THE EVOLUTION AND SOCIO-ECOLOGY OF TWO POPULATIONS OF THE VLEI RAT OTOMYS IRRORATUS

by

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For Dineshree and the Chinee mouse gang.

"... Besides, Individualism has really the higher aim..."
(The Picture of Dorian Gray: Oscar Wilde 1891)

PREFACE

This study was carried out in the Department of Biology, University of Natal, Durban, from March 1991 to December 1993, under the supervision of Professor J. Cooke and Dr K. Willan.

This thesis represents original work by the author and has not been submitted in any form to another university. Where use was made of the work of others it has been duly acknowledged in the text.

Neville Pillay

ACKNOWLEDGEMENTS

ABSTRACT

This work investigated two important evolutionary processes - speciation and adaptive variation - in two chromosomally-distinct allopatric Natal Midlands populations of the vlei rat Otomys irroratus. The two populations, at Kamberg and Karkloof, differ in the presence of a tandem fusion between chromosomes seven and 12 in the Kamberg karyotype. Speciation studies considered possible reproductive isolating mechanisms. In studies of adaptive variation, socio-ecological characteristics of both populations were investigated.

Data on breeding and postnatal development provided evidence of post-zygotic barriers. Interpopulation pairs had reduced breeding success compared to intrapopulation pairs, and some hybrids died before weaning. Surviving hybrids had reduced growth rates, and almost all were sterile.

In tests of pre-mating reproductive isolation, ethological barriers were emphasized. Individuals preferred same-population mates, suggesting the existence of mate recognition, which was achieved by means of population-specific courtship behaviour and communication, particularly olfactory, tactile and visual cues. No evidence of population-specific acoustic signals was found, although acoustic cues were associated with agonistic interaction, complementing other communicatory cues to promote increased aggression during interpopulation pairings.

Laboratory studies of behaviour and morphology and field work (trapping and habitat assessment) provided information about socio-ecological parameters. The Kamberg habitat was

harsher than the Karkloof one, as revealed by differences in
seasonal and spatial availability of food and cover. Cover
was the key determinant of the level of sociability of both
populations. Sparse, patchy cover selected for a partially
communal social system in Kamberg O. irroratus: females were
intrasexually tolerant and males were intrasexually highly
aggressive. This, in conjunction with male-biased sexual
dimorphism, implied that mating was polygynous. Abundant,
uniform cover selected for a dispersed social system in
Karkloof O. irroratus: females were intrasexually less
tolerant than males. Ritualized aggression between males and
a low degree of male-biased sexual dimorphism suggested that
males may have overlapping home ranges in nature and that
mating is promiscuous. Females possibly mated with dominant
males, however.

Contrasting social systems suggest that adaptation to
local environmental circumstances has occurred in allopatry,
and that Kamberg and Karkloof O. irroratus are undergoing
adaptive speciation. Post-zygotic and pre-mating
reproductive barriers appear to have evolved independently in
both populations, and could potentially impede gene flow
between the populations should they become sympatric. The
presence of the tandem fusion in the Kamberg karyotype which,
together with genetically-determined factors, may have caused
hybrid sterility, suggests that this population is a
chromosomally-determined incipient sibling species.

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CHAPTER 1

INTRODUCTION

MOTIVATION FOR THE STUDY

There has been much debate concerning the role of chromosomal change in speciation. While some authors maintain that there is no link between the two processes (inter alia Paterson 1985, Vrba 1985, John & Miklos 1988), others suggest that chromosomal mutation may initiate speciation (inter alia Capanna et al. 1985, Baker & Bickham 1986, Moritz 1986, Nevo 1991). Most of those in favour of the latter hypothesis maintain that chromosomal rearrangements are likely to inhibit interbreeding success between karyotypically dissimilar populations, primarily as the result of hybrid breakdown acting as a post-zygotic reproductive isolating mechanism. However, there is no consensus about the mode of chromosomal speciation. Some, like White (1978) associate chromosomal changes with sympatric (i.e. stasipatric) speciation. Other workers (Bickham & Baker 1980, Mayr 1982, Sites 1983) endorse instead an allopatric origin of chromosomal evolution and remain strongly opposed to the sympatric model, finding little evidence for its support.

Meester (1988) proposed a model of speciation in which chromosomal rearrangements may give rise to sibling species without overt phenotypic differentiation, if chromosomal change is not accompanied by gene mutations. Based on the ideas of other workers like Capanna et al. (1985) and Baker &

Bickham (1986), the model predicts that, should chromosomal speciation occur, post-zygotic reproductive isolation, caused by chromosomal imbalance in the hybrids, is likely to precede pre-mating reproductive isolation. Pre-mating barriers may then develop as a result of different mutation rates, selection pressures or random genetic effects in the sibling species during subsequent evolution (Dobzhansky et al. 1968, Rubinoff & Rubinoff 1971, Capanna et al. 1985). Furthermore, selection may operate against the energetic wastage of failed reproduction (i.e. hybrid breakdown), which would favour reinforcement or reproductive character displacement of previously developed post-zygotic barriers with pre-mating ones (Butlin 1987).

The Meester model is broad enough to encompass both sympatric and allopatric speciation. Meester (1988) concedes, however, that it is more parsimonious to propose that chromosomal speciation fits the allopatric model, particularly Mayr's (1982) peripatric model. This predicts that chromosomal divergence occurs in geographically isolated small populations which arise as a result of founder events.

This model of sibling speciation formed the basis of the Small Mammal Speciation Programme, currently being undertaken in the Biology Department, University of Natal. Initiated by J. Meester in the mid-1980's, the programme has attempted to study chromosomal and genotypic variation and the nature of reproductive isolation in sibling species (Meester et al. 1992). The programme has concentrated on southern African members of the murid subfamily Otomyinae - a taxon comprising eight morphologically fairly conservative rodents (Meester

1988, Meester et al. 1992), occupying various habitats in southern Africa (De Graaff 1981, Skinner & Smithers 1990). The taxon displays species-specific karyotypes (reviewed in Meester 1988), and may be divided into broadly mesophilic and xerophilic groups on biochemical grounds (Taylor et al. 1989).

Chromosomally, the vlei rat Otomys irroratus (Brants, 1827) is perhaps the most interesting of the otomyine species. Whereas all other otomyines studied appear to have intraspecifically uniform chromosome number and morphology (Meester et al. 1992), the vlei rat demonstrates remarkable intra- and interpopulation karyotypic variability (Robinson & Elder 1987, Contrafatto et al. 1992a, 1992b). Contrafatto et al. (1992b) have shown that 13 allopatric populations of O. irroratus examined thus far may be divided into three groups: Group A comprises five populations which have mostly acrocentric chromosomes, with three populations carrying what appears to be a tandem fusion between chromosomes seven and 12; Group B contains three populations which have totally heterochromatic short arms on the first seven pairs of autosomes; and Group C consists of five populations in which only four pairs of large autosomes are biarmed.

It therefore seems that active chromosomal speciation is occurring in O. irroratus at present, although there appears to be little or no interpopulation genetic or morphological variation. Allozyme electrophoretic data of 12 of the 13 O. irroratus populations (above) revealed that the gene pool of the taxon is more or less continuous, with low genetic distances between populations (Nei's genetic distance = 0.000

- 0.117), and that interpopulation genotypic differences are not reflected in either geographic location or karyotypic variation (Taylor et al. 1992). Phenotypically, multivariate morphometric analysis has revealed minor interpopulation differences in skull morphology (Hoffmann 1990), but renal morphology is unvarying between populations (Kearney 1990).

The karyotypic variation, together with the absence of significant phenotypic and genotypic differences, makes the vlei rat ideally suited to test the Meester model of chromosomal speciation. Moreover, O. irroratus breeds readily in captivity, and population-specific reproductive performance is highly consistent and predictable (N. Pillay & K. Willan Unpubl. data). On the basis of these findings, breeding (Pillay 1990, Pillay et al. 1992) and behavioural (Pillay 1990) studies were undertaken of three allopatric populations - Committee's Drift and Hogsback in the Eastern Cape Province and Karkloof in the Natal Midlands. The populations are karyotypically dissimilar, falling into two of the chromosomal groups recognized by Contrafatto et al. (1992b). Both Hogsback and Karkloof belong to Group A, with the Hogsback karyotype having a tandem fusion of chromosomes seven and 12, which is absent in the Karkloof karyotype. Committee's Drift belongs to group B. Pillay et al. (1992) found evidence of post-zygotic reproductive isolation between the Hogsback population and the other two populations, mainly as a result of the sterility and high pre-weaning mortality of hybrids. The presence of the tandem fusion in the Hogsback karyotype was apparently responsible for the sterility (Pillay et al. 1992), so that the Hogsback

population represents an incipient sibling species, according to Meester (1988). At the same time, increased aggression in inter- compared to intrapopulation encounters, as well as comparatively delayed mating in interpopulation pairings, provided evidence of pre-mating isolation between the Committee's Drift population and the other two populations (Pillay 1990).

It has recently become evident that two karyotypic forms of O. irroratus exist in the Natal Midlands. Apart from the population in the Karkloof forest, a representative of the Hogsback karyotype occurs in the Kamberg Nature Reserve. Although both the Kamberg and Karkloof populations share Group A karyotypes (i.e. mostly acrocentric chromosomes), they differ in respect of their diploid numbers: Kamberg - 2N = 24 - 27; Karkloof - 2N = 30 - 32. More importantly, the Kamberg karyotype possesses a tandem fusion between pairs seven and 12, as does the Hogsback population (Contrafatto 1992a, 1992b). Figure 1 shows representative karyotypes of the Kamberg and Karkloof populations. In the Karkloof karyotype, pairs a1 and a2 are supernumerary autosomes found only in this population (Figure 1). Intrapopulation differences in diploid number are due to variation in the number of small heterochromatic biarmed autosomes (pairs 8 and 9; Figure 1) which are analogous to B-chromosomes (Contrafatto 1992b).

Comparative studies of the Kamberg and Karkloof populations were undertaken, as the presence of the tandem fusion in Kamberg O. irroratus presented another opportunity to evaluate the Meester (1988) model of chromosomal
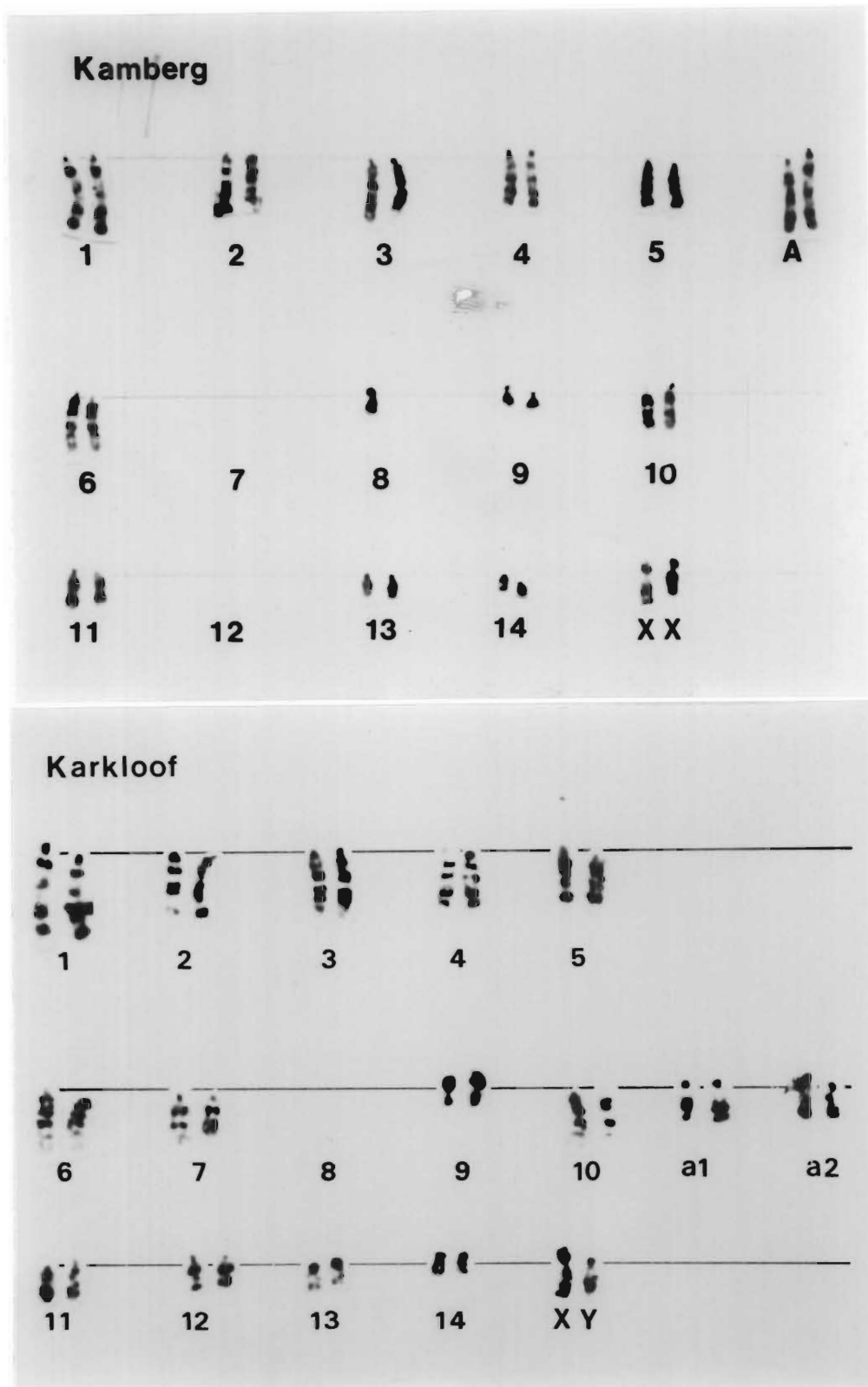
Figure 1. Representative karyotypes of Kamberg (2N = 27) and Karkloof (2N = 32) O. irroratus (Courtesy of G-C. Contrafatto). A tandem fusion (A) is present in the Kamberg karyotype. Autosomes a1 and a2 are unique to the Karkloof population.

speciation. The role of the tandem fusion in causing reduced
hybrid breeding success, which would act as a post-zygotic
isolating mechanism, was assessed. In addition, pre-mating
isolating mechanisms were investigated, as the chromosomal
speciation model (Meester 1988) predicts that post-zygotic
barriers will precede pre-mating ones.

Apart from the obvious karyotypic differences, the
Kamberg and Karkloof populations differ from the others
considered by Pillay (1990) in that they occur less than 50
km apart. In comparison, the Hogsback population studied by
Pillay (1990) occurs approximately 600 km away from the
Karkloof population. The close geographic proximity of the
Kamberg and Karkloof populations suggests that gene flow is
possible between them. This hypothesis stems mainly from
biochemical studies (Taylor et al. 1992). On the basis of
electrophoretic data of several southern African
O. irroratus populations, Taylor et al. (1992) maintained
that the taxon has a panmictic population structure, so that
gene flow is possible between populations, particularly those
in close proximity to one another. If gene flow is occurring
between the Kamberg and Karkloof populations, pre-mating
barriers can probably arise by reproductive character
displacement following secondary contact between the
populations (Butlin 1987).

In a treatise on evolutionary trends in the mole rat
Spalax ehrenbergi in Israel, Nevo (1991) emphasized the need
for a multidisciplinary approach in resolving species
groupings within this superspecies. He adds that
evolutionary studies in general should attempt to integrate

the twin processes of evolution: speciation and adaptation. The latter process considers the rather sensitive and often disputed study of adaptive variation. Non-adaptationists consider adaptationist views of the world to be nothing more than 'story-telling', which fail to recognize the significance of non-adaptive factors (e.g. genetic drift) in evolutionary change; see Gould (1982) and Krimbas (1984) for critiques on this subject.

While acknowledging the views of non-adaptationists, Nevo (1991) nevertheless maintains that organism - environment relationships provide the most credible explanations for many phenotypic, and sometimes genotypic, traits. Adaptation is also an important evolutionary force which may bring about speciation. Based on these ideas, Nevo (1991) proposed the concept of adaptive speciation, in which closely-related organisms differ in respect of their adaptation to local environmental conditions. Nevo (1982, 1991) maintains that explanations for adaptive speciation should be considered within the framework of the three basic tenets of science, i.e. they must be testable, falsifiable and predictive.

Several differences were found between the environmental conditions (e.g. rainfall, temperature) at the Kamberg and Karkloof localities. Given that the environment selects for population attributes, like behaviour and life histories (Hansson 1985, Hansson & Henttonen 1985, Nevo 1991), it is possible that Kamberg and Karkloof O. irroratus may be undergoing adaptive speciation, as are populations of S. ehrenbergi in Israel (Nevo 1991).

Adaptive variation may be investigated in any number of ways, and is best studied in a comparative fashion. A feature of previous studies on adaptation has been a multidisciplinary approach, usually involving the ecology of a species. For example, adaptive variation may be explained by eco-physiological (Nevo et al. 1979, Nevo 1991) or eco-geographical (Hansson 1985, Hansson & Henttonen 1985) rules. In the present study, the socio-ecological features of the Kamberg and Karkloof populations are considered. As a relatively new field, socio-ecology offers a novel approach by which to analyze adaptive variation. It is broadly defined as the comparative study of social structure in relation to ecology (Crook 1970), and considers mainly the functional significance of behaviour (see below). Besides environmental differences between the Kamberg and Karkloof localities, several population-specific behavioural characteristics were identified, permitting assessment of the socio-ecological characteristics of both populations.

APPROACH TO THE STUDY

This study is concerned with speciation and adaptive variation in Kamberg and Karkloof O. irroratus. Accordingly, the thesis is divided into two parts. The first part considers possible reproductive isolating mechanisms between the populations. Post-zygotic reproductive isolating mechanisms were identified by ascertaining whether or not hybrids were sterile or inviable; inviability is defined as the impairment of development and/or the failure of young to survive to adulthood (Mayr 1963). The breeding performance

of intrapopulation pairs was established and the results
formed the baseline for assessment of the reproductive
potential of interpopulation, backcross and hybrid-cross
pairs. In addition, the postnatal development of the progeny
of intra- and interpopulation pairs was studied in order to
compare the growth and developmental patterns, as well as the
fitness, of hybrid young in relation to those of 'purebred'
young.

Three broad classes of pre-mating barriers exist, namely
ecological, ethological and mechanical (Mayr 1963). Of
these, ethological components constitute the largest and most
effective class of isolating mechanisms. Ethological
barriers are often complex, and composed of several
modalities. In this study, a hierarchical approach is
adopted, in which a lack of mate recognition is regarded as
the most fundamental ethological barrier. Mate recognition
may be achieved when males and females recognize the
courtship behaviour of the other individual, so that mating
occurs. Courtship in turn relies on population-specific
communicatory cues, comprising essentially olfactory,
tactile, visual and auditory stimuli.

Throughout this study, recognition between conspecific
mates during courtship is viewed as indicating the existence
of a specific-mate-recognition-system (SMRS), as defined by
Paterson (1980, 1985). At the same time, contrasting SMRSs
are regarded as potential pre-mating isolating mechanisms.
The term SMRS is associated with the recognition concept of
speciation (Paterson 1985), and Mayr's (1982) isolation or
biological species concept is followed in this study.

Despite the apparent contradiction, SMRSs and reproductive isolation may be considered 'opposite sides of the same coin', in the sense that recognition of the signal-response chain between individuals will allow mating to occur, while, conversely, breakdown in this chain will be associated with speciation by reducing the chances of mating.

The second part of the thesis involves the socio-ecological characteristics of Kamberg and Karkloof O. irroratus. This aspect of the study was concerned primarily with social phenomena, which were established by means of laboratory studies of inter- and intrasexual social interaction involving animals from the same population. In addition, mate choice by females and sexual dimorphism in body size were also examined. However, sufficient field work (trapping and habitat assessment) was carried out to allow comparison of the ecological requirements of the two populations, and to provide a basis for interpretation of social trends observed in captivity (Eisenberg 1967).

In summary, the aims of the study were to ascertain: (i) whether either or both pre-mating and post-zygotic barriers would reduce or eliminate successful breeding between animals from Kamberg and Karkloof if environmental factors allowed contact between the two populations; and (ii) the existence of population-specific socio-ecological attributes. The study also addresses important issues in O. irroratus speciation, by relating the results of this study to those of previous cytogenetical (Contrafatto et al. 1992a, 1992b, Taylor et al. 1992) and recent molecular investigations

(mtDNA; Raubenheimer In prep.) of the populations in question.

Many parts of this thesis involve behavioural analyses which are used to provide information about social structure and about ethological isolating barriers to reproduction. The interpretation of data obtained here has therefore more often been functional than causal. This is to be expected since both evolution and social phenomena require functional explanations for behavioural acts (Huntingford 1984). The term function, as it applies to behaviour, refers to the critical consequence through which selection acts to maintain a behavioural character in its existing form (Hinde 1975); this, more loosely defined, refers to the adaptive significance of behaviour (McFarland 1987).

GENERAL BIOLOGY OF O. IRRORATUS

In this section, aspects of the general biology of O. irroratus are given which are essential to the interpretation of results presented in this thesis. More detailed information on the taxon is available elsewhere (inter alia Roberts 1951, Davis 1972, De Graaff 1981, Skinner & Smithers 1990).

Otomys irroratus is a medium-sized, stockily built rodent, which has a shaggy pelage, blunt face, deeply-grooved incisors, and a short well-haired tail; the ears are rounded and well-haired (Roberts 1951, De Graaff 1981, Skinner & Smithers 1990). The coat colour is essentially buffy-brown dorsally, with the throat, cheeks, sides and ventral parts

paler, although individuals of populations from comparatively xeric areas have a paler, ashy-grey pelage (Pillay 1990).

Perrin & Curtis (1980) showed that the digestive tract is specialized for herbivory, and the kidney has a medulla/cortex ratio and other characteristics typical of mesic physiological adaptation (Pillay et al. In press).

Otomys irroratus occurs widely on the southern savanna highveld, coastal montane and submontane grasslands, and in Cape Macchia (Davis 1974). It is confined mainly to the eastern parts of southern Africa but is found also in parts of the S.W. Cape Province, and an isolated population exists in eastern Zimbabwe and the adjacent parts of Mozambique (De Graaff 1981, Skinner & Smithers 1990; Figure 2); the Kamberg and Karkloof localities are shown in the insert in Figure 2.

The vlei rat is mesophilic - its preferred habitats comprise areas of lush vegetation with wet soil and standing water, as found along watercourses and marshes (inter alia Roberts 1951, Davis 1973, De Graaff 1981, Willan 1982, Skinner & Smithers 1990). It is not restricted to such areas, however, and may occur in montane grasslands, away from surface water (Davis 1973, Willan & Bigalke 1982). It is also known to inhabit areas where the rainfall is comparatively low and unpredictable, as in the Eastern Cape Province (Perrin 1980).

