6 months and 18 months ($r = 0.427$), suggesting that the observed similarities in the factors involved were not spurious, but indicated similarities in the underlying components.

Litter differences in component two scores were shown by GLM analysis to be close to significance at 18 months, unlike component one scores. Entire males scored the highest at 6 months, suggesting that they are the most likely to show a range of behavioural characteristics during tests. Gender differences were not apparent at 18 months, however this may be because the sample size of entire males was reduced to three, due to most males having been neutered in the interim.

At six months, component three showed significant litter and gender differences. The component was characterised by a *playful/bold-timid/friendly* characteristic. The willingness to *play with a novel toy* may be similar to the *boldness* characteristic (willing to approach a novel object) identified by McCune (1995). McCune's study, based on cats in cattery, revealed this to be the characteristic most influenced by paternal genetics. However, this study used only a small number of sires. A larger sample may reveal other genetic paternal effects.

Component three at 18 months was hard to interpret but there were similarities with component 3 at 6 months; interacting during the approach test was the most heavily weighted factor. *Playing with the toy* was excluded from PCA at 18 months. No significant effects of litter or gender were found for component 3 at 18 months and it showed no difference between litters. It can therefore be disregarded for the purposes of this discussion.

Component four (six months) showed no differences between litters and was therefore disregarded.

6.4.2. Differences between Shirley and other areas

The hypothesis that in areas where neutering is high an increased proportion of kittens will be sired by feral toms, with effects on the temperament of the kittens, was tested indirectly in this chapter. Here the temperament of kittens born in an area where neutering rates are known to be particularly high (see **Chapter 2**), was compared to the temperament of kittens born in other areas. The hypothesis predicts that kittens born in Shirley would be more likely to show *unfriendly* or *fearful* characteristics, having inherited, to a measurable extent, these characteristics from their fathers.

No significant differences were found between the categories of area defined here: Shirley and other areas. However there was a just non-significant trend ($p = 0.054$) in the direction predicted by the hypothesis: Component one scores revealed that at 6 months old, cats from Shirley were less likely to be friendly than cats from elsewhere. However, it should be remembered that the sample size of litters from Shirley was

small (N = 10), and the exclusion of the most unfriendly kitten from the Shirley sample would have reduced the differences between the areas considerably (p = 0.34). Also, when Shirley litters were compared only with areas where the was evidence of lower neutering rates, the differences were diminished (p = 0.32). Differences in component one scores between the areas had disappeared when the 18 month testing was carried out, which is to be expected considering that litter differences were no longer significant.

Further studies carried out on homed cats could include a boldness test similar to McCune's, where the willingness to investigate a novel situation without human presence is tested. The main problem to be overcome is one of experimental design, because the tester would have to observe from a position hidden from the cat. This would be difficult to arrange in people's homes.

7 General Discussion

The first three experimental chapters of this thesis investigated aspects of the population structure, population dynamics, ranging behaviour and mating systems of cats in the Southampton population. The final experimental chapter described a study of temperament in kittens. These elements of the study were combined to examine the possible effects of a regime of high neutering on the population genetics of cat temperament. In this chapter, I will summarise and discuss the main findings of the study, and will attempt to draw them together in the context of the Southampton cat population. Suggestions for future work are made, continuing from the findings of this study.

7.1. Population processes

The door to door surveys carried out in the first year of this study revealed that neutering rates among owned cats were much higher than had previously been appreciated. In Shirley, where the main "blanket" survey was carried out, 96.8% of adult males and 98.7% of adult females had been neutered. Retrospective analysis showed that this was a recent phenomenon; neutering had reached the level where there were not enough entire females to keep the population stable in the early 1980's. By 1995, the litters produced were sufficient only to maintain the population at approximately 25% of its present level.

Despite the dramatic increase in neutering, the data indicate that the Shirley population was stable between 1990 and 1998, at a level comparable with previous estimates of UK cat ownership; e.g. similar to Tabor (1983), higher than Chipman (1990). The population stability was mainly accountable for by human mediated immigration of cats from other areas. This realisation prompted door to door surveys in other regions of the Southampton area.

The results of subsequent surveys revealed that neutering rates varied between regions. No reproductive cats were identified in a suburban area close to Southampton. Here

the age structure gave signs that the "population" was in decline, and there was a high level of reliance on shelter organisations to supply cats (71% of cats under 5 years old). Rural areas contained enough breeding cats to maintain their populations. Other areas of Southampton showed lower neutering rates than Shirley; there were enough entire cats to maintain local populations, but still not enough to provide the surplus for immigration into other areas. Anecdotal evidence was gathered while talking to cat owners in the course of the temperament testing study. Residents of Sholing, Thornhill and Merryoak (see **Fig. 3.1.**) were often of the opinion that there were plenty of cats born locally. The relatively high number of respondents to advertisements from these areas pointed to higher breeding rates. In the Shirley area, one householder told me that a feral cat had given birth to eight litters in the her garden in the last in the last four years. This was not a pet cat so was not included in the fecundity analysis. In Sholing, I encountered the Hill family (see **Chapter 5**) their oldest female cat had given birth to 17 kittens and one of her daughters had produced 2 litters and was still entire. Relatively high breeding rates in areas that were not identified early in this study may account for the continued presence in the city of enough cats to maintain the population at its present level. Another factor to consider is the occasional incidence of a household where one or more fecund females reside. These cats can produce a large number of kittens, but because of their low density are prone to sampling error. The omission of one such householder from a survey due to chance, or lack of co-operation, could affect the results substantially.

The size of the owned cat population may presently be stable. However, the size of the potentially breeding population (also known as the effective population) has declined in direct proportion to the neutering rate. Before neutering became widespread more females would have reproduced, but it may be speculated that survival rates were lower, veterinary care would not have been so widespread and deliberate culling by humans may have taken place. At the time of the study, survival rates in Shirley were higher than has been shown in previous research in other urban areas; e.g. Manhatten, USA (Nassar and Mossier, 1982). In Southampton, at the time of the study, fewer cats reproduce than in 1978-1980, but the life expectancy of kittens is high.

It was demonstrated in **Chapter 5**, that three out of five subsequent litters born in a single household were sired by the same male as the previous litter. In two of these three cases, the second litter was mothered by a kitten from the first litter. If the results from these small samples prove to be representative it would point to a local high level of inbreeding. It may be hypothesised that such results would be less likely if the density of toms was higher, and thus competition between toms was greater, giving a lower level of father-offspring mating. More work is necessary to investigate how representative this sample is of the population as a whole, and whether the local density of toms (mediated by neutering rate), effects the probability of successive litters being sired by one male.

Given the low density of reproducing cats, and the suggested increase in the incidence of inbreeding, it is worth considering the possibility that cat reproduction may be increasingly limited to isolated pockets of closely related individuals, with the associated consequences of loss of genetic variability. However, population substructure was tested for, but not found, with respect to microsatellite alleles (**Chapter 5**), which are selectively neutral. This does exclude the possibility that differentiation will occur in the future, but there are reasons to believe that this scenario is not realistic; from the results of the population dynamics study (**Chapter 2**) and to a lesser extent by the radio-tracking study (**Chapter 4**), for reasons explained below.

The most obvious mechanism for gene flow is human mediated dispersal. Overall, the population surveys conducted within Southampton showed that 67% of cats were brought in from another region of the city or further away. Interestingly, cats that experienced this type of migration were much less likely to reproduce than cats that remained in their natal areas. Only 27% of reproduction was accounted for by immigrant females, making their fecundity estimable at 18.2% of the fecundity of females that remained in their natal area. This trend is partly attributable to shelter organisations, which ensure the neutering of all cats that they take care of. Despite the interesting link between human mediated migration and neutering, effective migration rates of 27% are ample to preserve homogeneity within the owned population when considering selectively neutral traits (Crow and Kimura, 1970). However,

temperament, (as with coat colour, see **Introduction**), may be assumed not to be selectively neutral, see below.

These lines of research suggest that the Southampton cat population is operating at, or close to, panmixis with respect to traits that do not effect suitability to a pet lifestyle. If this is the case, the impact of a reduction in effective population size on cat population genetics will be greatly reduced, because the cats are all drawn from one larger Southampton population. Cats from regions of Southampton are not genetically isolated. There is also some transfer of entire cats between cities (**Chapter 2**).

7.2. Spatial use by tom cats.

The density of tom cats was estimated to be 1 per 11 ha in the Shirley area, extrapolating from the 80% of householders in the area that were interviewed (**Chapter 2**), but higher than this in other areas of the city where neutering rates were lower. The radio-tracking study tackled the question of whether competition between owned males for mating opportunities would be likely under such conditions. The three cats over two years old that were used throughout the study had ranges (100% MCP) of 7.3ha to14.3 ha. Their core areas, which for some mammals are the exclusively defended areas part of their ranges (Harris *et al,* 1990), were 2.6-6.3 ha. In terms of linear distance, the adult cats reached a maximum of 400-800m from their home base. The ranges demonstrated here are large enough for an entire female to be within the range of two or more toms, even in Shirley. The core ranges correspond approximately to the estimated densities of toms in areas of the city where neutering rates were relatively low; the possible significance of this is discussed below.

Toms consistently reach 500m or more from their home base, in the course of a night. If they were also able to sire litters at households at a substantial distance from their home bases, by means of occasional long distance forays, this would be another mechanism for gene flow. There was one incidence in the study of a tom siring kittens in two households, these were 300m apart (**Chapter 5**). It is not clear how much of a barrier is presented by urban features such a major roads. All the cats crossed minor roads, and Ebony was observed to cross the A334 at night; a busy single lane road

during the daytime. Sam lived within range of a dual carriageway, but was not observed to cross it. It is possible that multi-lane roads do constitute a major barrier to movement by cats, especially as many such roads in cities are lined with fencing to prevent intrusions by animals. The movement of feral cats across the city environments would also be affected by these constraints.

A trend for home range size to increase with age was demonstrated here. This is consistent with previous findings on farm cats, which start to disperse at 1- 2 years old (Liberg, 1983) and the positive correlation between age and home range in urban toms (Chipman, 1990).

7.3. The cat mating system, investigated by microsatellite analysis.

The use of microsatellites, combined with the data from **Chapter 2** and **Chapter 4**, shed light on the mating system of owned cats in an urban environment. In upper Shirley, the four genotyped litters were sired by three or four different males. These were not the two or possibly three genotyped toms that lived within realistic range of the litters' birthplaces. Therefore, there must have been 6 or 7 potentially reproductive males within range of the 17.7 ha area defined by the birthplaces of the four litters. Two of the litters were born slightly outside the area covered by the door to door surveys, and the other two close to the edge. It is not surprising that the toms were not identified, but that the number of toms present in the area was higher than predicted by the door to door surveys.

The radio-tracking study indicated that the maximum range of toms is great enough for there to be more than two toms within reach of each oestrus female at the estimated densities for the Shirley population. The inferred presence of more toms in an area than would be predicted from the population surveys may be explicable by pet toms with large home ranges. An alternative explanation is that the unidentified toms were feral cats living in the Shirley area. It is not possible to test this directly with available data, but it is worth noting the suggested predominance of a guarding strategy (below). A successful guarding strategy would give a tom a much higher chance of siring any local litters than a visiting male. If this proved to be robust, it would mediate against

the hypothesis of "visiting" toms siring the Shirley kittens, and would therefore strengthen the "feral tom" hypothesis.

As mentioned previously, three out of five subsequent litters were sired by the same male that sired the previous litters in the same household. The four litters in upper Shirley born over a three month period were sired by three or four males; i.e. one male did not dominate mating opportunities. These results point very tentatively to a strategy more orientated to guarding than roaming (See **Introduction**). A roaming strategy is the one most commonly associated with cats. However, a guarding strategy might be predicted if the females, or groups of females, were spaced at a low density (Liberg and Sandell, 1988), such as was found in Shirley. In order to test this hypothesis it would be useful to obtain further data investigating the areas, if any, that toms attempt to defend exclusively. Obtaining radio-tracking data from toms living close together would be necessary. Interestingly, the size of core areas approximated to estimated densities of entire males, implying that these could have been exclusive areas. A quantative difference in behaviour of toms was demonstrated inside and outside of core areas (**Chapter 4**); movement was significantly faster outside cores, leading to speculation that movement inside cores was patrolling or guarding, and movement outside cores was exploration.

7.4. Temperament in cats

The temperament testing study was carried out on a large sample of homed kittens. The results supported previous research into the components that make up temperament, or "personality" in cats. The primary characteristic identified at 6 months of age, the one that explained the most variance, was sociable/unsociable. The next most important characteristic was a less clearly defined active/inactive trait. This is similar to the findings of Cook and Bradshaw (1996) and Feaver *et al* (1986). The third component was identified as boldness, and may be similar to that described by McCune (1995). The diminished litter differences measured at 18 months, such that the effects were not significant, partly reflects the expected changes brought by experience and maturation. But the study does not rule out the continued influence of genetic and early environmental effects; these may interact with ownership styles

(Turner, 1991) in more or less predictable ways, but since kittens from different litters have different owners, these will tend to mask within-litter similarities.

Importantly, this study also provided a means to test indirectly the primary hypotheses posed by the project: that high levels of neutering amongst owned cats leads to an increased proportion of owned kittens being sired by feral males, and that the temperament of these kittens will tend to be more fearful of or unfriendly towards humans. It was not possible to test directly the relationship between reproductive success and temperament, because variance in male reproductive success appeared to be low If it had been higher, this would have allowed the reproductive success of fathers to be related to temperament of their offspring. Alternatively, identifying a sample of kittens as being the progeny of either feral or pet toms would also have allowed the hypothesis to tested directly. However, the dual hypotheses described here lead to the prediction that kittens from areas of high neutering should tend to be more fearful/unfriendly to humans, than kittens from areas of lower neutering. When this was tested using GLM for the results at 6 months, the result was a non-significant trend in the predicted direction. When Shirley cats were compared with cats from all other areas the result was close to significance but the sample size of Shirley born litters was small. The mean Shirley score would have been substantially altered by the omission of one highly fearful cat. These results cannot therefore be regarded as robust, but do reveal an interesting trend for further exploration.

7.4.1. Cat temperament and population genetics

The panmixis of the population with respect to microsatellite loci may seem to mediate against regional differences in temperament. However throughout this study, temperament has been assumed to affect profoundly the probability of a cat remaining in the pet population, or joining the more fecund, but less mobile feral population. This interaction between feral and pet populations could lead to regional differentiation with respect to temperamental traits even when none is apparent for selectively neutral traits.

7.5. Summary

The owned cat population in Southampton appears presently to be stable despite high rates of neutering. The keys to the stability of the population are the high fecundity of the remaining entire females and the high survival rates of their offspring, coupled with human mediated migration of cats between regions, although there is also evidence for an increase in the adoption of feral cats. Effective migration is much lower than total migration, because cats that experience human mediated migration have a greatly increased chance of being neutered. Never the less, effective migration appears adequate to keep the city's population operating at panmixis, indicating that the effects of reduced population size caused by neutral drift are not likely to be measurable. Further increases in neutering rate could lead to a reduction in the population, such as that experienced in Australia (Anon. 1998).

The home ranges of tom cats were found to be large enough to allow potential overlap even in areas such as Shirley, where the density of toms was approximately one per 11 ha. The exclusive territorial behaviour of toms in a urban environment has still to be explored fully. The data indicate that cats cover ground more quickly outside their core areas than inside them.

Microsatellite analysis suggested that, in the upper Shirley area, males are not able to dominate mating opportunities within an area the size of a maximum home range. It is presently unclear whether the toms who were reproductively active in the area were "visitors" or whether, as seems likely, the total density of toms was increased by the presence of a number of feral toms.

Temperament testing of kittens at six months uncovered components of "personality" that were compatible with those reported by previous studies of homed kittens. Litter differences were not significant when the kittens were re-tested at 18 months. Inter area comparisons were made to test the prediction that kittens from high neutering areas would tend to be less sociable to humans. At 6 months there was a non-significant trend in this direction.

7.6. Further research

Many lines of further research suggest themselves at the end of this study. These would aim to build on the results obtained here by obtaining more data and refine the techniques and approaches used here. They are included here in the hope that they may prove to be useful suggestions for future researchers.

The population dynamics work carried out in this study described aspects of the Southampton population at the time of the study, and changes that have occurred in recent years. Predictions of future population trends would benefit from a modelling approach. This could be used to examine the effects of changes of neutering rates within demographic regions on the population as a whole. Modelling is presently being used to examine the interactions between feral and owned populations, and the effects of neutering on these interactions.

There is potential to expand the work on home ranges and territoriality. Radio-telemetry data from toms living close together would give a valuable insight into which areas of the range are defended exclusively. The stumbling block to such an experiment would be recruiting enough tom cat owners at a high enough density for there to be potential for home range overlap. This is not easy when neutering rates are as high as they presently are in Shirley. A concerted effort could be made to recruit the required sample of toms in an area of relatively low neutering, such as Sholing, Merryoak or another city. However, this would not directly tackle the questions relating to Shirley. Recruiting older toms for radio tracking would be informative, to investigate whether home ranges expand further after 3 years of age.

The radio-tracking carried out in this study was focussed on toms, because previous research indicated that toms would be more mobile than females, which tend to have much smaller home ranges (Liberg and Sandell, 1988). However, females sometimes move from their natal group when in oestrus, and it was shown in this study that resident males do not always sire kittens born in the same house. Recently, Barrett (1997) reported that some female home ranges were comparable to male home ranges. Radio-tracking of females could therefore be fruitful, to elucidate their role in the

structure of the mating system. Further work on mating systems using molecular techniques would also benefit from the use of an urban area with lower neutering rates. This would reduce the logistical problems of obtaining a large enough sample of cats, within an area small enough to allow a comprehensive census of the local population to be obtained by door to door surveys. An additional benefit would arise from the comparisons that could be made with the mating system in the upper Shirley area, where neutering rates were very high, as described here.

Further work on ranging behaviour and mating systems would ideally be carried out in the same area, allowing an integrated study to be made. One potential problem would be that the continued presence of a researcher in a restricted area would require the co-operation and tolerance of the local residents over an extended period. My own experience suggests that such tolerance would generally be forthcoming.

Feral cats were not used as subjects in this study, because of the problems of identifying cats as truly feral, and approaching them. If these problems could be overcome, and feral cats living in housing areas could be identified and used for DNA profiling and radio-tracking studies, the rewards could be great. Such data would allow behaviour and reproductive success of feral cats to be compared directly with pet cats.

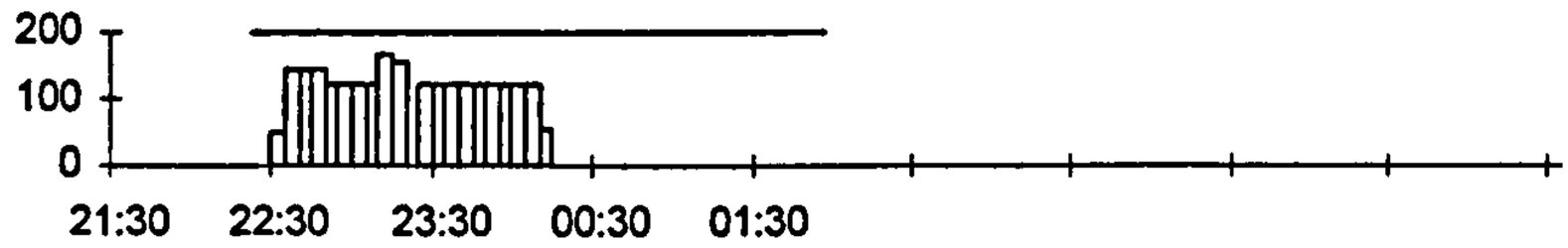
Further studies on the temperament of homed kittens could be designed to take account of the different environments encountered by the kittens, for example, by the use of questionnaires. This may enable litter differences to be teased out and identified in older kittens.

Appendix 1.

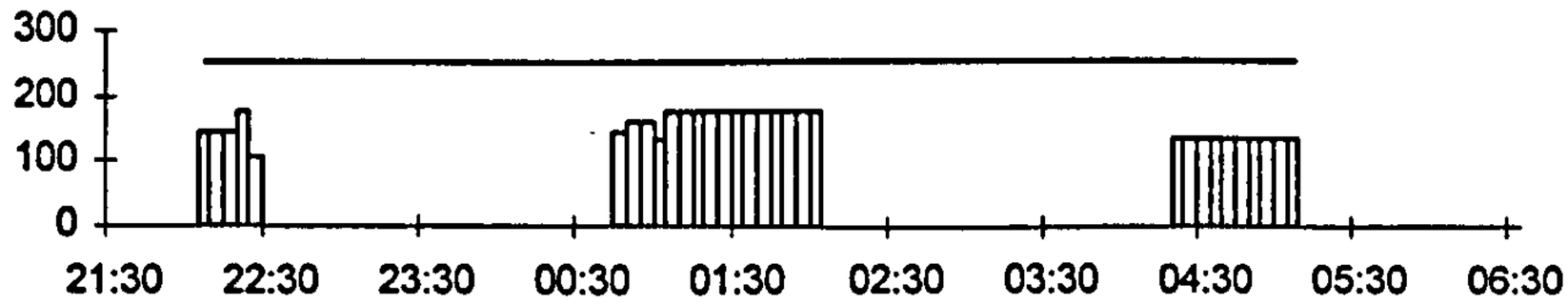
Distance from home (m) against time (x axis) shown over all continuous tracking sessions for the three main cats. Each vertical bar represents one five-minute time interval. The horizontal bar represents the time period that tracking was carried out.