Otomys irroratus usually nests above ground in open 'platform' nests under the cover of dense vegetation, but it may utilize the abandoned burrows of other small mammals (Roberts 1951, De Graaff 1981, Skinner & Smithers 1990). The species is mainly crepuscular, with some activity during both
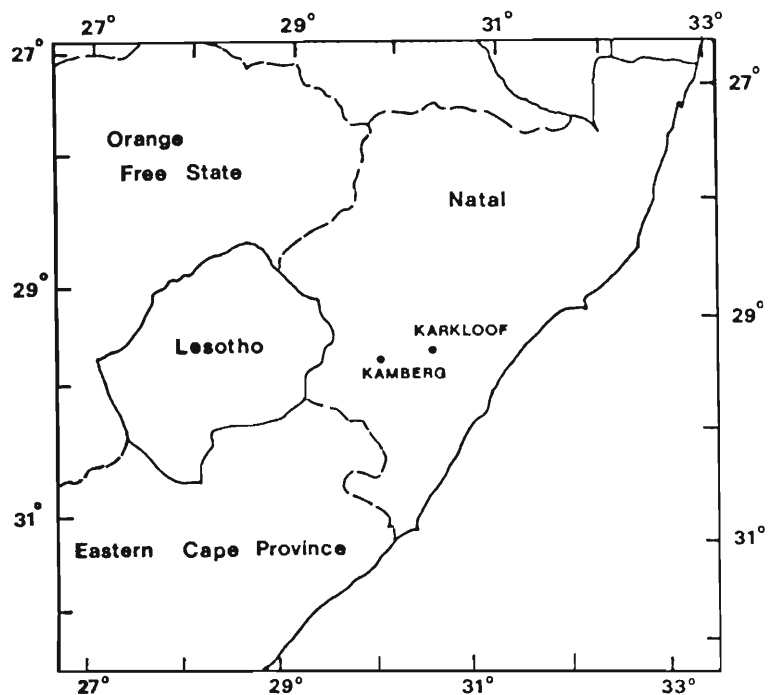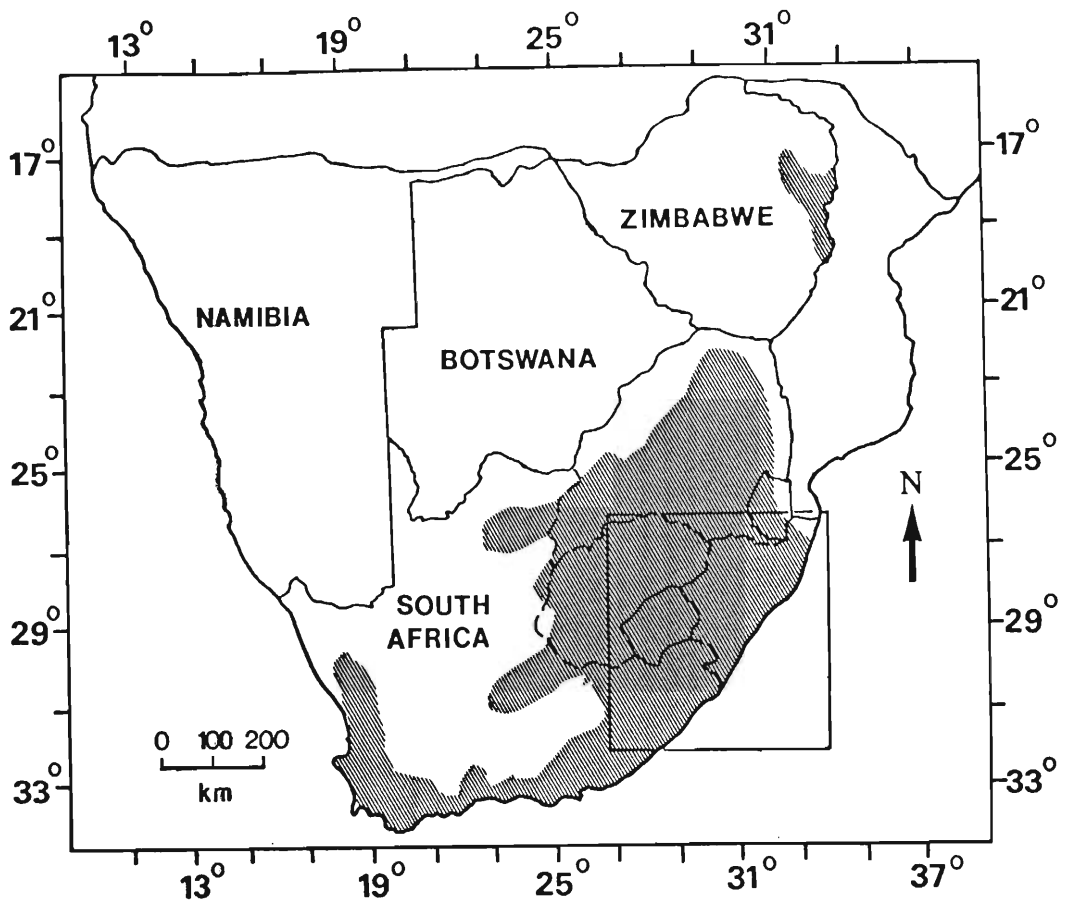
Figure 2. Southern African distribution of O. irroratus (after De Graaff 1981, Skinner & Smithers 1990). The approximate locations of the Kamberg and Karkloof localities are shown in the map of Natal (insert). Dotted line = provincial boundary, solid line = international boundary.

day and night (Davis 1972, Perrin 1981a). It is a strict herbivore (Davis 1973, Perrin & Curtis 1980), with a diet in nature consisting almost exclusively of grass, sedges and herbs.

In most parts of its range, O. irroratus usually breeds during the rainy season (Davis 1973, Willan 1982, Brown 1988), although animals in the Fish River Valley are apparently iteroparous (Perrin 1980). Gestation period is about 40 days, which is comparatively long for a small rodent (Willan & Meester 1989). Females display post-partum oestrous about a week after parturition (Pillay 1990). Litter sizes are small, with a mean litter size ranging from 1.48 in Eastern Cape O. irroratus (Perrin 1980) to 2.33 in a Transvaal highveld population (Davis & Meester 1981). Young nipple-cling during the first two weeks of life, suggesting that maternal care is well developed (Pillay et al. 1993); nipple-clinging is an adaptation to the surface nesting habits of the taxon (Willan 1990).

MODES OF SPECIATION IN O. IRRORATUS

As mentioned earlier, there is no concordance between the rates of genetic and chromosomal mutation in O. irroratus. Allozyme electrophoresis has demonstrated that O. irroratus populations show high heterozygosity, a lack of inbreeding and relatively low genetic distances between populations (Taylor et al. 1992). In contrast, chromosomal data indicate that the vlei rat is chromosomally polytypic (Robinson & Elder 1987, Contrafatto et al. 1992a, 1992b), with low heterozygosity among populations (Contrafatto et al. 1992b).

Hence, chromosomal change seems to be occurring more rapidly than genetic mutation in the vlei rat.

On the basis of the low genetic distances between the different O. irroratus cytotypes, and in particular that between the Hogsback population which has a tandem fusion (above; Pillay 1990) and other O. irroratus populations, Taylor et al. (1992) proposed that speciation in O. irroratus may be occurring in one of two ways. Chromosomal mutation (e.g. the tandem fusion), which could potentially cause reproductive isolation, may have arisen in sympatry, as postulated by White's (1978) stasipatric model of speciation. Alternatively, O. irroratus populations with novel karyotypes, like Hogsback, may have become geographically isolated in peripheral isolates as a result of recent founder events. These populations may be undergoing peripatric speciation, and may not yet have diverged allozymically; this prediction is based on Mayr's (1963) founder effect principle.

Population genetics studies have shown that the conditions for stasipatric speciation involve a sub-divided or fragmented population structure and high initial genetic distances between founder and ancestral populations, while peripatric speciation is characterized by a continuous population structure and low interpopulation genetic distances (Templeton 1980, Thompson & Sites 1986). Using these predictions, Taylor et al. (1992) suggest, on the basis of the panmictic population structure and low genetic distances between O. irroratus populations, that the mode of speciation in this taxon is most consistent with the founder

effect or peripatric model. Therefore, it is most parsimonious to assume that chromosomal, genetic and geographic divergence occurred as a series of founder events.

SOCIO-ECOLOGY

The field of socio-ecology is concerned with the adaptive significance of social systems, and is defined as the study of social structure in relation to ecology (Crook 1970). The evolution of social systems is influenced by both intrinsic (genetic) species characteristics and extrinsic (environmental) parameters (Crook 1970, Wilson 1975, Barash 1989). Importantly, social systems are dynamic and capable of evolutionary change, since genetically-determined patterns of social behaviour can be modified or altered by the environment (Crook 1970, Barash 1977, 1989).

Social systems may be territorial (asocial) on the one hand or communal (colonial) on the other, although a host of intermediates occur between these extremes. An important environmental determinant of social structure is the distribution and availability of resources - evenly distributed or clumped resources lead respectively to territoriality or communality (Wilson 1975). The relationship between resources and social structure is not always predictable, however (Ostfeld 1985, 1990), mainly due to the difficulties encountered in designing sound scientific experiments to test theoretical concepts. In addition, interpretation of socio-ecological phenomena is often ambiguous, and usually open to criticism because of a scarcity of supporting empirical evidence. Consequently,

generally applicable socio-ecological laws are lacking at present (Barash 1989).

In order to overcome these inadequacies, socio-ecologists have attempted to assess social structure in more general ecological terms, and have related levels of habitat stability or habitat harshness to degrees of sociability (Happold 1976, Willan 1982, Barash 1989). When resources are scarce and seasonally varying, the environment is harsh or unstable and animals living here are predicted to be communal, while habitats which are stable or less harsh select for territorial behaviour (Crook 1970, Barash 1989). The terms habitat stability and harshness are often used interchangeably (Thiery 1982, Willan 1982), but are not synonymous, since the concept of harshness encompasses that of stability (MacArthur & Wilson 1967, Brown 1988). In this study, the relative harshness of the Kamberg and Karkloof localities were assessed in order to establish the influence of habitat harshness on the social organization of each population.

There are several approaches to socio-ecological studies (Barash 1977), although the most useful involve a comparative method (Crook 1970, Jarman 1982, Barash 1989). Moreover, the most valuable comparative studies are those undertaken at the intrageneric and intraspecific levels, as they minimize the effects of phylogenetic differences, revealing the true adaptive significance of the social system under consideration (Barash 1989).

Previous behavioural and ecological work on O. irroratus has revealed that the taxon exhibits a dispersed (asocial)

social structure, incorporating temporal territoriality and adult isolation (Davis 1973, Willan 1982, Brown 1988). Willan (1982) predicted, however, that over the entire geographic range of O. irroratus, the degree of sociability within the taxon would increase with habitat instability/harshness. The present study sought to test this hypothesis.

ARRANGEMENT OF THE THESIS

Apart from the present introductory chapter, a general methods chapter (Chapter 2) and a general discussion and conclusions chapter (Chapter 9), the content of the thesis is divided into two sections, comprising four and two chapters respectively. The first section (Chapters 3 - 6) considers possible reproductive isolating mechanisms, while the second section (Chapters 7 & 8) is concerned with socio-ecological factors. The chapters within both these sections are written as manuscripts, with the first three chapters (3 - 5) having been submitted for publication in their present form. Because of this format there may be some repetition of introductory material or methodological details. The tables and figures are numbered in sequence for each chapter and not for the complete thesis. One reference section is given at the end of the thesis for convenience, and the pages are numbered sequentially.

CHAPTER 3

BREEDING AND POSTNATAL DEVELOPMENT [1]

INTRODUCTION

By means of a series of laboratory breeding trials,
Pillay et al. (1992) demonstrated the existence of reduced
reproductive success among three chromosomally distinct
allopatric populations (Committee's Drift, Hogsback,
Karkloof) of the vlei rat Otomys irroratus (Brants, 1827)
(Muridae, Otomyinae). Of significance in that study was that
a high proportion of hybrids from interpopulation pairings
involving Committee's Drift animals died before weaning, and
that almost all hybrids from Hogsback-cross pairings were
sterile. The presence of a tandem fusion in the Hogsback
karyotype was apparently responsible for this sterility.

The results of the breeding study (Pillay et al. 1992)
appeared to confirm the Meester (1988) model of chromosomal
speciation, which posits the formation of sibling species as
a consequence of chromosomal rearrangements which act as a
post-zygotic reproductive isolating mechanism.

In the course of ongoing studies on speciation in
populations of O. irroratus (see Meester et al. 1992), the
interbreeding potential of two allopatric Natal Midlands
populations (Kamberg and Karkloof) was ascertained. These

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[1]   This chapter, together with abstract,
    acknowledgements and references, is essentially the
    text of the paper submitted to the journal Acta
    Theriologica under the full title 'Post-zygotic
    reproductive isolation in two populations of the
    African vlei rat'.

populations were selected for study on the basis of their close geographic proximity (i.e. less than 50 km apart), suggesting that gene flow between them is possible in nature. More importantly, however, the populations are karyotypically dissimilar, with a tandem fusion in the Kamberg cytotype (Contrafatto et al. 1992b) which is absent in the Karkloof karyotype (Contrafatto et al. 1992a). Hence, the study presented another opportunity to evaluate the Meester (1988) model, and allowed testing of the hypothesis that a tandem fusion may dramatically reduce hybrid breeding success, as reported in the hybrids from interpopulation pairings involving Hogsback individuals (Pillay et al. 1992). In addition, chromosome and/or gene imbalances in hybrids are known to impair their growth and development (Dobzhansky and Levene 1951; Braverman et al. 1992), potentially rendering them inviable (Godfrey 1958, Lovecky et al. 1979); hybrid inviability (i.e. impaired development and/or failure to survive to adulthood) may act as a post-zygotic reproductive isolating mechanism (Mayr 1963). To ascertain whether or not hybrids in the present study were inviable, the postnatal development of young from intrapopulation and interpopulation pairs was compared.

MATERIALS AND METHODS

The populations represented in this study - Kamberg (29^{0}23'S, 29^{0}42'E) and Karkloof (29^{0}17'S, 30^{0}11'E) - are characterised by the following karyotypes: Kamberg - 2N = 24 - 27, all acrocentrics, tandem fusion between pairs seven

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BREEDING AND POSTNATAL DEVELOPMENT [1]

INTRODUCTION

By means of a series of laboratory breeding trials, Pillay et al. (1992) demonstrated the existence of reduced reproductive success among three chromosomally distinct allopatric populations (Committee's Drift, Hogsback, Karkloof) of the vlei rat Otomys irroratus (Brants, 1827) (Muridae, Otomyinae). Of significance in that study was that a high proportion of hybrids from interpopulation pairings involving Committee's Drift animals died before weaning, and that almost all hybrids from Hogsback-cross pairings were sterile. The presence of a tandem fusion in the Hogsback karyotype was apparently responsible for this sterility.

The results of the breeding study (Pillay et al. 1992) appeared to confirm the Meester (1988) model of chromosomal speciation, which posits the formation of sibling species as a consequence of chromosomal rearrangements which act as a post-zygotic reproductive isolating mechanism.

In the course of ongoing studies on speciation in populations of O. irroratus (see Meester et al. 1992), the interbreeding potential of two allopatric Natal Midlands populations (Kamberg and Karkloof) was ascertained. These

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[1] This chapter, together with abstract, acknowledgements and references, is essentially the text of the paper submitted to the journal Acta Theriologica under the full title 'Post-zygotic reproductive isolation in two populations of the African vlei rat'.

populations were selected for study on the basis of their close geographic proximity (i.e. less than 50 km apart), suggesting that gene flow between them is possible in nature. More importantly, however, the populations are karyotypically dissimilar, with a tandem fusion in the Kamberg cytotype (Contrafatto et al. 1992b) which is absent in the Karkloof karyotype (Contrafatto et al. 1992a). Hence, the study presented another opportunity to evaluate the Meester (1988) model, and allowed testing of the hypothesis that a tandem fusion may dramatically reduce hybrid breeding success, as reported in the hybrids from interpopulation pairings involving Hogsback individuals (Pillay et al. 1992). In addition, chromosome and/or gene imbalances in hybrids are known to impair their growth and development (Dobzhansky and Levene 1951; Braverman et al. 1992), potentially rendering them inviable (Godfrey 1958, Lovecky et al. 1979); hybrid inviability (i.e. impaired development and/or failure to survive to adulthood) may act as a post-zygotic reproductive isolating mechanism (Mayr 1963). To ascertain whether or not hybrids in the present study were inviable, the postnatal development of young from intrapopulation and interpopulation pairs was compared.

MATERIALS AND METHODS

The populations represented in this study - Kamberg ($29^0$23'S, $29^0$42'E) and Karkloof ($29^0$17'S, $30^0$11'E) - are characterised by the following karyotypes: Kamberg - 2N = 24 - 27, all acrocentrics, tandem fusion between pairs seven

and 12; Karkloof - 2N = 30 - 32, all acrocentrics
(Contrafatto et al. 1992a, 1992b).

Breeding

Maintenance of laboratory stocks and the conditions
under which breeding took place are described elsewhere (see
Pillay et al. 1992). The breeding performance of 70
intrapopulation, 40 interpopulation, 48 backcross and 12
hybrid-cross pairs (specified below as male x female) was
ascertained. Interpopulation trials comprised bi-directional
pairings (i.e. pairings of a Kamberg male and Karkloof
female, and of a Karkloof male and Kamberg female).
Backcross trials comprised pairings between progeny resulting
from interpopulation matings with individuals from the parent
stocks. Hybrid-crosses comprised pairings between hybrids
resulting from the same cross category. With the exception
of hybrids and some individuals used in intrapopulation
breeding, all animals used in breeding trials were known
breeders. For convenience, young of intrapopulation pairs
are referred to as purebred and those of interpopulation
pairs as hybrids.

Pairs were held together for a total of 150 days or
until the birth of the third litter, whichever occurred
earlier, but pairs that were unproductive after 80 days were
separated. Cages were inspected daily and the general
condition of the pairs was assessed. Pairs that engaged in
damaging fights were separated. After parturition, the cage
bedding was examined for evidence of dead neonates.
Post-mortem examination was confined to the external body

surface of neonates. Surviving juveniles were allowed to remain with the parents for the first 30 days, and thereafter transferred to holding cages. The postnatal development of selected litters representing intra- and interpopulation pairs was studied (see below).

The following were recorded for each litter: date of birth; size; and pre-weaning infant mortality. The fecundity (here defined as the product of mean litter size and the number of litters born per 150 days) was calculated for each pair. The Mann-Whitney U test was used to test for significance between data sets (Sokal and Rohlf 1987).

Postnatal Development

The postnatal development of 121 young representing 15 Kamberg, 22 Karkloof, eight Kamberg x Karkloof and 10 Karkloof x Kamberg litters was studied. Young were measured (i.e. head-body, hind-foot, ear and tail lengths) and weighed every second day for the first two weeks of life, and at weekly intervals thereafter to 14 weeks of age. The timing of the following physical developmental events was also monitored: opening of the eyes; first response to auditory and olfactory stimuli, indicated by a startle reaction to sucking sounds and gentle blowing across the face by the observer; development of locomotor abilities; weaning, indicated by the young changing over from suckling to solid food and by the inability to express milk from the mother; and the onset of sexual maturity. In addition, observations were made on the development of patterns of amicable and agonistic behaviour among littermates, and between young and their parents.

RESULTS

Breeding

Table 1 summarizes the results of intrapopulation, interpopulation, backcross and hybrid-cross breeding trials. All intrapopulation pairs produced offspring. In contrast, interpopulation breeding success was reduced, with only 47.5 % of pairs producing young. Moreover, males of 10 of the 12 unsuccessful Kamberg x Karkloof pairs (Table 1) attacked and seriously wounded their mates. No damaging fights were observed in any other pairing.

Backcross and hybrid-cross breeding were severely impaired. All 57 hybrids which survived beyond weaning (Table 1) were used in either or both backcross and hybrid-cross breeding trials, but only one hybrid (a Karkloof x Kamberg female paired with a Karkloof male) produced young. This represented a hybrid breeding success of < 2 %.

Both mean litter size and mean fecundity of the interpopulation pairs were significantly lower than those of the intrapopulation pairs.

While low pre-weaning mortality was characteristic of progeny of intrapopulation pairs (3.9 - 6.2 %), hybrids experienced higher mortality (26 - 37.5 %; Table 1). More importantly, 68 % of the hybrid neonates (17 of 25) which died during the study were injured, suggesting that infanticide may have been the cause of death.

Postnatal Development

The timing of the growth and behavioural developmental parameters is given in Table 2. Except as regards the age at

Table 1. Reproductive data in respect of the pairings indicated. n = sample size, 2 SE given in brackets.

Pairings	Matings n	success	Litter size n	X̄	Fecundity[1] n	X̄	Pre-weaning mortality Total young	Number died	%
Intrapopulation									
Kamberg	30	30	83	2.27 (0.17)	30	6.40 (0.61)	193	12	6.2
Karkloof	40	40	105	2.47 (0.15)	40	6.03 (0.61)	256	10	3.9
Totals	70	70	188	2.37 (0.11)	70	6.19 (0.44)	449	22	4.9
Interpopulation									
Kamberg x Karkloof	20	8	15	2.07 (0.45)[2]	8	4.14 (1.10)[3]	32	12	37.5
Karkloof x Kamberg	20	11	28	1.89 (0.30)[4]	11	4.71 (0.58)[5]	50	13	26.0
Totals	40	19	43	1.95 (0.25)	19	4.47 (0.57)	82	25	30.5
Backcrosses involving young of:									
Kamberg x Karkloof	24	0	-	-	-	-	-	-	-
Karkloof x Kamberg	24	1	1	1.00 -	3	1.00 -	1	0	0.0
Totals	48	1	1	1.00 -	3	1.00 -	1	0	0.0
Hybrid-crosses involving young of:									
Kamberg x Karkloof	6	0	-	-	-	-	-	-	-
Karkloof x Kamberg	6	0	-	-	-	-	-	-	-
Totals	12	0	-	-	-	-	-	-	-

1 = product of mean litter size and the number of litters born per 150 days.
2 - 5 = significantly smaller at the 5 % significant level from appropriate values of the Kamberg and Karkloof intrapopulation pairings.

Table 2. Timing of the onset of postnatal developmental events for progeny resulting from the pairings indicated. With the exception of sexual maturity which is expressed in weeks, all other values represent days after birth. Kam = Kamberg, Kar = Karkloof, n = number of young studied.

Pairings	n	Eyes open	Hearing	Olfaction	Weaning	Sexual maturity Male	Female	Co-ordinated locomotion	Social behaviour Amicable	Agonistic
Intrapopulation										
Kamberg	33	0-2	0-2	0-2	12-14	7-10	5-7	0-4	0	4-6
Karkloof	49	0-2	0-2	0-2	12-14	6-11	6-8	0-4	0	4-8
Interpopulation										
Kam x Kar	17	0-2	0-2	0-2	11-14	6-10	6-8	0-4	0	4-6
Kar x Kam	22	0-2	0-2	0-2	12-14	6-10	5-8	0-4	0	5-8

sexual maturity, the development of males and females resulting from the same group of pairings (e.g. Kamberg or Kamberg x Karkloof) was not materially different, and no distinction is made between the sexes. The developmental

phenomena observed in the present study (Table 2) were remarkably similar to those described for O. irroratus from the Transvaal highveld (Davis and Meester 1981) and Eastern Cape Province (Pillay 1990); detailed descriptions of O. irroratus postnatal development are available from these studies. Moreover, the development of purebred and hybrid young in the present study was indistinguishable.

Of all the physical developmental parameters, only increase in body mass revealed differences between hybrid and purebred young, and therefore only body mass is considered here. Growth rates in respect of head-body, tail, hind-foot and ear lengths of all purebred and hybrid young were equivalent to those of O. irroratus from the Transvaal highveld (see Davis and Meester 1981).

In view of apparent sexual dimorphism in body mass in the Kamberg population (N. Pillay Unpubl. data), growth rates of male and female young are considered separately in Figures 1 and 2 respectively. Theoretical von Bertalanffy growth curves (von Bertalanffy 1960, Creighton and Strauss 1986) were fitted to the mean values representing increase in body mass of purebred and hybrid young of each sex (Figures 1 and 2). Age-specific data for the growth curves were derived from the von Bertalanffy growth equation: $W_t = W_a [1-e^{-k(t-t0)}]^3$ where W_t = mass at time t, W_a = asymptotic mass, k = rate of change of mass increments, and t0 = theoretical parameter indicating the commencement of growth. Table 3 provides the theoretical von Bertalanffy equations for increase in body mass of purebred and hybrid young.
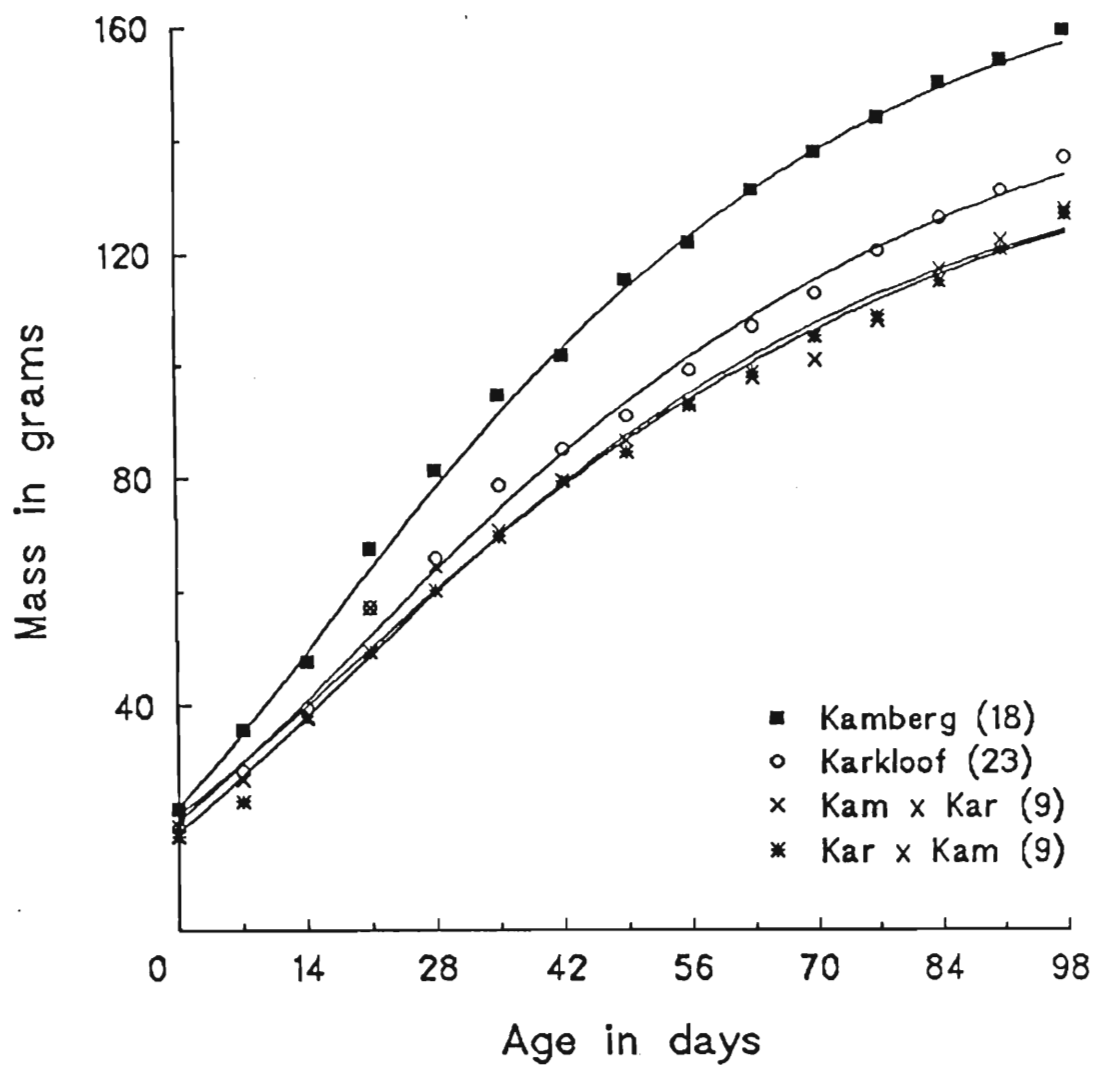
Fig. 1. Mass increase of males resulting from the pairings
indicated. Mean values indicated with symbols, and fitted von
Bertalanffy growth curves with lines. Sample size given in
brackets. Kam = Kamberg, Kar = Karkloof.
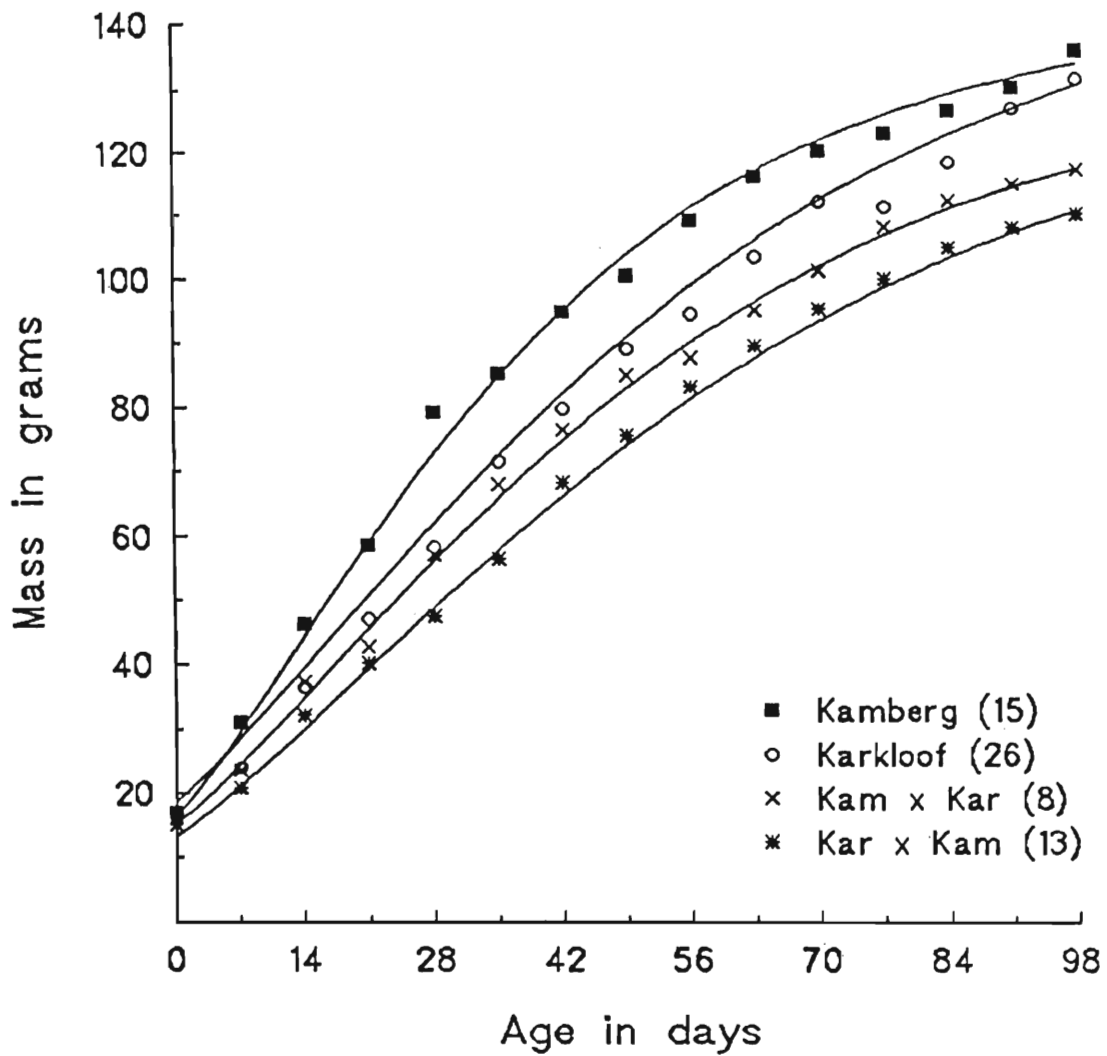
Fig. 2. Mass increase of females resulting from the pairings indicated. Mean values indicated with symbols, and fitted von Bertalanffy growth curves with lines. Sample size given in brackets. Kam = Kamberg, Kar = Karkloof.

Table 3. Von Bertalanffy growth equations[1] for increase in body mass of males and females resulting from the pairings indicated. Kam = Kamberg, Kar = Karkloof. Sample sizes as in Figure 1 (males) and Figure 2 (females).

Young	Males Growth equations	Females Growth equations
Intrapopulation		
Kamberg	$167.6[1-e^{-0.034(t+21.15)}]^3$	$144.3[1-e^{-0.028(t+19.67)}]^3$
Karkloof	$153.5[1-e^{-0.024(t+29.01)}]^3$	$142.0[1-e^{-0.025(t+28.78)}]^3$
Interpopulation		
Kam x Kar	$142.9[1-e^{-0.022(t+32.15)}]^3$	$131.6[1-e^{-0.026(t+21.60)}]^3$
Kar x Kam	$140.5[1-e^{-0.022(t+27.67)}]^3$	$130.3[1-e^{-0.023(t+27.37)}]^3$

1 - $W_t = W_a[1-e^{-k(t-t0)}]^3$ (see text for detail).

It is evident from Figures 1 and 2 that at all ages Kamberg males and females were respectively heavier than males and females produced by other pairs. With the exception of the pre-weaning development of Kamberg x Karkloof males, which was similar to that of Karkloof males, all hybrids were smaller than purebred young throughout development (Figures 1 and 2). This observation is supported by consideration of estimates of asymptotic mass (Table 3) which suggest that the parent types grow larger than their hybrids. In addition, slower growth rates (i.e. lower k values of the growth equations; Table 3) were recorded for the hybrids, although the growth rate of Kamberg x Karkloof females was within the range of purebred females.

DISCUSSION

Breeding

The results of the breeding study suggest reduced fertility at the interpopulation level: many (> 50 %) interpopulation pairs failed to produce young, while the overall reproductive success of those pairs that produced young was lower compared to intrapopulation pairs.

As in the present study, hybrids resulting from interpopulation pairings involving other O. irroratus populations also experienced increased pre-weaning mortality (see Pillay et al. 1992; above). In their study, Pillay et al. (1992) maintained that hybrid inviability was the result of chromosome or gene abnormalities which could cause physiological breakdown in the hybrids (Dobzhansky and Levene 1951, Burton 1990). However, hybrid mortality in the present study was apparently due to infanticide, and it is likely that genetically-determined maladaptive behaviour of neonates may have resulted in their being fatally wounded by the mother, as reported in other rodent species (Conley and Bell 1978, Harper 1981).

As a consequence of high levels of aggression which resulted in damaging fights, about half of the Kamberg x Karkloof pairs had to be separated shortly after pairing. It is tempting to speculate that such highly aggressive interaction during interpopulation pairings may have been the result of impaired recognition by either or both sexes of the courtship behaviour of the other individual. This issue is pursued below.

It is possible that the tandem fusion between chromosomes seven and 12 of the Kamberg karyotype (Contrafatto et al. 1992b) was responsible for the severely reduced breeding success of backcross and hybrid-cross pairs. Matings in which one of the parents has a tandem fusion may result in hybrids with a 75 % loss in gamete viability (Moritz 1986). Similarly, hybrid sterility as a result of a tandem fusion has been reported in other studies involving O. irroratus (Pillay et al. 1992), as well as in a study of the dik-dik Madoqua kirki (Ryder et al. 1989).

Only one out of the 57 hybrids used in backcross and hybrid-cross breeding trials produced young, indicating that more than 98 % of hybrids were sterile. However, it would have been expected that some hybrids would not have inherited the tandem fusion and may have produced young. Also, if the tandem fusion was responsible for sterility in those hybrids with the fusion, at least 25 % of the gametes of these hybrids would have been viable, and it is probable that some would have had breeding success. This is particularly true of those hybrids (with tandem fusions) involved in backcross breeding, since these were paired with members of the parent stocks which were apparently fertile; in hybrid-crosses, both hybrids could have produced unbalanced gametes, reducing the chances for successful breeding. Hence, it may be argued that other factors either on their own (hybrids with no fusion) or in combination with the tandem fusion (hybrids with the fusion) were responsible for the breakdown in hybrid breeding. The literature indicates that genetically-determined morphological defects (inter alia Mayr

1963) and/or physiological and behavioural abnormalities (inter alia Dobzhansky et al. 1968) of hybrids might have reduced their mating success.

Unlike interpopulation hybrids, viable and fertile young were produced by pairings between individuals representing different cytotypes within both populations (e.g. between 2N = 24 and 2N = 27 Kamberg individuals, and between 2N = 30 and 2N = 32 Karkloof individuals). The differences in diploid number of the different cytotypes reflect intrapopulation variation in the number (from 0 to 3) of copies of a pair of B-chromosomes (Contrafatto et al. 1992b). With some exceptions, the influence of B-chromosomes on the phenotype is negligible (Volobujev 1981, Jones and Rees 1982), and it was predictable that phenotypically and reproductively fit purebred offspring would have been produced by cytotype cross pairings.

Postnatal development

Generally, the growth and development of hybrid and purebred young were indistinguishable. In terms of increase in body mass, however, hybrids were comparatively small throughout development, and experienced comparatively slow overall growth rates.

The impaired growth of hybrids may have been brought about by the disruption of chromosome and/or gene complexes during hybridization. This usually leads to physiological and biochemical breakdown in the hybrids, as reported in some Drosophila species (Dobzhansky and Levene 1951, Dobzhansky et al. 1968, Orr 1990, Braverman et al. 1992).

Interspecific pairings between deer (Peromyscus maniculatus) and oldfield (P. polionotus) mice produced hybrids with depressed growth rates (Rogers and Dawson 1971). In this case, placental development of the hybrid foetus was retarded, inhibiting the prenatal growth of hybrids. It was suspected that maternal-foetal immunological incompatibility inhibited hybrid development (Rogers and Dawson 1971). The Peromyscus hybrids never overcame the intra-uterine suppression of their development, and, as adults, hybrids were smaller than the parent types (Dawson 1971). Such incompatibility between mother and foetus may have also contributed to the comparatively slow development of hybrids in the present study. This hypothesis remains tentative, however, as no attempts were made to establish immunological incompatibility between hybrid young and their parents.

CONCLUSIONS

The results obtained in this study provide convincing evidence that gene flow between Kamberg and Karkloof O. irroratus would be hindered should these populations meet in nature. Reduced interbreeding success, as a consequence of lower hybrid fitness (i.e. increased pre-weaning mortality or inhibited growth), may function as a post-zygotic reproductive isolating mechanism.

As in other O. irroratus populations (e.g. Hogsback, Eastern Cape Province; Pillay et al. 1992), the presence of the tandem fusion in the Kamberg karyotype is particularly significant in the breakdown of reproduction, and the Kamberg population may be regarded as an incipient sibling species,

as predicted by Meester (1988). However, this conclusion requires testing by means of cytological studies of the gametes of hybrids. The production of sterile/inviable hybrids is energetically wasteful, and represents a reduction of the reproductive potential and inclusive fitness of individuals involved in interpopulation matings. Thus, selection is likely to rapidly favour pre-mating barriers to reproduction (inter alia Baker and Bickham 1980, Solginac 1981, Capanna et al. 1985). High levels of aggression, apparently causing Kamberg males to seriously injure Karkloof females, hint at the possibility of underlying differences in population-specific courtship behaviour. For successful courtship behaviour to occur (i.e. fertilization to be achieved), mating partners must recognize one another as potential mates. This recognition is achieved by means of a signal-response communication chain, defined as the specific-mate-recognition-system (Paterson 1980, 1985). It would therefore appear that there is a breakdown in mate recognition between individuals representing the two populations, and that potential pre-mating reproductive isolating mechanisms have also evolved in the Kamberg and Karkloof O. irroratus.

CHAPTER 4

CHOICE AND OLFACTORY DISCRIMINATION TESTS [1]

INTRODUCTION

Mutual recognition as potential mates by males and females of the same species is essential for the maintenance of species continuity; it is equally important that individuals recognize members of other closely related species as non-mates. Such recognition may be achieved during courtship. The courtship behaviour of conspecifics therefore depends upon a male-female communication system composed of species-specific signals and responses, which has been referred to as the specific-mate-recognition-system (SMRS; PATERSON 1980, 1985). The signal-response chain during courtship may involve auditory, olfactory, tactile and/or visual cues (KOEPFER 1987).

Where closely related species occur in sympatry, differences of the SMRS may function as premating barriers, restricting mating between males and females of different species; such species are then sexually isolated. The role of pre-mating isolation in allopatric species is not clear, because selection does not operate for recognition or isolation here (see DOBZHANSKY et al. 1968). Nonetheless, allopatric species may display pre-mating isolation when they

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[1] This chapter, together with abstract, acknowledgements and references is essentially the text of the paper submitted to the journal Ethology under the full title 'Evidence of pre-mating reproductive isolation in two allopatric populations of the vlei rat Otomys irroratus'.

become sympatric if courtship behaviour is modified in one or more of the following ways: (i) as a response to local environmental differences (PATERSON 1985, VERREL 1988); (ii) because of random genetic drift (RUBINOFF & RUBINOFF 1971); (iii) as result of the pleiotropic effects of genes (DOBZHANSKY et al. 1968); and (iv) by selection favouring enhancement (reinforcement) or replacement (reproductive character displacement) of existing post-mating differences (BUTLIN 1987), following secondary contact between the species.

In previous breeding studies involving two allopatric Natal Midlands Otomys irroratus populations (Kamberg and Karkloof), Kamberg males attacked and seriously wounded Karkloof females during interpopulation pairings (Chapter 3). It was hypothesized that these damaging fights were due to underlying differences in population-specific courtship which caused a breakdown in mate recognition between individuals of the two populations. This hypothesis is evaluated in the present study. Furthermore, it was predicted that interpopulation differences in modes of communication (which acted as SMRSs) rendered Kamberg and Karkloof animals behaviourally incompatible during interpopulation breeding trials. If this were so, it would indicate a measure of pre-mating reproductive isolation between the populations.

In the present study, male and female preferences for communicatory cues of opposite sex individuals of the same and different population was ascertained in two experiments based on the choice-chamber technique (DEMPSTER & PERRIN 1990). 1) 'Whole animal' choice tests allowed a test animal to choose between stimulus animals on the basis of auditory,

olfactory and visual cues but prevented physical contact between animals. 2) Olfactory discrimination tests permitted choice for stimulus odours only.

METHODS

Animals used in the study were either live-trapped at Kamberg ($29^0 23'$S, $29^0 42'$E) and Karkloof ($29^0 17'$S, $30^0 11'$E) in the Natal Midlands, South Africa, or laboratory reared descendants (F1) of wild-caught parents. Details of the maintenance of animals in captivity and the environmental conditions under which the present study was conducted are described elsewhere (PILLAY et al. 1992).

Whole animal choice tests

Experiments were conducted in an apparatus consisting of three linearly arranged chambers (Fig. 1); design of the apparatus was similar to that used by CARTER & BRAND (1986). The apparatus, constructed entirely of 2.5 x 1.5 cm wire mesh, was placed in a tray of woodshavings before each test. The outer chambers A and B (Fig. 1) housed the homotype (same population) and heterotype (different population) stimulus animals, which were of the opposite sex to the test animal in the centre chamber C. The centre chamber comprised three compartments, connected by two passages. The activity of the test animal in the middle chamber was video-recorded using a Hitachi KP-141 CCTV camera and a Hitachi VTL-30ED time lapse video recorder. Video recordings of behaviour were made under fluorescent white light during the light phase of the
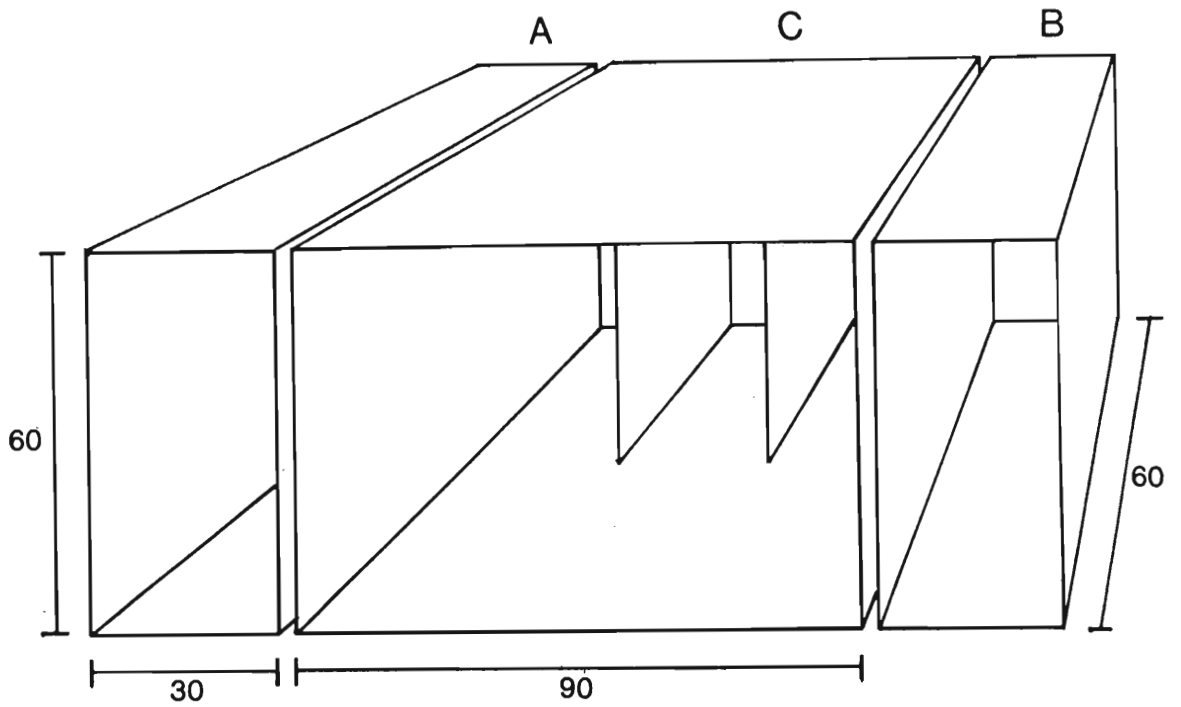
Fig. 1: Diagrammatic representation of the apparatus used in 'whole animal' choice tests. Chambers A and B housed the stimulus (homotype or heterotype) animals, and chamber C the test animal. Measurements are given in cm.

light cycle, and under incandescent red lights during the dark phase.

Prior to testing, a 24 h acclimation period allowed test and stimulus animals to become familiar with the test apparatus. Stimulus animals were introduced into the outer chambers which were detached and maintained separately from the centre chamber. A table of random numbers was used to determine the end chamber (A or B) in which homotype and heterotype animals were housed. The test animal was in the middle compartment of chamber C (see Fig. 1); entry to the adjacent compartments was restricted by blocking off the passages with wire-mesh. After the acclimation period, **testing began by reattaching the end chambers to the centre** chamber. Thereafter, the passage barriers in the centre chamber were removed and video recordings were made of the behaviour of the test animal. Tests usually started at 08h00 and lasted 24 h. Food was provided in the middle compartment only and only during the acclimation period, although quantities were sufficient to sustain animals for the entire test period. At the end of each test, the experimental apparatus was thoroughly washed with water and detergent, and allowed to air dry.

All animals were sexually experienced. Females were in oestrus, as confirmed by vaginal smears. Animals were used as test animals only once. Stimulus animals were used for a maximum of two experiments. Prior to testing, test and stimulus animals had never met in the laboratory.

In all tests, the time spent by test animals in chambers adjacent to homotype and heterotype individuals was recorded,

and mean values were calculated for each sex of both
populations. Data were analyzed in four time periods: (i)
first hour of testing; (ii) remainder of the light phase of
the light cycle (light period); (iii) dark phase of the light
cycle (dark period); and (iv) total 24 h. In addition, the
mean number of visits to chambers of stimulus animals by each
sex was calculated for the first hour. One-tailed paired
t-tests (ZAR 1984) were used to test the hypothesis that
animals preferred homotype rather than heterotype
individuals.

Olfactory discrimination tests

The choice chamber used in the olfactory discrimination
experiments comprised an asbestos arena and three detachable,
galvanised sheet iron test units (Fig. 2). Odour sources of
sexually experienced stimulus animals (homotype and
heterotype individuals of the opposite sex to the test
animal) were in either test unit A or B (Fig. 2), as
determined from a table of random numbers. Odour sources
comprised woodshavings soiled with faeces, urine and possibly
other body secretions collected from the cage bedding of
stimulus individuals whose cages had not been cleaned for at
least a week. Uncontaminated woodshavings, which served as a
control, were in test unit C (Fig. 2). Soiled and control
samples were collected immediately prior to testing and were
introduced into the respective test units in clean glass
Petri dishes (10 cm diameter).

Experiments were conducted by placing a sexually
experienced test animal in the arena and video recording
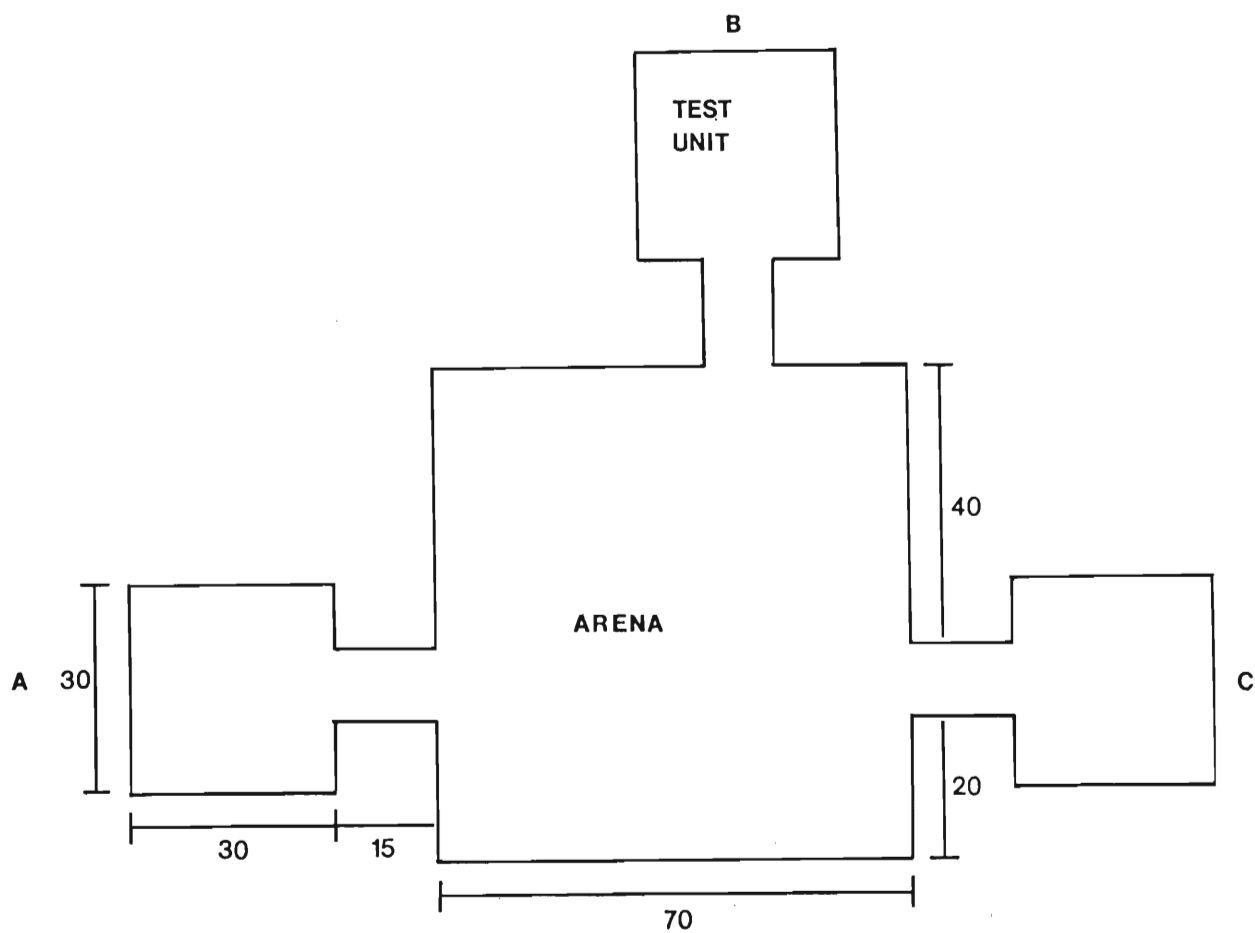
Fig. 2: Ground-plan of the choice chamber used in olfactory discrimination tests. Odour sources of stimulus (homotype or heterotype) animals were placed in test units A or B and of the control in test unit C. Measurements are given in cm. Heights: chamber = 60 cm; test unit = 50 cm.