Appendix 1.1: Ebony

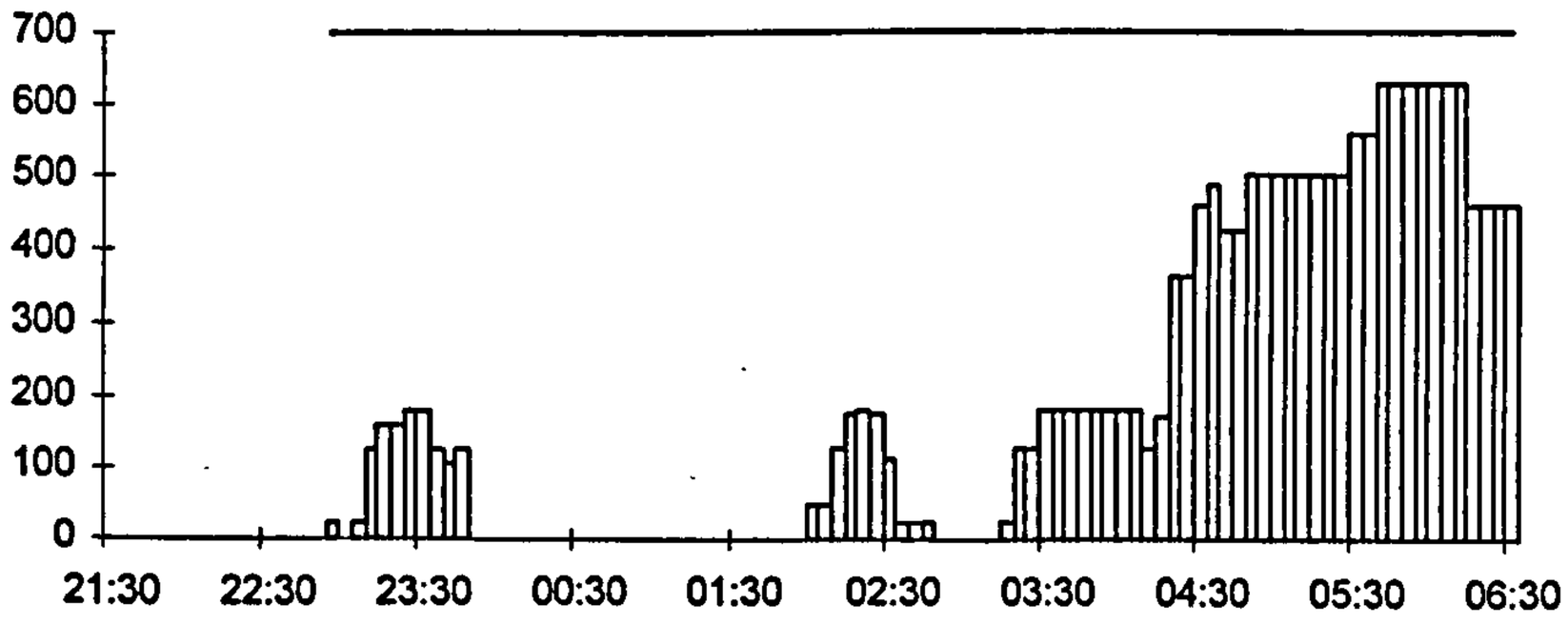
a. Night 1



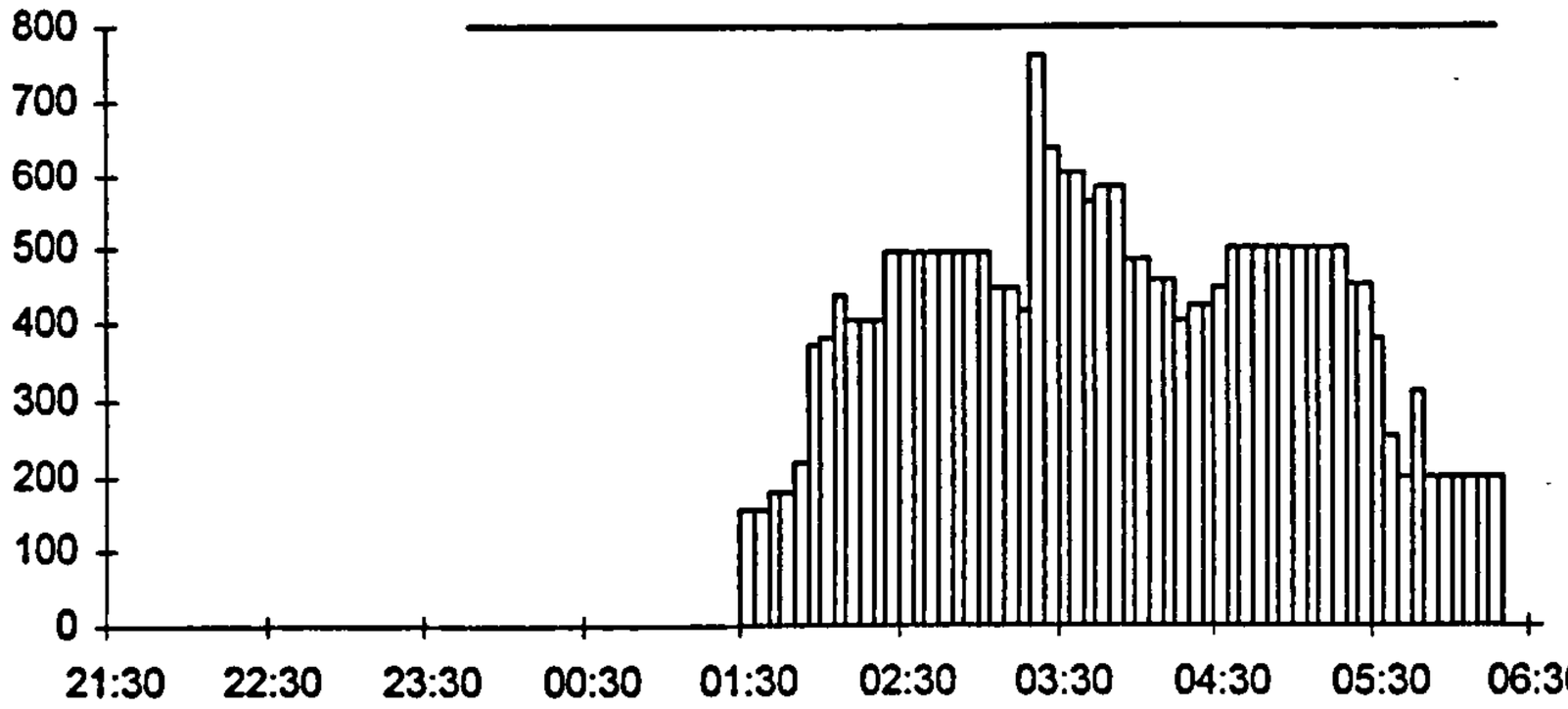
b. Night 2



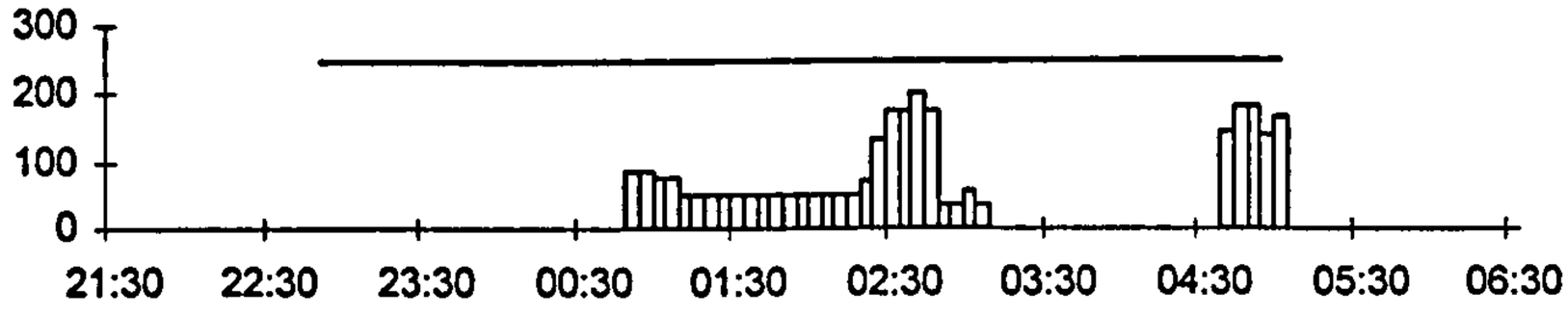
c. Night 3



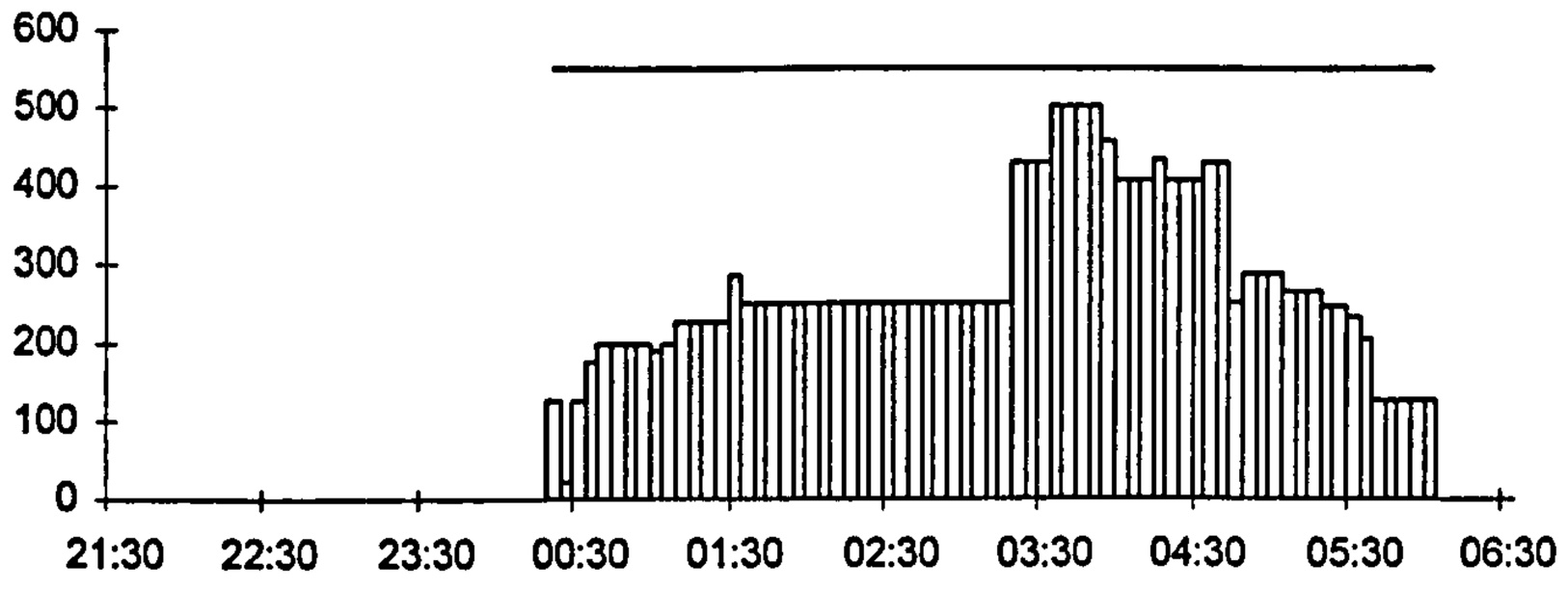
d. Night 4



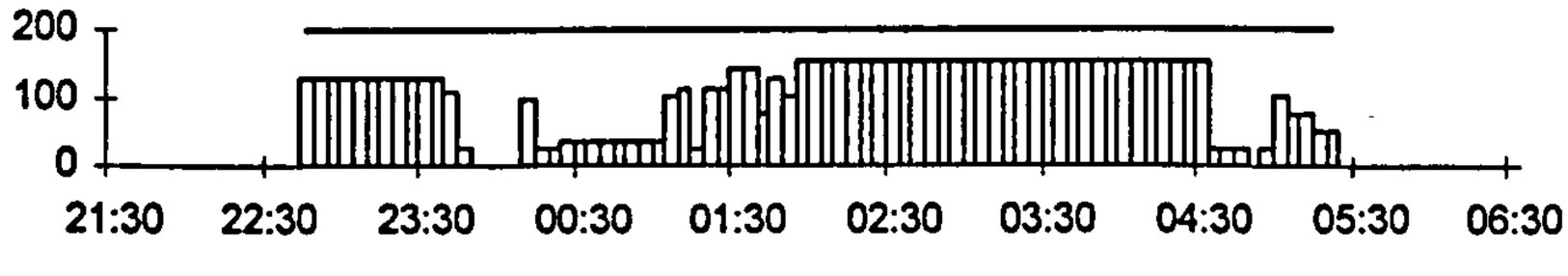
e. Night 5



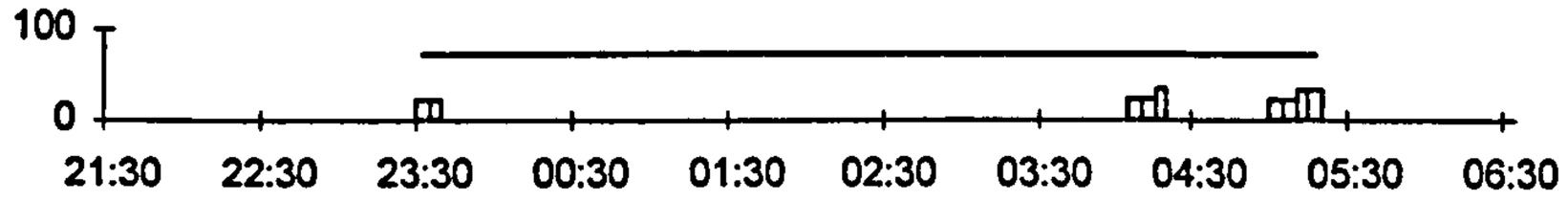
f. Night 6



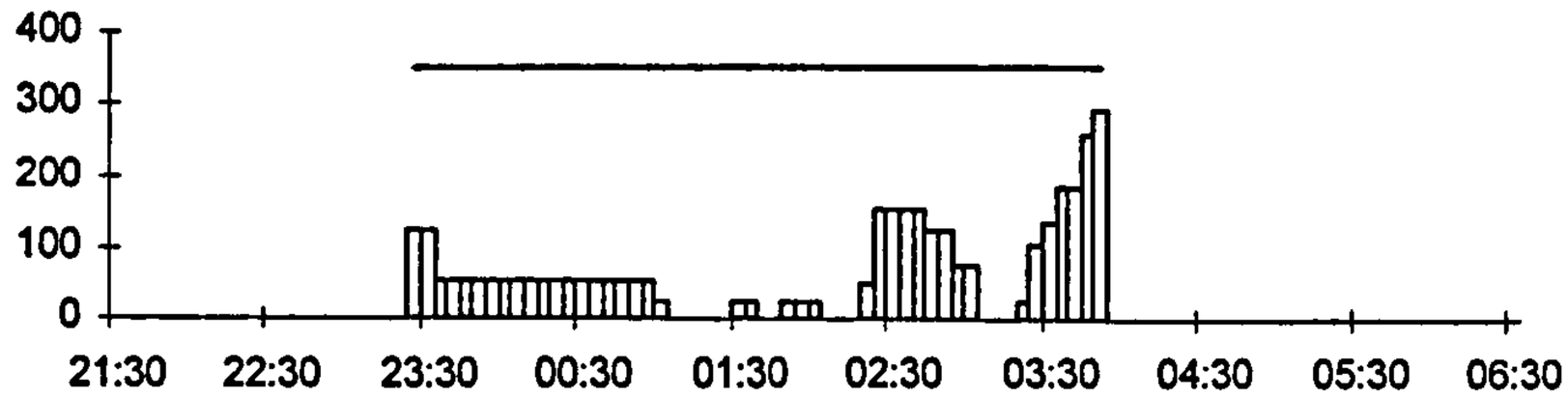
g. Night 7



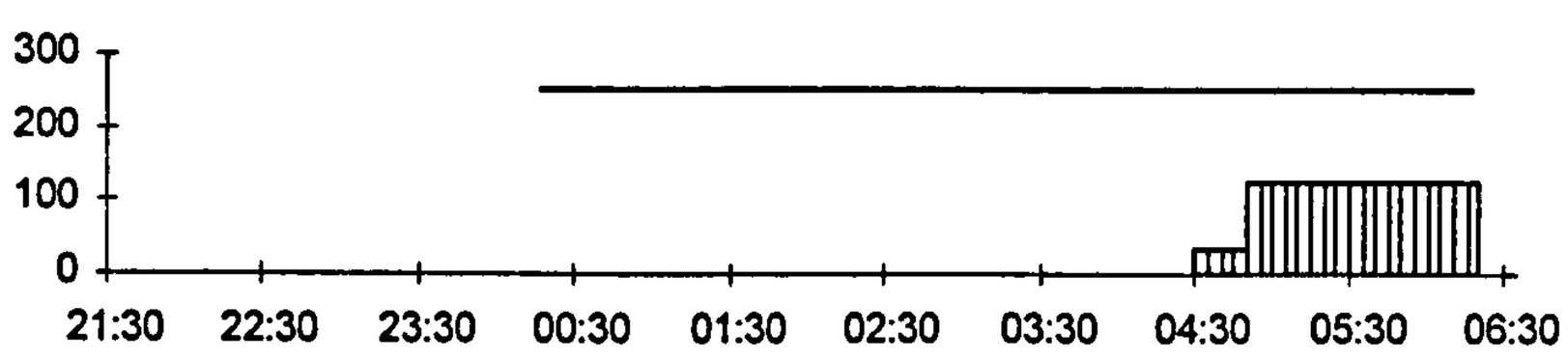
h. Night 8



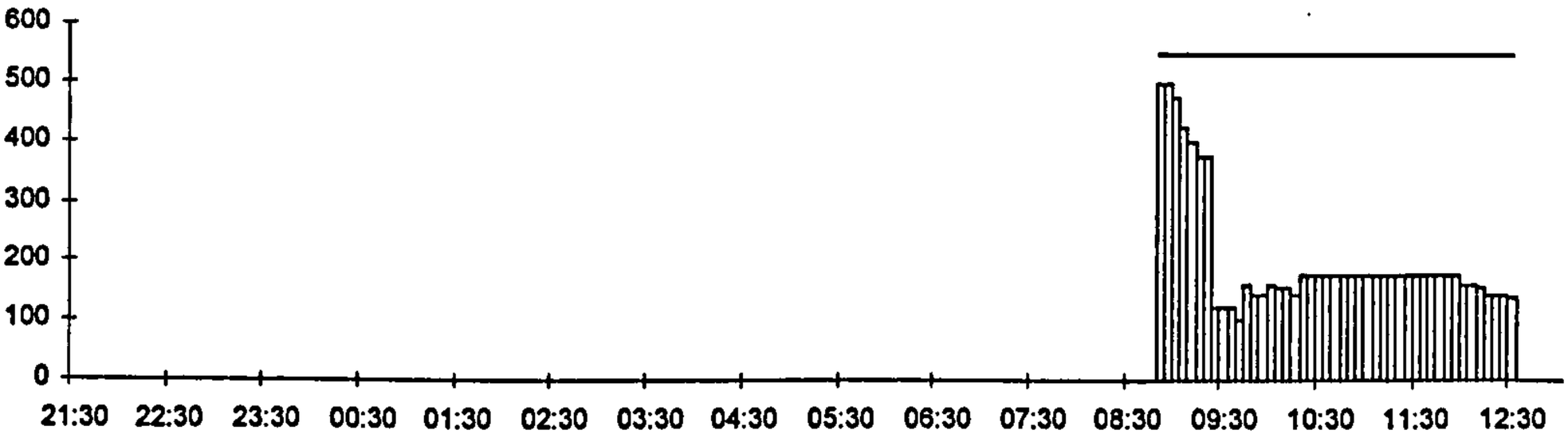
i. Night 9



j. Night 10

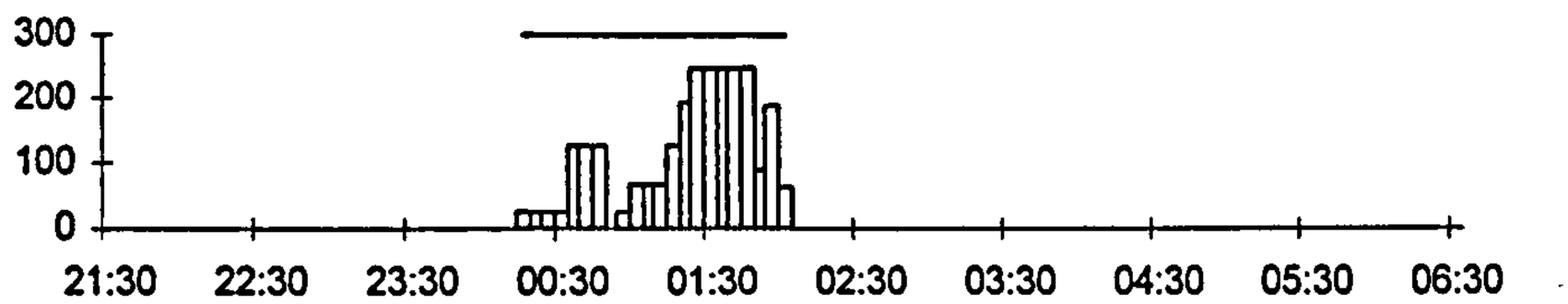


k. Night 11

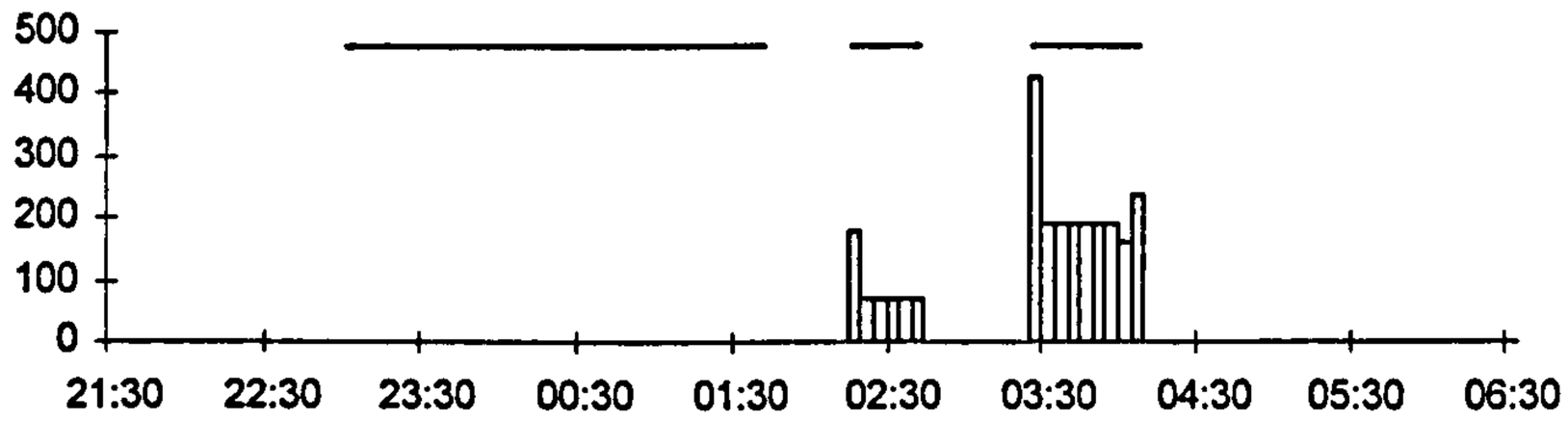


Appendix. 1.2: Sam

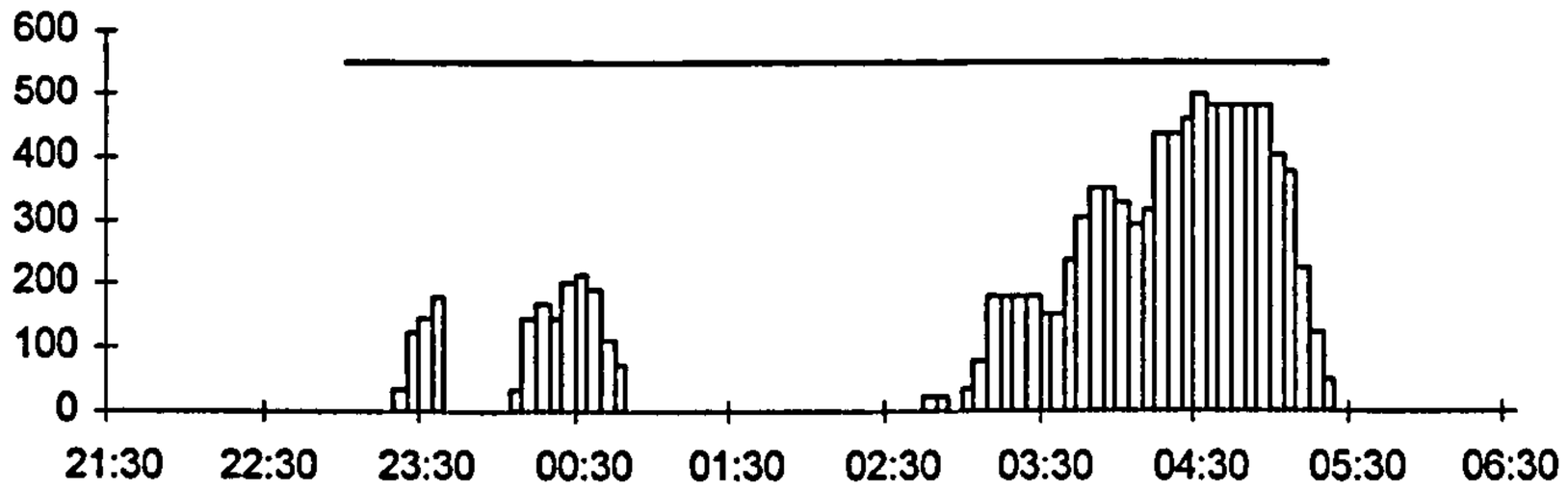
a. Night 1



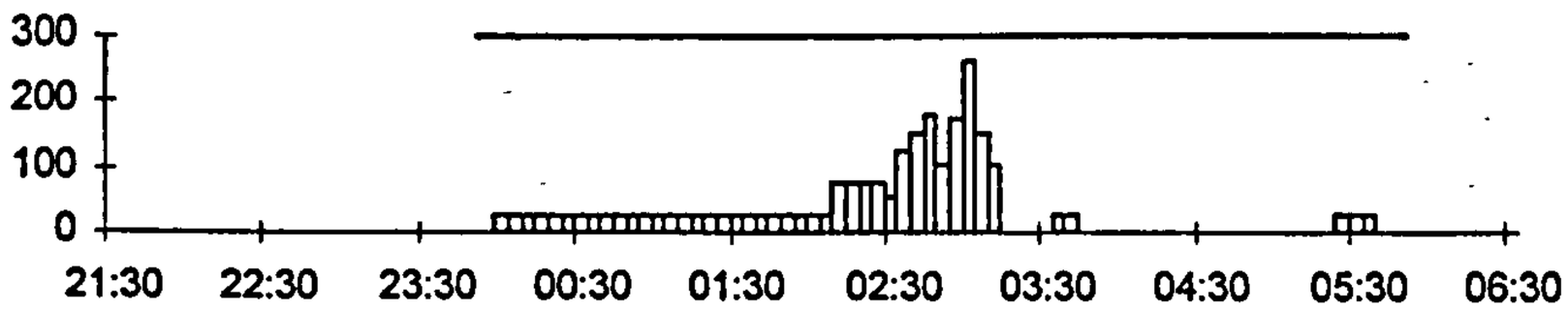
b. Night 2



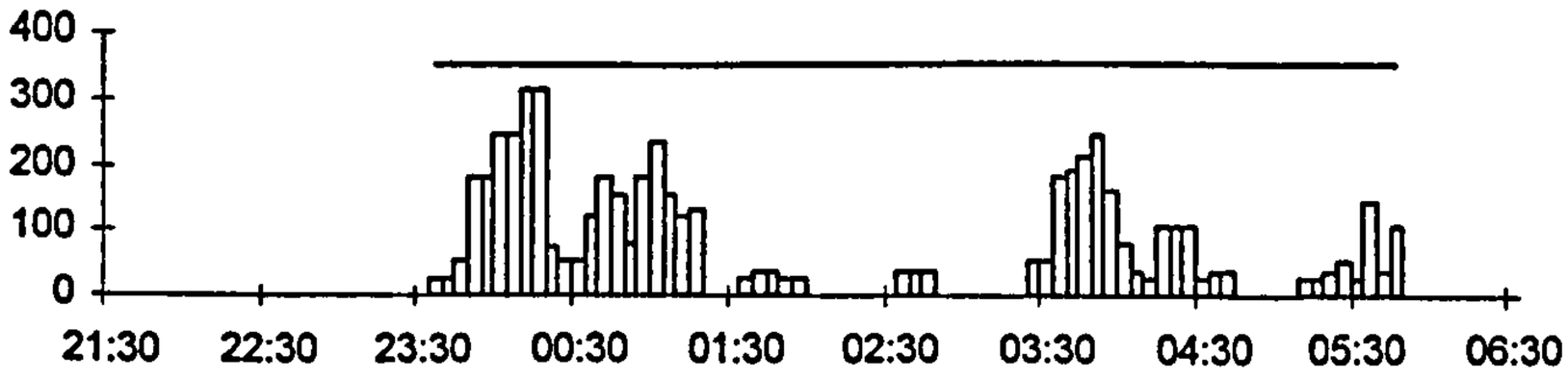
c. Night 3



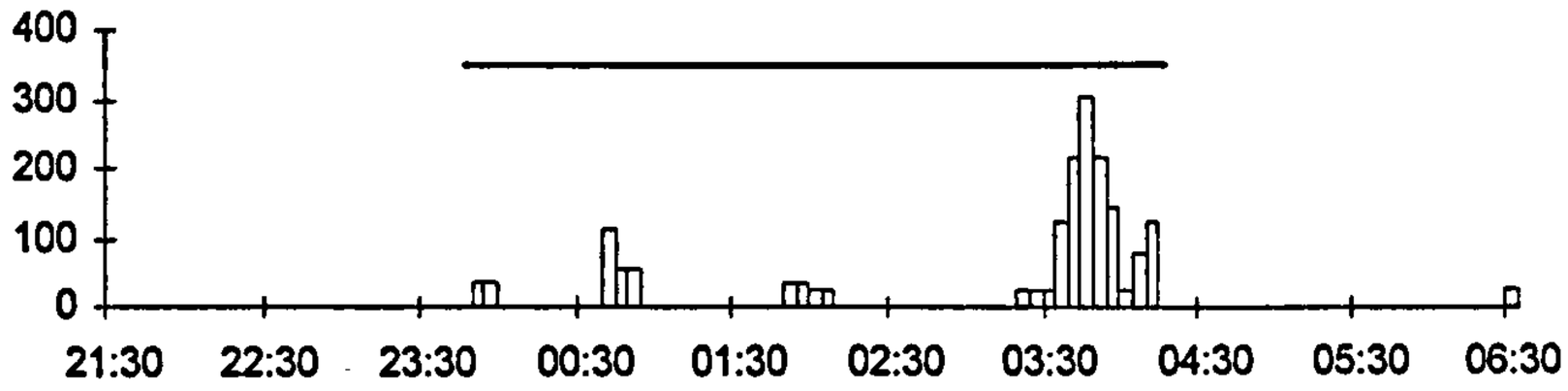
d. Night 4



e. Night 5

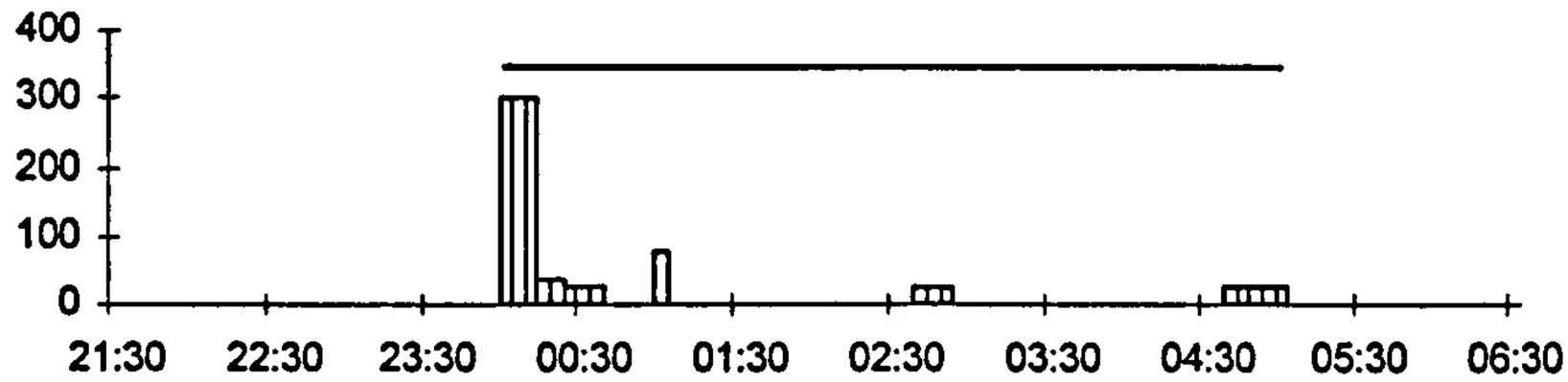


f. Night 6

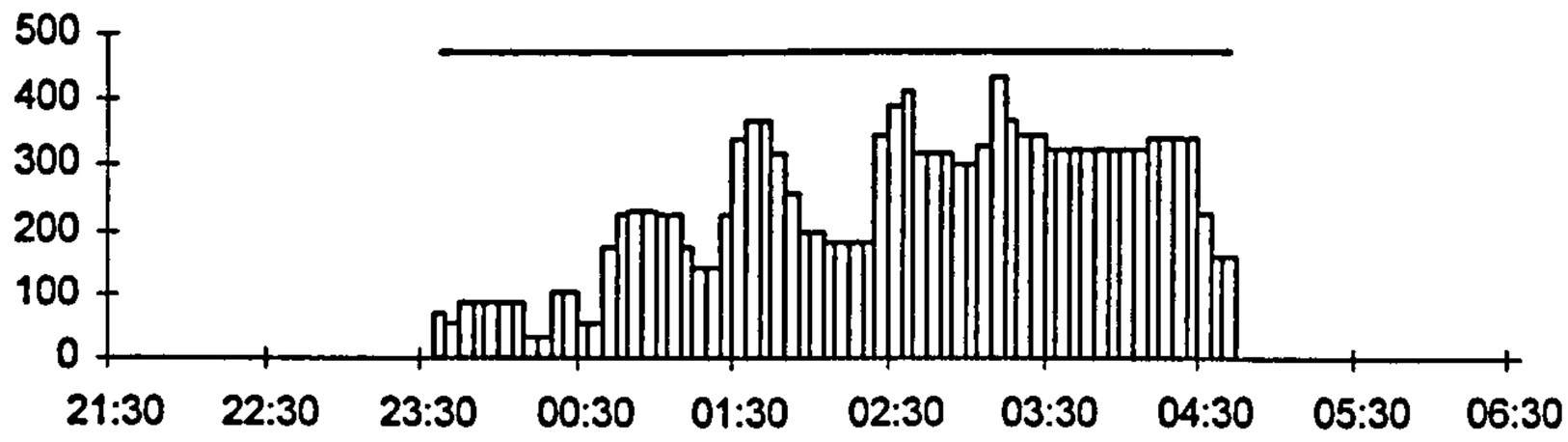

400
300
200
100
0
21:30
22:30
23:30
00:30
01:30
02:30
03:30
04:30
05:30
06:30

Appendix. 1.3. Marmalade

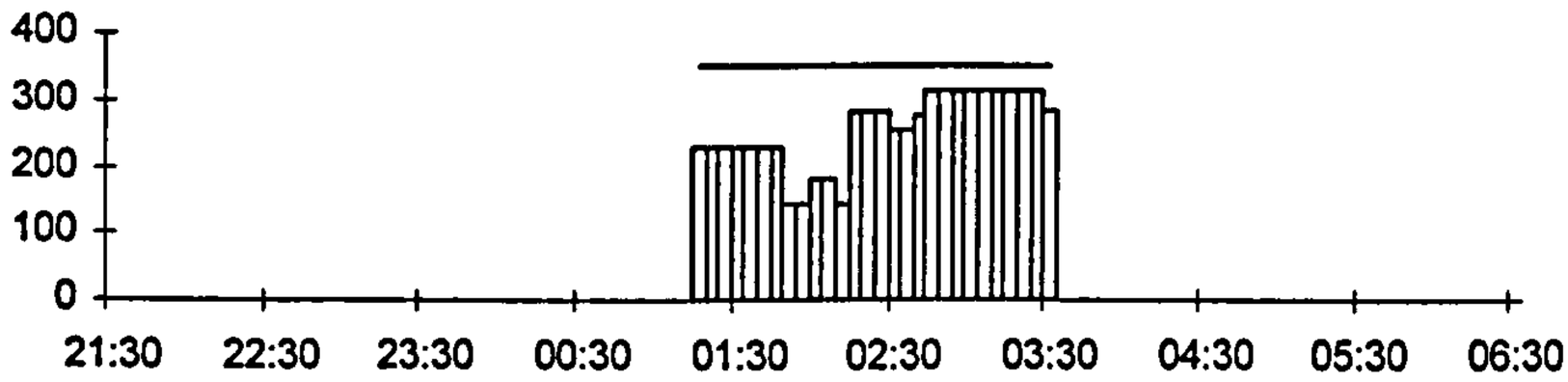
a. Night 1



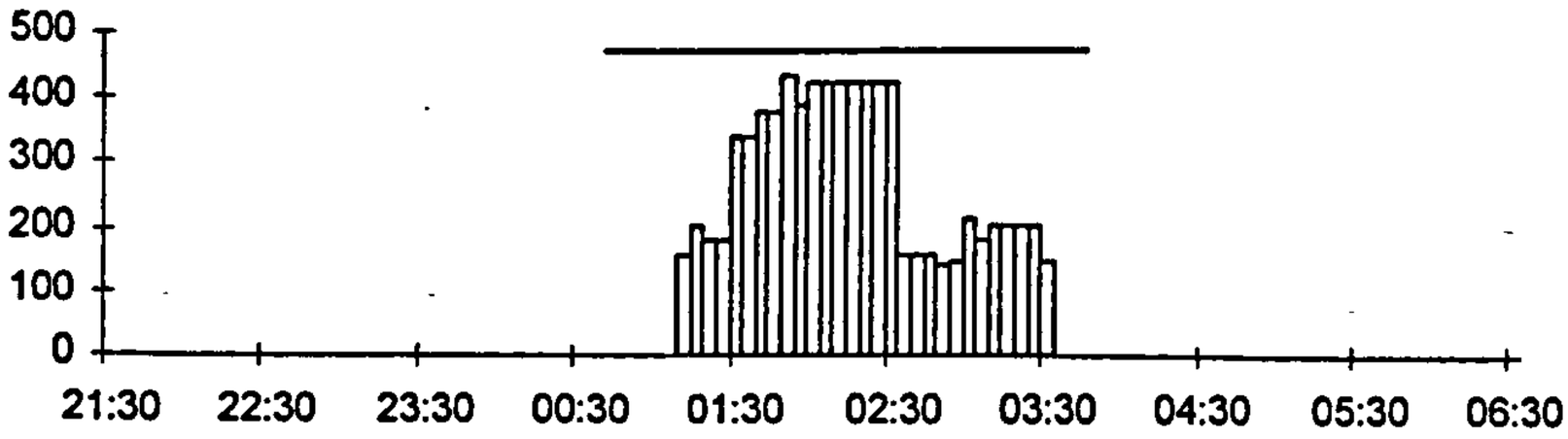
b. Night 2



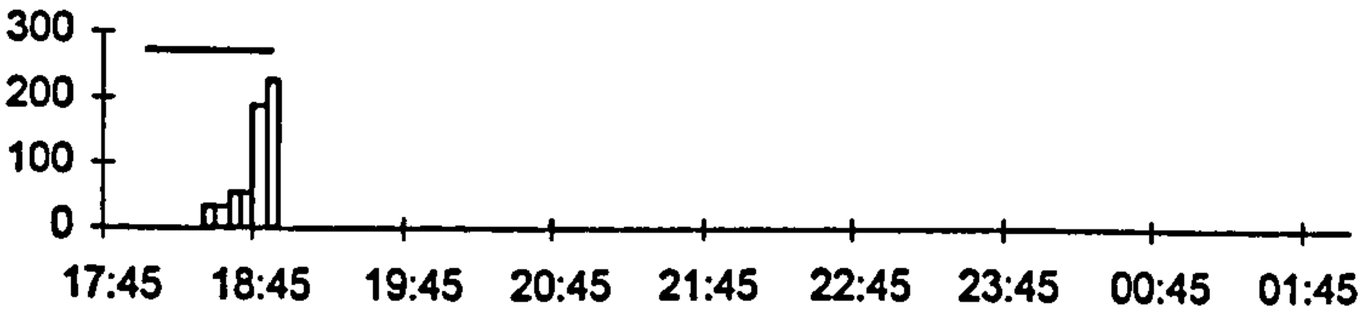
c. Night 3



d.Night4



e. Night 5



Appendix 2

Appendix 2.1. Extracting DNA from faeces

- Faeces were collected in sterile universal tubes and stored at -20°c.
- The protocol used for extraction was adapted from Tikel et al (1996). 0.15-0.25g of faeces was suspended in 1ml of lysis buffer (40 mM Tris; 2mM EDTA; 0.2 M NaCl; 10% SDS). 30ul of Proteinase K (10 mg/ml) was added, and the solution was incubated overnight.
- The lysate was phenol/chloroform extracted, using a modified form of the protocol in 5.5.2.1: using 800ul of phenol/chloroform and 800ul of isoamyl/alcohol. Also, an additional phenol/chloroform step was performed. Pellets were suspended in 50ul of TE.

Appendix 2.2.

2.2.1. Phenol chloroform extraction.

This protocol was carried out using 50 hair roots per sample, these were crushed in an Eppendorf tube and stored on ice prior to the extraction process.

- 300µl TEN/SDS(20%) (9:1); and 30µl Proteinase K (Sigma) were added to the hair roots. The mixture was homogenised using a Teflon homogeniser, and incubated overnight at 37°C.
- 200 µl phenol and 200µl chloroform: IAA (25:1) were added to the tubes. The tubes was shaken for 10 minutes and centrifuged for 10 minutes. The lower phase was then removed and discarded.
- 300µl chloroform: IAA (1:1) was added, the mixture was shaken for 10 minutes, and then centrifuged. The aqueous top phase (contains DNA) was removed and added to clean tubes.
- 30µl NaAc and 500µl EtOH (100%) was added to the tubes, which were shaken 10 times, and placed in a freezer at -20oC for 2h or overnight.
- The tubes were centrifuged for 15 minutes on 13,000 r.p.m. and the EtOH solution

carefully drained out.

- 500µl EtOH (70%) was added to each sample, the tubes were briefly shaken and centrifuged for 3 minutes.
- The EtOH was drained carefully from the samples, the tubes were centrifuged again briefly, and the excess removed with a pipette. The tubes were covered with foil, and left to dry for 30 minutes. TE buffer (20µl) was added to each sample, and the tubes were put in incubator on 37^0C for 30 minutes to ensure that the DNA dissolved.

2.2.2. Salting out method

- 5 µl Proteinase K (10 mg/ml) was added to 5-10 hair roots in an Eppendorf tube and the mixture was homogenised gently using a Teflon homogeniser. The homogeniser was then washed with 300 µl TEN/0.5% SDS (pH 7.6) into the tube. The mixture was incubated for 3h or o/n at 37oC.
- 100 µl 5M NaCl (saturated) was added to the homogenate (final concentration is 2M), the tube was shaken vigorously for 15 seconds (percipitates proteins) and centrifuged for 5 minutes. The supernatant was removed and added to clean Eppendorf tubes.
- One volume (400µl) of ice-cold EtOH (100%) was added. The tubes were put on 20^oC for 5 minutes (sufficient to precipitate small quantities of DNA). The samples were then centrifuged for 5 minutes. The EtOH was drained off carefully and the pellet was washed with 70% EtOH.
- The EtOH was drained carefully from the samples, the tubes were centrifuged again briefly, and the excess removed with pipettes. The tubes were covered with foil, and left to dry for 30 minutes. TE buffer (20µl) was added to each tube and the tubes were put in incubator on 37^0C for 30 minutes to ensure that the DNA dissolved.

2.2.3. Chelex Method

- Hair roots (1-10µl per sample) were submerged in 250µl of hair lysis buffer, and

incubated at 56°c for 2 hours. 2.5ul RNAse A (10mg/ml) was added one hour before the end of the incubation.

- The tubes were vortexed, 225µl of 5% Chelex (Biorad) was added to each sample and the tubes were incubated at 95°c for 15 minutes.
- The tubes were brought to room temperature and centrifuged for 1 minute.
- 350µl of the supernatant was transferred to freshly labelled tubes, being careful not to pick up any beads.

Varying volumes of the supernatant were used as the DNA source in PCR reactions.

2.2.4. Single Hair Immersion method.

- M.Q. H_2O (6.44µl) was transferred into a 0.5 ml eppendorf tube.
- A single hair for each sample was used, the root area was washed with MQ H_2O using a P200 Gilson pipette. Approximately 5mm was off the root end of the hair using flame-sterlised scissors, and submerged in the H_2O.
- The remaining reactants were added, and the PCR reaction was carried out.

Appendix 2.3. Protocol for preparation of M13 sequencing ladders.

- Single stranded M13mp18 (0.2 µg/µl) solution (supplied in the Sequenase Kit, Pharmacia) was used:

DNA control (M13)	10 µl
H_2O	4 (3 µl*)
Sequencing buffer	4 µl
Sequenase primer (40)	2 µl
Total vol.	20 (*19 uL)

*when ^{35}S is not fresh, add less H_2O to compensate

- Two sets of the solution were made, one for A and one T track markers
- Water was heated in an ice box to 65° C (3/4-1/2 full of water)
- Contents of solution were mixed together and heated at 65° C for 2 min.

- These solutions were cooled slowly to < 30°C, by placing ice-box in a cold room. It takes about 1.5 to 2 hours for the temperature to the water to fall below 30°C, once below 30°C chill these solutions on ice till required.
- Whilst the solutions cooled, further tubes were labelled (A and T) and 20 uL of ddATP termination mix added to A tube and similarly ddTTP termination mix added to the T-tube. These solutions are kept at room temperature.
- Labelling mix from the kit was diluted 1:5:

Labellling mix	2 µl
HPLC H_2O	8 µl
Total volume	10 µl

- When the solutions (steps 1 to 6) had cooled below 30°C, the T7 DNA polymerase from the kit was diluted:

T7 polymerase	1 µl
Dilution buffer	7 µl
Total volume	8 µl

- The termination tubes (A and G) were pre-heated and left in 37°C water for 5 min
- Labelling reaction. To each of the solutions (steps 1 to6) was added add:

Labelling solution	20 µl (*19 µl)
DTT, 0.1 M	2 µl
Diluted labelling mix	4 µl
^{35}S dATP	1µl (*2 µl)
Diluted enzyme sol.	4µl

* when ^{35}S is not fresh add more

- The reaction mixtures were mixed, centriguged briefly, and incubated at RT for 2-5 min
- 28 µl of the labelling reactions was transfered to the termination-mix tubes, mixed, and the reactions incubated at 37° C for 5 min
- Reactions were stopped by adding 32 µl of STOP solution (sequencing loading dye)
- These marker solutions (80 µl) were diluted by adding:

H_2O	50 µl

Sequencing dye	30 µl

- 5 to 6 µL per lane was as a size marker on microsatellite gels

Appendix 2.4. Alleles scored for all individuals at all loci. Maternal I.D. and the I.D of related individuals is shown. The data was presented in this format for analysis by Kinship 1.2.