(described above) its behaviour in response to the
two unfamiliar odour sources and the control sample. Tests
lasting 4 h took place during the light phase of the light
cycle. All animals were tested only once, and all female
test subjects were in oestrus. After each test, all
equipment was washed and dried, as described earlier.

The total time spent in each test unit by test animals
was recorded, and mean values were calculated for each sex of
both populations. Data for the first hour of testing were
analyzed separately. The mean number of visits to odour and
control sources by males and females of both populations was
calculated for the first hour. Initial examination of the
data indicated that in every case the frequency of visits to,
and the time spent in, the control unit by test animals was
comparatively low. Therefore, statistical comparisons
(one-tailed paired t-tests; ZAR 1984) were made only in
respect of preference for homotype and heterotype odour
sources by test animals.

RESULTS

Whole animal choice tests

Mean number of visits by each sex to compartments
adjacent to stimulus animals for the first hour of testing is
given in Table 1. Mean time spent adjacent to stimulus
animals for three time periods and for 24 h of testing are
presented in Table 2 and Fig. 3 respectively. In every case,
both males and females more frequently visited homotype than
heterotype stimulus individuals, although this preference was
statistically significant only in respect of females (Table 1).

Table 1: Mean ± 2 SE number of visits to compartments adjacent to homotype and heterotype individuals by the test animals indicated, for the first hour of testing. t = paired t-test values.

Test animals	n	Stimulus animals homotype	Stimulus animals heterotype	t
Males				
Kamberg	21	20.7±8.8	17.8±7.3	1.70 ns
Karkloof	25	32.3±8.5	28.0±9.9	0.29 ns
Females				
Kamberg	21	25.7±5.5	13.0±4.8	3.61 **
Karkloof	23	30.1±7.5	8.3±4.2	4.54 ***

** = p < 0.01, *** = p < 0.001

Table 2: Mean ± 2 SE number of minutes spent in compartments adjacent to homotype and heterotype individuals by the test animals indicated, during three time periods. t = paired t-test values. n = as in Table 1.

Test animals	First hour homotype	First hour heterotype	t	Light period homotype	Light period heterotype	t	Dark period homotype	Dark period heterotype	t
Males									
Kamberg	29.6± 5.4	26.0± 5.0	0.81 ns	443.7± 93.6	228.5± 67.4	3.80 **	240.8± 20.6	225.4± 20.9	1.47 ns
Karkloof	28.0± 7.9	24.9± 4.9	0.72 ns	449.7± 70.4	279.0± 69.3	3.16 **	276.0± 61.0	208.4± 65.2	2.49 *
Females									
Kamberg	32.0± 4.1	16.0± 2.9	6.34 ***	506.8± 38.1	231.5± 36.8	8.94 ***	308.0± 34.3	141.2± 31.0	5.72 ***
Karkloof	29.3± 9.5	7.1± 3.2	3.82 **	515.4± 57.9	203.5± 47.0	7.22 ***	329.3± 47.2	149.3± 38.3	4.61 ***

* p < 0.05, ** p < 0.01, *** p < 0.001
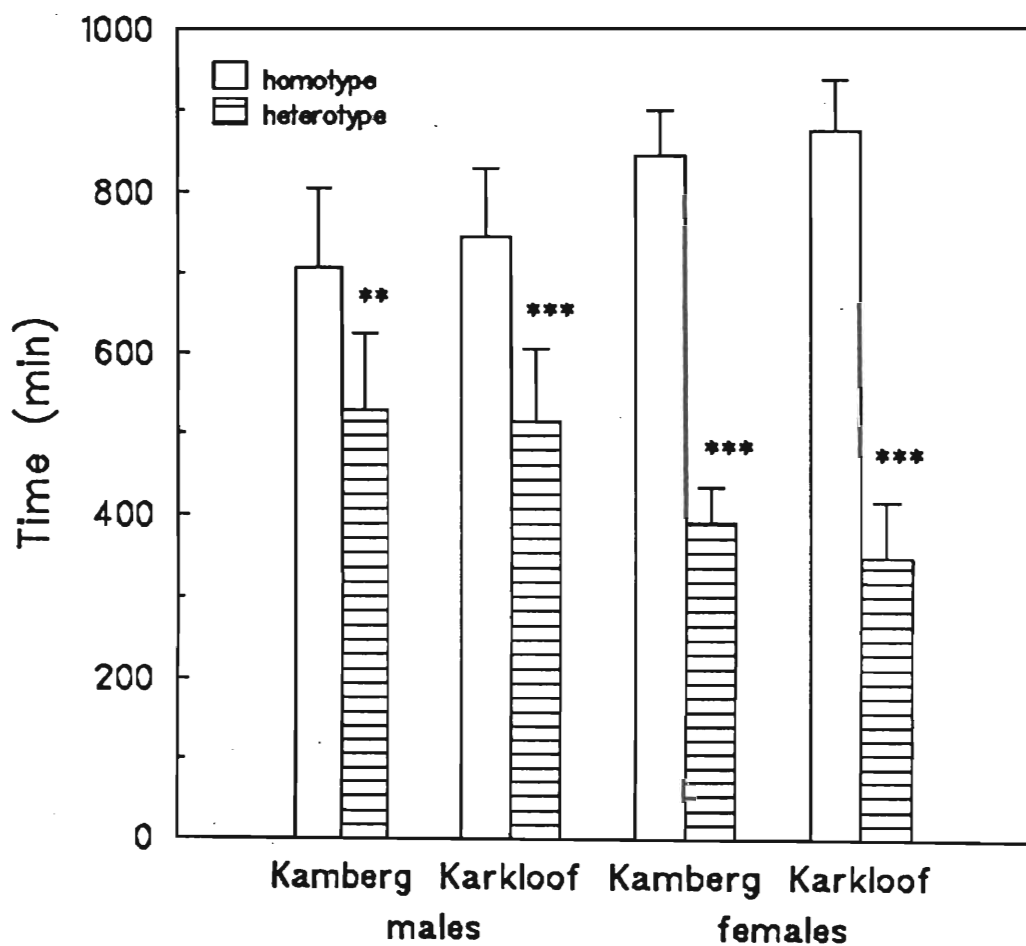
Fig. 3: Mean number of minutes spent in chambers adjacent to homotype and heterotype individuals by the test animals indicated, for the total 24 h of testing. n = as in Table 1. Vertical bars = 2 SE of the mean. ** = p < 0.01, *** = p < 0.001, paired t-tests.

The time spent adjacent to stimulus individuals by test animals was comparable to the number of visits. The majority of test animals spent significantly more time adjacent to homotype than heterotype stimulus individuals during each time period (Table 2) and for the total 24 h of testing (Fig. 3). All males were less discriminating during the first hour of testing, however (Table 2). In addition, Kamberg males did not show clear preference for homotype females during the dark period.

Olfactory discrimination tests

Mean number of visits to odour sources of stimulus animals by each sex for the first hour of testing is given in Table 3. Mean time spent with odour sources of stimulus animals by test animals for the first hour and the total 4 h of testing is illustrated in Fig. 4. Results obtained here were remarkably similar to those of the whole animal choice tests. In terms of both the frequency (Table 3) and duration (Fig. 4) of visits, test animals preferred the odour sources of homotype rather than heterotype individuals. Once again, male preference of homotype odour was not statistically significant during the first hour of testing.

DISCUSSION

The results obtained from experiments in the present study bear out the hypothesis that Kamberg and Karkloof O. irroratus distinguish between homotype and heterotype mates. Similar findings have been reported for other rodent species, where discrimination was made either between

Table 3: Mean ± 2 SE number of visits to test units with odours of homotype and heterotype individuals by the test animals indicated, for first hour of testing. t = paired t-test values.

| Test animals | n | Odour sources | | t |
		homotype	heterotype	
Males				
Kamberg	20	38.3±20.5	29.0±18.4	0.53 ns
Karkloof	20	42.6±22.9	33.1±21.2	1.05 ns
Females				
Kamberg	20	43.1±5.5	21.7±4.8	5.10 ***
Karkloof	18	37.4±12.1	2.1±2.2	4.50 ***

*** p < 0.001
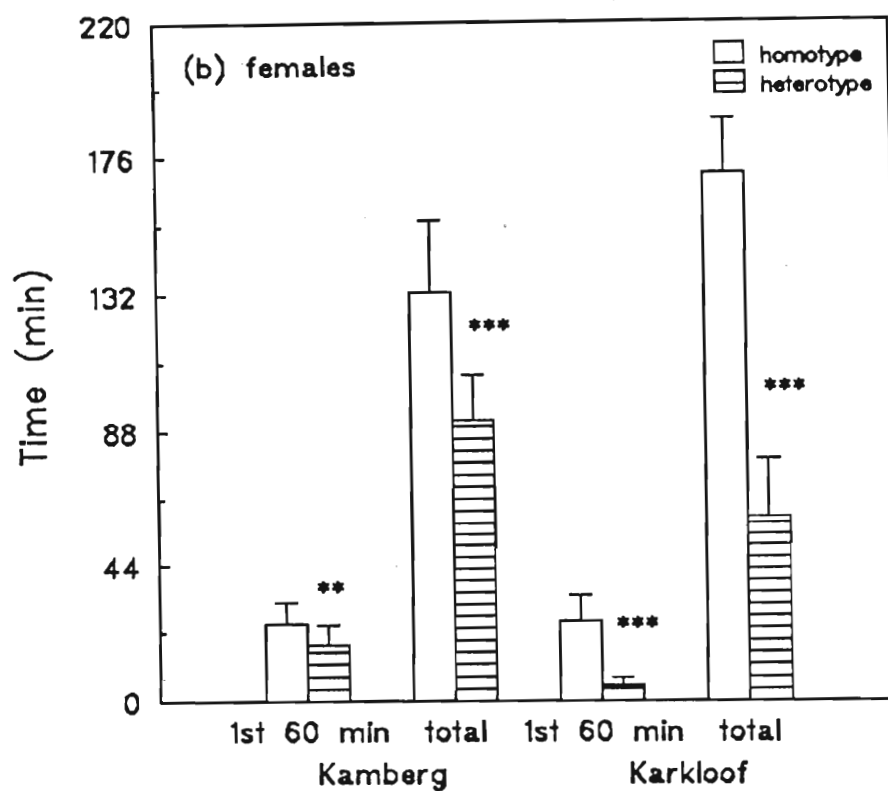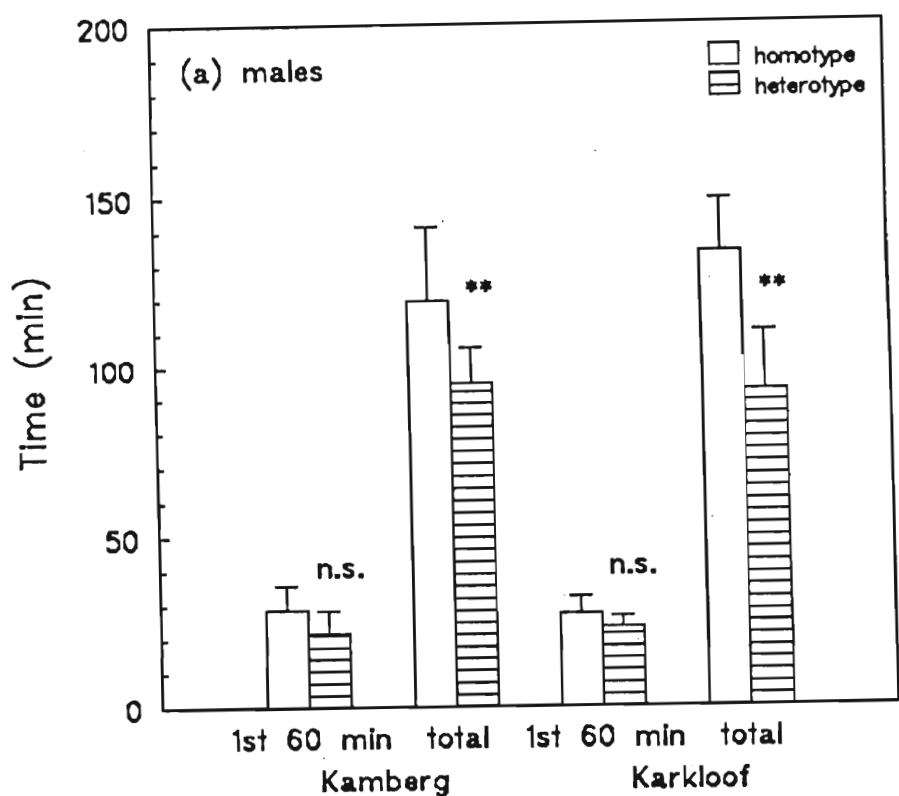
Fig. 4: Mean number of minutes spent in test units with odours of homotype and heterotype individuals by (a) males and (b) females, for the first hour and for the total four hours of testing. Vertical bars = 2 SE of the mean. n = as in Table 3. ** = p < 0.01, *** = p < 0.001, paired t-tests.

stimulus animals (inter alia SMITH 1965, NEVO & HETH 1976, CARTER & BRAND 1986) or between stimulus odours (inter alia MOORE 1965, NEVO et al. 1976, COX 1984).

From whole animal choice tests, it appears that mate recognition was achieved by means of multi-modal communicatory stimuli from living animals, excluding only tactile cues. However, the outcome of the olfactory discrimination tests was virtually identical to that of the whole animal choice experiments, suggesting the overriding importance of population-specific chemosignals as a basis for mate recognition.

It is of interest that whereas odour apparently played a significant part in mate recognition in the present study, there is no consensus concerning the role of chemosignals in the social interaction of O. irroratus. For example, O. irroratus in the Transvaal highveld evidently emphasizes olfaction during social interaction (DAVIS 1973), while studies in the Natal Midlands (WILLAN 1982) and in East Africa (KINGDON 1974) suggest that the taxon probably favours a combination of auditory and visual stimuli.

In all cases, females were the more highly discriminating, almost exclusively preferring homotype males or their odour. Despite demonstrating overall preference for females of the same population, males failed to show a clear preference at the beginning of both experiments. Such intersexual asymmetry has been demonstrated also by Peromyscus spp. in whole animal choice tests (SMITH 1965), as well as by Microtus ochrogaster in odour discrimination experiments (NEWMAN & HALPIN 1988). SMITH (1965) maintained

that the exploratory drive of <u>Peromyscus</u> males was highest at the start of testing, resulting in their being less discriminating than females during this time. In this case, increasing male selectivity corresponded with decreasing levels of exploratory behaviour. Likewise, initial high levels of exploratory behaviour may account for Kamberg and Karkloof males being comparatively less discriminating during the first hour of tests.

The hypothesis that equal preference for homotype and heterotype females by males was the result of increased exploratory drive is supported by consideration of <u>O. irroratus</u> mating strategy. Trapping studies at Kamberg and Karkloof (N. PILLAY Unpubl. data) suggest that the Karkloof population, like other <u>O. irroratus</u> populations (DAVIS 1973, WILLAN 1982, BROWN 1988), has a promiscuous mating system. In contrast, Kamberg animals apparently mate polygynously (N. PILLAY Unpubl. data). Clearly, males involved in polygamous matings will attempt to mate with several different females (ORIANS 1969, EMLEN & ORING 1977, THOMAS & BIRNEY 1979). PARKER (1974) maintains that such males are in a constant state of intrasexual competition, and that selection favours males which attempt to mate with many females in a short time period. Hence, it is possible that until they recognised homotype females as potential mates, males in the present study attempted to solicit both stimulus females. The result was that at the beginning of experiments males spent an equivalent amount of time with both homotype and heterotype females, or with the odour of both stimulus females.

The equal preference for homotype and heterotype females by Kamberg males during the dark phase of the light cycle is not readily explained. As opposed to the situation during the light period, it may be assumed that during the dark period, communication between test and stimulus animals was accomplished by using all cues besides visual stimuli. On this basis, it appears that Kamberg males emphasise visual cues in mate recognition. This argument is unconvincing, however, since Kamberg females distinguished between homotype and heterotype males throughout testing, regardless of the time period. Clearly, additional studies are needed to explain the lack of discrimination of Kamberg males during the dark period.

CONCLUSIONS

The results obtained here suggest the existence of population-specific courtship behaviour, resulting in Kamberg and Karkloof individuals preferring same-population mates. The outcome of the olfactory choice tests provides overwhelming evidence that population-specific odours (i.e. differences in SMRSs) formed the basis of mate recognition. There is little doubt that genetic divergence has occurred in allopatry to the extent that should the Kamberg and Karkloof populations meet in nature, pre-mating reproductive isolating mechanisms may prevent interpopulation matings. Pre-mating isolation is incomplete, however, as Kamberg and Karkloof interpopulation pairs bred in the laboratory (Chapter 3), but this is usually the case among allopatric populations (DOBZHANSKY et al. 1968, RUBINOFF & RUBINOFF 1971).

Therefore, it appears that both pre- and post-zygotic isolation mechanisms have evolved in Kamberg and Karkloof <u>O. irroratus</u>.

CHAPTER 5

INTRA- AND INTERPOPULATION MALE-FEMALE

ENCOUNTERS [1]

INTRODUCTION

Pre-mating reproductive isolating mechanisms are essential for preventing interbreeding between males and females of closely-related species. Of all pre-mating isolating mechanisms, the most effective ones involve ethological components (DOBZHANSKY et al. 1968), especially species-specific courtship behaviour (KOEPFER 1987). Differences in courtship behaviour permit conspecific males and females to recognize only one another as potential mates, thereby ensuring positive assortative mating (BEILES et al. 1984). In an evolutionary sense, positive assortative mating has the advantage of eliminating time and energy wastage incurred as a result of failed mating attempts with non-specifics; such matings bear high costs in respect of reproductive success and individual fitness.

Recognition between conspecific mates during courtship is normally mediated through a system of male-female signals and responses, defined as a specific-mate-recognition-system

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[1] This chapter, together with abstract, acknowledgements and references, is essentially the text of the paper submitted to the journal Zeitschrift für Säugetierkunde under the full title 'Evidence of pre-mating reproductive isolation in two allopatric populations of the vlei rat Otomys irroratus: intra- and interpopulation male-female encounters'.

(i.e. SMRS; PATERSON 1980, 1985). The signal-response chain involves mainly communicatory cues.

Previous research on mate recognition in two allopatric populations of the vlei rat Otomys irroratus has revealed positive preference for mates of the same population or their odours in two-choice laboratory experiments (Chapter 4). These populations represented the Kamberg and Karkloof localities. On the basis of the results from mate preference experiments, it was hypothesized that differences in population-specific courtship behaviour, and olfactory cues in particular, played a major role in mate recognition in Kamberg and Karkloof O. irroratus.

Courtship comprises all behaviour patterns leading to copulation (McFARLAND 1987), and includes the interaction of several modalities of communication (e.g. odour, sound, postural changes; CARTER and BRAND 1986; DEMPSTER et al. 1992). Since the design of the choice-chamber experiments was such that no physical contact was permitted between test and stimulus animals (Chapter 4), the exact nature of the courtship differences between the Kamberg and Karkloof populations is unknown. Neither is it known whether differences in courtship behaviour bring about mate recognition during interpopulation pairings, nor whether other cues (e.g. tactile and visual), apart from olfactory ones, play a role in mate recognition. On this basis, a series of observational studies were undertaken of male-female interaction during intrapopulation encounters. The present study aimed to establish whether or not other communicatory cues, besides olfactory ones, are important in

mate recognition in the Kamberg and Karkloof populations. The study also aimed to ascertain whether differences of the courtship behaviour (i.e. differences in SMRSs) between Kamberg and Karkloof animals rendered them incompatible during interpopulation encounters. Such a situation would indicate the existence of pre-mating barriers to reproduction between the two populations.

MATERIALS AND METHODS

Animals used in the study were either live-trapped at Kamberg ($29^0 23'$S, $29^0 42'$E) and Karkloof ($29^0 17'$S, $30^0 11'$E) in the Natal Midlands, South Africa, or laboratory reared descendants (F1) of wild-caught parents.

Details of the maintenance of animals in captivity and the environmental conditions under which the present study was conducted are described elsewhere (PILLAY et al. 1992). The interaction of 20 of each of Kamberg and Karkloof intrapopulation pairs and a total of 34 interpopulation pairs (specified below as male x female) was studied in neutral arena encounters. Interpopulation pairings were bi-directional (PILLAY et al. 1992), comprising 18 Kamberg x Karkloof and 16 Karkloof x Kamberg pairs.

Encounters were staged in asbestos enclosures 90 x 90 x 60 cm, furnished with coarse wood shavings. Between encounters, enclosures were washed with water and a 50 % ethyl alcohol solution to remove odours of the previous occupants.

Prior to observations, an enclosure was divided into two parts with a wire mesh partition. A male and a female in

pro-oestrus (confirmed by vaginal smears) were placed on either side of the partition at 18h00. Following a familiarization period of approximately 14 h (i.e. at 08h00 the next day, when females usually displayed oestrus; N. PILLAY Unpubl. data), the partition was removed and the first 20 min of interaction video-recorded. Testing coincided with the period of maximum diurnal activity (i.e. from 07h00 to 09h00 during the light phase of the light cycle). Recordings were made under fluorescent white light using a Hitachi KP-141 CCTV camera and a Hitachi VTL-30ED time-lapse video cassette recorder.

PILLAY (1990) showed that male-female interactions in staged encounters, involving three O. irroratus populations, differed at both the intra- and interpopulation level even after 12 days of pairing. On this basis, additional video recordings of the interactions of each pair in the present study were made every two days from the day of pairing (designated Day 0), for 12 days. Recordings under incandescent red lights were conducted for one hour during the dark phase of the light cycle (i.e. from 00h00 to 01h00; period of maximum nocturnal activity).

All animals were sexually experienced. Members of a pair had never previously met in the laboratory. Males were used in two encounters (i.e. one intrapopulation and one interpopulation encounter). Females were used only once.

Analysis of video recordings made during the first 20 min of interaction entailed first encoding 18 male and 15 female behaviour patterns (below). Thereafter, all behaviour patterns occurring in successive 10 s intervals were recorded,

using the one-zero time-sampling method (ALTMAN 1974). Acts were identified when animals changed their behaviour (after DEMPSTER et al. 1992).

The frequencies of all behaviour patterns of males and females of each pairing were calculated. Mean frequencies were obtained for each sex in respect of the different categories of interaction (i.e. intrapopulation or interpopulation pairings).

During the nocturnal sampling (i.e. recordings every second night for 12 nights), the occurrence of only agonistic and amicable behaviour patterns in each pair were recorded, and mean percentages were calculated for intra- and interpopulation pairings. Other behaviour patterns (e.g. exploratory, sexual behaviour) were not considered because the frequency of their occurrence was too small to warrant analysis.

Results obtained from diurnal and nocturnal recordings were treated separately because of the different time scales involved (i.e. 20 min and 7 nights). Data from diurnal experiments were tested for significance using the Mann-Whitney U test (SOKAL and ROHLF 1987). Data obtained from the nocturnal study illustrated trends that were sufficiently clear to obviate the need for statistical analysis.

RESULTS

Behaviour patterns identified in encounters in this study, as well as equivalent behaviour patterns for rodent species in other studies, are presented in Table 1. Most

Table 1. Behaviour patterns identified in encounters in the present study and the equivalent behaviour patterns for rodent species in other studies. Those behaviour patterns that were combined to allow comparison between diurnal intrapopulation and interpopulation encounters are indicated as subsets A - D; see text.