CAT	Group	Mat ID	Fca8	Fca77	Fca23	Fca35	Fca43	Fca78	Fca90	Fca96
Burg	1		141/147	142/144		142/142	127/131	188/199	107/107	208/208
Gillf	2		135/135	144/149	137/141	136/136	127/127	195/201	91/105	208/216
Gillm	2		135/135	144/149	137/147	136/136	127/127	195/201	91/105	208/208
AmM	3		121/133	144/144	137/137	136/136	127/127	194/205	91/91	208/208
Sheba	3	AmM	121/121	144/144	137/147	136/136	117/	188/194	91/113	208/208
Red	3	AmM	121/121	144/149	137/141	136/142	117/127	195/205	113/91	208/208
WS	3	AmM	121/133	144/149	137/147	136/136	117/127	188/194	91/113	208/208
WLL	3	AmM	121/133	144/149	137/147	136/136	119/127	195/205	91/113	208/208
AmD	4		121/121	146/	137/147	136/		195/188	109/113	208/208
AmK	3	AmM	121/133	144/146	137/137	136/136	127/127			208/208
C17	5		121/133	144/148	131/141	142/142	119/127		102/113	208/210
Pick	6		133/135	144/144	131/133	136/142	127/127	188/195	105/113	208/212
Palm	7		135/135	144/147	131/145	136/136	119/127	195/199	91/107	214/214
Dear	8		121/137			142/142			113/113	
MeadD	10		137/143		147/147		117/127		105/115	
Mish	9	MeadM	135/135	144/144	137/147	136/136	117/127	194/195	105/115	183/208
Cush	9	MeadM	135/135	144/144	137/137	136/142	127/127	195/201	91/105	183/208
MeadM	9		135/135	144/144	137/137	136/136	117/127	195/195	91/115	208/208
MD1	9	MeadM	121/135	144/144	137/137	136/136	127/127	195/201	91/105	183/208
MD2	9	MeadM	121/135	144/144	137/147	136/136	127/127	194/195	91/105	208/208
Tom	11		121/135	144/149	137/147	136/136	119/119	195/199	113/113	208/208
Gerry	11		135/135	149/149	137/137	136/136	119/119	199/199	91/91	208/208
Mo	12		121/145	144/147	141/147	142/142	117/127	195/200	105/105	202/216
MoM	12	MoM		147/147	128/141	136/142	117/133	194/200	105/113	202/220
Dix	13	DixM	121/137	149/151	137/141	136/142	117/127	201/	91/115	208/232
DixM	13		137/137	149/153	137/141	136/136	117/117	191/199	91/91	208/208
Ntom	14		133/135	144/151	137/141	136/142	117/127	197/201	113/115	208/212
Holly	15		121/137	144/151	137/147	136/142	121/121	188/194	91/105	208/218
Leon	15	Holly	135/137	151/151	137/143	136/142	117/121	194/201	91/115	208/232
Tull	15	Holly	121/137	144/151	141/147	136/142	117/121	194/201	115/91	208/208
NTK	15	Tull	121/135	144/151	141/141	136/142	117/117	194/201	115/115	208/208
Tibs	16	Mog	121/121	144/145	141/147	136/136	127/127	201/	91/91	208/208
Mog	16		121/121	144/140	143/147	136/136	127/127	186/	91/105	208/216
Polly	17		121/135			136/142	127/127	195/203	113/113	208/218
Chas	17	Polly	133/135	144/148	131/133	136/136	127/127	194/203	102/113	220/218
Emma	17	Polly	121/133	144/144	133/141	136/136	127/127	194/195		208/220
Splod	17	Polly	121/133	144/146	131/133	136/142	127/127	199/203	102/113	208/218
Thorn	17	Polly	121/135	144/146	147/131	136/146	127/127	194/203	102/113	208/208
Rowe	18	SmM	141/147	144/144	141/131	136/142	127/127	188/199	105/113	208/208
SmM	18		121/141	144/148	131/149	142/142	127/121	199/199	105/113	183/208
Sm	18	SmM	141/141	148/148	141/149	136/142	119/121		105/113	208/208
Hwill	19		121/139	144/144	137/147	136/136	119/127	201/201	105/113	208/216

HNW	19	121/137	144/144	147/147	136/136	117/127	185/192	113/113	208/216
Magic	20	121/143	144/149	137/143	136/136	127/127		91/113	
Ben	20 Bguin	143/145	144/144	137/143	136/136	121/127	190/194	91/113	208/208
Bguin	20	145/145	144/144	137/137	136/142	121/121	194/194	91/105	208/208
Bsaf	20 Bguin	133/145	144/151	137/137	136/140	127/121	194/201	91/113	208/210
Lguin	20 Bsaf	133/135	144/149	137/147	140/142	121/127	194/201	91/91	208/208
Jaffa	20 Bsaf	137/145	149/151	137/147	136/142	121/127	194/194	105/113	208/208
Lsaf	20 Bsaf	135/145	144/151	137/147	136/136	127/127	188/194	91/105	210/220
hk1	20 Bsaf	133/137	149/151	137/147		127/127	188/194	91/105	208/220
Marm	21	119/121	144/144	137/147	136/136	117/127	191/195	91/107	208/208
StM	22	139/147	144/148		136/136	121/121	201/201	91/117	214/220
Strud	22 StM	133/147	144/147		136/136	117/121	187/201	91/91	208/214
MessM	23	121/133	144/149	131/137	136/142	127/127	191/199	91/113	183/208
Mtuft	23 MessM	121/133	144/149	137/151	136/136	127/127	191/201		
Mfluf	24	121/139	147/147	127/137	136/142	127/127	201/201	102/113	206/206
MTum	24	121/128	141/145	137/137	136/142	127/127	201/201	102/113	208/208
Macey	25	121/137	144/147	131/131	136/142	127/127	191/201	91/107	216/216
TellM	26	121/137	144/144	137/147	136/142	117/127	188/199	105/113	208/220
Silvi	26 TellM	121/137	144/144	137/141	136/136	117/127	194/199	105/105	208/220
Cassy	26 TellM	137/137	144/144	137/137	136/142	117/117	199/199	91/113	220/220
Tell	26 TellM	121/137	144/144	137/147	136/136	117/127	188/194	91/105	208/220
TellU	26	121/137	142/151	137/147	136/136	117/127	188/199	105/105	208/216
MadM	31	135/137	144/151	133/129	142/142	119/117	194/194	105/113	208/214
Phoeb	31 MadM	121/137	144/144	133/137	136/142	127/119	194/201	105/113	208/208
Wisky	31 MadM	135/137	149/151	147/133	142/142	117/121	194/201	91/105	208/208
Lilly	32	121/137	144/148	141/141	136/142	117/127	193/199	105/113	183/208
Bsam	33	119/141	144/148	137/145	136/142	117/119	199/199	105/115	210/210
Bsuz	34	121/133	144/148	137/141	136/142	127/127	199/199	91/113	208/208
Molly	34 Bsuz	133/133	144/144	137/141	136/142	127/127	199/199	91/113	208/210
Zig	34 Bsuz	133/133	144/144	141/147	136/142	127/127		113/117	208/210
Max	34 Bsuz	121/121		131/141	136/142	127/127		91/91	208/208
Suzy	35	121/133	144/150	131/131	136/136	117/127	194/201	105/105	208/208
S67	36	133/137	142/149		136/136	127/127	195/	113/116	208/216
Trav	37	137/143	144/151	129/137	136/136	117/127	194/194	102/113	208/208
Dunn	38	121/135	144/144	137/139	136/142	117/127	199/199	105/107	183/208
Weed	39	121/135	144/146	143/143	136/136	127/127		102/105	208/208
Belli	40	131/135	142/151	137/147	136/136	127/133	195/201	113/113	183/208
Lang	41	137/144	144/144	127/147	136/136	127/127	194/199	91/113	208/208
How	42	121/135	144/144	129/137	136/136	127/127	201/201	109/115	
Ar6a	43	137/137	144/144	137/137	136/136	127/127	199/199	91/109	208/208
B&W	44	121/133	144/151	137/131	136/136	117/127	194/201	91/105	208/208
Bac	45	121/137	144/149	131/141	142/142	117/117	199/201	113/113	183/208
Rol1	46	121/133	146/	137/141	142/142	119/127	199/199	102/113	212/216
Rol2	47	137/137	144/144	137/137	136/142	121/133	197/197	91/117	208/208
Bar	48	121/147	144/152	137/141	136/136	119/127	195/199	91/119	208/216
Ghand	49	121/147		137/143	136/136	117/119	188/199	91/105	
Scruf	50	121/121	149/149	137/137	142/142	127/127	188/199	113/117	208/208
Lever	51	121/141	144/149	129/141	136/150	119/127	188/194		183/214
Lums	52	121/137	144/151	137/141		127/127	197/197	91/115	208/208

Tiger	53	137/137	144/144	137/147	136/142	117/117	188/199	91/115	208/208
Eb	54	135/135	144/147	142/146	136/136	121/127	195/201	93/113	208/208
Gray	55	120/137	144/144	147/149	142/142	119/127	188/201	105/105	202/208
HinT	56		142/144	141/129	136/142	127/127		91/107	208/208
Jake	57	121/135	144/150	139/141	136/142	127/123	193/199	91/105	208/208
Joyse	58	121/148	144/144	137/137	142/142	127/127	195/195	91/115	208/208
Laide	59	121/137	144/144	141/147	142/142	127/127		111/117	208/208
Duke	60	121/141	146/	137/141	136/136	127/127	194/201	91/107	208/208
Rambo	61	137/145		137/143	136/142	127/127	197/198	91/107	183/208
SirG	62	135/141	144/147	147/143	136/142	119/127	188/201	105/109	208/208
Siv	63	121/137		131/137	136/142			105/113	
Staff	64	121/133	144/144	131/137	136/142	127/127	191/199	107/113	208/208
War	65	121/137	144/144	143/147	136/136	121/127		113/113	224/224
Whit	66	121/121	144/144	137/137	136/142	121/127		105/111	208/208
Zoe	67	121/139	144/144	137/147	136/136	119/127	188/201	91/105	208/208
Harry	68	121/133		133/141	136/146	115/117			

Appendix 2.5. Allele frequency data as presented to Genepop 1.2. Alleles have been given 2 digit identifiers. Population delimiters were used or removed, as appropriate. One individual per family group has been used.