This study	Other studies
Explore arena	Digging + alert (SWANSON 1974)
Self-groom	Grooming (SWANSON 1974)
Mark	Marking (WILLAN 1982)
Watch	Watch (DEMPSTER et al. 1992)
Inactive	Inactive (BURLEY 1980)
Naso-nasal contact	Nose-nose contact (SWANSON 1974)
A) Agonistic	
Aggressive approach	Aggressive approach (WILLAN 1982)
Chase	Chasing (SWANSON 1974)
Upright sparring	Upright + sparring (SWANSON 1974)
Submissive flee	Flee (GRANT and MACKINTOSH 1963)
B) Amicable	
Amicable approach	Amicable approach (WILLAN 1982)
Groom invitation	Nosing (BARNETT 1975)
Allogroom	Social grooming (EISENBERG 1967)
Huddle	Huddling (WILLAN 1982)
C) Follow-mount (males only)	
Naso-anal contact	Naso-anogenital contact (SWANSON 1974)
Follow	Follow (BURLEY 1980)
Attempted mount	Attempted mount (EISENBERG 1967)
Mount	Mount (BURLEY 1980)
D) Present (females only)	
Move away	Move away (EISENBERG 1967)
Dart	Darting (BURLEY 1980)
Present and lordosis	Present posture + lordosis (BURLEY 1980)

behaviour patterns were displayed by both sexes, although follow-mounting and presenting were sex-specific activities.

Mean frequencies of behaviour patterns recorded during the first 20 min of intrapopulation encounters of males and

females are given in Table 2 and Table 3 respectively. Included in both tables are the results of intrasexual comparisons (Mann-Whitney U test) of all behaviour patterns.

Generally, neither males (Table 2) nor females (Table 3) exhibited many interpopulation differences in the behaviour patterns recorded. In both samples, males and females spent a large proportion of time at opposite ends of the arena, and hence the frequency of inactive behaviour was higher than that of other behaviour patterns. Except for the comparatively high levels of following and attempted mounting acts by Karkloof males, all other behaviour patterns that were significantly different represented agonistic and amicable interactions (Tables 2 and 3). Compared to their Karkloof counterparts: (i) Kamberg males were involved in **significantly less upright sparring, more frequently** favouring amicable approach, allogroom, and huddle behaviour (Table 2); and (ii) Kamberg females were significantly less **submissive and more frequently participated** in grooming invitation, allogrooming and huddling activities (Table 3).

Mean frequencies of behaviour patterns recorded during diurnal interpopulation and intrapopulation encounters are compared in respect of males in Figure 1 and females in Figure 2. Preliminary examination of the data indicated a low frequency of some behavioural acts during interpopulation encounters. These acts were therefore combined with other motivationally similar acts (Table 1). Significantly higher levels of aggression and significantly lower levels of amicability were recorded in interpopulation than in intrapopulation encounters. Furthermore, there was a marked

Table 2. Mean frequencies of behaviour patterns for male
Kamberg and Karkloof <u>O. irroratus</u> during the first 20 min of
intrapopulation encounters. 2 SE given in brackets.
U-stat = Mann-Whitney U test values.

Behaviour patterns	Kamberg	Karkloof	U-stat	
Explore	17.4 (2.65)	13.4 (3.19)	57.5	
Self-groom	0.5 (0.68)	1.1 (0.82)	64	
Mark	13.8 (4.38)	12.7 (3.24)	51.5	
Watch	3.2 (2.00)	3.3 (1.49)	58.5	
Inactive	54.4 (9.32)	60.4 (7.08)	56	
Naso-nasal	18.5 (3.40)	17.7 (5.56)	57	
Aggressive approach	1.9 (1.73)	1.6 (1.00)	51.5	
Chase	12.5 (4.43)	16.5 (2.55)	67	
Upright spar	2.4 (1.44)	6.8 (1.97)	87	*
Flee	1.1 (0.96)	1.5 (0.80)	62	
Amicable approach	19.8 (1.97)	14.9 (2.77)	74	*
Groom invitation	3.1 (1.82)	1.3 (0.99)	67.5	
Allogroom	4.5 (0.99)	1.4 (1.31)	79.5	*
Huddle	14.2 (4.71)	5.3 (2.70)	86	*
Naso-anal	12.6 (3.10)	12.2 (2.18)	51.5	
Follow	6.6 (2.53)	10.5 (2.94)	74	*
Attempted mount	8.5 (13.2)	12.7 (1.98)	75	*
Mount	4.4 (2.71)	4.4 (1.27)	61.5	

* = values which differed at the 5% significance level.

Table 3. Mean frequencies of behaviour patterns for female
Kamberg and Karkloof <u>O. irroratus</u> during the first 20 min of
intrapopulation encounters. 2 SE given in brackets.
U-stat = Mann-Whitney U test values.

Behaviour patterns	Kamberg	Karkloof	U-stat	
Explore	12.6 (2.77)	12.9 (3.41)	50	
Self-groom	5.3 (2.07)	4.8 (1.86)	55	
Mark	7.9 (2.28)	7.5 (2.26)	55.5	
Watch	13.9 (3.72)	17.7 (4.19)	66	
Inactive	64.4 (9.32)	66.4 (7.08)	56	
Naso-nasal	18.5 (3.40)	17.7 (5.56)	57	
Aggressive approach	2.3 (1.23)	1.6 (1.00)	69	
Upright spar	8.1 (2.60)	9.0 (2.55)	52.5	
Flee	4.3 (1.52)	7.5 (0.90)	89.5	*
Amicable approach	2.3 (1.23)	1.0 (2.22)	69	
Groom invitation	**5.2 (2.37)**	**2.0 (1.16)**	**77**	*
Allogroom	6.0 (3.16)	1.4 (1.31)	78	*
Huddle	14.2 (4.71)	5.3 (2.70)	86	*
Move away	3.6 (1.61)	5.1 (1.28)	68.5	
Dart	2.4 (2.10)	3.1 (2.34)	52.5	
Present	1.5 (1.04)	0.7 (0.85)	62.5	

* = values which differed at the 5% significance level.
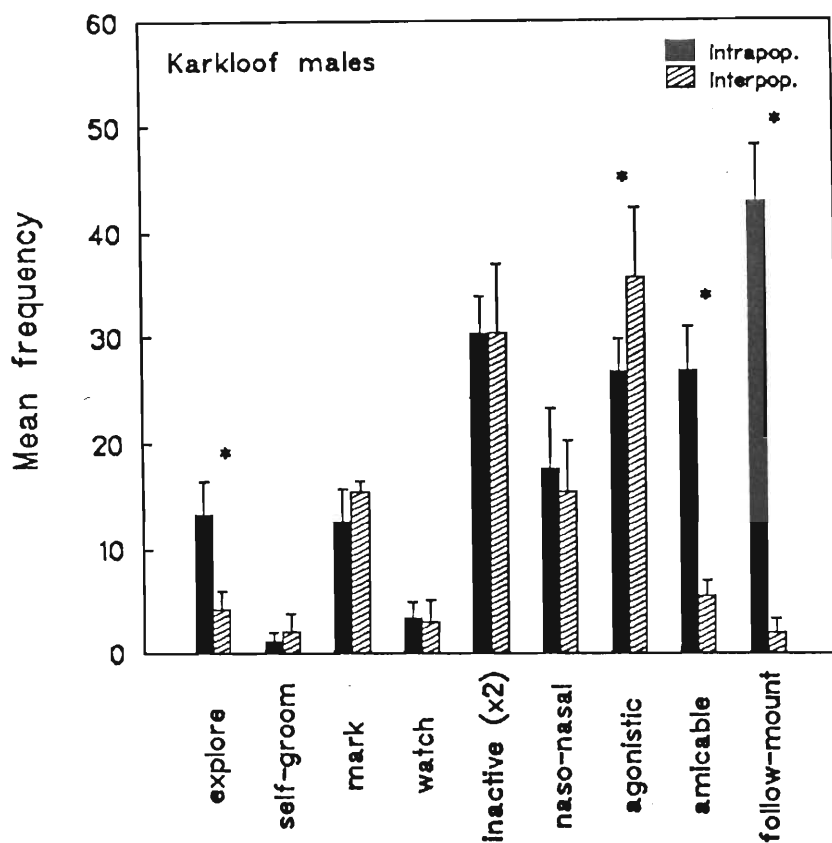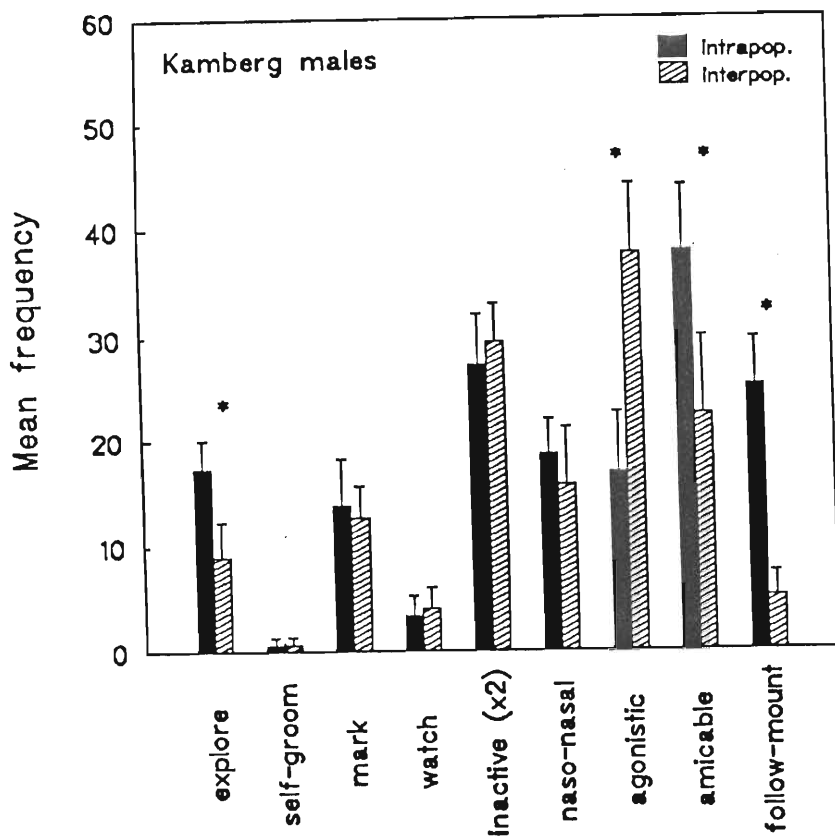
Figure 1. Comparison of mean frequencies of behaviour patterns for males during the first 20 min of intrapopulation and interpopulation encounters. Error bars = 2 SE of the mean. * = values which differed at the 5% significance level, Mann-Whitney U test.
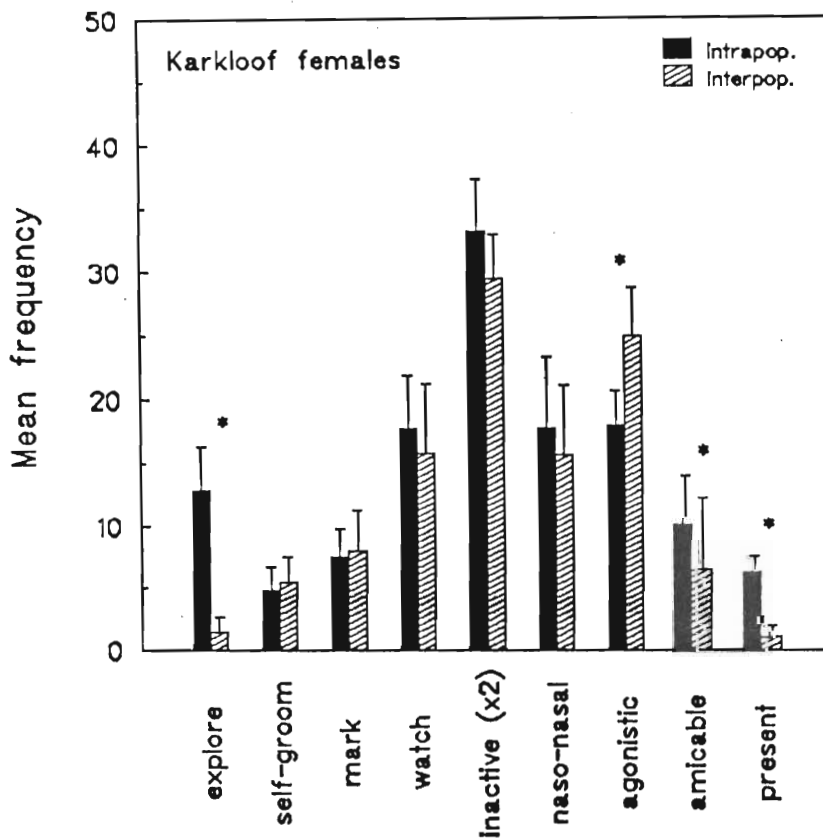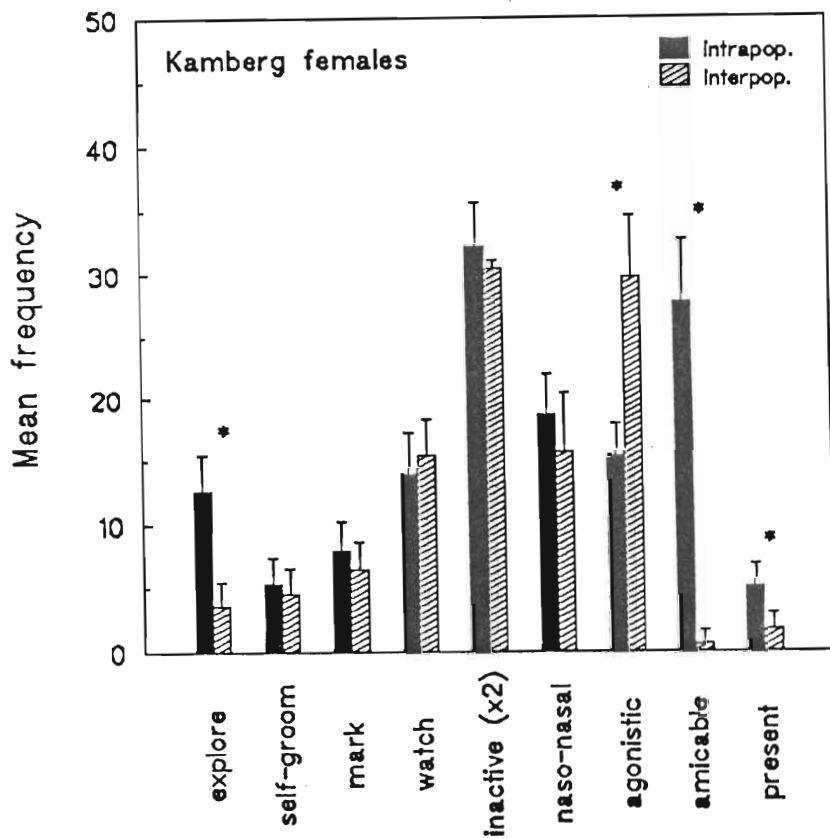
Figure 2. Comparison of mean frequencies of behaviour patterns for females during the first 20 min of intrapopulation and interpopulation encounters. Error bars = 2 SE of the mean. * = values which differed at the 5% significance level, Mann-Whitney U test.

decrease in sexual activity during interpopulation encounters (i.e. fewer follow-mount acts by males and fewer presenting acts by females). Interestingly, exploratory behaviour by all animals was significantly lower during inter- than intrapopulation encounters.

Mean percentages of nocturnal interaction devoted to agonistic and amicable behaviour are plotted against time in Figure 3. Males of nine Kamberg x Karkloof pairs and four Karkloof x Kamberg pairs attacked and seriously wounded their mates during the first two days of encounters. These pairs were separated, resulting in reduced sample sizes for the interpopulation pairings from Day 2 onwards (see Figure 3).

For every pairing, levels of agonistic interaction were highest soon after animals were paired, and none of the pairs immediately displayed high levels of amicability (i.e. Day 0; Figure 3). It is evident in all cases that levels of agonistic interaction decreased during encounters, with a corresponding increase in levels of amicability. The most important feature illustrated in Figure 3 is the variation in the time taken to the point of intersection of the curves representing agonistic and amicable interaction. Kamberg intrapopulation pairs displayed equal levels of amicable and agonistic interaction sooner than any other pairing (i.e. before Day 2), while the Karkloof pairs reached this stage just before Day 3. In contrast, the curves representing agonistic and amicable interaction of the interpopulation pairings intersected at approximately Day 6 (Figure 3).
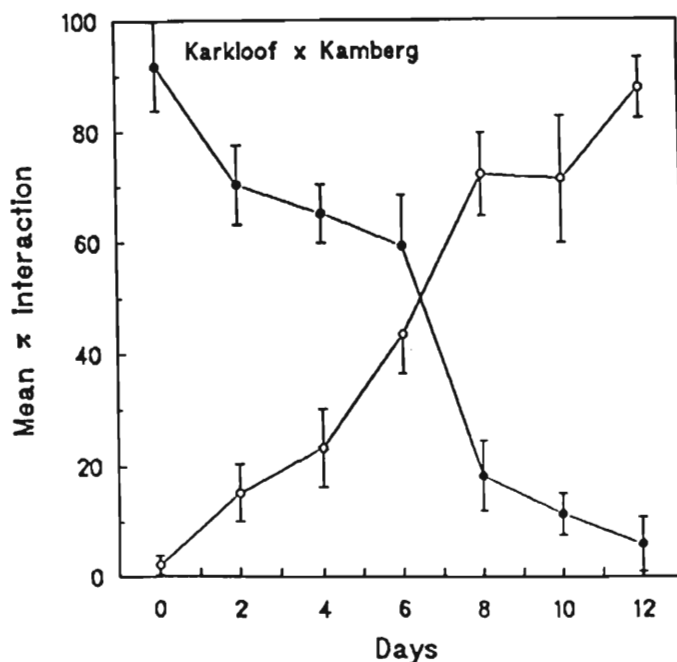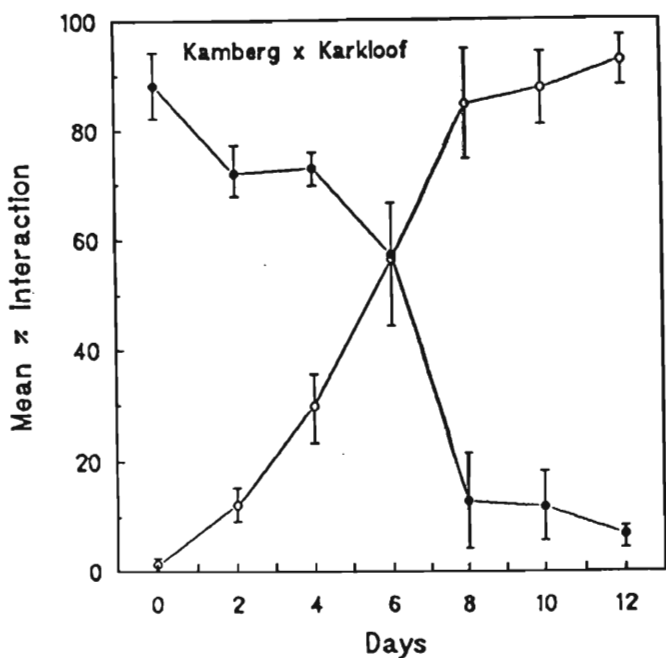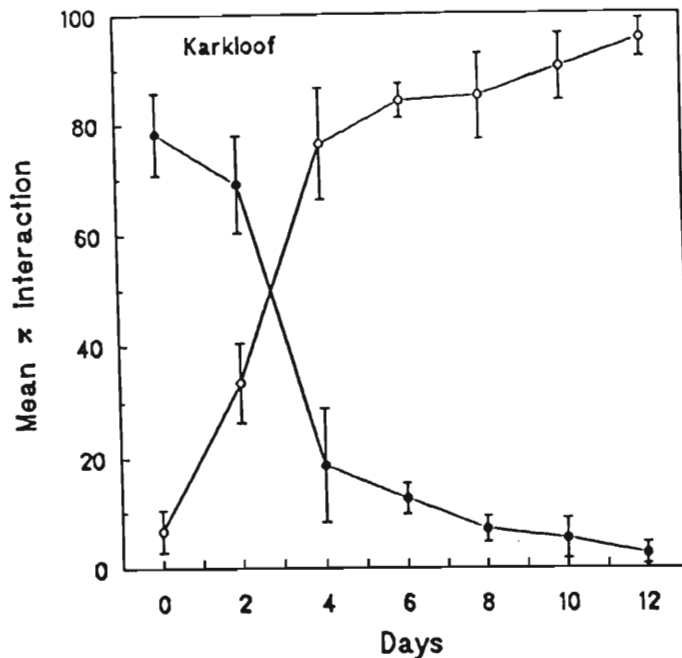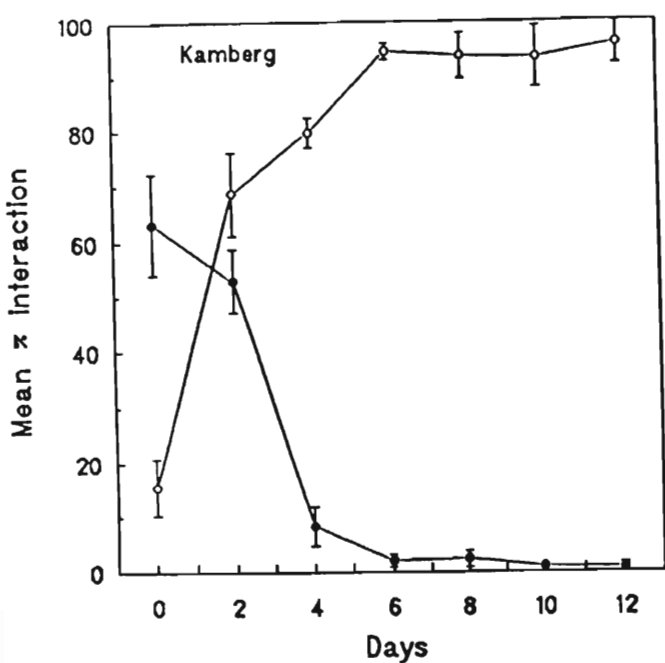
Figure 3. Mean percentage nocturnal agonistic (filled circles) and amicable (open circles) interaction for the intrapopulation and interpopulation pairings indicated. Error bars = 2 SE above and/or below the mean. Sample size = 20 each for the intrapopulation pairings. Sample size for Kamberg x Karkloof and Karkloof x Kamberg pairs = respectively 18 and 16 on Day 0, and 9 and 12 from Day 2 to 12.

By day 12 interpopulation pairs still displayed higher levels of agonistic interaction than intrapopulation pairs (Figure 3).

DISCUSSION

Interpretation of male-female interactions observed in this study necessitates first outlining some relevant aspects of the social system of the Kamberg and Karkloof populations. Results of extensive laboratory and limited field studies (Chapter 8) suggest that the social structure of the Karkloof population is similar to that of other O. irroratus populations (DAVIS 1973; WILLAN 1982; BROWN 1988). Breeding females are intrasexually more aggressive than males, and appear to be intrasexually highly territorial. Reproductively active males have intrasexually overlapping home ranges and have intrasexual dominance hierarchies. The home ranges of males overlap those of females, and males compete with one another for mating opportunities. The Kamberg population differs in some important respects from the others. Kamberg males are intrasexually highly aggressive and probably occupy exclusive (intrasexually non-overlapping) territories in nature (Chapter 8). Kamberg females are intrasexually highly amicable and appear to occupy intrasexually overlapping territories (Chapter 8).

High levels of aggressive interaction are characteristic of species which display territoriality, like O. irroratus. Such behaviour leads to mutual avoidance between conspecifics (inter alia RUFER 1967; SWANSON 1974; HAPPOLD 1976; DELANY and HAPPOLD 1979; WHITE and FLEMING 1987), thereby enabling

animals to maintain their territories. It follows that
agonistic interaction between the sexes will occur when they
first meet. For mating to occur, however, potential mates
must eventually reach a stage of mutual amicability.
Therefore, the mating behaviour of territorial taxa comprises
aggressive, fighting and fleeing drives on the one hand, and
sexual (amicable) drives on the other (SPIETH 1958; TINBERGEN
1954). These conflicting drives often result in complex and
prolonged courtship behaviour (NEVO 1969; PARKER 1974).

In comparison with the Kamberg pairs, Karkloof and both
interpopulation pairs displayed higher levels of aggression
and lower levels of amicability during the first 20 min of
encounters, and attained primarily amicable relationships
later. In addition, interpopulation pairs maintained higher
levels of aggression to the end of their encounters. It
would appear therefore that the Karkloof, Kamberg x Karkloof
and Karkloof x Kamberg pairs required longer periods to
overcome aggressive tendencies (NEVO 1969; PARKER 1974),
suggesting that mate recognition between members of these
pairs was delayed.

Intrapopulation pairings

Karkloof males followed and attempted to mount females
from the same population more frequently than did Kamberg
males, and Karkloof females were more aggressive than any
other individuals tested in intrapopulation encounters
(Tables 2 and 3). In contrast, Kamberg intrapopulation
encounters displayed comparatively high levels of amicable
interaction (e.g. huddling, grooming). These differences

between the two populations may have been due to underlying differences in population-specific mating strategy.

On the basis of the social organization of both populations, it has been hypothesized that the mating systems of Kamberg and Karkloof populations are polygynous and promiscuous respectively (Chapter 8): Kamberg males apparently have exclusive access to several females, while Karkloof males compete within a hierarchical framework for access to receptive females. Similar relationships between social organization and mating strategy have been predicted in several rodent species (MADISON 1980; OSTFELD 1985, 1986), although it is acknowledged that such generalizations may not always hold true.

Females of species which are involved in promiscuous matings would tend to meet males (i.e. potential mates) comparatively frequently, and selection would be predicted to favour reduced attractiveness of males to females (ALDER et al. 1981). As such, females would respond aggressively to male solicitation; increased female aggression may function as a mate choice mechanism based on male quality (FERKIN 1987). At the same time, male-male competition for receptive females would be intense, and the most successful males would be those which rapidly achieve copulation with receptive females (ALDER et al. 1981).

In polygynous species, males maintain intrasexually exclusive territories, and males would have previously established their territories on the basis of intrasexual competition (SHAPIRO and DEWSBURY 1986). In order to ensure mating with a genetically-fit male, females simply mate with

the male within whose territory they occur (EMLEN 1976; SHAPIRO and DEWSBURY 1986). Therefore, polygynously-mating females may be less discriminating during mate choice than promiscuously-mating females, potentially resulting in reduced aggression and higher amicability during the courtship of polygynous species.

Hence, male-female interaction may be more aggressive, and mating attempts by males more frequent, in rodent species with a promiscuous rather than a polygynous mating strategy.

Interpopulation pairings

In terms of the levels of intersexual aggression, frequency of sexual activity, and the time taken for pairs to attain equivalent levels of aggression and amicability in the present study, the Kamberg x Karkloof and Karkloof x Kamberg pairings were distinct from the intrapopulation pairings. In addition, 13 interpopulation pairs were separated following damaging fights. These results bear out the hypothesis that both Kamberg and Karkloof individuals discriminate between mates from the same and the other population. Similarly, increased sexual activity and reduced aggression in intra- relative to interspecific encounters were apparently indicative of conspecific mate recognition in Gerbillurus species (DEMPSTER et al. 1992).

The somewhat reduced exploratory behaviour observed in all interpopulation pairings is not readily explained. Studies of male-female interactions involving Microtus pennsylvanicus and M. pinetorum (CRANFORD and DERTING 1983) indicated that, unlike intraspecific pairs, interspecific

pairs favoured aggressive and contact-orientated behaviour over exploratory activity. High levels of agonistic interaction were also characteristic of interpopulation encounters in the present study, and it is likely that decreased exploratory behaviour is merely the result of pairs engaging in increased agonistic interaction.

Failure to recognize specific auditory, olfactory, tactile and/or visual cues may result in high levels of aggression during interspecific encounters of closely-related rodent species, and may lead to delayed recognition between potential mates (inter alia BAUER 1956; GODFREY 1958; SCOTT 1966; KOEPFER 1987). Consequently, differences in behaviour between intra- and interpopulation pairings, as exemplified by increased aggression during interpopulation pairings, may be directly attributable to contrasting courtship behaviour, and, in particular, population-specific modes of communication; the constituent units of O. irroratus courtship behaviour include various communicatory cues together with varying levels of amicable and agonistic behaviour (WILLAN 1982; PILLAY 1990).

In olfactory discrimination experiments, Kamberg and Karkloof individuals preferred odours of same-population mates (Chapter 4), clearly demonstrating that olfactory cues are important in mate recognition. In the present study, the importance of olfactory cues in courtship was indicated by high frequencies of naso-nasal and naso-anal contact in all encounters.

Except for inactive behaviour and possibly exploratory behaviour, all other behaviour patterns observed in

encounters constituted tactile and visual signals (DAVIS 1972; WILLAN 1982; PILLAY 1990). Hence, tactile and visual communication appear to be important in the courtship behaviour of both populations. Population-specific behaviour patterns were revealed in intrapopulation pairings: Kamberg encounters were characterized by more amicable approach, groom, and huddle behaviour than Karkloof encounters. In contrast, Karkloof pairs performed more follow-mount, upright sparring, and submissive behaviour than Kamberg pairs. These differences in courtship behaviour may have rendered representatives of both populations behaviourally incompatible during interpopulation encounters.

It is intriguing that females never performed naso-anal sniffing behaviour in the present study, but did so during investigations of the copulatory behaviour of both populations (N. PILLAY Unpubl. data). This variability in female behaviour may be explained in terms of differences in the length of the familiarization period in each study. Before the start of encounters, pairs were kept apart by means of wire-mesh for approximately 14 h in this study, and for at least five days in the copulatory behaviour study. It is possible therefore that females perform naso-anal contact behaviour with males to which they have been exposed for longer periods.

CONCLUSIONS

As in previous studies of mate recognition involving Kamberg and Karkloof populations (Chapter 4), the results of the present study indicate the existence of

population-specific courtship behaviour. The effects of differences in courtship behaviour, which is a important SMRS, are clearly reflected in interpopulation encounters. In this study, underlying disparities in population-specific olfactory, tactile and visual cues resulted in high levels of agonistic interaction. The role of aggression as a pre-mating reproductive isolating mechanism has been identified in chromosomal races of _Spalax ehrenbergi_ (NEVO et al. 1986) and in populations of _Mus musculus_ (CORTI et al. 1989). Similarly, aggression may function as a pre-mating barrier to breeding between Kamberg and Karkloof individuals, should the populations meet in nature.

CHAPTER 6

AUDITORY COMMUNICATION

INTRODUCTION

Acoustic communication is well developed in <u>Otomys</u> <u>irroratus</u> (Davis 1972, Willan 1982, Pillay 1990). The taxon apparently favours low frequency sounds, and no evidence of ultrasonic vocalization has been found. All <u>O. irroratus</u> acoustic signals are important during the social interaction of members of the species, and are associated mainly with agonistic behaviour (Davis 1973, Willan 1982).

No attempts have yet been made to spectrographically analyze <u>O. irroratus</u> sounds, and it is therefore uncertain whether the entire acoustic repertoire of the taxon has been identified. Moreover, Baker (1987) maintains that some sounds, although acoustically indistinguishable, display characteristic tracings on a sonagraph. Another shortcoming of earlier research has been the assumption, on behavioural rather than spectrographic grounds, that <u>O. irroratus</u> does not vocalize ultrasonically.

In the present study, acoustic signals produced by Kamberg and Karkloof <u>O. irroratus</u> were spectrographically analyzed. Facilities for detecting audible (i.e. $\leq$ 18 kHz) and ultrasonic sound were available, so that recordings over a wide frequency range were possible. The aim of the study was to describe the structure of recorded sounds, as well as to ascertain the behavioural conditions under which each sound is produced.

Species-specific vocalization may act as a pre-mating reproductive isolating mechanism, as was demonstrated in chromosomal species of mole-rats (Heth & Nevo 1981, Nevo et al. 1987). The results of previous research on specific mate recognition suggest that Kamberg and Karkloof individuals distinguish between mates from the same and the other population on the basis of olfactory (Chapter 4) as well as tactile and visual cues (Chapter 5). Since nothing is known about acoustic signals in mate recognition in Kamberg and Karkloof O. irroratus, this study also aimed to establish whether or not population-specific sounds exist.

Dempster (1990) maintained that if acoustic signals are important in species recognition, a greater number of vocalizations would be expected during intra- than interspecific encounters. Although not obvious in Dempster's study, it is possible that species recognition might translate into mate recognition should a greater a number of acoustic signals occur in intra- relative to interspecific sexual encounters (Nevo et al. 1987). This hypothesis is evaluated in the present study by comparing the number of signals produced in intra- and interpopulation male-female dyads.

MATERIALS AND METHODS

Details of the maintenance of animals in captivity and the environmental conditions under which the present study was conducted are described in Chapter 2. Acoustic signals produced in 11 Kamberg and 12 Karkloof intrapopulation, and 15 Kamberg x Karkloof and nine Karkloof x Kamberg

interpopulation male-female dyadic encounters were recorded. In earlier studies on courtship, male O. irroratus produced very few sounds (Pillay 1990) and, in order to increase the likelihood of obtaining male vocalizations, recordings were also made during each of five Kamberg and Karkloof intrapopulation male-male encounters. The experimental procedure employed in all encounters is described elsewhere (Chapter 5). All females were in oestrus (confirmed by vaginal smears) at the start of encounters so as to increase the possibility of recording sounds during sexual activity.

Recordings of audible sound were made using a Sony TCM-6DX portable cassette recorder at a tape speed of approximately 8 cm/sec, and an Ambico V-0624 zoom microphone which was sensitive to frequencies of up to 30 kHz. In addition to these recordings, a QMC S100 bat detector connected to a Marantz CP230 tape recorder (tape speed 4.75 cm/sec) was used to detect ultrasonic vocalizations in 50 % of encounters. The bat detector was operated in a broadband frequency mode because it was not known whether O. irroratus produces ultrasonic signals, nor within what frequency range the signals would be if such signals were produced.

Acoustic analysis was performed using a Uniscan sonagraph (UNIGON II). A frequency range of 0 - 40 kHz was selected for sounds of high frequency, and 0 - 10 kHz for those of low frequency.

The following variables were identified in sonagrams of each sound (after Koeppl et al. 1978): (i) fundamental (lowest) and maximum frequencies; (ii) duration of each

signal, including its components (syllables); (iii) number of harmonics - the components of a sound that are multiples of the fundamental frequency; and (iv) number of syllables - sound elements that are observed in the sonagram as patterns temporally separated from other such units.

The behavioural circumstances under which sounds were produced are briefly described below.

RESULTS

A total of 671 acoustical signals were recorded, of which 156 were worthy of spectrographic analysis, as they had negligible background noise. Three main types of sound were recognized: two were vocal (chit and alarm squeal) and the other non-vocal (tail-quivering). With the exception of some **chit vocalizations which approached a maximum frequency of 24 kHz (below), no ultrasonic signals were detected.**

Mean values of the appropriate variables obtained for each acoustic signal of Kamberg and Karkloof individuals are presented in Table 1. Included in the table are the results of interpopulation comparisons (Mann-Whitney U test) of the parameters of every signal. Sounds produced by males and females of the same population were statistically indistinguishable, and data were therefore pooled.

Mean values describing the parameters of each acoustic signal were similar among all animals studied (Table 1). No significant interpopulation differences were apparent in respect of any of the parameters considered.

Table 1. Mean ± 2 SE values of the parameters describing the acoustic signals produced by the subjects indicated. No differences were found at the 5 % significance level, so P is not given. n = number of calls analyzed. U-stat = Mann-Whitney U test values.

Subjects	n	Frequency (kHz)		duration (s)	harmonics	syllables
		fundamental	maximum			
High frequency chit						
Kamberg	23	0.00±0.00[1]	21.19±0.79	0.78±0.09	-	4.65±0.79
Karkloof	30	0.00±0.00[1]	20.72±0.80	0.83±0.07	-	5.23±0.96
U-stat		-	385	400.5	-	384.5
Low frequency chit						
Kamberg	22	0.00±0.00[1]	15.96±0.46	0.76±0.10	-	5.18±1.00
Karkloof	32	0.00±0.00[1]	16.27±0.35	0.74±0.07	-	4.22±0.73
U-stat		-	376	360		438
Alarm squeal						
Kamberg	8	1.51±0.08	11.60±0.66	1.21±0.08	6.75±0.98	4.75±1.39
Karkloof	11	1.50±0.08	12.41±0.49	0.98±0.08	6.36±1.15	4.13±1.10
U-stat		48.5	62	58	47	55
'Slurred' alarm squeal						
Kamberg	6	1.35±1.31	8.14±0.85	1.01±0.12	4.76±1.40	4.67±2.17
Karkloof	6	1.39±1.06	8.37±1.20	0.88±0.11	3.76±1.96	3.51±1.52
U-stat		20	20	27	28.5	22.5
Tail-quivering						
Kamberg	17	0.00±0.00[1]	3.43±2.79	0.95±0.09	-	12.05±2.43
Karkloof	12	0.00±0.00[1]	3.32±2.70	1.18±0.16	-	14.08±2.78
U-stat		-	121.5	123	-	121

1 - Zero values are given because no distinction was made between fundamental frequency and the zero beat frequency.

Sonagrams representing chit, alarm squeal and tail-quivering signals appear as Figures 1 - 3. Chitting was used extensively in all agonistic encounters and in 33 % of sexual encounters. During aggressive encounters, subordinates (non-aggressors) produced chits more often than dominants (aggressors). Similarly, females (non-aggressors) chitted more frequently than males (aggressors), almost always when males approached or attempted to mount. Two distinct forms of chit vocalizations were identified (i.e. high and low frequency chits). High frequency chit calls were recorded when interacting dyads were in close proximity
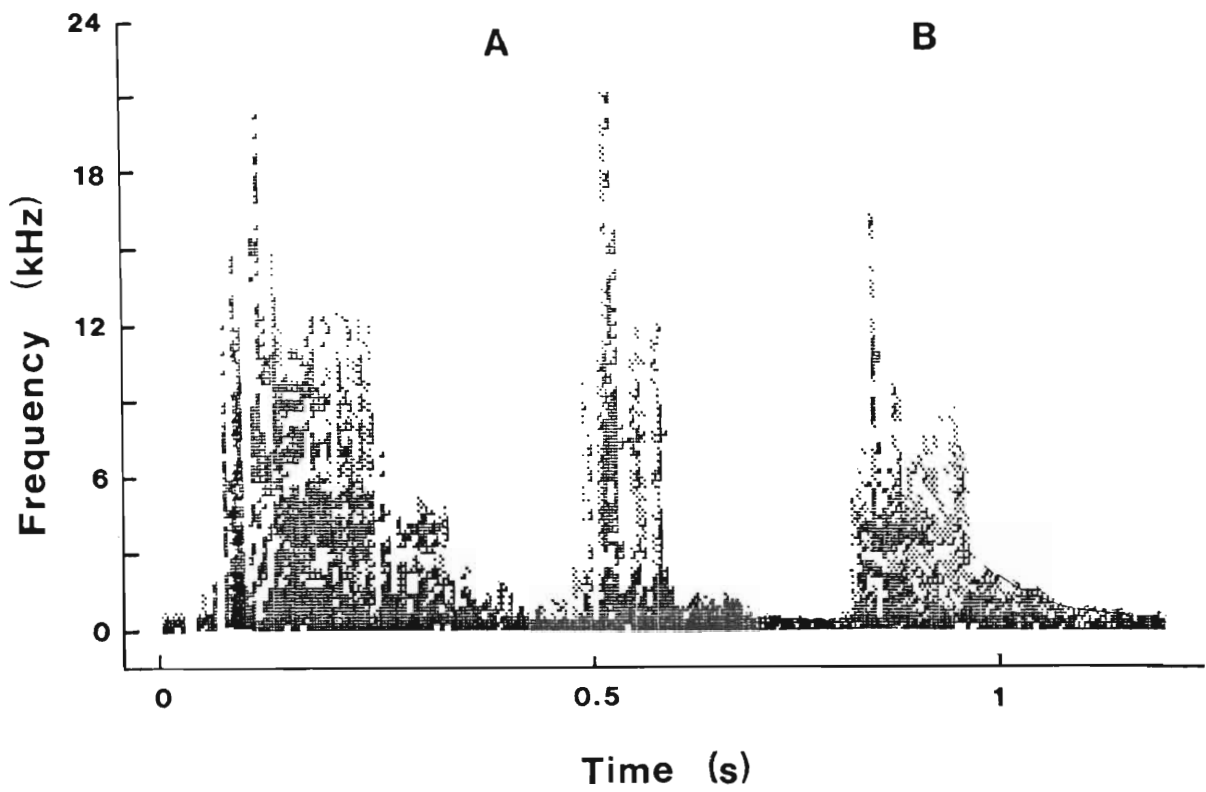
Figure 1. Representative spectrogram of high frequency (A) and low frequency (B) chit calls of O. irroratus.
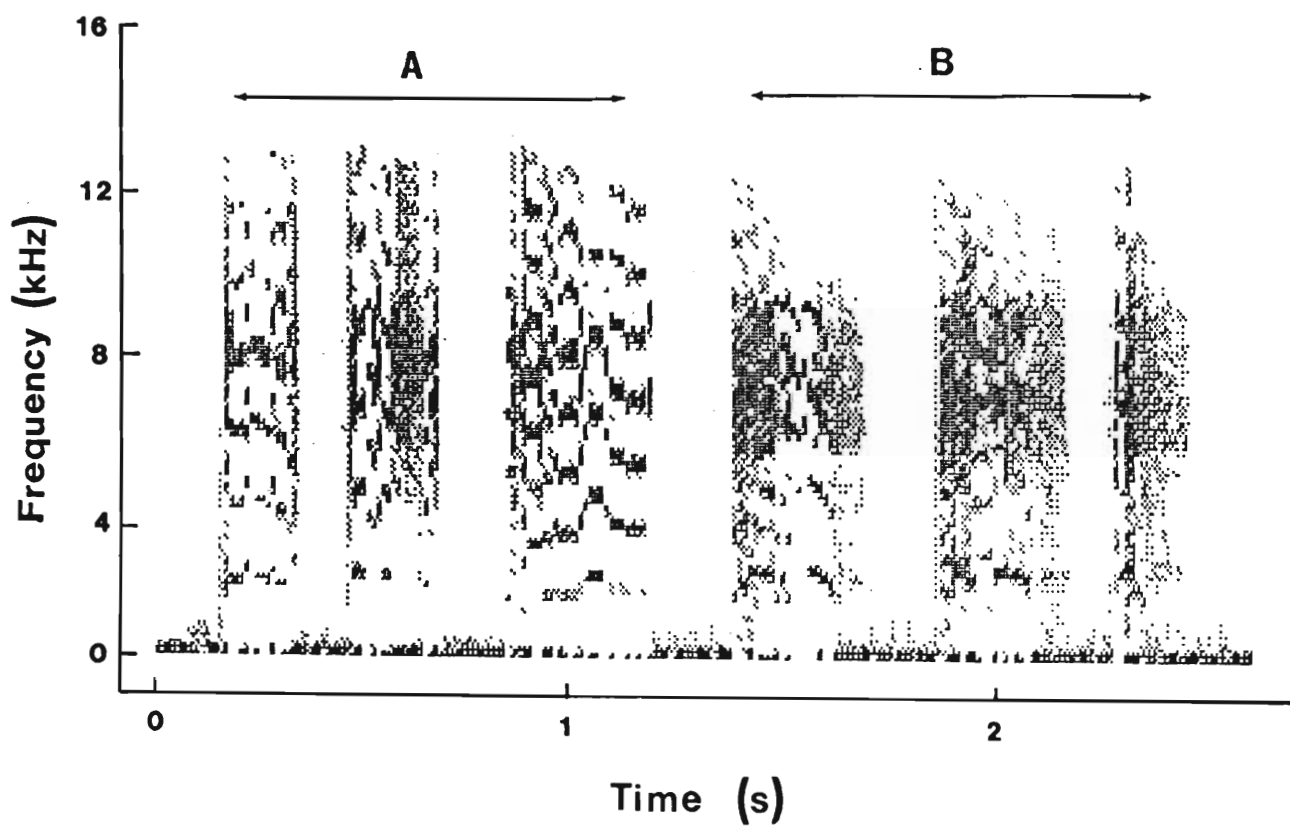
Figure 2. Representative spectrogram of normal (A) and 'slurred' (B) alarm squeal calls of O. irroratus.
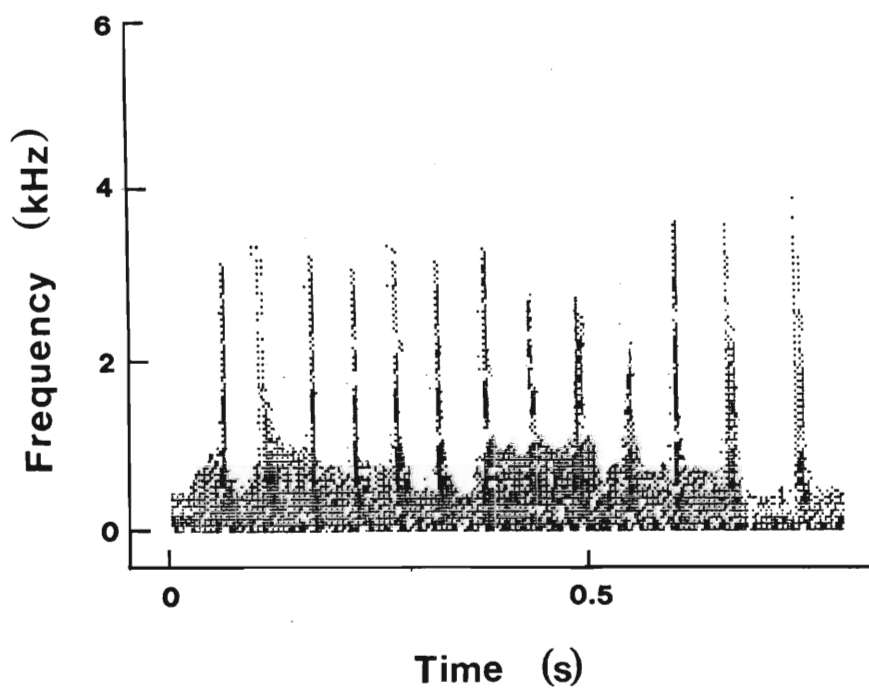
Figure 3. Representative spectrogram of tail-quivering signals of <u>O. irroratus</u>. Tail-quiver signals appear as several vertical syllables, and a broad band of background noise of 1 kHz is present.

and usually engaged in upright sparring. Low frequency chits were emitted when individuals were further apart or when the aggressor approached the non-aggressor. In 73 % of cases, chitting by the non-aggressor checked the advance or caused the retreat of the aggressor. Chits were of short duration and lacked harmonics (Figure 1).

Like chit calls, alarm squeals were recorded during both agonistic and sexual encounters. This call was issued by the non-aggressor that was under direct physical attack by the aggressor. Most dyads avoided physical contact, resulting in a low incidence (n = 31) of alarm squeal call behaviour. Two forms of alarm squeal calls were recognized (Figure 2). They were of equal fundamental frequency and duration but differed in terms of the maximum frequency. The first form of the call was characterized by clearly defined harmonics, while the second form appeared as a 'slur' on the sonagram. Behaviourally, the first form was produced by the non-aggressor when a dyad engaged in locked fighting. Subsequent to the production of an alarm squeal, the aggressor ceased its attack on the non-aggressor. If the dyad remained in close proximity immediately after fighting, the non-aggressor usually issued 'slurred' alarm squeals.

Tail-quivering was used by the aggressor in agonistic encounters, with both sexes tail-quivering during sexual interaction. Occasionally, animals produced chit and tail-quivering sounds in rapid succession, resulting in the production of compound signals as presented in Figure 4. Tail-quivering was characterized by several vertical syllables which were the result of the tail striking the
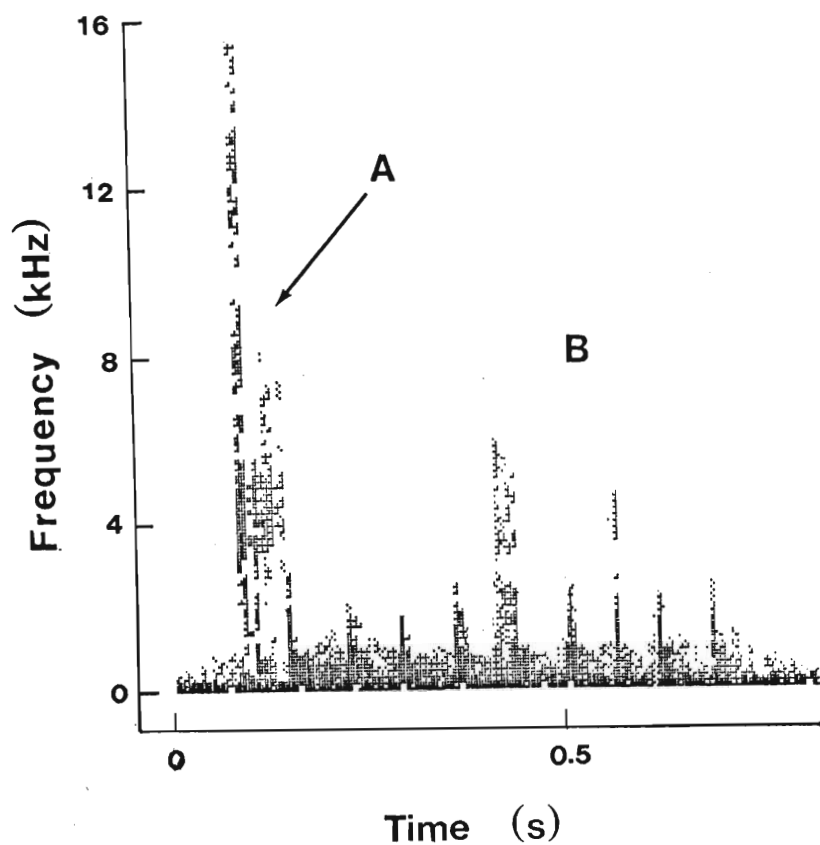
Figure 4. Representative spectrogram of a compound acoustic signal of <u>O. irroratus</u>. A low frequency chit (A) is followed by tail-quivering (B).

substrate. In all instances, tail-quivering by one individual caused the other individual to adopt an upright body posture, possibly in anticipation of imminent attack.