Title line: "by subpop"
ADH fca8
ADH fca77
ADH Fca23
ADH Fca35
ADH Fca43
ADH Fca78
ADH Fca90
ADH Fca96
Pop
HinT , 0000 4244 4129 3642 2727 0000 9107 0808
Laide , 2137 4444 1447 4242 2727 0000 1117 0808
Lang , 3744 4444 2747 3636 2727 9499 9113 0808
Harry , 1233 0000 3341 3646 1517 0000 0000 0000
B&W , 2133 4451 3731 3636 1727 9401 9105 0808
Rowe , 4147 4444 4131 3642 2727 8899 0513 0808
pop
Jake , 2135 4450 3941 3642 2723 9399 9105 0808
Ntom , 3335 4451 3741 3642 1727 9701 1315 0812
DixM , 3737 4953 3741 3636 1717 9199 9191 0808
Holly , 2137 4451 3747 3642 2121 8894 9105 0818
Tibs , 2121 4445 4147 3636 2727 0100 9191 0808
Belli , 3135 4251 3747 3636 2733 9501 1313 8308
pop
MeadD , 3743 0000 4747 0000 1727 0000 0515 0000
HNW , 2137 4444 4747 3636 1727 8592 1313 0816
Tiger , 3737 4444 3747 3642 1717 8899 9115 0808
Mish , 3535 4444 3747 3636 1727 9495 0515 8308
pop
Staff , 2133 4444 3137 3642 2727 9199 0713 0808
Tom , 2135 4449 3747 3636 1919 9599 1313 0808
MadM , 3537 4451 3329 4242 1917 9494 0513 0814
Bac , 2137 4449 3141 4242 1717 9901 1313 8308
Siv , 2137 0000 3137 3642 0000 0000 0513 0000
pop
Eb , 3535 4447 4246 3636 2127 9501 9313 0808
Bsam , 1941 4448 3745 3642 1719 9999 0515 0010
Zoe , 1239 4444 3747 3636 1927 8801 9105 0808
Zig , 3333 4444 4147 3642 2727 0000 1317 0810
Ben , 4345 4444 3743 3636 2127 9094 9113 0808
Marm , 1921 4444 3747 3636 1727 9195 9107 0808
Macey , 2137 4447 3131 3642 2727 9101 9107 1616
Molly , 3333 4444 3741 3642 2727 9999 9113 0810

Weed , 2135 4446 4343 3636 2727 0000 0205 0808
pop
Mtuft , 2133 4449 3751 3636 2727 9101 0000 0000
Mfluf , 2139 4747 2737 3642 2727 0101 0213 0600
Duke , 2141 4600 3741 3636 2727 9401 9107 0808
pop
Lilly , 2137 4448 4141 3642 1727 9399 0513 8308
Suzy , 2133 4450 3131 3636 1727 9401 0505 0808
Scruf , 2121 4949 3737 4242 2727 8899 1317 0808
Gray , 2037 4444 4749 4242 1927 8801 0505 0208
Ghand , 2147 0000 3743 3636 1719 8899 1905 0000
Burg , 4147 4244 0000 4242 2731 8899 0707 0808
Palm , 3535 4447 3145 3636 1927 9599 9107 1414
Strud , 3347 4447 0000 3636 2721 8701 9191 0814
barrow, 2147 4452 3741 3636 1927 9599 9119 0816
Rol1 , 2133 4600 3741 4242 1927 9999 0213 1216
Rol2 , 3737 4444 3737 3642 2133 9797 9117 0808
pop
Rambo , 3745 0000 3743 3642 2727 9798 9107 8308
SirG , 3541 4447 4743 3642 1927 8801 0509 0808
Whit , 2121 4444 3737 3642 2127 0000 0511 0808
C17 , 2133 4448 3141 4242 1927 0000 0213 0810
ArnM , 2133 4444 3737 3600 2700 9405 9191 0808
Lever , 2141 4449 2941 3650 1927 8894 0000 8314
Mo , 2145 4447 4147 4242 1727 9550 0505 0216
S67 , 3337 4249 0000 3636 2727 9500 1316 0816
Ar6a , 3737 4444 3737 3636 2727 9999 9109 0808
Lums , 2137 4451 3741 0000 2727 9797 9115 0808
Trav , 3743 4451 2937 3636 1727 9494 0213 0808
Gillf , 3535 4449 3741 3636 2727 9501 9105 0816
Pick , 3335 4444 3133 3642 2727 8895 0513 0812
Dear , 2137 0000 0000 4242 0000 0000 1313 0000
Chas , 3135 4448 3133 3636 2727 9403 0213 2018
Dunn , 2135 4444 3739 3642 1727 9999 0507 8308
How , 2135 4444 2937 3636 2727 0100 0915 0000
Joyse , 1248 4444 3737 4242 2727 9595 9115 0808
War , 2137 4444 4347 3636 2127 0000 1313 2424

Appendix 2.6. Reconstructed paternal genotypes for litters where 2 of more full siblings were genotyped. A forward slash between alleles indicates that either one or the other was a paternal allele, a + symbol indicates that both alleles were present in the father.

i. Shirley/Freemantle area

Litter	Fca8	Fca77	Fca23	Fca35	Fca43	Fca78	Fca90	Fca96
Foyle (1)	131+133	146+(144/148)	133/141/147	136+146	127	194+199	102	208+220
Tell (2)	137+(121)	144	141+137	136	117	194+199	105+91	220
Morris (3)	121/145	144	147	142	127	194	105	216
Gil (4)	135	144/149	137/141/147	136	127	195/201	91/105	208

ii. Hill household

Litter	Fca8	Fca77	Fca23	Fca35	Fca43	Fca78	Fca90	Fca96
2	135+137	149	147	136/142	127/121	188 + 194	91 + 105	208+ 220
3+4	133	151	137	140		121	113	210

Appendix 2.7. Significance thresholds and type 2 error rates for likelihood ratios for full sibling vs. maternal half sibling. Significance levels were calculated by simulation using allele frequencies for the whole data set.

Settings for primary hypothesis:

Rm:	0.5
Rp:	0.5

Settings for null hypothesis:

Rm:	0.5
Rp:	0

Results of significance simulations

2000 simulated pairs used to calculate values

p<:	Ratio	Type II error
0.05	3.32E+00	0.51
0.01	9.90E+00	0.7225
0.001	4.79E+01	0.914

Appendix 2.8. Significance thresholds and type 2 error rates for likelihood ratios for father-offspring vs. unrelated. Significance levels were calculated by simulation using allele frequencies for the whole data set.

Settings for primary hypothesis:

Rm: 0

Rp: 1

Settings for null hypothesis:

Rm: 0

Rp: 0

Results of significance simulations

1000 simulated pairs used to calculate values

p<:	Ratio	Type II error
0.05	0.00E+00	0
0.01	1.25E+01	0.109
0.001	2.13E+02	0.484

Appendix 2.9. Significance thresholds and type 2 error rates for likelihood ratios for paternal half siblings vs. unrelated. Significance levels were calculated by simulation using allele frequencies for the whole data set

Settings for primary hypothesis:

Rm: 0

Rp: 0.5

Settings for null hypothesis:

Rm: 0

Rp: 0

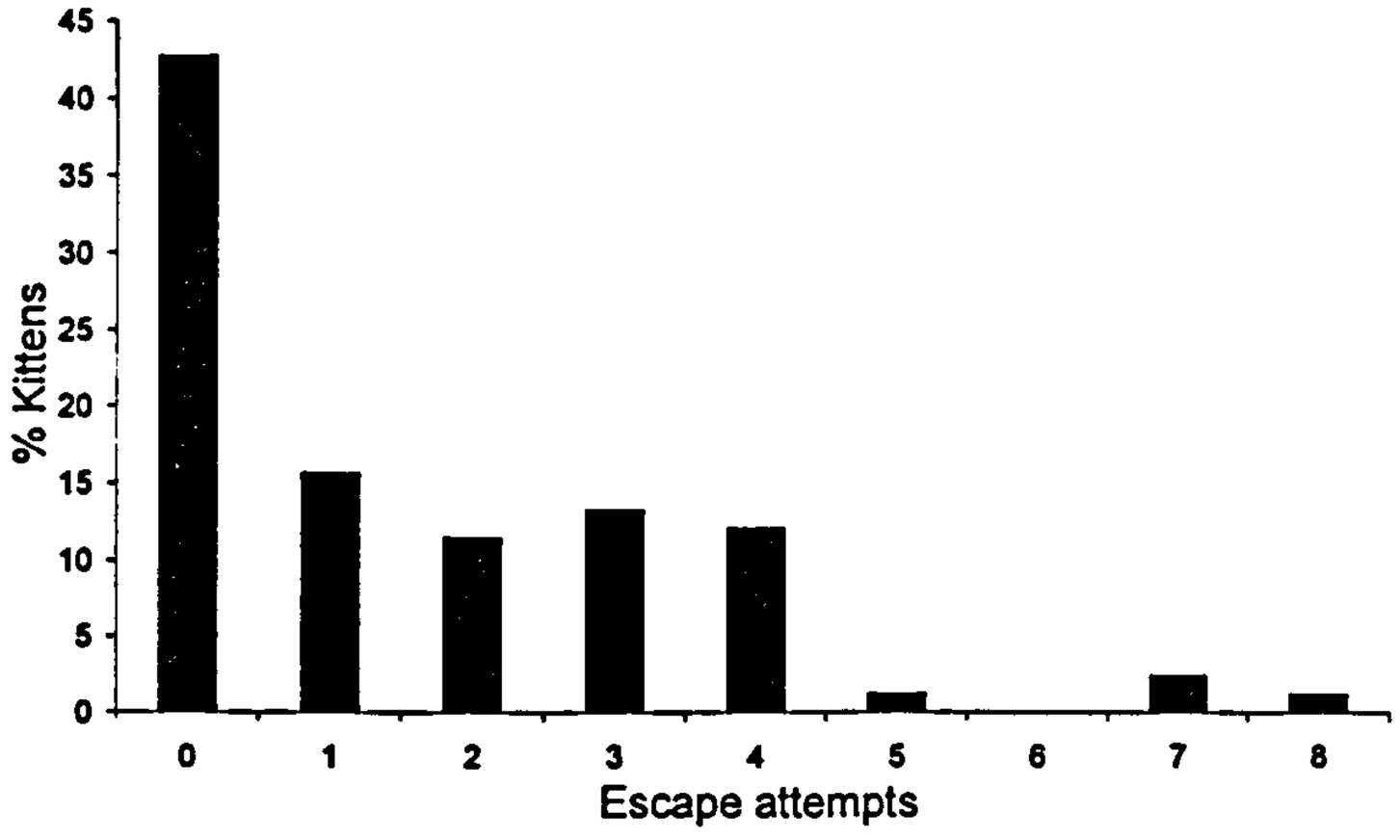
Results of significance simulations

1000 simulated pairs used to calculate values

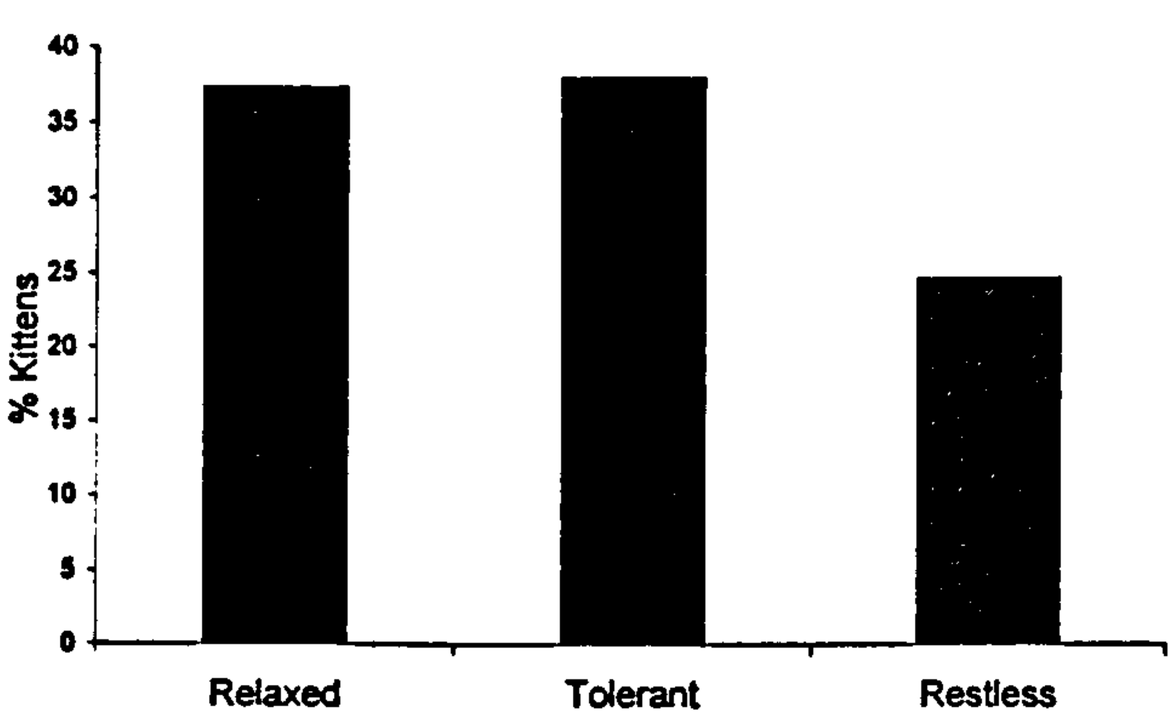
p<:	Ratio
0.05	3.25E+00
0.01	1.08E+01
0.001	3.13E+01

Appendix 3.1. Distribution of data from temperament testing at 6 months. Data from tests one and two is pooled.

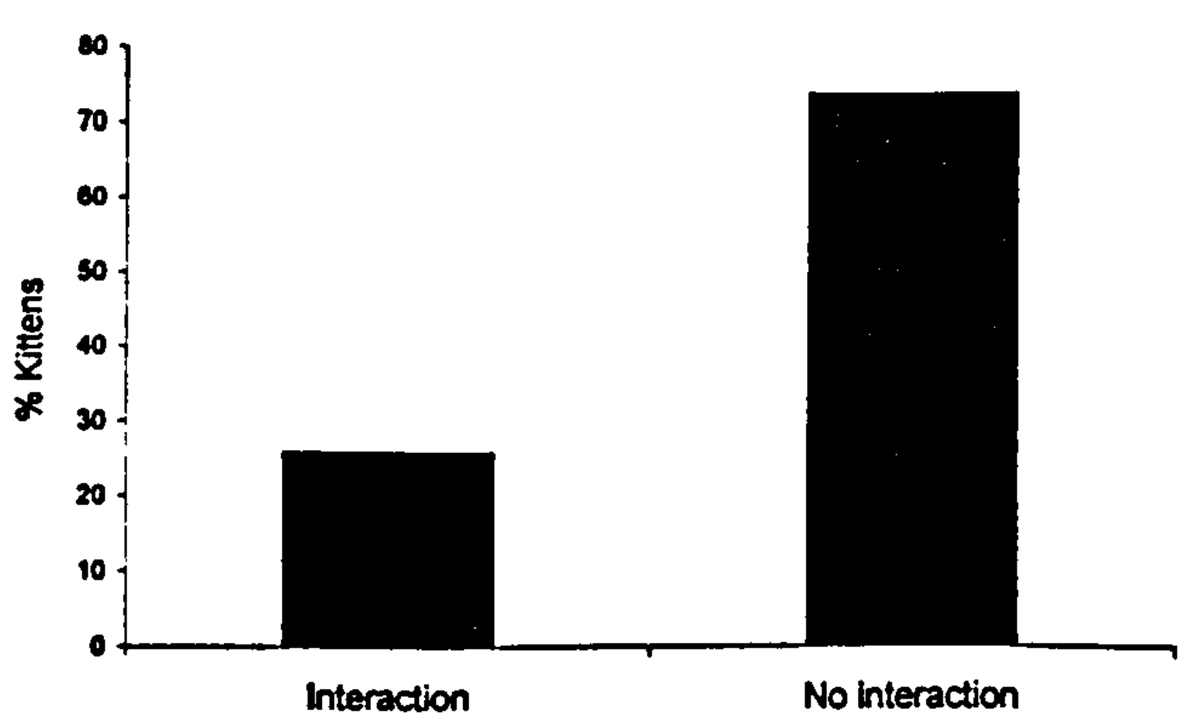
Appendix 3.1.1. Escape attempts during familiar person handling test (6 months) Values of 8 represent the maximum recorded value plus one (see Methods, **Chapter 6**).



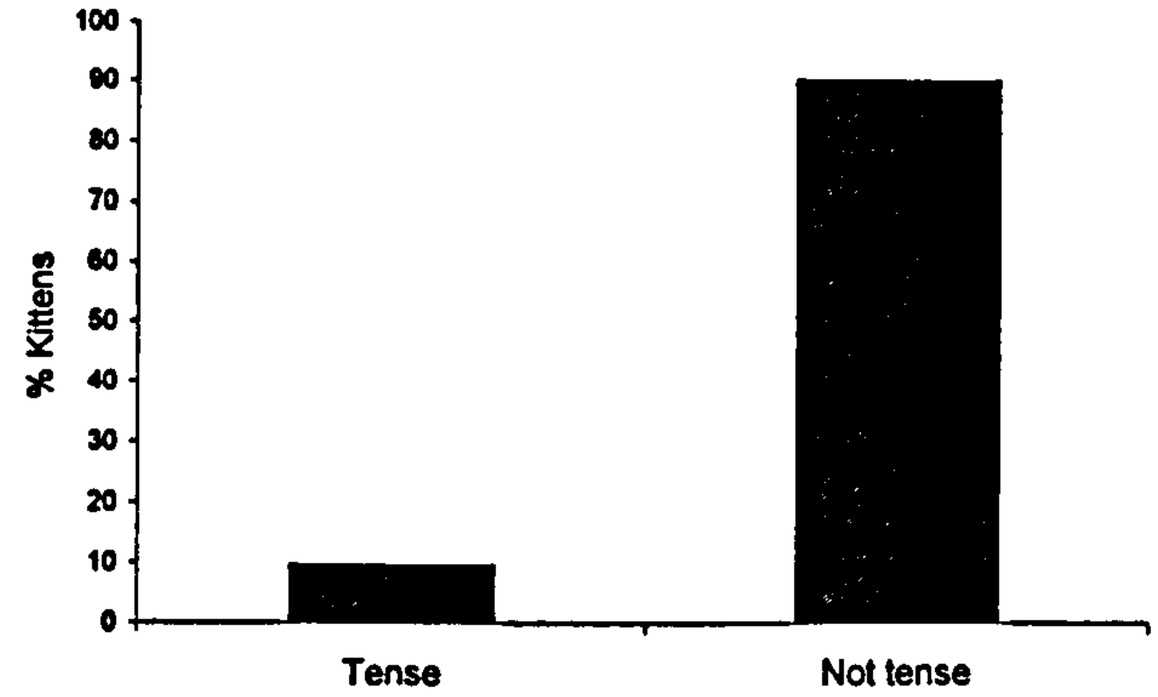
Appendix 3.1.2. State of activity during familiar person handling test (6 months).



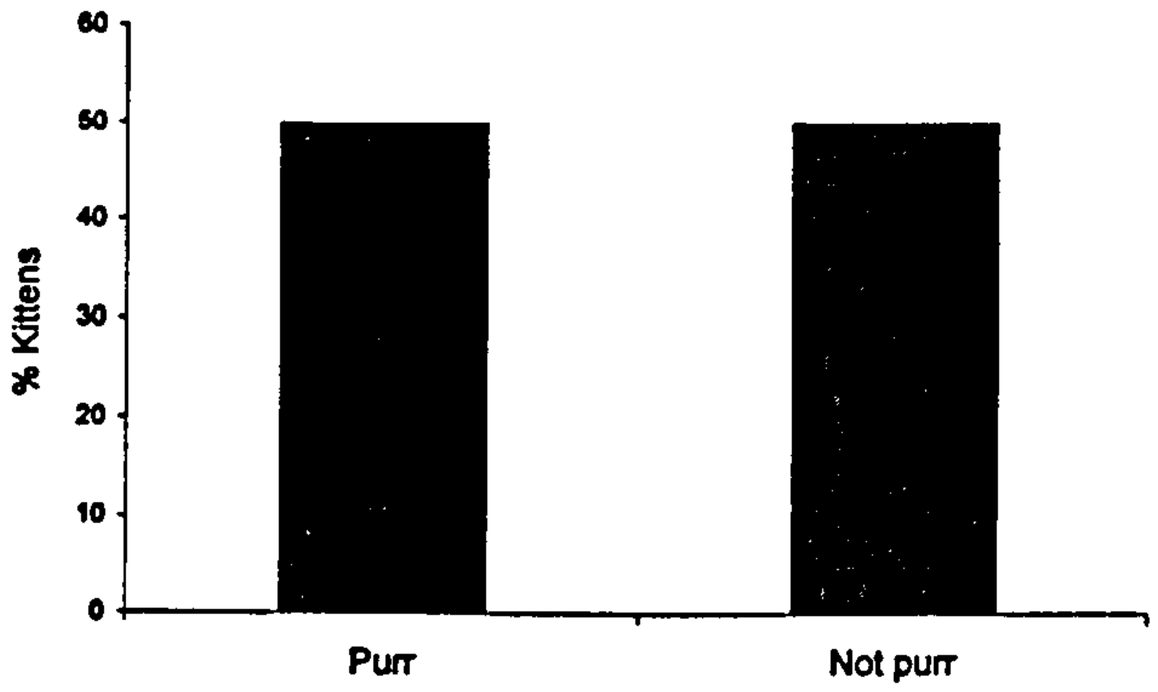
Appendix 3.1.3. Interactive in familiar person handling test (6 months).



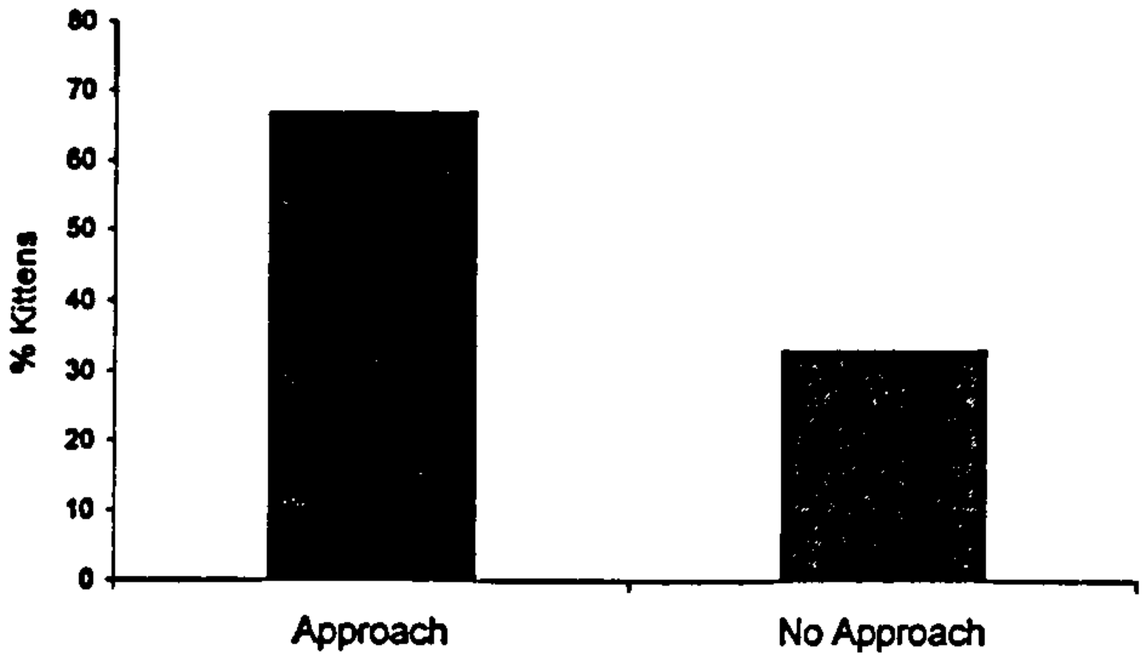
Appendix 3.1.4. Tenseness in familiar person handling test (6 months).



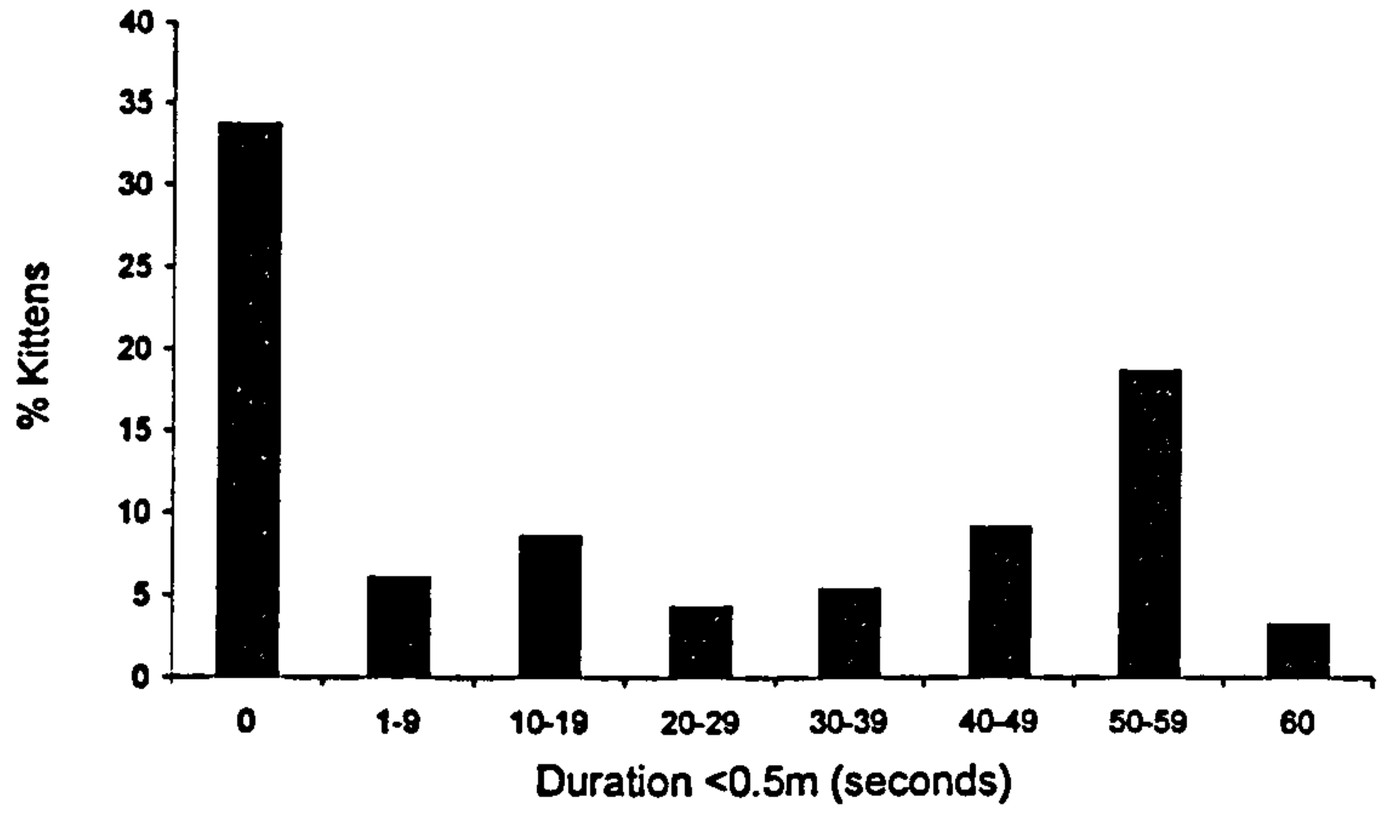
Appendix 3.1.5. Purring in familiar person handling test (6 months).



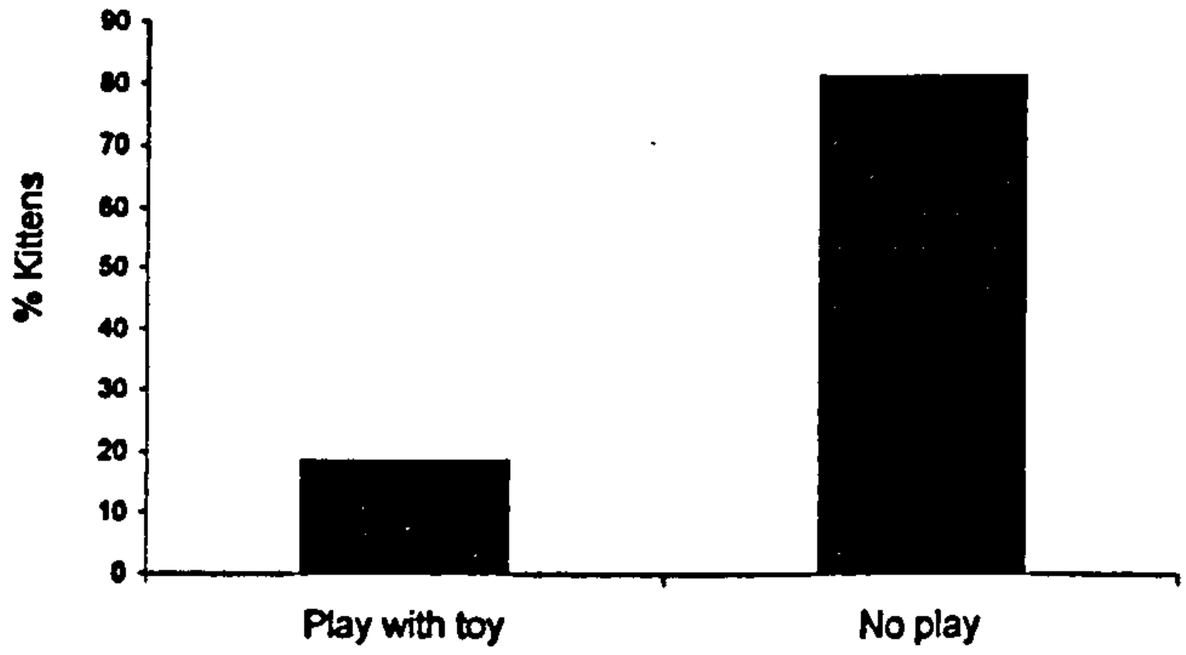
Appendix 3.1.6. Approach to <0.5m in approach test (6 months).



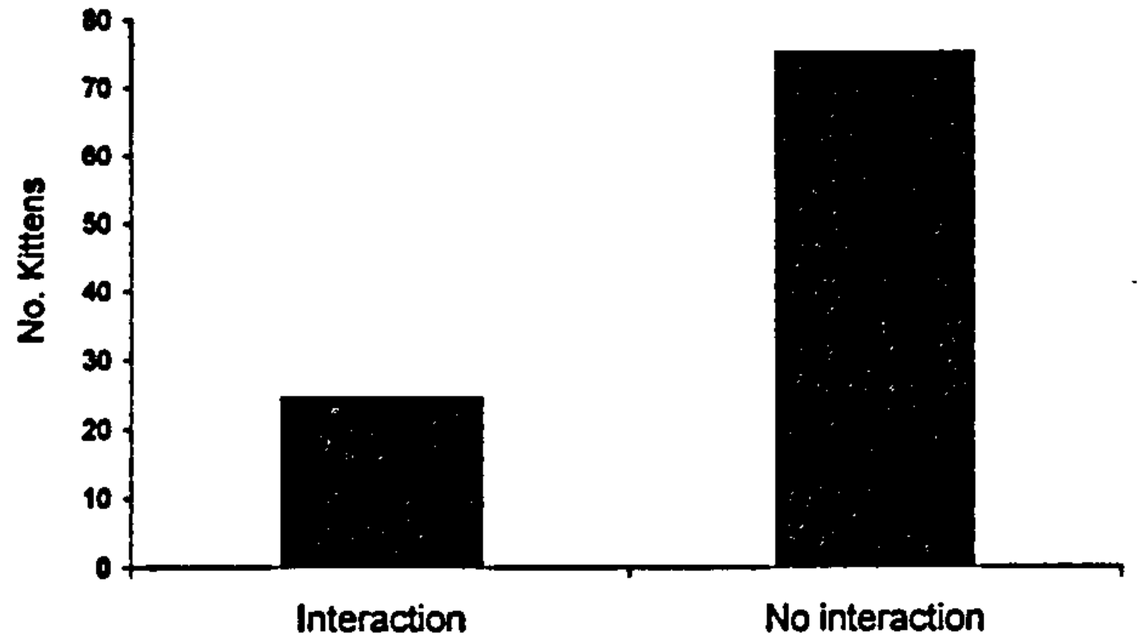
Appendix 3.1.7. Duration <0.5m in approach test (6 months).



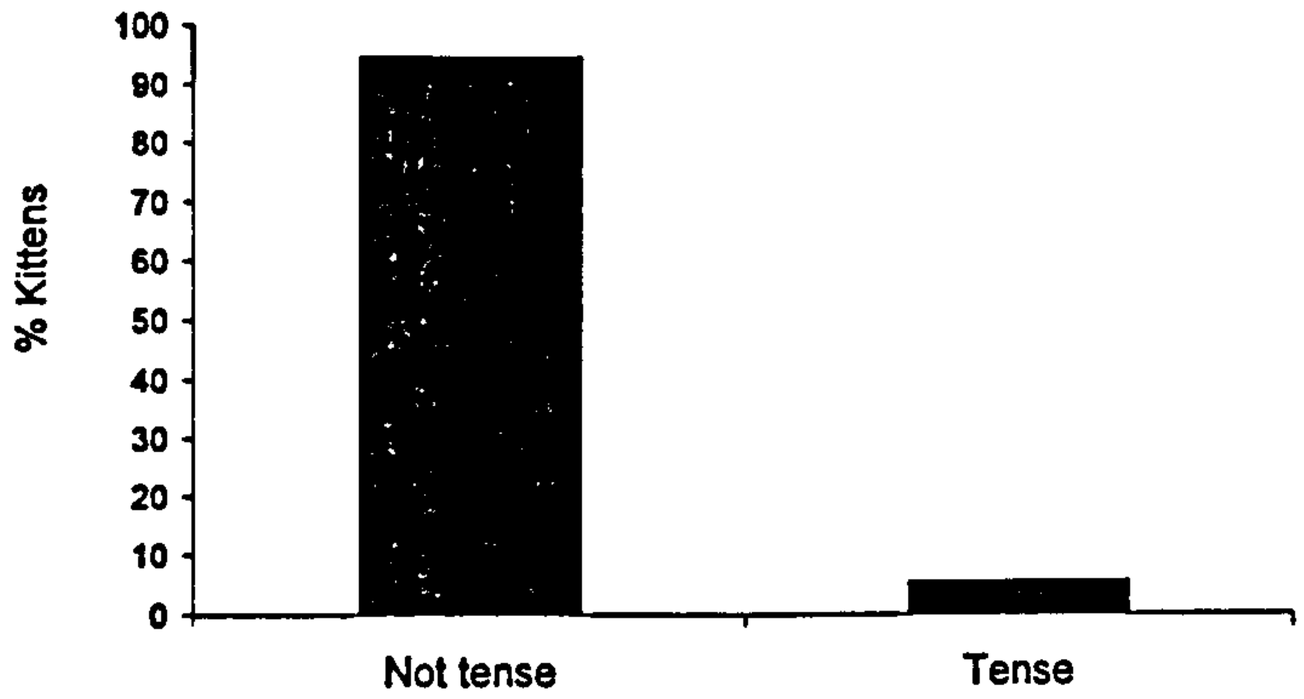
Appendix 3.1.8. Play with toy in approach test (6 months).



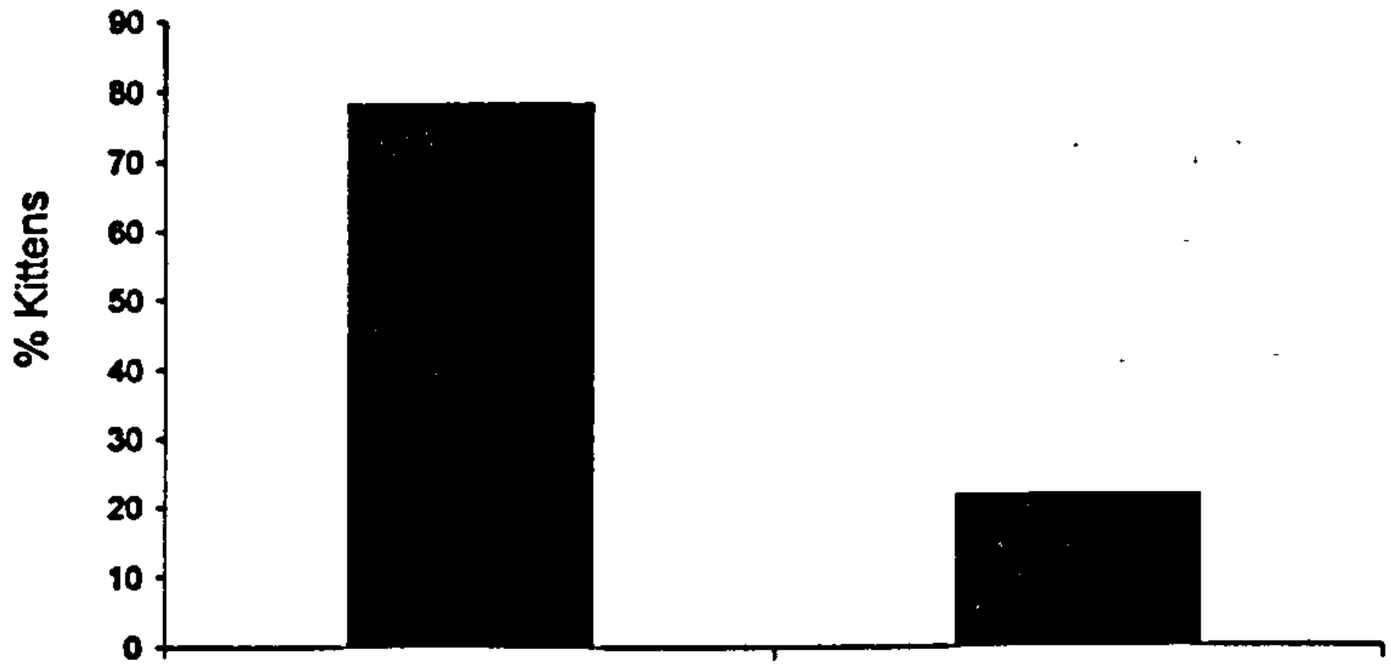
Appendix 3.1.9. Interact with test person in approach test (6 months).



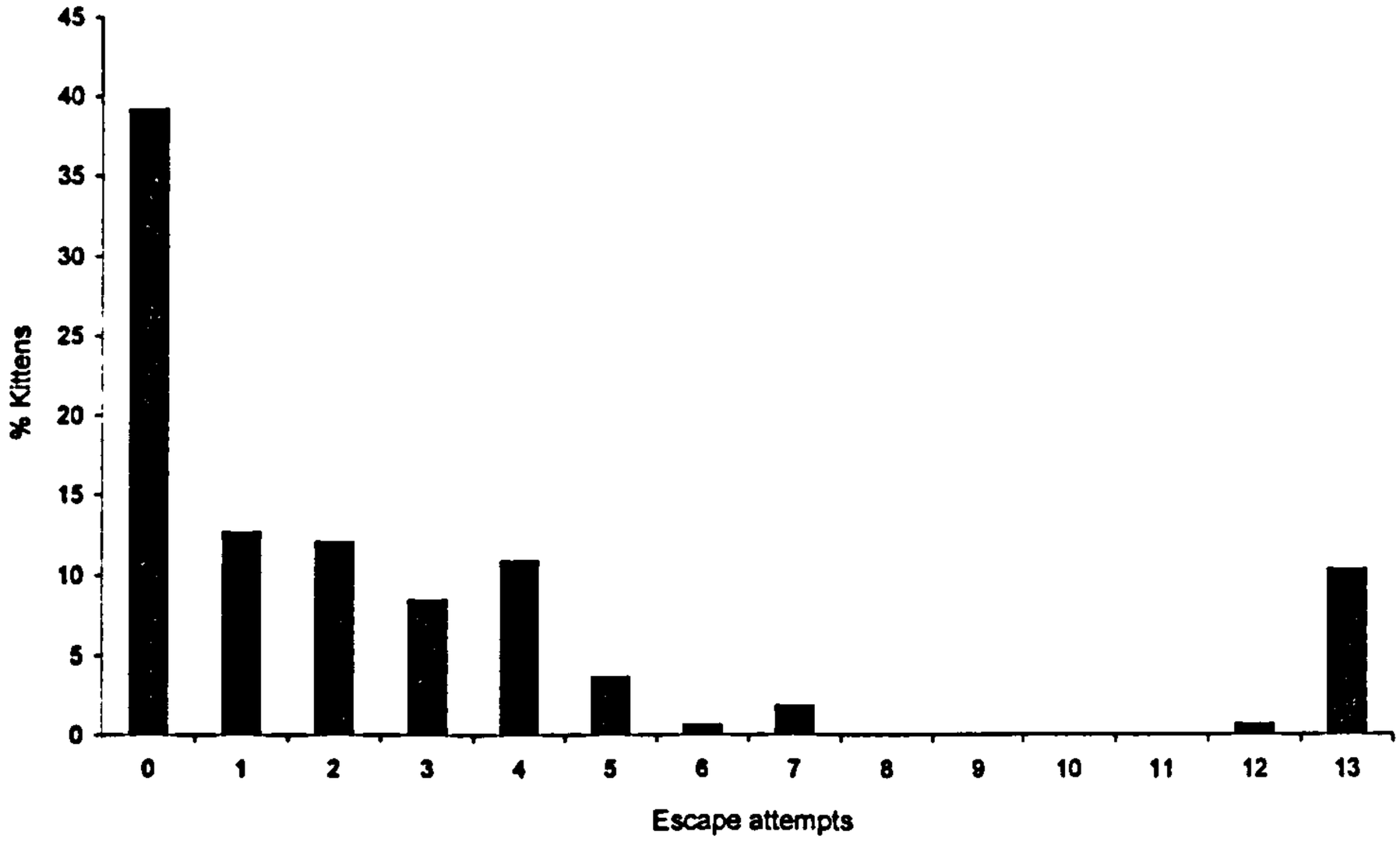
Appendix 3.1.10. Tenseness in approach test (6 months).



Appendix 3.1.11. Purring during approach test (6 months).

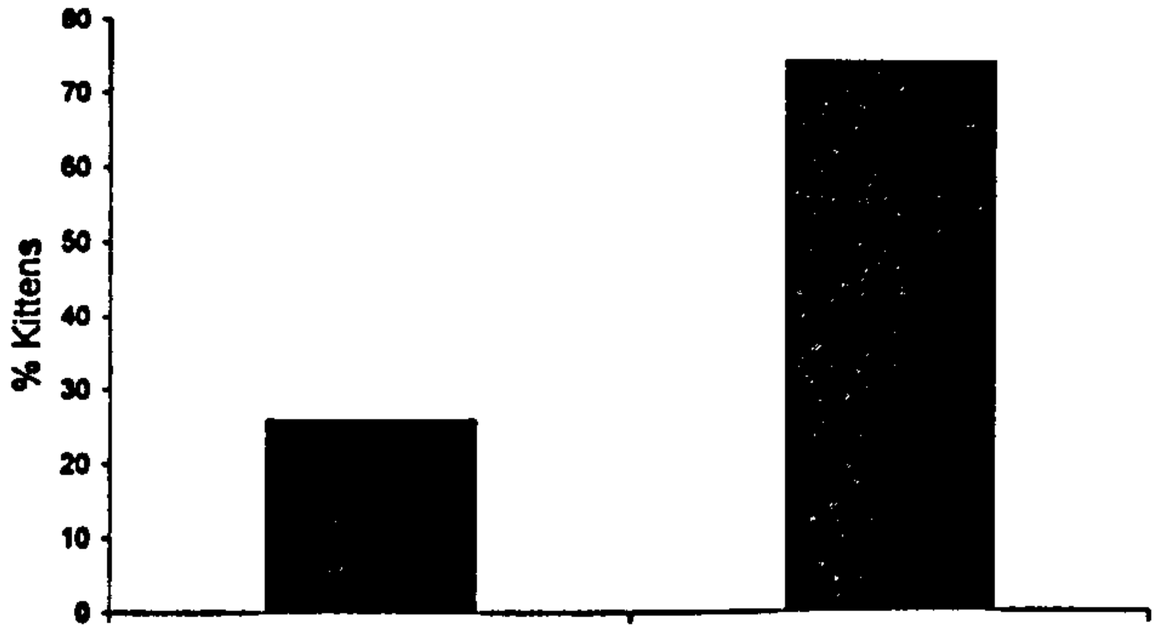


Appendix 3.1.12. Escape attempts during unfamiliar person handling test (6 months). Values of 13 represent the maximum recorded value plus one (see **Methods**).

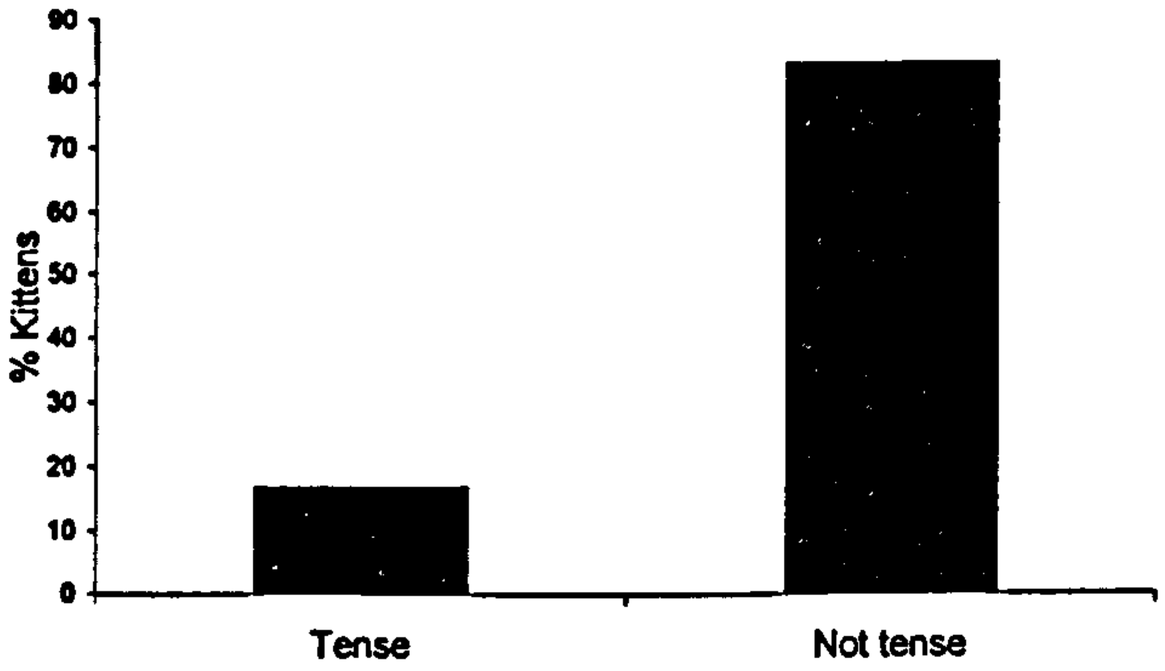


Appendix 3.1.13. State of activity during unfamiliar person handling test (6 months).

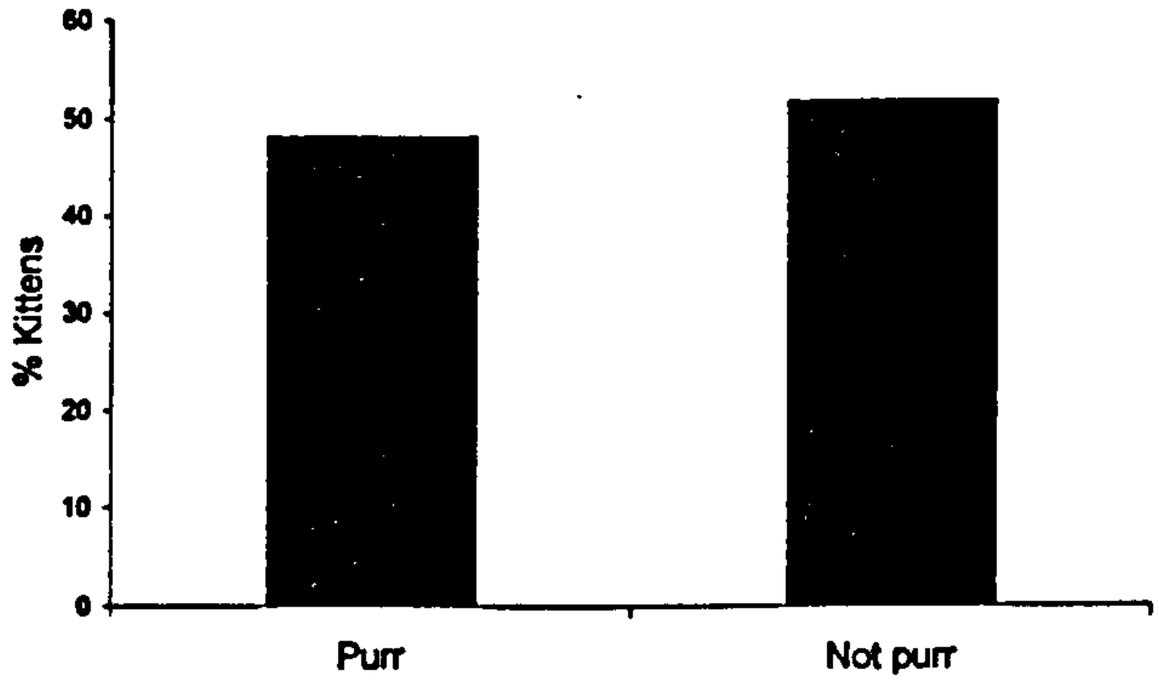
Appendix 3.1.14. Interaction in unfamiliar person handling test (6 months).



Appendix 3.1.15. Tenseness in unfamiliar person handling test (6 months).