Median numbers of high and low frequency chit calls, and tail-quivering signals recorded during intra- and interpopulation male-female dyads are presented in Table 2; median instead of mean values are given because of the large variation in the number of signals among encounters. Alarm squeals were not considered here because of their infrequent occurrence in all encounters. More chit calls were produced during inter- than intrapopulation dyads, while an almost equivalent number of tail-quivering signals was detected in all dyads.

Pair-wise comparisons of all possible dyads (e.g. Kamberg vs Kamberg x Karkloof) in respect of each signal, using the χ^2 2 x 2 contingency test for comparison of medians (median test table; Zar 1984), confirmed the above observations. Interpopulation dyads produced significantly more high and low frequency chits than intrapopulation dyads, while there was no significant difference in the number of tail-quivering signals between any two dyads. In terms of the number of signals produced, there was no significant difference between the two intrapopulation dyads. Kamberg x Karkloof dyads produced significantly more low frequency chits than Karkloof x Kamberg dyads.

DISCUSSION

All three major acoustic signals recorded in this study were produced also by O. irroratus from Transvaal highveld (Davis 1972) and Natal Midlands (Willan 1982) localities. As

Table 2. Median numbers of acoustic signals produced during the male-female intra- and interpopulation encounters indicated. Range given in brackets.

| Dyadic encounters | Chit | | tail-quiver |
	high frequency	low frequency	
Intrapopulation			
Kamberg	21 (16-41)	23 (0-34)	14 (7-23)
Karkloof	18 (15-46)	36 (17-41)	12 (6-21)
Interpopulation			
Kamberg x Karkloof	72 (41-101)	46 (0-62)	15 (7-21)
Karkloof x Kamberg	68 (42-83)	57 (2-120)	11 (5-48)

none of the previous researchers had access to a sonagraph, they were not able to distinguish two forms of alarm squeals. The importance of spectrographic analysis of acoustic signals is illustrated by the fact that acoustic distinction between 'slurred' and normal alarm squeals could be made only when each squeal was played back on the cassette recorder and simultaneously matched with its tracing on the sonagraph.

Lack of ultrasonic vocalizations by animals in this study, as well as in studies of other O. irroratus populations (Davis 1972, Willan 1982), may be directly attributable to the grassland habitats preferred by O. irroratus (De Graaff 1981). High frequency calls are used for communication over short distances (Stoddart & Sales 1985), and are markedly attenuated by grass (Smith 1979). Hence, O. irroratus appears to be adapted to produce comparatively low frequency sounds, which are less attenuated by grass. This conclusion is supported by the fact that

dyads produced high frequency chit calls when they were in close proximity and low frequency chits when they were further apart.

In contrast to other rodents (<u>inter</u> <u>alia</u> Koeppl <u>et</u> <u>al</u>. 1978, Stoddart & Sales 1985), <u>O. irroratus</u> produces a limited number of acoustic signals, all of which are associated mainly with aggressive interaction, confirming the findings of Davis (1972) and Willan (1982). The social structure of Kamberg males and all Karkloof individuals encompasses territoriality and adult isolation; Kamberg females appear to be non-territorial and intrasexually amicable (discussed in Chapter 8). Aggressive/territorial animals tend to emphasize agonistic sounds during social encounters, as reported in the slender mongoose <u>Herpestes</u> <u>sanguineus</u> (Baker 1982).

Functional significance of acoustic signals

Previous research has suggested that <u>O. irroratus</u> has evolved ritualized aggression, and that acoustic signals in conjunction with visual and tactile cues may be important in preventing damaging fights (Davis 1972, Willan 1982, Brown 1988, Pillay 1990). The function of each acoustic signal in ritualized aggression is discussed in the following paragraphs.

Most of the sounds recorded during encounters were chit calls. The importance of these calls may be related to the mode of dispersion of <u>O. irroratus</u>. Over large parts of its range, the taxon prefers mesic, well vegetated habitats (De Graaff 1981). These habitats are often of limited size (Willan 1982) and population densities may be as high as 80

individuals per ha during the breeding season (Brown 1988).
In species which are conspecifically highly aggressive and
territorial and which occur at high densities, like
O. irroratus, it is predictable that selection would favour
mutual avoidance. Hence, low frequency chit calls, which may
be transmitted in densely vegetated habitats (Smith 1979),
would provide individuals with information as to the
approximate location of conspecifics (Willan 1982), thereby
preventing contact between highly aggressive neighbours.
When animals do establish contact, high frequency chits would
prevent animals from physically attacking one another,
clearly demonstrating the adaptive function of this acoustic
signal.

Koeppl et al. (1978) more easily located ground
squirrels (Spermophilus spp.) that were squealing than those
which produced other calls. This was possible because of the
low frequency and abundant harmonics of squeal calls.
Further, it was suggested that these calls allowed colony
members to locate one another. In the present study, alarm
squeals were of longer duration and lower frequency than chit
calls and comprised several harmonics. While it is tempting
to speculate that alarm squeals allow individual O. irroratus
to locate each other, this may not be feasible for two
reasons. First, in contrast to the highly sociable
Spermophilus spp., O. irroratus is highly aggressive and
territorial, and, except during mating, individuals avoid one
another. Second, alarm squeals were produced when animals
were in close proximity to one another.

An alternative explanation for the function of alarm squeals may be to ensure the location and retrieval of young by the mother (Koeppl et al. 1978). Unweaned O. irroratus pups separated from the mother normally issue alarm squeal calls (Davis & Meester 1981), and are then retrieved by the mother (Pillay et al. 1993). Sonagrams of squeals of the young are identical to those of the adults (N. Pillay Unpubl. data). It is possible therefore that squeal calls by the young constitute attention/distress signals, and are retained in the adult vocal repertoire where they may be important in appeasement behaviour; alarm squealing by the non-aggressor usually prevented damaging fights. In addition, 'slurred' alarm squeals by non-aggressors ensured that aggressors did not mount further attacks on them, supporting the hypothesis that these calls serve as appeasement signals.

Tail-quivering is known to provide auditory as well as visual cues in some rodents (Clark & Schien 1966, Ewer 1968). Ewer (1968) also maintains that such behaviour may be well developed in species which rarely engage in biting attack. Tail-quivering may therefore be important in ritualized aggression in O. irroratus.

Evolutionary considerations

There were no significant differences between the data sets representing acoustic signals produced by Kamberg and Karkloof subjects. This was entirely predictable, in view of the fact that all signals were associated with agonistic interaction and not courtship behaviour. Signals which favour ritualized aggression, and which permit the

establishment and maintenance of the spacing of individuals, are unlikely to be subject to adaptive variation. Selection would therefore operate to maintain such signals. By the same token, selection is more likely to favour variation in signals which function as specific-mate-recognition signals during courtship (Paterson 1985). In this case, differences in population-specific acoustic signals either arise as a response to local environmental conditions in allopatry (Catchpole & McGregor 1985), or act as pre-mating barriers in sympatry (Stratton & Uetz 1981; Nevo et al. 1987).

Interpopulation dyads produced significantly more chit vocalizations than intrapopulation dyads, contradicting the hypothesis that animals produce more vocalizations in response to stimuli from same- rather than different-population mates (Dempster 1990). Chit calls were associated with aggressive interaction, and a greater number of these calls during interpopulation encounters may complement interpopulation differences in olfactory, visual and tactile cues in promoting aggression. Aggressive behaviour is a potential pre-mating isolating mechanism between Kamberg and Karkloof individuals (Chapter 5).

Unless differences existed which were too subtle for detection, the evidence presented here indicates that population-specific acoustic signals do not exist among the Kamberg and Karkloof populations. Hence, the acoustic signals recorded in this study served neither as mate recognition signals, nor as pre-mating reproductive isolating mechanisms, although they work indirectly to promote aggressive interaction during interpopulation male-female encounters.

CHAPTER 7

POPULATION-SPECIFIC HABITAT CHARACTERISTICS AND

POPULATION ECOLOGY

INTRODUCTION

Socio-ecological studies have focused on the adaptive significance of social systems, and have attempted to relate the social organization of a species to its ecological circumstances (Crook 1965, 1970, Barash 1989, Standen & Foley 1989 and references therein). In such relationships, the environment is particularly important because it is the underlying determinant of the social behaviour of individuals, thereby influencing the social organization of species (Crook et al. 1976, Barash 1989, Standen & Foley 1989). Knowledge of the environmental characteristics (especially those concerning the habitat) of a species are therefore central to the study of its socio-ecology. Habitat characteristics of primary interest are stability (i.e. the predictability and degree of seasonal variation in the carrying capacity; Happold 1976; Willan 1982) and the length of the growing season (Barash 1974, 1989), although the spatial distribution and availability of food and cover are also important (inter alia Crook 1965, 1970, Jarman 1974, Andersen et al. 1976).

On the basis of these concepts, field studies were undertaken to describe and compare the habitat characteristics of Kamberg and Karkloof Otomys irroratus. Because of their importance in determining social organization, the availability of food and cover were

investigated, while other factors which influence both food and cover were also examined (e.g. soil characteristics). Data obtained here were used to ascertain: (i) the factors that influenced the ecological distribution of O. irroratus in both localities, which in turn provides information with regard to population-specific habitat requirements; and (ii) the relative harshness of each habitat. Habitat harshness rather than habitat stability is considered in this study because the concept of harshness is broader and includes that of stability, as defined by Brown (1988); this issue is discussed in detail later (see DISCUSSION). Habitat requirements and habitat harshness are key variables in the study of socio-ecology (Crook et al. 1976, Happold 1976).

In addition to habitat characteristics, several population characteristics were studied. These comprised indices of home range size, sex ratio, abundance and ecological distribution. The aim of this study was to provide a basis for the studies of social structure and socio-ecology which are discussed in Chapters 8 and 9.

MATERIALS AND METHODS

Study areas

The Kamberg Nature Reserve (Kamberg) and Hans-Merensky Forest (Karkloof) are part of the Grassland Biome recognized by Rutherford & Westfall (1985). Acocks (1988) described the vegetation at Kamberg as Highland sourveld (veld type 44a) and that at Karkloof as Natal mist belt 'Ngongoni veld (veld type 45). Although much of the Kamberg vegetation still fits the description provided by Acocks, this is not the case at Karkloof. As a result of large-scale afforestation at

Karkloof, the 'Ngongoni veld is restricted to a few isolated patches. Clearings in the forest where trees have succumbed as result of abiotic (e.g. snow) or biotic (e.g. ring-barking by rodents) damage, as well as abandoned dirt roads overgrown with grass, serve as 'corridors' linking the patches of 'Ngongoni veld. On the basis of visual signs, like grass cuttings and nests, it appears that O. irroratus densities are very high in the corridors during late summer and autumn, suggesting that these are used as dispersal routes.

Kamberg receives more rainfall than Karkloof: mean annual rainfall from 1986 to 1991 was 1186 mm at Kamberg (Natal Parks Board Unpubl. data) and 1023 mm in the Karkloof Forest (R. Parker Pers. comm.). However, condensing mist may substantially increase the annual precipitation at Karkloof (R. Parker Pers. comm.). At both localities, rainfall is seasonal (Figure 1), with approximately 80 % falling in summer. Frost is common for at least six months (April - September) at both localities (Bowland & Perrin 1993, R. Parker Pers. comm.), and severe frost is known to occur at Kamberg. Snow occurs occasionally at both localities. Temperatures at Kamberg range from a mean annual minimum of 4.5^{0}C in July to a mean annual maximum of 13.2^{0}C in January (Humphrey 1983). Mean temperatures at Karkloof are higher, and 8.4^{0}C (July) and 18^{0}C (February) were calculated as the mean annual minimum and maximum respectively (Oatley in litt.) .

Two sites per locality were selected for study. Selection was based on preliminary ad libitum trapping, which indicated that O. irroratus was locally abundant. The
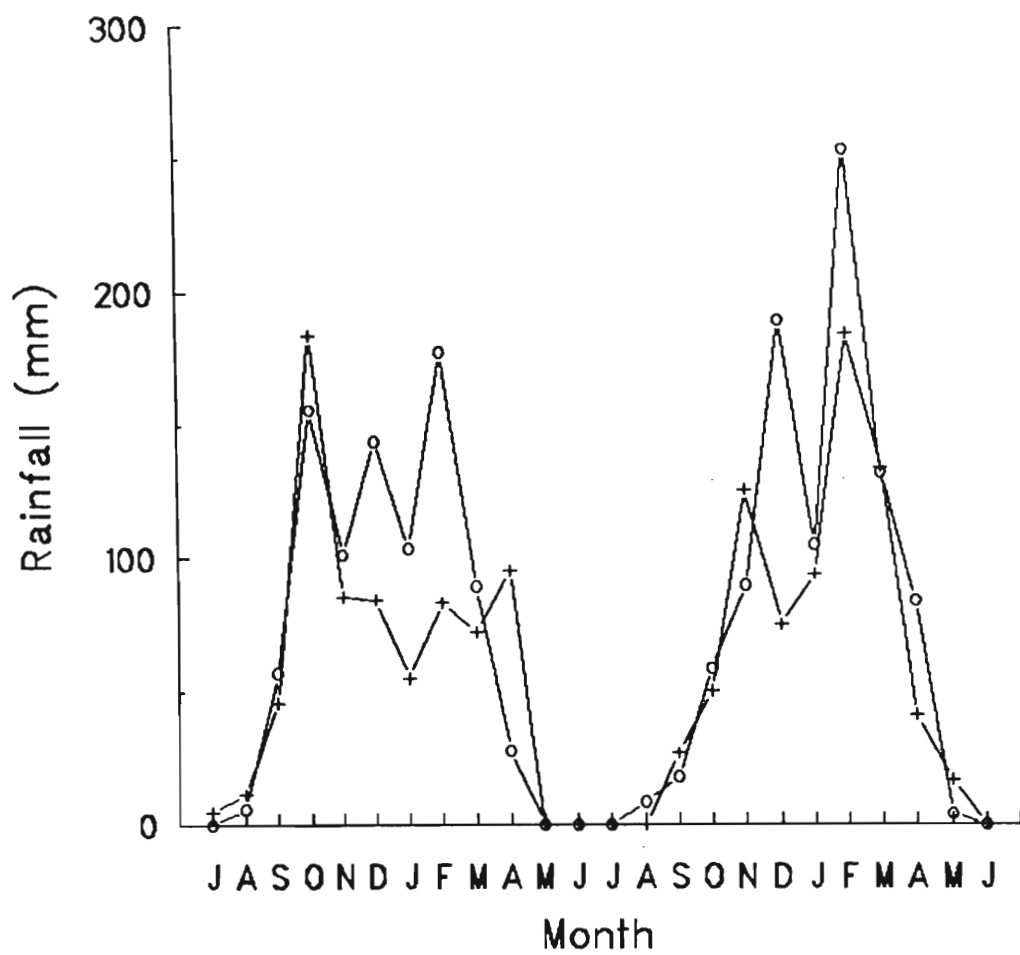
Figure 1.   Mean monthly rainfall (July 1991 - June 1993)
recorded at the Kamberg Nature Reserve (o-o) and
Hans-Merensky (Karkloof) Forest (+-+).

Kamberg sites, referred to as A (29^{0}23'08"S; 29^{0}42'21"E,
altitude 1660 m above sea level.) and B (29^{0}23'38"S;
29^{0}42'19"E, 1700 m a.s.l.), were approximately 2 km apart,
both on grass-dominated level ground/valley bottoms. The
nearest common water source (vlei/wetland) was ± 1 km away
from both sites. The Karkloof sites, referred to as C
(29^{0}17'09"S; 30^{0}11'31"E, 1576 m a.s.l.) and D (29^{0}17'52"S;
30^{0}11'11"E, 1550 m a.s.l.), were approximately 3 km apart,
both on gently sloping (5^0 - 10^0) south-facing hillsides. The
sites were densely vegetated with tall grass. At the edge of
each site were pine trees (Pinus sp.) of 3 - 4 m height at
site C and 11 - 15 m at site D. A perennial stream was
present at both of the Karkloof sites. At site C, a stream
opened into a dam at the bottom of the hill, while a stream
flowed at the edge of site D.

Plant species collected at the study sites are listed in
Table 1. The comparatively low floristic diversity at both
localities (i.e. 44 species at Kamberg and 45 at Karkloof),
together with the high number of exotics indicate that both
habitats are disturbed (Willan 1982), possibly as a result of
regular burning at Kamberg (J. Vermeulen Pers. comm.) and the
afforestation at Karkloof. Three grasses dominated the
vegetation at each locality: Eragrostis curvula and Themeda
triandra at both localities, and Enneapogon scorparius at
Kamberg and Bothriochloa bladhii at Karkloof.

Population characteristics

Otomys irroratus populations were sampled at the Kamberg
and Karkloof localities in summer 1992 (December), autumn

Table 1. Plant species present at the Kamberg and Karkloof study areas. Family names given in capitals. * = exotic.

Kamberg	Karkloof
ARACEAE	
Zantedeschia aethiopica	Zantedeschia aethiopica
BORAGINACEAE	
Heliotropium amplexicaule *	
COMPOSITAE	
Berkheya speciosa	Cirsium vulgare *
Bidens pilosa *	Conyza pinnata
Conyza obscura	Conyza scariba
Conyza pinnata	Helichrysum aureo-nitens
Conyza scariba	Helichrysum latifolia
Helichrysum aureum	Helichrysum longifolium
Helichrysum aureo-nitens	Helichrysum umbraculigerum
Helichrysum griseum	Senecio isatideus
Helichrysum latifolium	Senecio madagascariensis
Helichrysum palidum	Tagetes minuta
Senecio bupleuroides	
Senecio heliopsis	
Senecio madagascariensis	
Tagetes minuta	
CYPERACEAE	
	Cyperus obtusiflorus
	Kyllinga erecta
DENNSTAEDTIACEAE	
Pteridium aquilium	Pteridium aquilium
EUPHORBIACEAE	
Acalypha penduncularis	Acalypha penduncularis
	Acalypha punctata
GERANIACEAE	
Geranium sp.	
HYPOXIDACEAE	
Hypoxis angustifolia	Hypoxis angustifolia
IRIDACEAE	
	Crocosmia pottsii
LAMIACEAE	
Leonotis intermedia	Leonotis intermedia
LILIACEAE	
Bulbine frutescens	Ledebouria floribanda
OXALIDACEAE	
	Oxalis smithiana

Table 1. Continued.

Kamberg	Karkloof
	PINACEAE
	Pinus elliotti *
	Pinus patula *
	POACEAE
Aristida canescens	Agrostis montevidensis *
Agrostis montevidensis *	Bothriochloa bladhii
Diheteropogon filifolius	Cymbopogon excavatus
Diheteropogon amplectens	Diheteropogon filifolius
Enneapogon scorparius	Enneapogon scorparius
Eragrostis capensis	Eragrostis curvula
Eragrostis curvula	Eragrostis racemosa
Eragrostis racemosa	Harpochloa falx
Harpochloa falx	Heteropogon contortus
Hyparrhenia hirta	Hyparrhenia hirta
Hyparrhenia dichroa	Miscanthus capensis
Paspalum dilatatum	Panicum natalense
Setaria sphacelata	Paspalum dilatatum
Sporobolus fimbriatus	Paspalum paspaloides
Stipa trichotoma *	Setaria sphacelata
Themeda triandra	Stipa trichotoma *
Trachypogon spicatus	Themeda triandra
	ROSACEAE
Rubus sp.*	Rubus sp.*
	RUBIACEAE
Pentanisia prunelloides	
	RANUNCULACEAE
Ranunculus multifidus	Ranunculus multifidus
	TYPHACEAE
	Typha latifolia
	VERBENACEAE
Verbena bonariensis	Verbena bonariensis

1993 (April), and winter 1993 (July), using standard live-trapping techniques (Krebs 1966). Sites B (Kamberg) and D (Karkloof) were burnt in May/June 1993, and therefore were not sampled in July.

Census of rodent populations was conducted by means of information gathered from trapping along traplines. Although

it would have been desirable to have used the more conventional trapping grids in this study, the trapping sites were either too small (Kamberg) or too interrupted by watercourses (Karkloof) for setting up trapping grids. Moreover, census of rodents along traplines has also been employed in other rodent studies (_inter alia_ Willan 1982, Bonadventura 1992, Douglass _et al_. 1992).

At each site, PVC live traps (Willan 1979) were placed in two permanently marked parallel rows 15 m apart. In each row, 15 trap-stations were placed 10 m apart. Two traps, baited with a rolled oats/currants/maize seed mixture, were placed at every station. Traps were set for five days during each season and were cleared at dawn and dusk. Captured _O. irroratus_ were individually toe-clipped and released where caught, after the mass to the nearest gram, sex, reproductive condition and station of capture had been recorded. Individuals were deemed to be adult if they weighed $\geq$ 90 g (after Willan 1982). Males were considered sexually mature when the testes had descended into the scrotal sac and were of full adult size. Sexually active females were those that were gravid (ascertained by palpation), lactating (milk expressed from the nipples) or had the vaginal orifice open (perforate).

Four variables were calculated from trapping data obtained in every trap-site during each season. These were: 1. Abundance/number of _O. irroratus_ on the traplines, expressed as a percentage caught per station night of effort (Willan 1982). Conventional estimation of population density, such as removal methods (Southwood 1966), could not

be performed because of the low numbers of animals caught during any one trapping period.

2. Distribution of individuals along the traplines, calculated by using Green's index (Ludwig & Reynolds 1988). Green's index is computed as $GI = [(S^2/X)-1]/(n-1)$, where X = mean number of captures per station, S^2 = the variance, and n = total number of individuals caught. GI is favoured over other distribution formulae as it can be used to compare data sets with different sample sizes (Ludwig & Reynolds 1988). GI values equal 0 when distribution is random, and equal 1 for clumped distribution, while values approximate $-1/(n-1)$ when distribution is uniform.

3. Index of adult home range size, calculated as the average distance (Av.D) travelled between successive captures (Brant 1962). This method was chosen in order to make comparisons between the results of the present study with those of Brown (1988) who used Av.D. to ascertain indices of home range size in an Eastern Cape O. irroratus population. In addition, Av.D. values correlate well with the home range sizes of several rodents (Wolfe 1968, Slade & Swihart 1983, Bonaventura et al. 1992).

4. Overall and adult sex ratio (expressed as the number of females per male) regardless of the reproductive condition of each sex, and the percentage adults sexually active.

The Student's t-test and chi-square test were used to analyze differences between means and frequencies respectively (Sokal & Rohlf 1987).

Habitat characteristics

During each season, two vegetation transects, which overlapped the trapping transects, were sampled per study site. Two 1 m² quadrats were each placed about 1 m away from every trap-station. Maximum plant height was measured and percentage cover of green plants visually estimated within each quadrat. In addition, the percentage cover provided by the dominant grasses (Table 1) was visually estimated in summer. This was not possible in autumn and winter, however, as most grasses were devoid of floral structures, reducing the accuracy with which individual grass species could be identified. In all cases, cover estimates were divided into six classes: (1) 0 - 10 %; (2) 11 - 30 %; (3) 31 - 50 %; (4) 51 - 70 %; (5) 71 - 90 %; (6) 91 - 100 %.

Soil depth was ascertained by hammering a 1.5 m pointed steel rod (1.5 cm diameter) into the ground until it could penetrate no further; one reading per station was obtained. Other soil characteristics were obtained from a single 5 cm deep scrape per station. The soil was initially differentially weighed before and after drying to establish soil moisture content and thereafter sieved (mesh size = 0.5 cm) to obtain clay-loam fractions.

An index of cover provided by the vegetation at each trap-station was ascertained by measuring light penetration to ground level, using a LI-COR Model LI-188B integrating photometer fitted with a quantum sensor. The use of a light meter was favoured over other techniques (see Phillips 1959) because the meter provided a quantitative measure of cover which allowed statistical analysis of the data obtained

(below). Other rodent studies have also used a light meter to obtain an assessment of cover (see Willan 1982, Marchand 1984).

The photometer used in this study is responsive to photosynthetically active radiation (wavelength = 400 - 700 nm), and the sensor is cosine-corrected to reduce errors when readings are made in diffuse radiation conditions. The photometer was integrated over 10 s intervals. Readings were taken by first measuring incident light levels above a grass canopy, and immediately thereafter recording light levels at the base of the grass, directly below the first reading. Light penetration was calculated by expressing the reading at the base as a percentage of the reading above the canopy. Five sets of readings were taken within 5 m of every station, in small mammal runways, and always on cloudless days between 13h00 - 14h00. Station means were calculated.