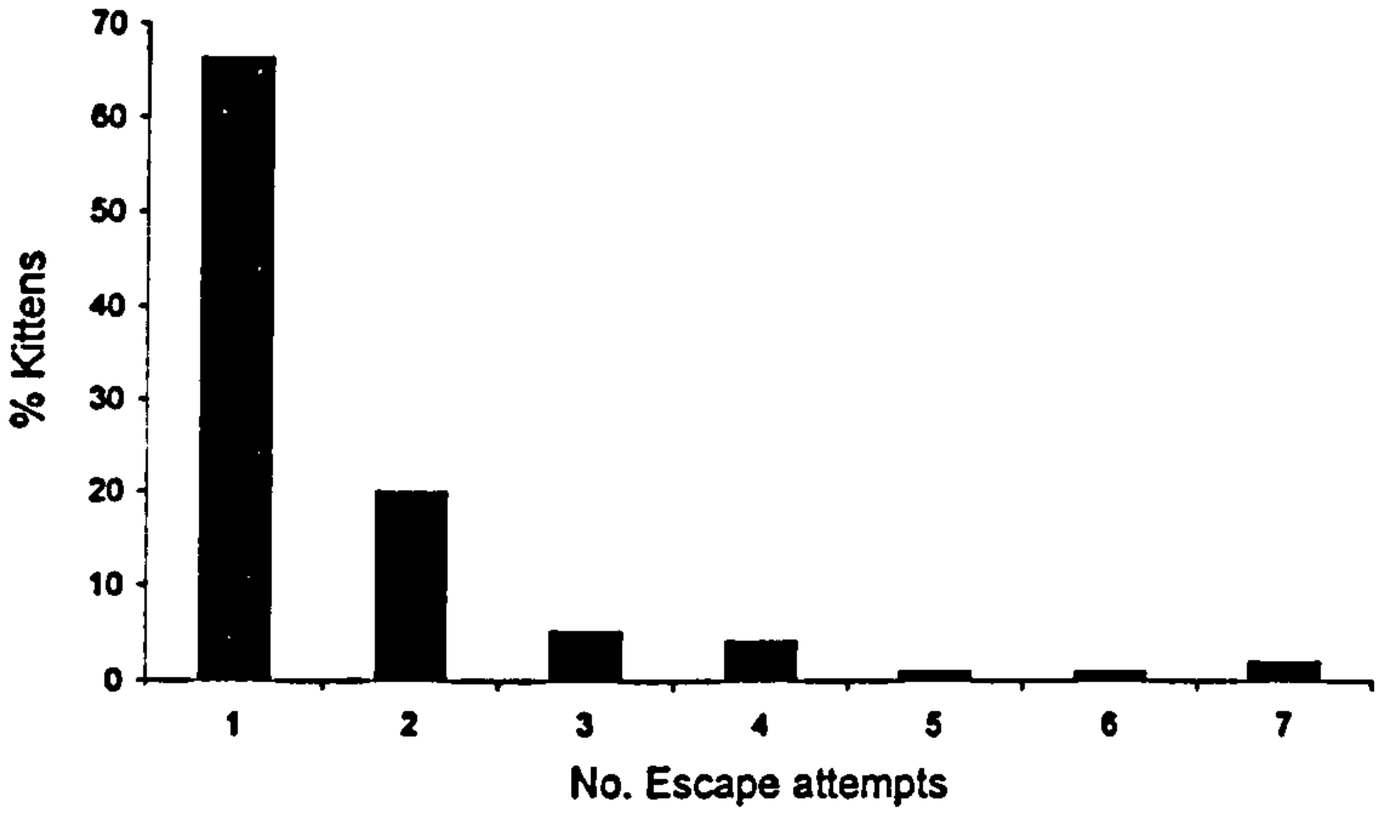


Appendix 3.1.16. Purring in unfamiliar person handling test (6 months).

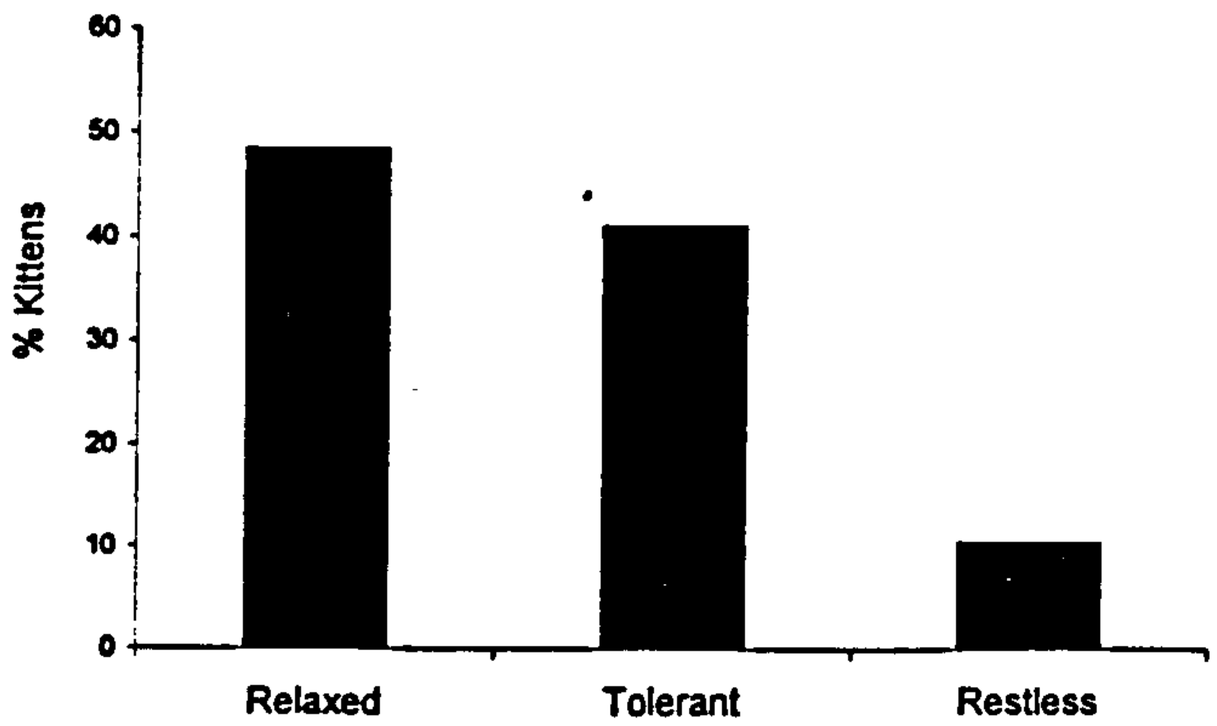


Appendix 3.2. Distribution of data from temperament testing at 18 months. Data from tests one and two is pooled.

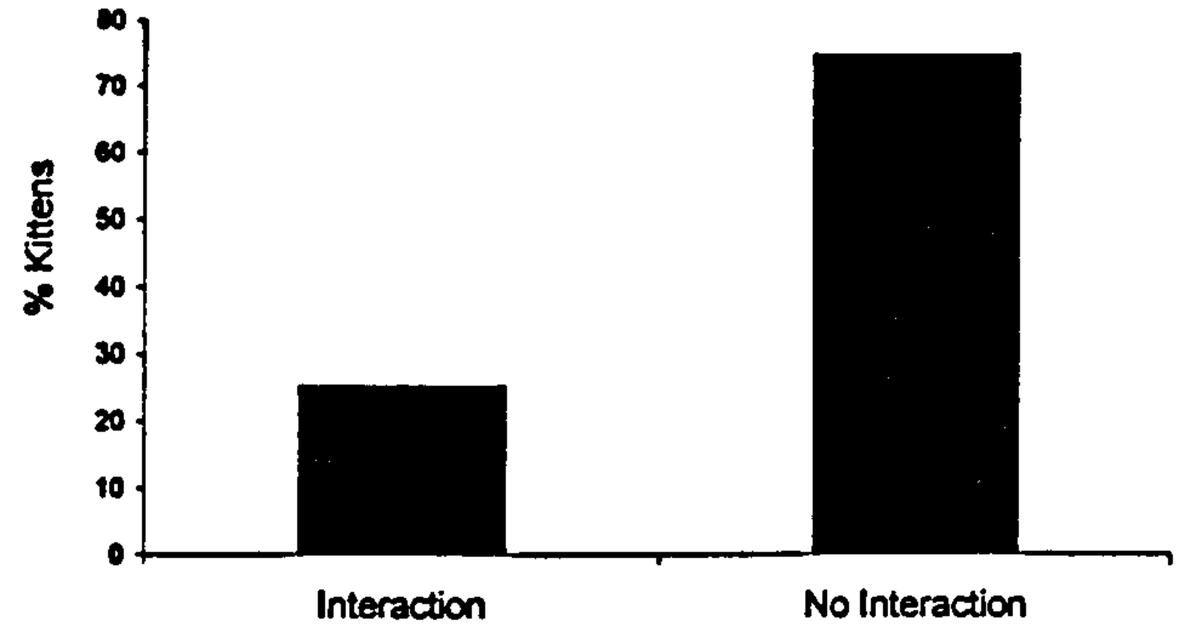
Appendix 3.2.1. Escape attempts during familiar person handling test (18 months). Values of 7 represent the maximum recorded value plus one (see **Methods**).



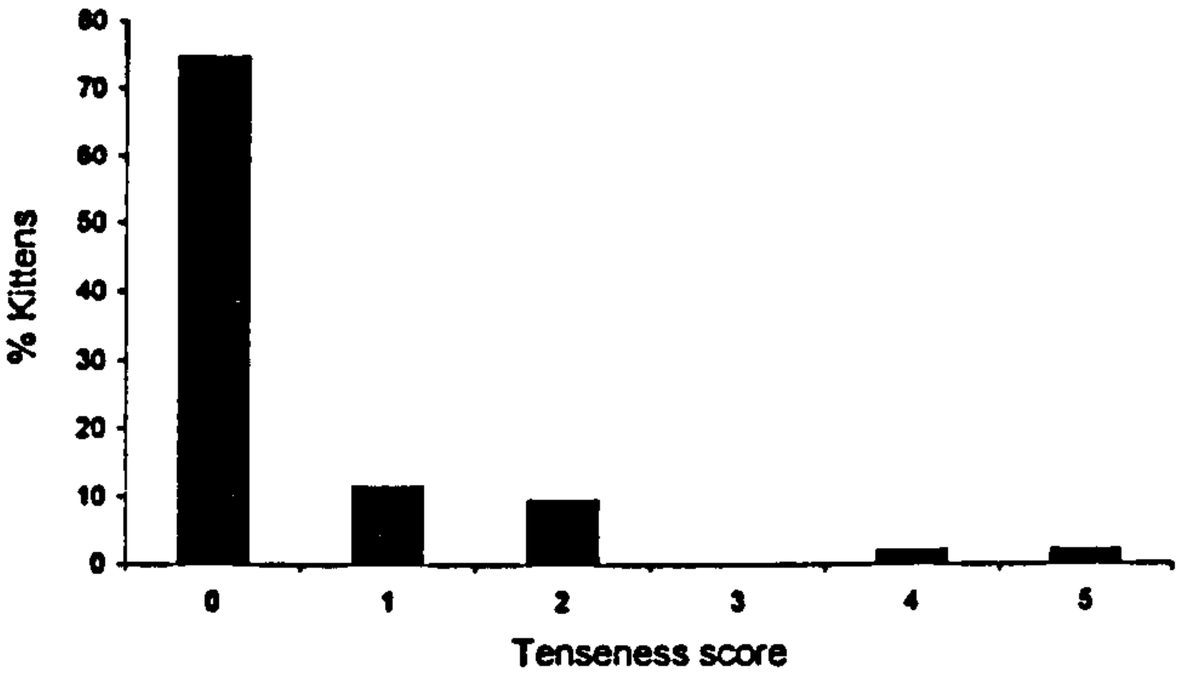
Appendix 3.2.2. State of activity during familiar person handling test (18 months).



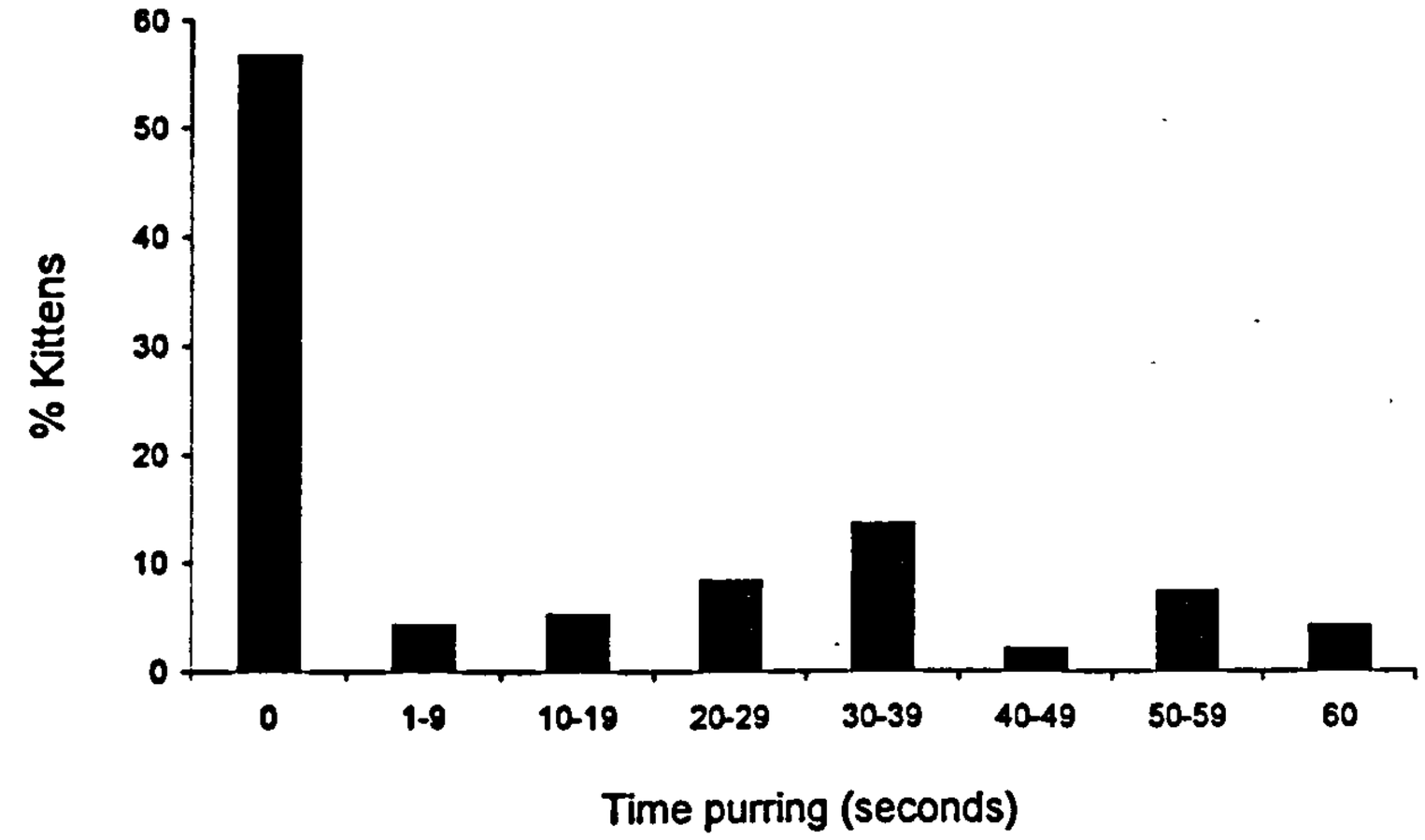
Appendix 3.2.3. Interaction in familiar person handling test (18 months).



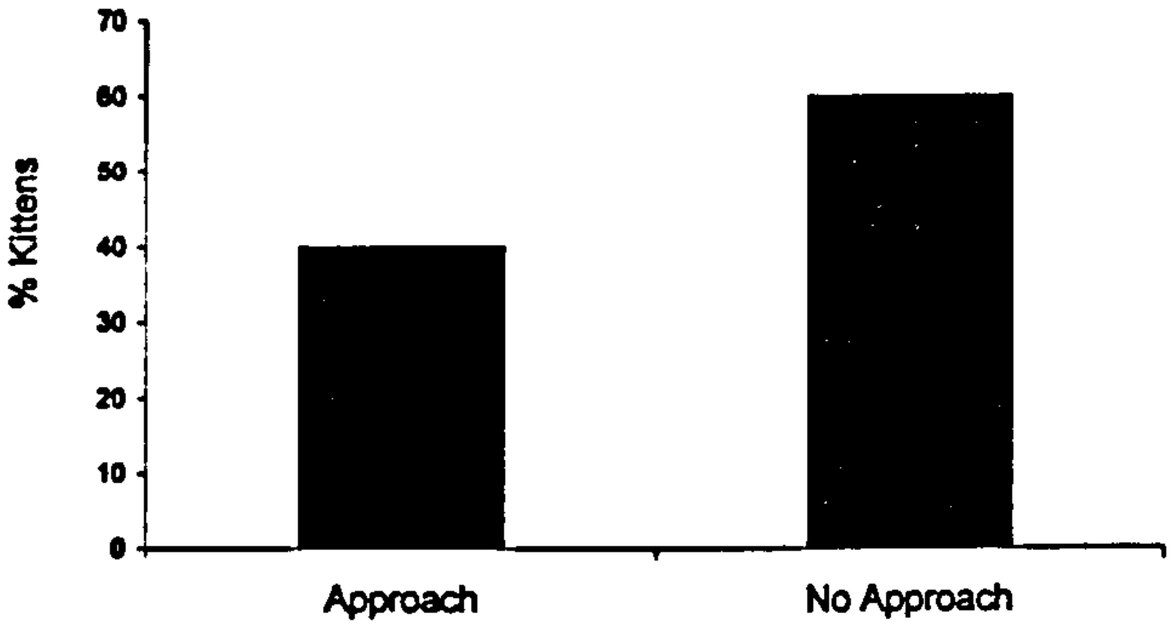
Appendix 3.2.4. Tenseness in familiar person handling test (18 months).



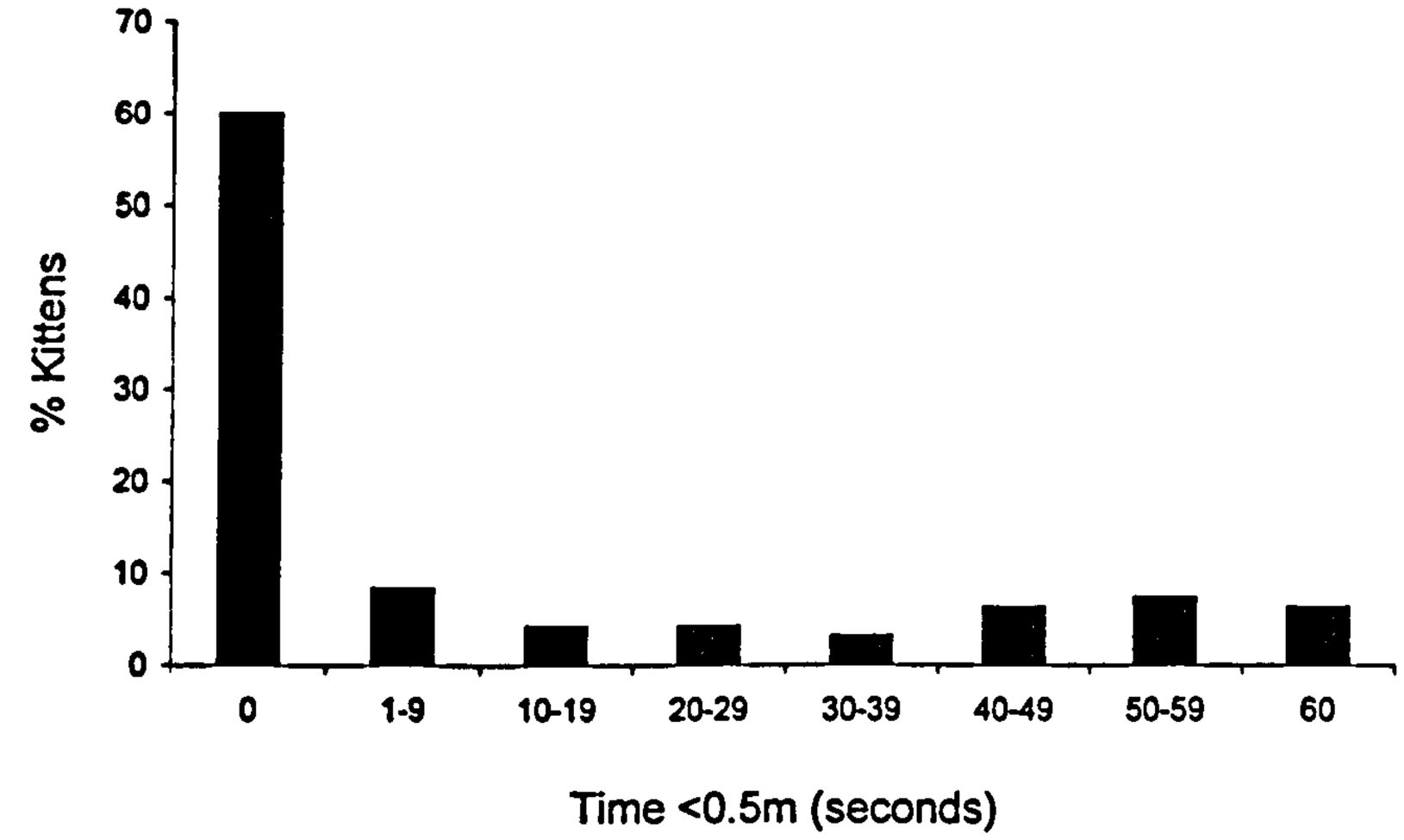
Appendix 3.2.5. Purring in familiar person handling test (18 months).



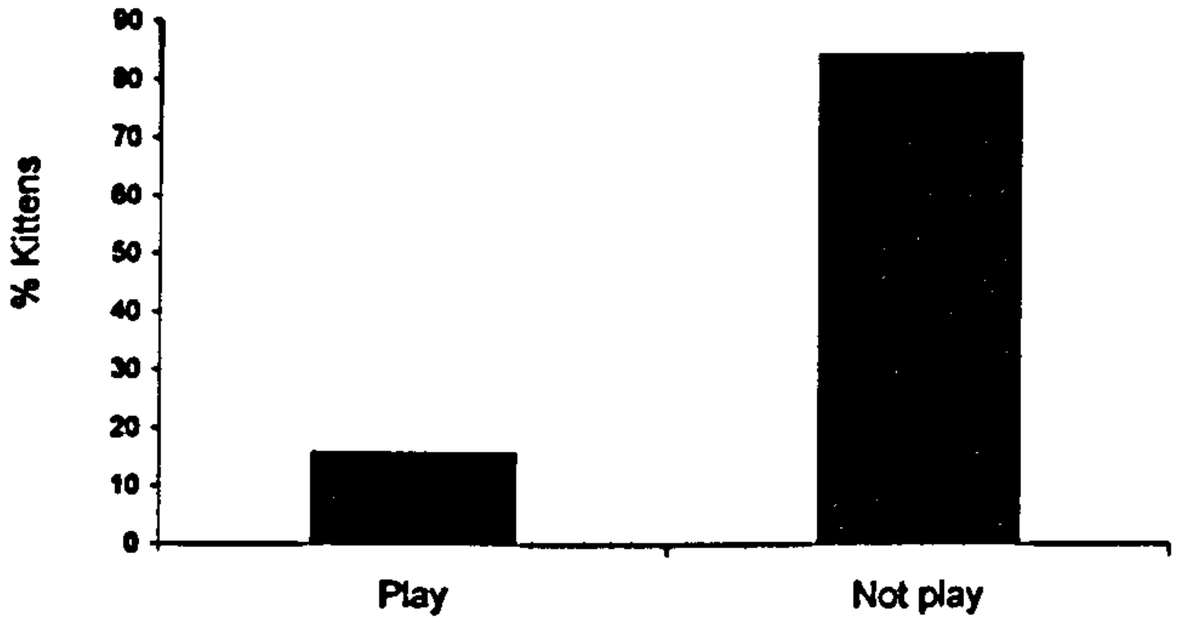
Appendix 3.2.6. Approach to <0.5m in approach test (18 months).



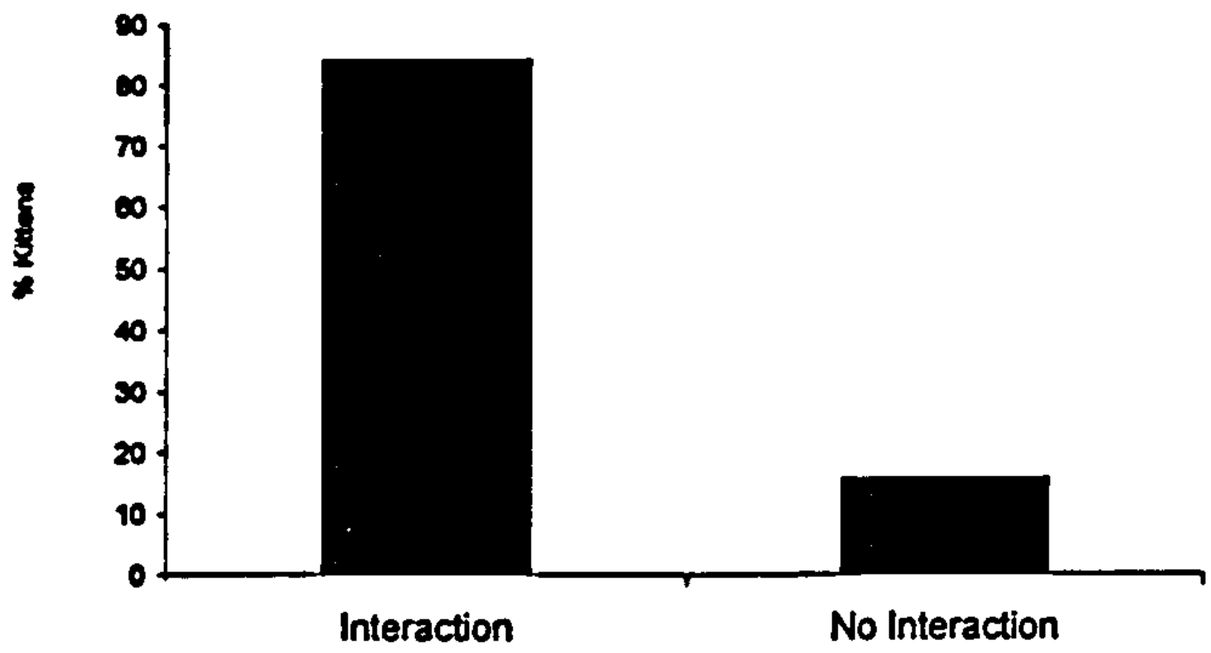
Appendix 3.2.7. Duration <0.5m in approach test (18 months).



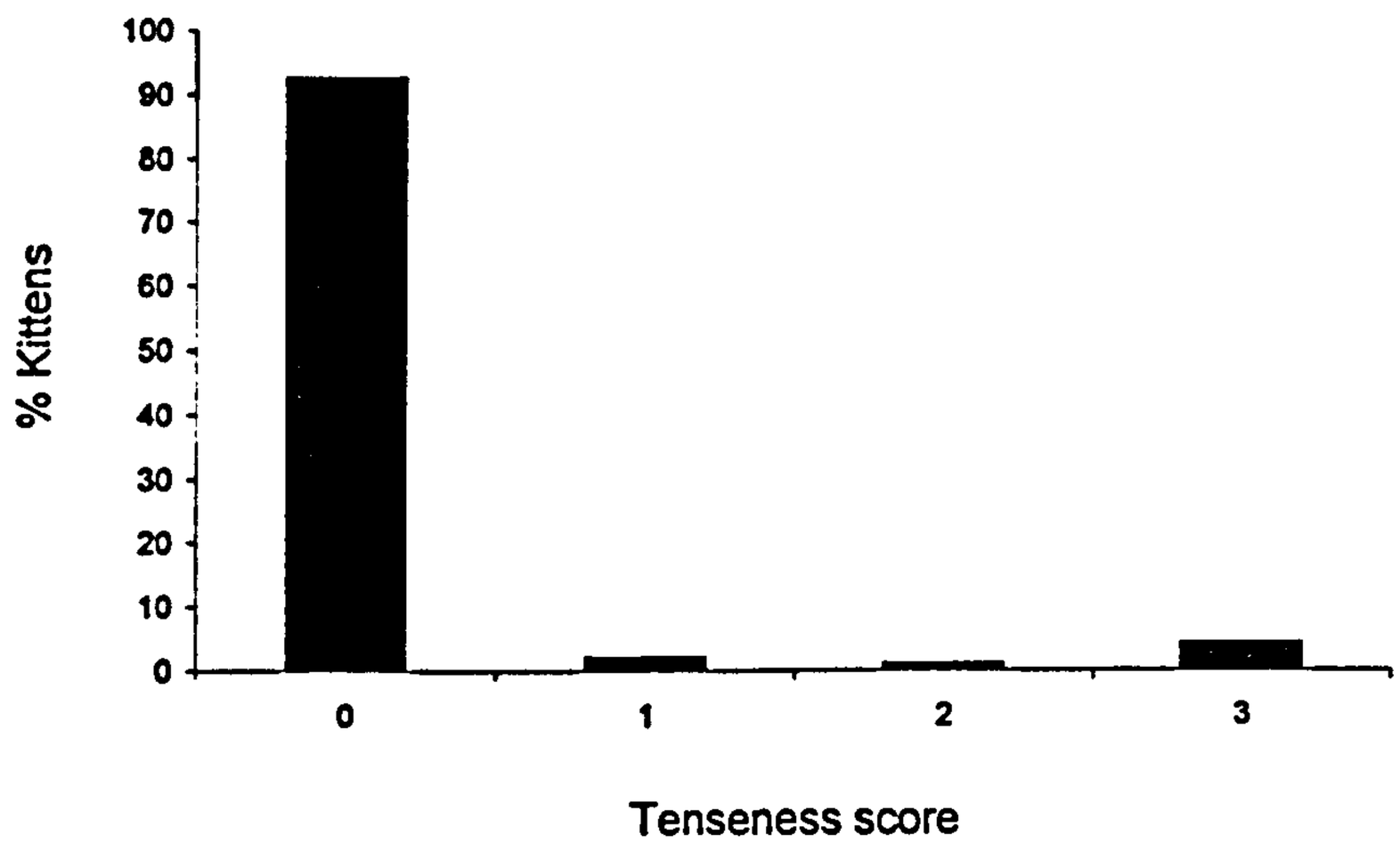
Appendix 3.2.8. Play with toy in approach test (18 months).



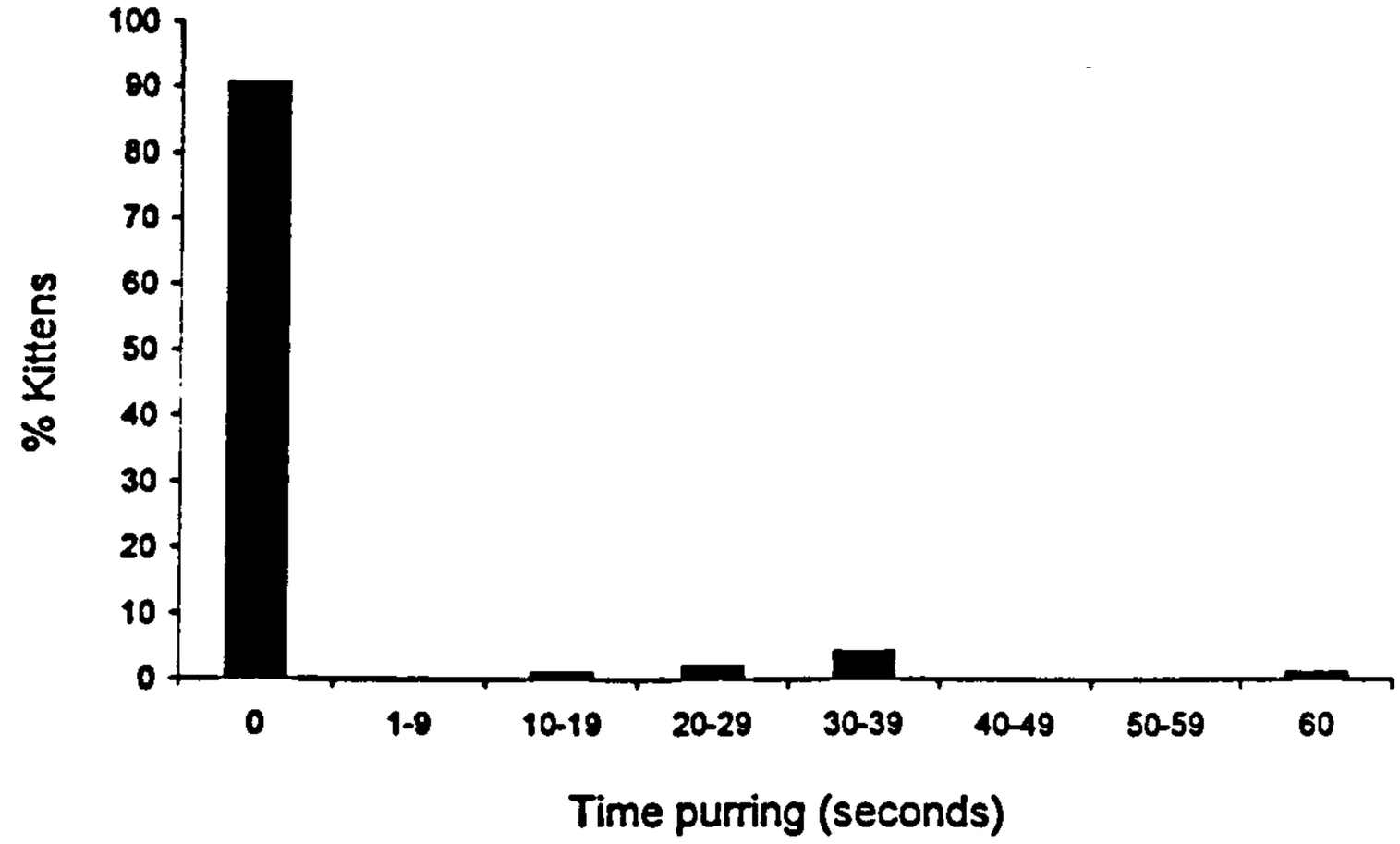
Appendix 3.2.9. Interact with test person in approach test (18 months).



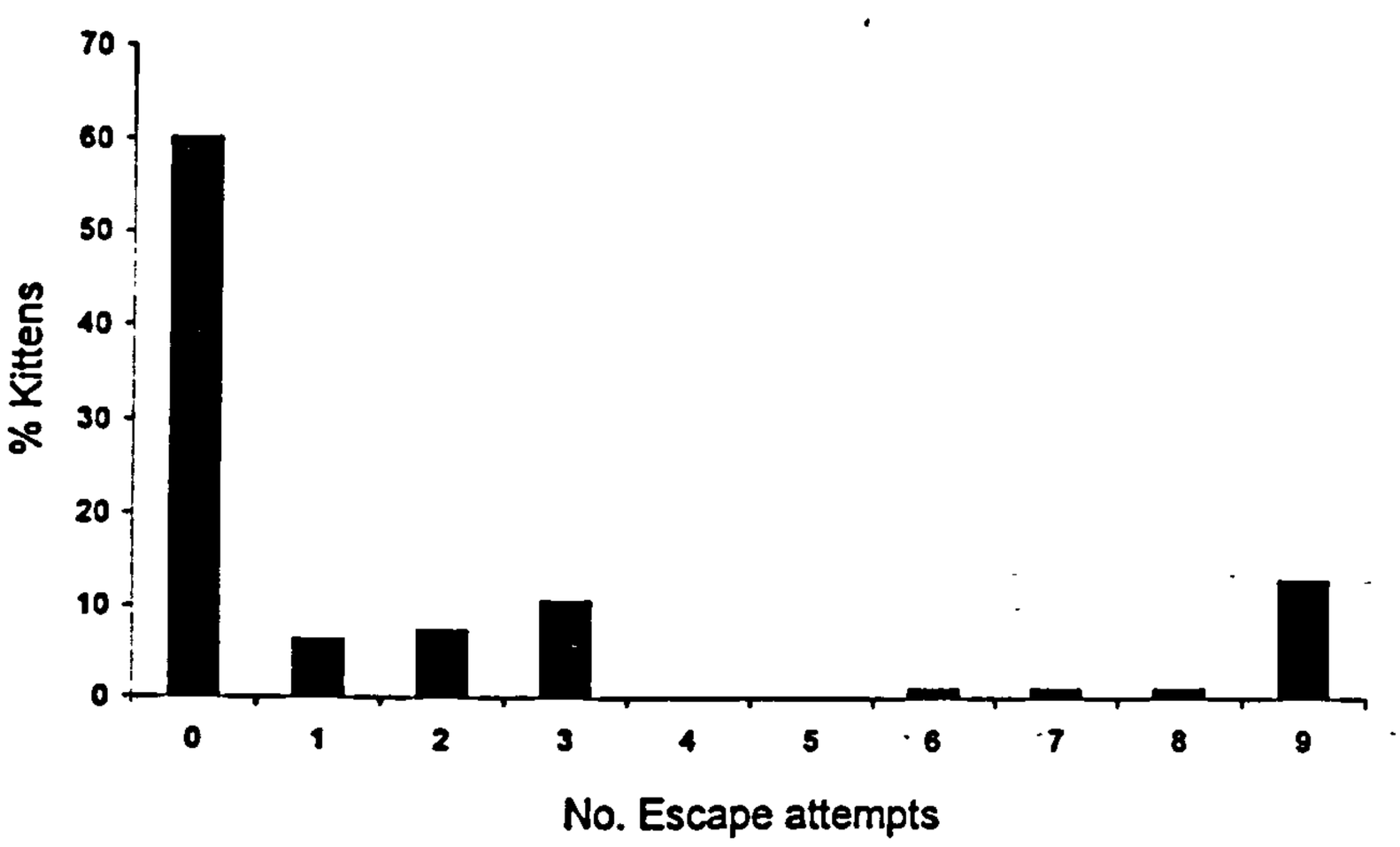
Appendix 3.2.10. Tenseness in approach test (18 months).



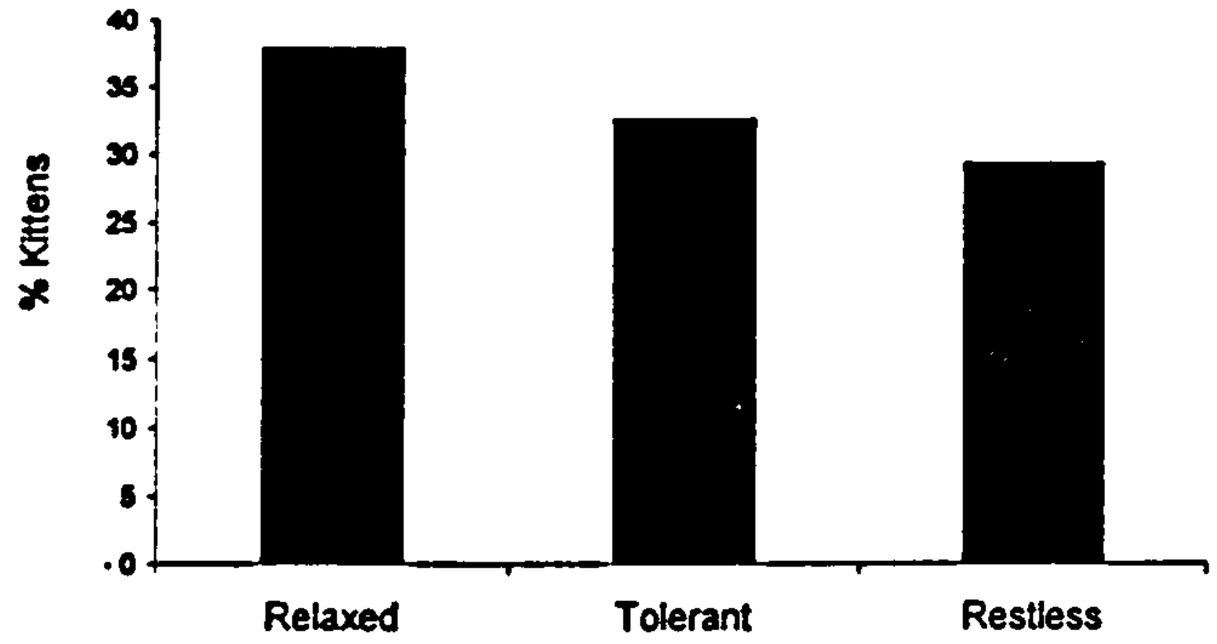
Appendix 3.2.11. Purring during approach test (18 months).



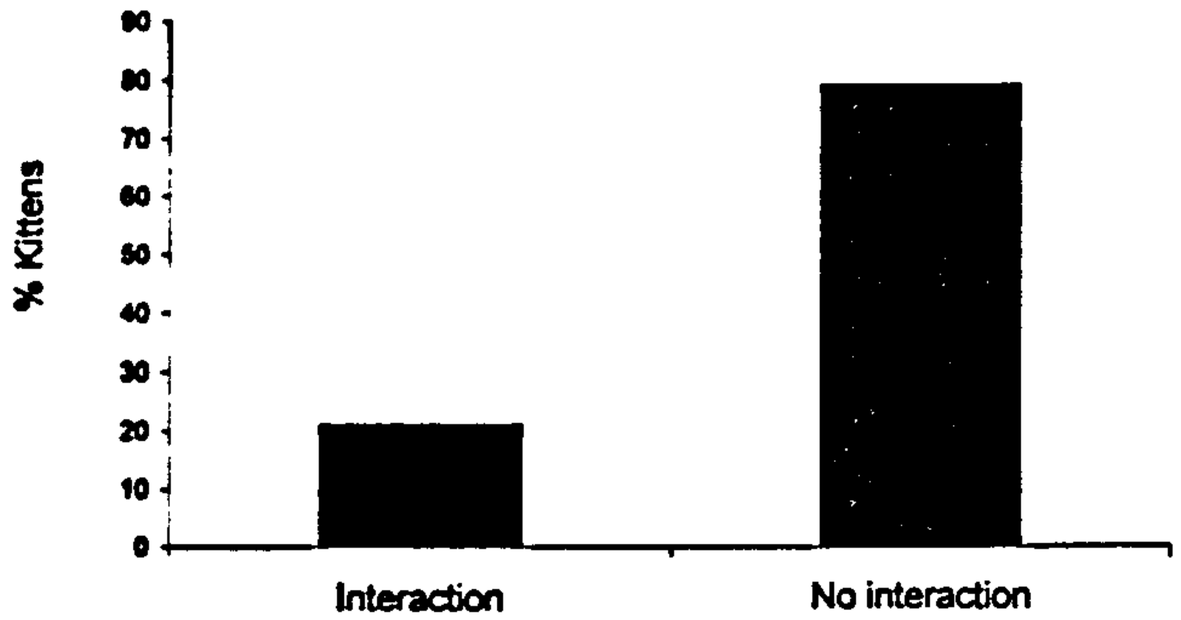
Appendix 3.2.12. Escape attempts during unfamiliar person handling test (18 months). Values of 9 represent the maximum recorded value plus one (see **Methods**).



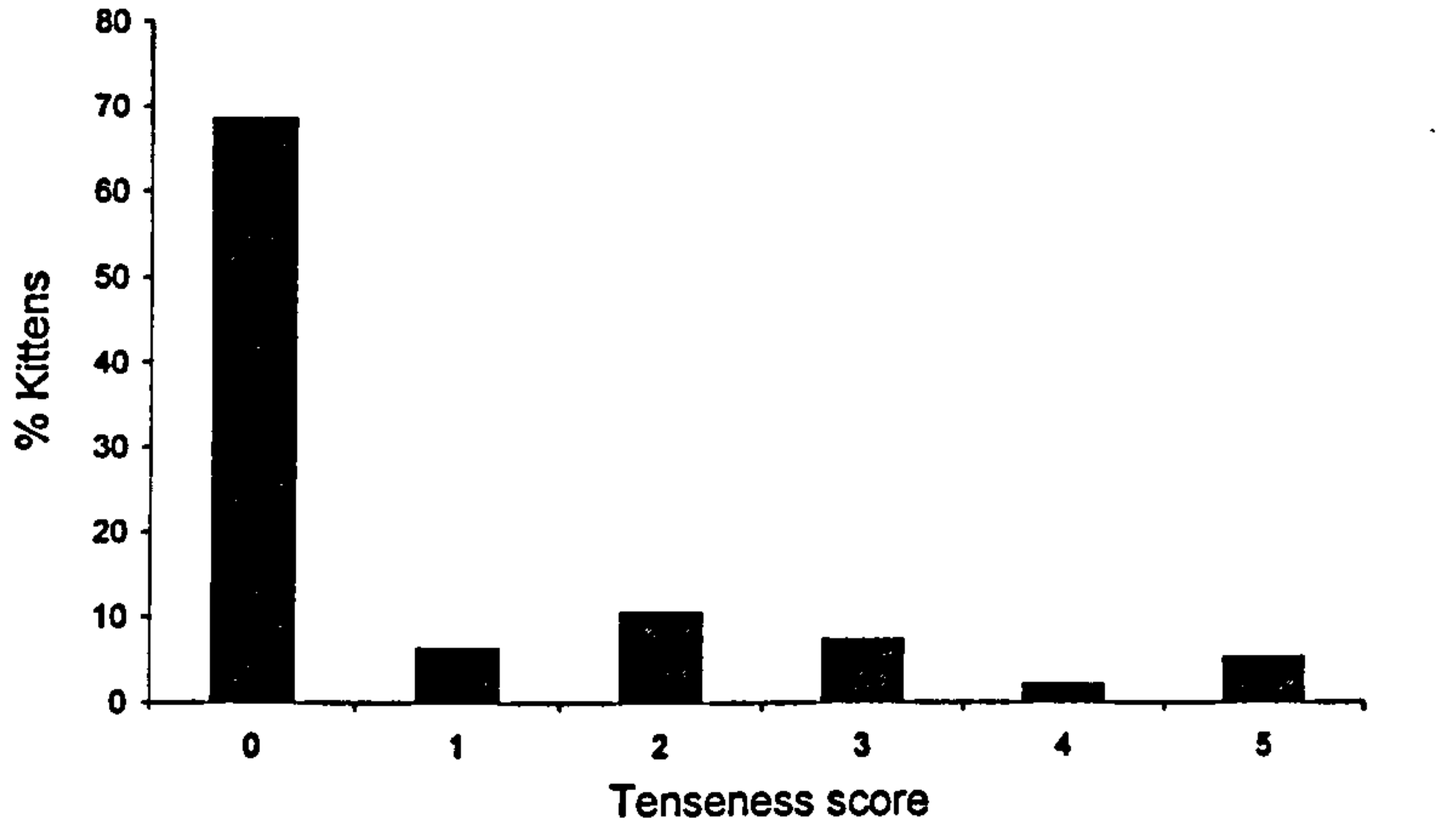
Appendix 3.2.13. State of activity during unfamiliar person handling test (18 months).



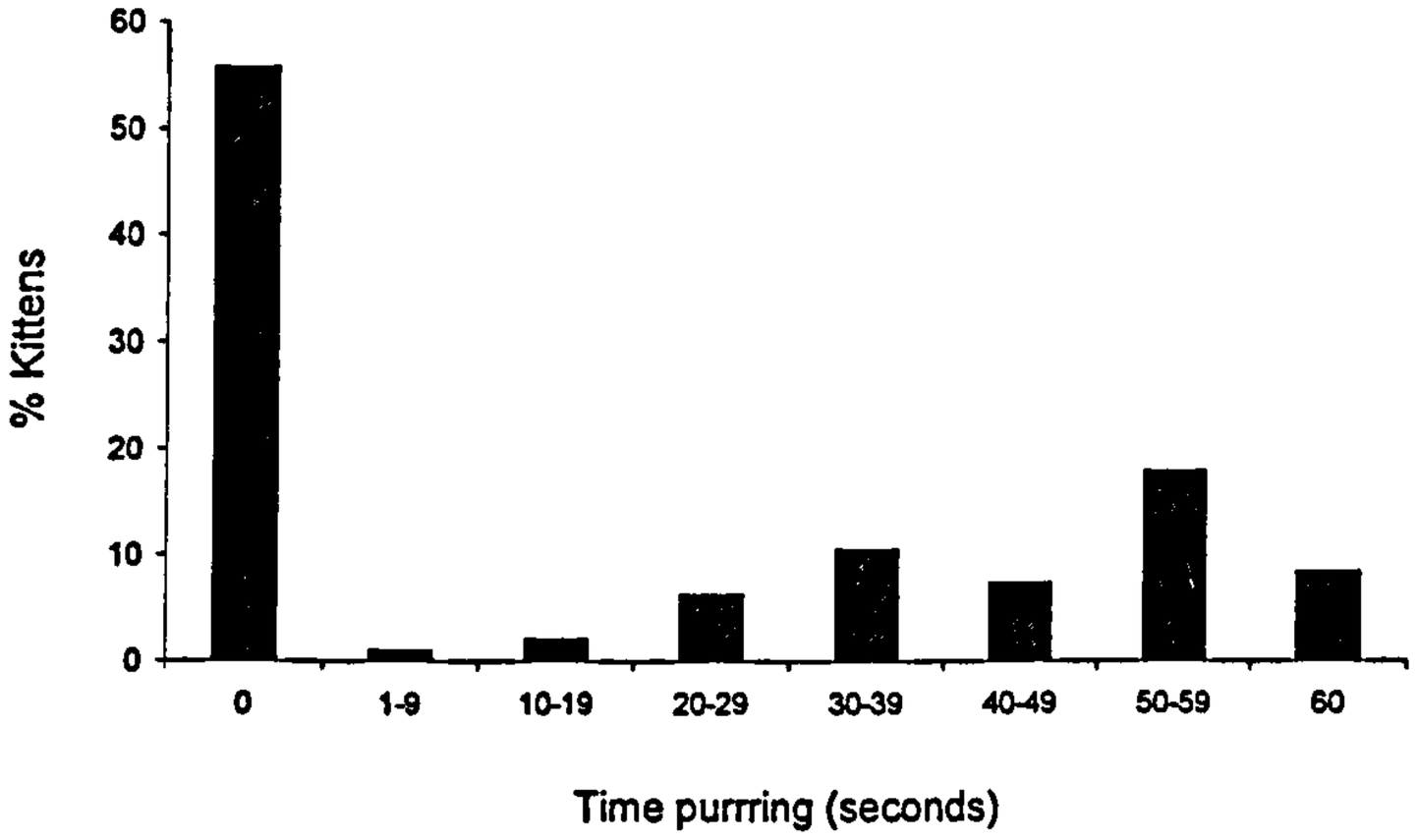
Appendix 3.2.14. Interaction in unfamiliar person handling test (18 months).



Appendix 3.2.15. Tenseness in unfamiliar person handling test (18 months).



Appendix 3.2.16. Purring in unfamiliar person handling test (18 months).



REFERENCES

Adkins C.A. and Stott, P. (1998). Home ranges, movements and habitat associations of red foxes *Vulpes vulpes,* in suburban Toronto, Ontario, Canada. *Journal of Zoology, London* **244**, 335-346.

Amos, B., Schlotterer, C. Diethard, T. (1993). Social structure of pilot whales revealed by analytical DNA profiling. *Science*, **260**, 670-672.

Amos, B. (1992). Analysis of polygamus mating systems using DNA fingerprinting. *Symp. zool. Soc. Lond*, **64**, 151-165.

Anon. (1994). The Metropolitan Domestic Cat: A survey of the population characteristics and hunting behaviour of the domestic cat in Australia. Reark Research.

Anon. (1995). Cat and dog ownership and product trends. *Anthrozoos*, **8**, 55.

Anon. (1998). Social Trends. National Statistics Institute Report, p.221.

Baldwin, J.A. (1975). Notes and speculation on the domestication of the cat in Egypt. *Anthropos.* **70**, 428-48.

Barratt, D.G. (1997). Home range size, habitat utilisation and movement patterns of suburban and farm cats. *Ecography*, **20**, 271-280.

Beaver, B.V. (1976). Feline behavioural problems. *Vet. Clinics Nth. Am.* **6**, 333-340.

Blumenburg, B. and Lloyd, A.T. (1980). Mutant allele frequencies in the domestic cat: a preliminary discussion of selection with particular reference to the United Kingdom and Eire. *Genetica*, **54**, 17-28.

Bradshaw, J.W.S. (1992). *The Behaviour of the Domestic Cat*. CAB International.

Bradshaw, J.W.S. and Cook, S.E. (1994). "Behavioural styles" in cats, detected from post-meal behaviour at four months, one year and two years of age. *End of Trial Report To WCPN.*

Bradshaw, J.W.S. and Cook, S.E. (1996). Patterns of pet cat behaviour at feeding occasions. *Applied Animal Behaviour Science*. **47**, 61-74.

Bradshaw, J.W.S. and Cook, S.E. (1997). Feral kittens: socialisation and suitability as pets. *Final Report to the Cats Protection League.*

Brown, S.L. and Bradshaw, J.W.S (1996) Social behaviour in a small colony of neutered feral cats. *FAB Journal*. **March 1996,** 35-37.

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

Burke, T. (1989). DNA fingerprinting and other methods for the study of mating success. *Trends in Ecology and Evolution*, 4(5), 139-144.

Burke, T., Hanotte, O., Bruford, M.W. and Cairns, E. (1991). Multilocus and single locus minisatellite analysis in population biological studies. In DNA fingerprinting: approaches and applications. Ed. T. Burke, G. Dolf. A.J. Jeffreys and R. Wolf. 155-168. Birkhauser Verlag, Basal/Switzerland.

Burt, W.H. (1943). Territoriality and home range concepts as applied to mammals. *Journal of Mammology*, **24**, 346-352.

Calhoon, R.E. and Haspel, C. (1989). Urban cat populations compared by season, subhabitat and supplemental feeding. Journal of Animal Ecology, **58**, 321-328.

Carding, A. (1975). The growth of the pet population in Western Europe and the implications for dog control in Great Britain. Pp. 66-68 in: R.S. Anderson, (ed.), *Pet Animals and Society*. London: Bailliere Tindall.

Caughley, G.(1977). *Analysis of vertebrate populations*. Wiley.

Chakraborty, R., Meagher, T.R. and Smouse, P.E. (1988). Parentage analysis with genetic markers in natural populations. 1. The expected proportion of offspring with unambiguous paternity. *Genetics*, **118**, 527-536.

Chalmers, N. (1983) The development of social relationships. In T.R. Halliday and P.J.B. Baker (ed.) *Animal Behaviour, Volume 3: Genes development and learning.* Blackwell Scientific, pp. 114-148.

Chipman, P. (1990). Influences on the Home Range Sizes of Domestic Cats *(Felis Catus)*, in an urban Environment. MSc. thesis, Manchester Polytechnic.

Clark, J.M. (1975). The effects of selection and human preference on coat colour gene frequencies in urban cats. *Heredity*, **35**, 195-210.

Clark, J.M. (1976). Variation in coat colour gene frequencies and selection in the cats of Scotland. *Genetica*, **46**, 401-412.

Clutton-Brock, J. (1994). The animal that walks by itself. In: *1994 Yearbook of Science and the Future*, pp. 156-77. Chicago: Encyclopedia Britannica, Inc.

Collard, R.R. (1967). Fear of strangers and play behaviour in kittens with varied social experience. *Child Development*, **38**, 877-91.

Cook, S.E. and Bradshaw, J.W.S. (1996). Reliability and validity of a holding test to measure "friendliness" in cats. **In:** "The Animal Contract", ISAZ 1996 Conference Abstract.

Cook, S.E. and Bradshaw, J.W.S. (Submitted). Stability in responses of pet cats to being held by an unfamiliar person, from weaning to three years of age.

Cooley, W.W. and Lohnes, P.R. (1971) *Multivariuate data analysis.* Wiley, New York.

Corbett, L.K. (1978). A comparison of the social organisation and the feeding ecology of the domestic cat *(Felis catus)*, in two contrasting environments in Scotland. *Carnivore Genetics Newsletter*, **3**, 269.

Crow, J.F. and Kimura, M. (1970). An Introduction to Population Genetics Theory. New York: Harper and Row.

Dards, J.L. (1978). Home ranges of feral cats in a Portsmouth dockyard. *Carnivore Genetics Newsletter*, **3**, 242-55.

Dards, J.L. (1983). The behaviour of dockyard cats: interactions of adult males. *Applied Animal Ethology*, **10**, 133-153.

Davies, N. B., Hatchwell, B.J., Robson, T. and Burke, T. (1992). Paternity and parental effort in dunnocks *Prunella modularis*: how good are male chick-feeding rules. *Anim. Behav.*, **43**, 729-745.

Dixon, K.R. and Chapman, J.A. (1980). Harmonic mean measure of animal activity centers. *Ecology*, **61(5)**, 1040-1044.

Dixon, A., Ross, D., O'Malley, S.L.C. and Burke, T. (1994). Paternal investment inversely related to paternity in the reed bunting. *Nature*, **371**, 698-700.

Doncaster, C.P. and Macdonald, D.W. (1996). Intraspecific variation in movement behaviour of foxes (*Vulpes vulpes*): a reply too White, Saunders and Harris. *Journal of Animal Ecology*, **65**, 126-127.

Doncaster, C.P. and Macdonald, D.W. (1997). Activity patterns and interactions of red foxes (*Vulpes vulpes*) in Oxford city. *J. Zool., Lond.* **241**, 73-87.

Edwards, A., Hammond, H. A., Jin, L., Caskey, C.T. and Chakraborty, R. (1992). Genetic variation at five trimeric tandem repeat loci in four human population groups. *Genomics*, **12**, 241-253.

Feaver, J.M., Mendl, M.T. and Bateson, P. (1986). A method for rating the individual distinctiveness of domestic cats. *Animal Behaviour*, **34**, 1016-1025.

Fitzsimmons, N.N. (1998). Single paternity of clutches and sperm storage in the promiscuous green turtle (Chelonia mydas). *Molecular Ecology*, **7**, 575-584.

Gautestad, A.O. and Mysterud I.M. (1995). The home range ghost. *Oikos*, **74**, 195-204.

Gilbert, D.A., Packer, C., Pusey, A.E., Stephens, J.C. and O'Brien, S.J. (1991). Analytical DNA fingerprinting in lions: parentage, genetic diversity and kinship. *Journal of Heredity*, **82**, 378-386.

Goodnight, K.F. and Queller, D.C. (1995). Relatedness 4.2b. Goodnight Software. Available at URL http://www.rice.edu/wasps.

Goodnight, K.F. and Queller, D.C. (1996). Kinship 1.12 Goodnight, K.F. and Queller, D.C. (1995). Relatedness 4.2b. Goodnight Software. Available at URL http://www.rice.edu/wasps.
Goodnight Software. Available at URL http://www.rice.edu/wasps.

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

Hamilton, J.B. (1969). Relationship of castration, spaying, and sex to survival and duration of life in domestic cats. *Journal of Gerontology*, **20**, 96-104.

Harris, S., Cresswell, W.J., Forde, P.G., Trewhella, W.J., Woolard, T. and Wray, S. (1990). Home -range analysis using radio-tracking data- a review of problems and techniques particularly as applies to the study of mammals. *Mammal Review,* **20**, 97-123.

Hart, B.L. and Barrett, D.V.M. (1973). Effects of castration on fighting, roaming, and urine spraying in adult male cats. *Journal of the American Veterinary Medical Association*. **163**, 290-293.

Hart B.L. and Hart L.A. (1984). Selecting the best companion animal: breed and gender specific behavioural profiles. In: The pet connection: its influence on our health and quality of life. Ed. R.I.L. Anderson, B.L. Hart and L.A. Hart. pp. 195-196. University of Minnesota press, Minneapolis.

Hespel, C. and Calhoon, R.E. (1987). Home ranges of free ranging cats, *Felis catus*, in Brooklin, New York. *Canadian Journal of Zoology,* **67**, 178-181.

Hoelzel, A.R. and Green, A. (1992). Analysis of population level variation by sequencing PCR-amplified DNA. *In: Molecular Genetic Analysis of Populations: A Practical Approach*. Ed. Hoelzel A.R. pp 159-187. IRL Press at Oxford University Press.

Hoger, H. (1994). Mutant allele frequencies in domestic cat populations in Austria. *Journal of Heredity*, **85**, 139-42.

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.

Hurni, H. and Rossbach, W. (1987). The laboratory cat Chap. 29. In **UFAW** handbook on the care and management of laboratory animals, 6th edition. Ed. T. Poole and R. Robinson, pp 476-492. Longman Scientific and Technical, Harlow.

Izawa, M., Doi, T. and Ono, Y. (1982). Grouping patterns of feral cats *(Felis catus)*, living on a small island in Japan. *Japan Journal of Ecology*, **32**, 373-82.

Izawa, M., Doi, T. and Ono, Y. (1991). Notes on the spacing pattern of feral cats at high density. *Bull. Kitakyushu Mus. Nat. Hist.*, **10**, 109-113.

Izawa, M. and Ono, Y. (1986). Mother-offspring relationships in the feral cat population. *Journal of the Mammal Society of Japan*, **11**, 27-34.

Jeffreys, A.J., Wilson, V. and Thein, S.L. (1985). Hypervariable "minisatellites" regions in human DNA. *Nature*, **314**, 67-73.

Jennrich, R.I. and Turner, F.B. (1969). Measurement of non-circular home range. *Journal of Theoretical Biology*, **22**, 227-237.

Karsh, E.B. (1983). The effects of early handling on the development of social bonds between cats and people. In *New perspectives on our lives with companion animals*, ed. A.H. Katcher and A.M.Beck. Philadelphia: University of Pennsylvannia Press.

Karsh, E.B. and Turner, D.C. (1988). The human- cat relationship. *In: The Domestic Cat: The Biology of its Behaviour*. Ed: D.C. Turner and P. Bateson, pp. 159-178. Cambridge: Cambridge University Press.

Kenward, R. (1987). *Wildlife Radio Tagging*. Academic Press , New York, N.Y.

Kerby, G. and Macdonald, D.W. (1988). Cat society and the consequence of colony size. In: *The Dometic Cat: The Biology of its Behaviour*. Ed: D.C. Turner and P. Bateson, pp. 67-82. Cambridge: Cambridge University press.

Kie, J.G., Baldwin, J.A. and Evans, C.J. (1994). Calhome: Home Range Analysis Program.

Kristensen, T. (1981). Feral cat control in Denmark. In: The Ecology and Control of Feral Cats, pp.68-72. Ed. Universities Federation for Animal Welfare. Potters Bar: UFAW.

Lade, J.A., Murray, N.D., Marks, C.A. and Robinson, N.A. (1996). Microsatellite differentiation between Philip Island and mainland Australian populations of the red fox *Vulpes vulpes*. *Molecular Ecology*, **5**, 81-87.

Langeveld, M. and Niewold, F. (1985). Aspects of a feral cat *(Felis catus L.)* population on a Dutch island. In: Proc. XVIIth Congress International Union of Game Biologists.

Larkin, R.P. and Halkin, D.H. (1994). A review of software packages for estimating animal home ranges. *Wildlife Society Bulletin*, 22, 274-287.

Leyhausen, P. (1988). The tame and the wild another Just-So Story? In: *The Domestic Cat: The Biology of its Behaviour*. Ed. D.C. Turner and P. Bateson, pp. 57-66. Cambridge: Cambridge University Press,

Liberg, O. (1980). Spacing patterns in a population of rural free roaming domestic cats. *Oikos*, **35**, 336-349.

Liberg, O. (1981). Predation and social behaviour in a population of domestic cats: an evolutionary perspective. Ph.D. thesis, University of Lund, Sweden.

Liberg, O. (1983). Courtship behaviour and sexual selection in the domestic cat. *Applied Animal Ethology*, **10**, 117-132.

Liberg, O. and Sandell M. (1988). Spatial organisation and reproductive tactics in the domestic cat and other felids. In: *The Domestic Cat: The Biology of its Behaviour*. Ed. D.C. Turner and P. Bateson, pp. 83-98. Cambridge: Cambridge University press.

Lorenz, K. (1937). The companion in the bird's world. *Auk*, **54**, 245-73.

Macdonald, D.W. and Apps, P.J. (1978). The social behaviour of a group of semi-dependent farm cats, *Felis catus*: a progress report. *Carnivore Genetics Newsletter*, **3**, 256-68.

Macdonald, D.W., Apps, P.J., Carr, G.M. and Kerby, G. (1987). Social dynamics, nursing coalitions and infanticide among farm cats, *Felis catus*. *Advances in Ethology*, **28**, 1-66.

McCune, S. (1992). Temperament and the welfare of domestic cats. D.Phil. Thesis, Cambridge University.

McCune, S. (1995). The impact of paternity and early socialisation on the development of cats' behaviour to people and novel objects. *Applied Animal Behavioural Science*, **45**, 109-114.

Martin, P. and Bateson, P. (1986). *Measuring Behaviour, an introductory guide*. Cambridge: Cambridge University Press.

Martinez, J.G., Burke, T., Dawson, D., Soler, J.J., Soler, M. and Moller, A.P. (1998). Microsatellite typing reveals mating patterns in the brood parasitic great spotted cuckoo (Clamator glandarius). *Molecular Ecology*, **7**, 289-297.

Maynard-Smith, J. and Parker, G.A. (1976). The logic of asymetrical contests. *Animal Behaviour*, **24**, 159-175.

Mendl, M. and Harcourt, R. (1988). Individuality in the domestic cat. *In: The Domestic Cat: The Biology of its Behaviour*. Ed. D.C. Turner and P. Bateson, pp. 41-56. Cambridge: Cambridge University Press.

Meier, M. and Turner, D.C. (1985). Reactions of house cats during encounters with a strange person: evidence for two personality types. *Journal of the Delta Society*, **2**, 45-53.

Mellersh, C. and Sampson, J. (1993). Simplifying detection of microsatellite length polymorphisms. *Biotechniques*, **15(4)**, 582-584.

Mennotti-Raymond, M.A., David, V.A., Claibourne Stephens, J. Lyons, L.A. and O'Brien, S.J. (1997a). Genetic individualization of domestic cats using feline STR loci for forensic applications. *Journal of Forensic Science*, **42(6)**, 1039-1051.

Mennotti-Raymond, M.A., David, V.A. and O'Brien, S.J. (1997b). Pet cat hair implicates murder suspect. *Nature*, **386**, 274.

Menotti-Raymond, M.A. and O'Brien S.J. (1995). Evolutionary conservation of ten microsatellite loci in four species of Felidae. *Journal of Heredity*.**86**, 319-322.

Mertens, C. and Turner, D.C. (1988). Experimental analysis of human-cat interactions during first encounters. *Anthrozoos*, **2**, 83-97.

Mirmovitch, V. (1995). Spatial organisation of urban feral cats *(Felis catus)* in Jerusalem. *Wildlife Research*. **22**. 299-310.

Morin, P.A. and Woodruff, D.S. (1992). Paternity exclusion using multiple hypervariable microsatellite loci amplified from nuclear DNA of hair cells. *In: Paternity in Primates: Genetic Tests and theories*. Ed. Martin, R.D., Dixon, A.F. and Wickings, E.J. pp 63-81. Basel, Karger.

Morin, P.A., Wallis, J., Moore, J.J. and Woodruff, D.S. (1994). Paternity exclusion in a community of wild chimpanzees using hypervariable simple sequence repeats. *Molecular Ecology*, **3**, 469-478.

Morris, P.A. (1988). A study of home range and movements in the hedgehog (*Erinaceus europaeus*). *Journal of Zoology*, **214**, 433-449.

Nasser, R. and Mosier, J.E. (1982). Feline population dynamics: a study of the Manhattan, Kansas, feline population. *Am. J. Vet. Res.*, **43(1)**, 167-170.

Natoli, E. (1985). Spacing patterns in a colony of urban stray cats *(Felis catus L.)* in the historic centre of Rome. Applied Animal Ethology, **14**, 289-304.

Natoli, E. and DeVito, E. (1988). The mating system of feral cats living in a group. In: The*Domestic Cat: The Biology of its Behaviour*. Ed. D.C. Turner and P. Bateson, pp. 99-109. Cambridge: Cambridge University Press.

Natoli, E. and De Vito, E. (1991). Agonistic behaviour, dominance rank, and copulatory success in a large multi-male feral cat, *Felis catus L.*, in central Rome. *Animal Behaviour*, **42**, 227-241.

Neville, P.F. and Remfry, J. (1984). Effect of neutering on two groups of feral cats. *The Veterinary Record*, **114**, 447-450.

Newman, K. and Wetton, J.H. (1996). Highly polymorphic microsatellites in the house sparrow *Passer domesticus*. *Molecular Ecology*, **5**, 307-309.

Newell, S.L. and Bradshaw, J.W.S. (1989). Social interactions between neutered feral cats. Paper presented at *Vth International Conference on the relationship Between Humans and Animals*. Monaco: November 1989.

Panaman, R. (1981). Behaviour and ecology of free ranging female farm cats (*Felis Catus L.*). *Zeitschrift fur Tierpschologie*, **56**, 59-73.

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

Packer, C. and Pusey, A.E. (1982). Cooperation and competition in coalitions of male lions: kin selection and game theory. *Nature*, **296**, 740-742.

Packer, C., Pusey, A.E., Rowley, H., Gilbert, D.A., Martenson, J. and O'Brien, S.J. (1991b). Case study of a population bottleneck: Lions of the Ngorongoro crater. *Conservation Biology*, **5 (2)**, 219-230.

Paetkau, D. and Strobeck, C. (1994). Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*, **3**, 489-495.

Paetkau, D., Calvert, W., Stirling, I. and Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4(3)**, 347-354.

Patronek, G.J., Beck, A.M. and Glickman, L.T. (1997). Dynamics of dog and cat populations in a community. Journal of the American Veterinary Association. **210 (5)**, 637-641.

Pemberton, J.M., Ambon, S.D., Guiness, F.E., Clutton-Brock, T.H. and Dover, G.A. (1992). Behavioural estimates of male mating success tested by DNA fingerprinting in a polygynous mammal. *Behavioural Ecology*, **3(1)**, 62-75.

Pontier, D. and Natoli, E. (1996). Male reproductive success in the domestic cat (*Felis catus L.*): A case history. *Behavioural Processes*, **37**, 85-88.

Price, E.O. (1984). Animal domestication. *Quarterly Revue of Biology*, **59**, 1-30.

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)**, 225-288.

Ragni, B. and Randi, E. (1986). Genetic variability and biochemical systems of domestic and wild cat populations *(Felis silvestris silvestris). J. Mamm.* **72(1)**, 79-88.

Randi, E. and Ragni, B. (1991). Genetic variability and biochemical systematics of domestic and wild cat populations (*Felis silvestris*: Felidae). *Journal of Mammalogy*, **72(1)**, 79-88.

Raymond, R. and Rousset, F. (1995a). An exact test for population differentiation. *Evolution*, **49(6)**, 1280-1283.

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

Rees, P. (1981). The ecological distribution of feral cats and the effects of neutering a hospital colony. In: The Ecology and Control of Feral Cats, pp. 12-22. Ed. UFAW: Potters Bar: UFAW.

Reichholf, J. (1983). Frequency and mobility of different colour types of domestic cats *Felis silvestris f. catus* in Southeastern Barvaria. *Saugetierkundl. Mitt.* **31,** 79-80.

Reisner, I.R., Houpt, K.A., Erb, H.N. and Quimby, F.W. (1994). Friendliness to humans and defensive aggression in cats: the influence of handling and paternity. *Physiology and Behaviour*, **55**, 1119-1124.

Rico, R., Rico, I. and Hewitt, G. (1996). 407 million years of conservation of microsatellite loci among fish species. Proc. R. Soc. Lond. B. **263**, 549-557.

Ridley, M. (1993). *Evolution*. Blackwell Scientific Publications.

Robinson, I.H., (1992). Social behaviour of the cat. In: *The Waltham Book of Dog and Cat Behaviour*, pp. 79-95. Ed. C. Thorne. Pergamon Press: Oxford.

Robinson, R. (1984). Cat. *In: Evolution of domestic animals*, pp. 217- 225. Ed. I.L. Mason. London: Longman.

Robinson, R. and Manchenko, G.P. (1981). Cat gene frequencies in cities of the U.S.S.R. *Genetica*, **55**, 41-46.

Robinson, R. and Silson, M. (1969). Mutant gene frequencies In cats of southern England. *Theoretical and Applied Genetics*, **39**, 326-329.

Roelk, M.E., Martenson, J.S. and O'Brien, S.J. (1993) The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Current Biology*, **3 (6)**, 340-350.

Ruiz-Garcia, M. (1993). Analysis of the evolution and genetic diversity within and between Balearic and Iberian cat populations. *Journal of Heredity*, **84**, 173-180.

Samuel, M.D., Pierce, D.J. and Garton, E.O. (1985). Identifying areas of concentrated use within the home range. *Journal of Animal Ecology*, **54**, 711-719.

Searle, A.G. (1949). Gene frequencies in Londons cats. *Journal of Genetics*, **49(3)**, 214-220.

Serpell, J.A. (1988). The domestication and history of the cat. In: *The Domestic Cat: The Biology of its Behaviour*. Ed, D.C. Turner and P. Bateson, pp. 151-158. Cambridge: Cambridge University Press.

Scaller, G.B. (1972). *The serengeti lion: a study of predator-prey relations*. Chicago: University of Chicago Press.

Smithers, R.H.N. (1968). Cat of the Pharaohs. *Animal Kingdom*, **61**, 16-23.

Strassmann, J.E., Goodnight, K.F., Klingler, C.J. and Queller, D.C. (1998). The genetic structure of swarms and the timing of their production in the queen cycles of neotropical wasps.

Sunnocks, P., De Barrow, P.J., Lushai, G., Maclean, N. and Hales, D. (1997). Genetic structure of an aphid studied using microsatellites: cyclic parthenogenesis, differentiated lineages and host specialization. *Molecular Ecology*. **6**, 1059-1073.

Taberlet, P., Camarra, J.-J., Griffin, S., Uhres, E. Hanotte, O., Waits, L.P., Dubois-Paganon, C, Burke, T. and Bouvet, J. (1997). Noninvasive genetic tracking of the endangered Pyrenean brown bear population. *Molecular Ecology*. **6**, 869-876.

Tabor, R. (1981). General biology of feral cats. The Ecology And Control of Feral Cats. pp. 5-11. Ed UFAW. Potters Bar: UFAW.

Tabor, R. (1983). The wildlife of the domestic cat. London: Arrow Books.

Tabor, R. (1989). The changing life of feral cats (*Felis catus L.*) at home and abroad. *Zoological Journal of the Linnean Society*, **95**, 151-161.

Todd, N.B., (1977). Cats and commerce. *Scientific American*, **237**, 100-107.

Thompson, D.M., Brown N.N. and Clague C.E. (1992). Routine use of hair root or buccal swab specimens for PCR analysis: advantages over using blood. *Clinica Chimica Acta*, **207**, 169-174.

Tickel, D., Blair, D. and Marsh, H.D. (1996). Marine mammal faeces as a source of DNA. *Molecular Ecology*, **5**, 456-457.

Turner, D.C. (1991). The ethology of the human-cat relationship. *Schweiz. Arch. Tierheilk*, **133**, 63-70.

Turner, D.C., Feaver, J., Mendl, M. and Bateson, P. (1986). Variations in domestic cat behaviour towards humans: a paternal effect. *Animal Behaviour*, **34**, 1890-2.

Turner, D.C. and Mertens, C. (1985). Home range size, overlap, and exploitation in domestic farm cats (*Felis catus*). *Behaviour*, **99**, 22-45.

Turner, D.C. and Stammbach-Geering K. (1990). Owner assessment and the ethology of the human-cat relationship. *Waltham symposium; Pets, benefits and practice*, 25-30.

Van Aarde, R. and Blumenburg, B. (1980). Genotype correlates of body and adrenal weight in a population of feral cats, *Felis catus. Carnivore*, **3**.

Vinogradov, A.E. (1994). Locally associated alleles of cat coat genes. *Journal of Heredity*, **85**, 86-91.

Walsh, P.S., Metzger D.A., Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR based typing from forensic material. *Biotechniques,* **10**, 506-513.

Warner, R.E. (1985). Demograpghy and movements of free ranging domestic cats in rural Illinois. *Journal of Wildlife Management,* **49(2)**, 340-346.

White, C.L. and Harris, S. (1994). Encounters between red foxes *(Vulpes vulpes):* implictions for territory maintenance, social cohesion and dispersal. *Journal of Animal Ecology*, **63**, 315- 327.

White, G.C. and Garrot, R.A. (1990). *Analysis of Wildlife Radio-Tracking Data.* Academic Press Inc. San Diego.

Wilson, D.S., Clark, A.B., Coleman, K. and Dearstyne, T. (1994). Shyness and boldness in humans and other animals. *Trends in ecology and evolution*, 9, 442-445.

Worton, B.J. (1987). A review of models of home range for animal movement. *Ecological Modelling*, **38**, 277-298.

Worton, B.J. (1989). Kernel methods for estimating the utilisation distribution in home range studies. *Ecology*, **70(1)**, 164-168.

Wray, S., Cresswell, W.J., White, P.C.L. and Harris, S. (1992). What, if anything, is a core area? An analysis of the problems of describing internal home range configurations. *Wildlife Telemetry: Remote Monitoring and Tracking of Animals* (eds I.G. Priede and S.M. Swift), pp. 256-271. Ellis Horwood, Chichester.

Yamane, Y., Doi, T. and Ono, Y. (1996). Mating behaviors, courtship rank and mating success of male feral cats (*Felis catus*). Journal of Ethology, **14**, 3-44.

Zajc, I., Mellersh, C., Kelly, E.P. and Sampson, J. (1994). A new method of paternity testing for dogs, based on microsatellite sequences. *Veterinary Record*, **135(23)**, 545-547.

Zeuner, F.E. (1963). *A History of Domesticated Animals*. London: Hutchinson.