Multiple regression analyses (Zar 1984) were used to ascertain the habitat variables which influenced the distribution of O. irroratus at both localities during each season. Following the procedures described in other rodent trapping studies (Douglass 1976, M'Closkey 1976, Douglass et al. 1992), the number of O. irroratus caught per trap-station was regressed against the measured habitat variables at that station. Because of the low number of O. irroratus captures per trap-site, the data gathered from each of the two trap-sites (i.e. A & B or C & D) per locality were pooled in the multiple regression analysis. In addition, all data sets used in the multiple regression analyses were arcsine

transformed to produce values whose frequencies were normally distributed (Pimentel 1979, Sokal and Rohlf 1987).

RESULTS

During 5400 trap-nights of effort per locality, a total of 61 and 99 individual O. irroratus were trapped at Kamberg and Karkloof respectively. At all study sites, the striped mouse Rhabdomys pumilio was the most common small mammal species. The mean ratio of Rhabdomys:Otomys was 5.4:1 over all seasons. Other species that were infrequently caught were the Natal multi-mammate mouse Mastomys natalensis and the grey pygmy climbing mouse Dendromus melanotis at Kamberg, and the water rat Dasymys incomtus and greater musk shrew Crocidura flavescens at both localities.

Population characteristics

Seasonal variation in the distribution and abundance of O. irroratus is given in Table 2. The population density at Karkloof was higher than that at Kamberg during all seasons, as suggested by the values of abundance (Table 2). Generally, abundance was high in summer and autumn and decreased in winter. Green's index indicates that the distribution of O. irroratus at the Kamberg study sites was clumped during all three seasons, while at Karkloof individuals were either uniformly or randomly distributed in summer and autumn and clumped in winter (Table 2).

Table 2. Abundance and distribution of O. irroratus at the Kamberg and Karkloof trap-sites during the seasons indicated. n = number of individual captures.

Season/ Locality	Site	n	Abundance (%)[1]	Distribution Green's[2] index	Predicted[3] distribution
Summer					
Kamberg	A	15	5.0	0.31	Clumped
	B	10	3.3	0.41	Clumped
Karkloof	C	25	8.3	-0.04	Uniform
	D	17	5.7	-0.07	Uniform
Autumn					
Kamberg	A	14	4.7	0.27	Clumped
	B	16	5.3	0.33	Clumped
Karkloof	C	28	9.3	-0.08	Uniform
	D	19	6.3	0.02	Random
Winter					
Kamberg	A	6	2.0	0.58	Clumped
Karkloof	C	10	3.3	0.63	Clumped

1 - Abundance = trapping success per station night of effort.
2 - Green's index - calculated as $[(S^2/X)-1]/(n-1)$, see text.
3 - The presumed distribution, based on Green's index values, of O. irroratus along the traplines.

Overall and adult sex ratios strongly favoured females in Kamberg at all times (Table 3), but significant departures from parity were recorded only in the adult sex ratio in summer at site A ($\chi^2 = 4.5$, $p < 0.05$). All sex ratios were approximately equal to one at the Karkloof study sites.

All individuals were sexually active in summer. In autumn, nearly all Kamberg adults captured were in breeding condition, while only three of the 26 individuals (i.e. two males and a female) caught at Karkloof were in reproductive condition (Table 3).

Table 3. Overall and adult sex ratio (number of females relative to one male) and percentage sexually active adult animals at the Kamberg and Karkloof study sites during the seasons indicated.

Season/ Locality	Site	Sex ratio		% sexually active[1]	
		Overall	Adult	Males	Females
Summer					
Kamberg	A	1.50	7.00	100	100
	B	2.33	6.00	100	100
Karkloof	C	0.92	0.88	100	100
	D	0.89	1.40	100	100
Autumn					
Kamberg	A	1.80	2.50	100	100
	B	1.29	4.00	100	75
Karkloof	C	0.75	1.29	29	11
	D	1.11	1.00	0	0
Winter					
Kamberg	A	2.00	3.00	0	0
Karkloof	C	1.50	1.50	0	0

1 - scrotal males; perforate, gravid or lactating females.

Mean body mass and mean Av.D. of males and females at Kamberg and Karkloof are presented in Table 4. No statistical comparisons were made in respect of either body mass in winter, or mean Av.D. values during all seasons, because sample sizes were low (Table 4). In order to confirm the existence of seasonal variation in body mass, as well as the existence of sexual dimorphism, the mass of palpably pregnant females was excluded from the data set since the additional foetal mass would have biased the analysis. Generally, body mass of both sexes decreased seasonally. Male-biased sexual dimorphism in body mass was evident only in the Kamberg population and only in summer ($t_4 = 4.47$,

Table 4.   Mean ± 2 SE body mass of, and average distance travelled (Av.D.) by, adult Kamberg and Karkloof males and females during the seasons indicated.   Ranges given in brackets.   n = sample size.

Season/	Body mass (g)		Av.D. (m)	
Locality	Males	Females	Males	Females
Summer				
Kamberg	188.0±24.0 (176-200) n = 2	143.3±9.1 (132-154) n = 4	18.8±3.1 (17.2-20.3) n = 2	12.6±2.0 (10.0-16.3) n = 6
Karkloof	145.5±6.7 (123-163) n = 13	136.8±8.7 (124-148) n = 5	14.2±2.3 (10.0-17.3) n = 6	11.6±2.0 (10.0-14.1) n = 4
Autumn				
Kamberg	169.7±8.5 (164-178) n = 3	138.3±11.1 (124-150) n = 4	18.3±0.9 (13.2-14.1) n = 2	12.2±1.4 (10.0-14.1) n = 5
Karkloof	140.3±8.2 (115-158) n = 10	135.3±7.1 (115-152) n = 13	11.2±1.6 (10.3-13.1) n = 4	10.1±0.22 (11.2-12.1) n = 4
Winter				
Kamberg	153.0±8.0 (149-157) n = 2	127.6±13.9 (124-142) n = 3	- -	- -
Karkloof	133.0±18.0 (124-142) n = 2	124.7±11.6 (115-135) n = 3	- -	- -

$p < 0.05$) and autumn ($t_5 = 3.52$, $p < 0.05$).   During all seasons, Kamberg males and females were heavier than their Karkloof counterparts, with significant differences recorded only between males and only in summer ($t_{13} = 4.46$, $p < 0.001$) and autumn ($t_{11} = 3.47$, $p < 0.05$).

In winter, all or most individuals were restricted to comparatively favourable microhabitats along the traplines (see Habitat characteristics, below), displaying zero Av.D.

These values are therefore not considered here. Data presented in Table 4 indicate that mean Av.D. of Karkloof O. irroratus decreased from summer to autumn, and that of Kamberg O. irroratus was seasonally unvarying. Kamberg males displayed the highest mean Av.D.

Habitat characteristics

Locality-specific seasonal habitat variables are given in Table 5. Plant height varied seasonally at both localities; seasonal variation was generally greater at Kamberg than at Karkloof. However, the presence of Hyparrhenia dichroa, a grass exceeding 2 m in height, resulted in a higher mean value for plant height at Kamberg during winter. The proportion of green plants also decreased seasonally at both localities, with the highest and lowest modal class values recorded respectively at Karkloof in summer (91 - 100 %) and Kamberg in winter (11 - 30%). Higher overall modal class values were recorded at Karkloof than at Kamberg. Measurements of soil depth, soil moisture content and clay-loam fractions were constant during all seasons at both localities. However, the soil at Kamberg always contained more clay and was drier than that at Karkloof. Light penetration to ground level was lowest in summer and highest in winter. Light penetration values at Karkloof were consistently lower than at Kamberg.

Tables 6 and 7 provide the results of stepwise multiple regression analyses for the number of captures of O. irroratus versus the habitat parameters at both localities during each season. Correlation coefficients are included to

Table 5. Locality-specific seasonal habitat parameters. With the exception of % cover of green plants for which modal class values are given (see text), all other values represent mean values. Data for summer and autumn sampling represent pooled values at sites A & B at Kamberg and sites C & D at Karkloof. Sample sizes for both localities: plant characters = 240 quadrats/season in summer and autumn and 120 quadrats in winter; soil characters and light penetration = 60 readings in summer and autumn and 30 readings in winter. 2 SE given in brackets.

Parameters	Kamberg			Karkloof		
	Summer	Autumn	Winter	Summer	Autumn	Winter
Plant height (cm)	84.3 (10.2)	77.5 (8.1)	126.0 (58.3)	136.7 (11.5)	123.5 (11.7)	107.1 (18.2)
% cover green plants	71-90	51-70	11-30	91-100	71-90	31-50
Soil depth (cm)	97.5 (14.2)	88.4 (17.4)	94.3 (13.3)	97.0 (11.0)	95.9 (12.9)	92.0 (21.8)
% soil moisture	27.2 (2.5)	32.4 (4.4)	32.8 (5.1)	43.0 (1.8)	40.0 (2.5)	37.0 (2.3)
Clay-loam fraction (%)	0.2 (0.1)	0.2 (0.1)	0.3 (0.1)	0.5 (0.1)	0.4 (0.1)	0.5 (0.1)
Light penetration (%)	5.2 (2.3)	7.3 (4.8)	13.6 (9.1)	0.6 (0.2)	2.5 (1.6)	5.3 (2.3)

Table 6. Multiple regression analysis of abundance of O. irroratus versus habitat parameters at Kamberg during the seasons indicated. Add F = additive variance, Cum F = cumulative variance, R = correlation coefficient, Sig. = level of significance (ns: p > 0.05; s: p < 0.05;).

Season	Multiple regression			Correlation coefficients		
	Parameter	% Add F	% Cum F	Parameter	R	Sig
Summer	Light pen.		37	Light pen.	-0.87	s
	Plant height	27	54	Plant height	0.76	s
	% cover T. triandra	24	78	% cover E. curvula	0.85	s
	% cover E. curvula	15	93	% cover T. triandra	-0.64	s
	Soil moisture	3	96	Soil moisture	0.13	ns
				Soil depth	0.90	s
Autumn	Light pen.		36	Light pen.	-0.77	s
	Plant height	15	51	Clay-loam (%)	-0.19	ns
	Clay-loam (%)	2	53	Plant height	0.73	s
				Soil depth	-0.16	ns
				Soil moisture	0.74	s
Winter	Light pen.		25	Light pen.	-0.71	s
	Plant height	23	48	Plant height	0.68	s
	% cover green plants	3	51	% cover green plants	0.38	ns

Table 7. Multiple regression analysis of abundance of O. irroratus versus habitat parameters at Karkloof during the seasons indicated. See Table 6 for an explanation of the abbreviations.

Season	Multiple regression			Correlation coefficients		
	Parameter	% Add F	% Cum F	Parameter	R	Sig
Summer	Soil moisture		21	Soil moisture	0.68	s
	Plant height	15	36	% cover E. curvula	0.63	s
				Plant height	0.82	s
				Light pen.	-0.28	ns
Autumn	Soil moisture		44	Clay-loam (%)	0.64	s
	Light pen.	4	48	Light pen.	-0.28	ns
	Clay-loam (%)	2	50	Soil moisture	0.16	ns
Winter	% cover green plants		40	% cover green plants	0.74	s
	Plant height	13	53	Plant height	0.65	s
	Light pen.	5	58	Soil moisture	0.32	ns
	Soil moisture	1	59	Light pen.	-0.35	ns

indicate negative or positive associations for various variables. The order in which the correlation coefficients are listed in Tables 6 & 7 was ascertained using F-test values (Zar 1984).

During summer, the number of O. irroratus captures at Kamberg was primarily associated with percentage light penetration (negative correlation), plant height, and cover of T. triandra (negative correlation) and E. curvula. In addition, soil moisture content contributed small elements to the multiple regression. During autumn and winter, the distribution of animals was determined largely by light penetration (negative correlation), with plant height also influencing distribution during these seasons. The cover of green plants was important in winter, although its influence was not statistically significant (Table 6).

Otomys irroratus captures at Karkloof correlated with soil moisture content and plant height in summer, soil moisture content in autumn, and cover of green plants and plant height in winter (Table 7).

DISCUSSION

The data set obtained in this study is large and for this reason the discussion is divided into four broad sections: habitat description; ecological distribution; population ecology; and comparative ecology. The characteristics of the habitats or populations at Kamberg and Karkloof are discussed separately in the first three sections, and are compared only in the last section.

Habitat description

At Kamberg, short grasses (i.e. < 60 cm; mainly T. triandra) dominated the vegetation, occupying approximately 70 % of site A and 65 % of site B. The remainder of the vegetation at both sites comprised E. curvula-dominated patches. Cover was better in these patches than in the surrounding vegetation. Therefore, overall light penetration readings recorded at stations along the Kamberg traplines were comparatively high (Table 5), and cover was poor. Food availability was high in summer, as indicated by modal values of the cover of green plants. The proportion of green plants decreased somewhat in autumn and substantially in winter, suggesting that food availability was reduced in the latter season. Such seasonal fluctuation in the food supply reflects the seasonal rainfall at Kamberg (Figure 1).

During all seasons, the vegetation at the Karkloof trap-sites (C & D) consisted of dense, tall, tussocky grasses, uniformly distributed over the trapline, and comprised mainly mixed communities of E. curvula, B. bladhii

and T. triandra. Much of the grass was green in summer and
autumn, and thus food was abundant. The low levels of light
penetration to ground level indicated that cover was better
during these seasons. Food availability and cover decreased
during winter, possibly because of the low winter rainfall at
Karkloof (Figure 1).

Ecological distribution

More than any other parameter considered, light
penetration to ground level dictated the distribution of
O. irroratus at Kamberg. Individuals aggregated in areas
where light penetration was low (Table 6) and cover was high,
resulting in a clumped distribution throughout all seasons
(Table 2). The positive correlation between distribution and
plant height at all times was entirely predictable since tall
plants usually provided better cover.

Kamberg O. irroratus was significantly positively
associated with E. curvula and significantly negatively
associated with T. triandra during summer. Such associations
are contrary to expectations since E. curvula has a low
nutritive value (van Oudtshoorn 1992), and is apparently
unpalatable to O. irroratus (Willan 1982). However, it
appears that the preference for E. curvula is directly
attributable to the cover provided by this grass, which was
better than that of other grasses at the Kamberg study sites
(see Habitat description; above). Several nests were located
in the E. curvula patches but none were found under the cover
of T. triandra, supporting the above observation. Another
possible explanation for the association with E. curvula is

that, as the grass is unpalatable to livestock and presumably to other large herbivores, rodent species nesting under the grass would suffer less disturbance than those nesting under more palatable grasses (Willan 1982). The presence of three large herbivore species in the vicinity of both Kamberg study sites may have also influenced the distribution of O. irroratus here.

In winter, the number of O. irroratus captures was strongly associated with cover (i.e. low light levels), and weakly related to green plants. This suggests that, despite obviously harsh feeding conditions, as suggested by the lack of green plants, habitat preference was influenced by cover rather than food availability. Otomys irroratus is apparently adapted to a poor quality diet (De Graaff 1981, Brown 1988), presumably enabling it to feed on comparatively dry vegetation during winter.

Karkloof O. irroratus captures were positively correlated with soil moisture in summer and autumn. As a result of its herbivorous diet, it was anticipated that O. irroratus would be attracted to areas with increased soil moisture, since such areas support lush vegetation (i.e. food; Caldwell 1975).

Unlike the situation at Kamberg, Karkloof O. irroratus was weakly associated with cover at all times (i.e. low and non-significant light penetration correlation values; Table 7). This situation apparently reflects the uniform distribution of dense vegetation at Karkloof (see Habitat description) which provided abundant cover and possibly

nest-sites. In winter, O. irroratus was significantly associated with green plants at Karkloof, indicating that because cover was not a limiting factor at Karkloof, animals could seek out areas where food was of better quality.

Nesting habits

The nesting behaviour of both O. irroratus populations is considered here because it is an important aspect of the habitat/microhabitat preferences of rodents (Davis 1973). Moreover, information of the nesting habits of each population is an important variable for assessing habitat harshness (Brown 1988). As in other O. irroratus populations (inter alia Davis 1973, Willan 1982), Karkloof individuals nested above ground in dense vegetation. Nests were poorly constructed, and were open and shallow 'cup-shaped'. Where available, the species also used the abandoned burrows of other animals (e.g. moles). Lack of nest-chambers in the burrows indicated that these were used only as refuge sites.

In terms of their nesting behaviour, the Kamberg population closely resembled the Karkloof population (i.e. nests on the surface or in burrows under dense cover). However, the nests of Kamberg animals were well constructed and more elaborate than those of Karkloof animals. For example, the surface nest of Kamberg O. irroratus was a deep cup, which was completely enclosed by the addition of a roof. Also, the presence of large quantities of plant material in the burrows suggested that burrows were used as nest-chambers. Of greater significance was the fact that grass used in the construction of both nest types was cut and split, indicating

that animals manipulated the grass; none of the other
O. irroratus populations (n = 10; N. Pillay & K. Willan
Unpubl. data) manipulate the grass used for nesting.
Confirmation that O. irroratus occupied these nests was
provided by species-specific grass chewings and droppings
inside, or at the entrances to, the nests. Furthermore,
captive Kamberg individuals regularly construct enclosed
nests with grass that has been finely chewed (N. Pillay
Unpubl. data).

It is possible that nest-building behaviour of Kamberg
O. irroratus may be an adaptation to colder circumstances.
Comparatively low mean annual temperatures were reported at
Kamberg. This factor, in the absence of a thermal buffer
provided by extensive and abundant cover, probably means that
Kamberg O. irroratus is exposed to extremely cold conditions,
especially during winter. Hence, the well-constructed nests
provide thermal insulation during cold periods. The extent
of the insulation provided by these nests is unknown, but
warrants further investigation.

Population ecology

Sixty-one and 99 individual O. irroratus were trapped at
Kamberg and Karkloof respectively. In comparison with other
studies involving the taxon (Davis 1973, Willan 1982), these
results represent uncharacteristically high trapping success,
perhaps reflecting the severe, on-going drought in southern
Africa, which possibly caused more animals to prefer the bait
over the drier grass/herbs.

Due to increased recruitment, O. irroratus at both localities was more abundant in autumn than in summer, as confirmed by the presence of high numbers of sub-adults and juveniles (Table 3). In contrast to trapping results in June 1991 and July 1992 (Pers. obs.), individual captures at both localities were low in the winter of 1993. These differences in trapping success also probably reflect the drought in southern Africa, which was particularly severe during autumn/winter 1993. Consequently, lack of suitable cover and reduced food availability may have respectively brought about increased predation and mortality.

The carrying capacity of the Karkloof study sites was higher than that at the Kamberg sites during all seasons, as indicated by the number of O. irroratus caught, which was consistently lower at the latter locality.

Unlike those of the Karkloof population, almost all Kamberg males and females were reproductively active in autumn (i.e. April 1993). Similar results were obtained during ad libitum trapping studies at both localities in April 1991 and May 1992 (Pers. obs., R. Parker Pers. comm.), implying that Kamberg O. irroratus has an extended breeding season, while this is not the case at Karkloof. The extended breeding season of the Kamberg population is contrary to expectations, since environmental conditions during autumn were not optimal for breeding, as was apparent from the poor feeding conditions (Table 5), as well as low autumn temperatures (Humphrey 1983).

Three factors indicate that Kamberg O. irroratus may be reproductively adapted to breeding in autumn. First, it

nests in well-constructed enclosed nests which obviously provide a favourable micro-climate for unweaned young, especially in autumn when temperatures are low and frost is on the ground. Second, it has a reduced litter size (mean: Kamberg = 2.27 vs Karkloof = 2.47; Chapter 3) which would place lower energetic demands on the mother (McClure 1987, Millar 1987), so that unborn young would be better able to survive poor maternal feeding conditions than would a larger litter. Third, O. irroratus is adapted to a poor quality diet (Brown 1988). Similarly, O. irroratus in the Fish River Valley nests under piles of cut reeds (K. Willan Pers. comm.), has a small litter size (mean = 2.06; Pillay et al. 1992), and is iteroparous (Perrin 1980), despite relatively harsh environmental conditions and unpredictable food availability at this Eastern Cape locality (Pillay 1990).

Home range size is directly related to the body size of animals (Harestad & Bunnell 1979), and inversely related to the abundance of food (Wilson 1975, Bonaventura et al. 1992). On this basis, two interpretations are possible with regard to the values for Av.D. in this study. First, adult males of both populations were heavier than females and had greater Av.D. values than females (Table 4). Second, because food availability, as indicated by the quantity of green plants, was lower at Kamberg, O. irroratus here had to forage over a larger area, and travelled greater distances than Karkloof O. irroratus.

Karkloof O. irroratus displayed greater Av.D. in summer than in autumn. Male rodents involved in polygamous matings may increase their home range size during the breeding season

so as to increase the probability of locating receptive females (Bonaventura et al. 1992). In addition, the high energetic demands made on mammalian females during pregnancy and lactation (Millar 1987) may cause females to forage over longer distances during the breeding season (Bonaventura et al. 1992). Similarly, Karkloof O. irroratus apparently mates promiscuously (see Chapter 8), is reproductively active in summer, and Av.D. values suggest that this population may have larger home range sizes during summer than at other times.

As measured by Av.D., Kamberg O. irroratus had home ranges of similar size in summer and autumn. This was probably due to individuals being reproductively active during both seasons.

In terms of body mass, Kamberg animals were larger than Karkloof animals, reflecting differences in locality-specific mean annual temperatures. Low mean annual temperatures probably selected for the larger body size of Kamberg O. irroratus, which, in addition to its thermally insulated nests, allows it to survive periods of thermal stress (i.e. cold in winter).

Like O. irroratus on the Transvaal highveld (Davis 1973), adults of both populations represented in this study displayed seasonal variation in body mass (i.e. highest values recorded in summer and lowest in winter). Seasonal loss of body mass is not universal in O. irroratus populations, however. For example, O. irroratus in two Eastern Cape localities (i.e. Amatole mountains; Brown 1988 and Fish River Valley; Perrin 1981b) did not drop

significantly in body mass in winter. The poor <u>O. irroratus</u>
winter feeding conditions on the Transvaal highveld (Davis
1973) and the Natal Midlands (Kamberg and Karkloof) are
probably due to the seasonal rainfall in these localities.
Rodents are known to mobilize fat reserves during
nutritionally harsh periods (Bobek 1973, Grodzínski 1985),
and it appears that the decrease in body mass of <u>O. irroratus</u>
in this study resulted from increased fat utilization during
the unfavourable autumn/winter period (see Perrin 1981b).

Male-biased sexual dimorphism in adult body mass and a
female-biased adult sex ratio were evident in the Kamberg
population. This suggests that sexual selection, probably as
a result of male-male competition for exclusive access to
several receptive females, is operating in the Kamberg
population (Emlen & Oring 1977, Boonstra <u>et al</u>. 1993a). This
issue is pursued in Chapter 8.

Comparative ecology

In this section, an attempt is made to compare the
habitat requirements of <u>O. irroratus</u> at Kamberg and Karkloof,
and to assess the relative harshness of the habitats in
question.

The concept of habitat harshness is poorly defined in
the literature (Thiery 1982, Brown 1988), and is often
confused with habitat stability and habitat predictability
(Willan 1982), concepts which are recognized as being part of
(MacArthur & Wilson 1967), or as defining (Brown 1988),
habitat harshness. In this study, Brown's (1988) definition
of habitat harshness is adopted, which recognises as a harsh

habitat one in which density-independent mortality, as well
as the energy requirements for maintenance and reproduction,
is higher than in a more favourable (less harsh) habitat;
habitat harshness can only be discussed in relative terms
(Thiery 1982). Many habitat characteristics potentially
affect harshness (Table 8), although plant growth, because it
directly affects food supply and cover availability, is
considered to be most important (inter alia Jarman 1974,
Happold 1976, Willan 1982).

It is not possible here to fully explain the mechanisms
underlying population-specific habitat requirements or
factors influencing habitat harshness, because of the low
number of sampling periods (i.e. three seasons per locality).
In addition, no attempt has been made to ascertain all
variables influencing density-dependent mortality or energy
requirements for maintenance and reproduction (Brown 1988;
Table 8). Measuring all these variables would have been a
massive undertaking, and was not possible given the time
constraints of the study. When locality-specific habitat
characteristics are compared, however, a few general trends
are detected, as set out below.

Habitat requirements and habitat harshness

Cover availability was the major determinant of the
distribution of O. irroratus at Kamberg during all seasons;
cover was apparently provided by E. curvula-dominated stands
which were patchily distributed along the study sites.
Unlike the Kamberg study sites, cover was not a limiting
resource at Karkloof, presumably because of the abundant and

Table 8. Some characteristics potentially affecting habitat harshness (after Brown 1988). DIM = density-independent mortality.

| Parameter | Habitat type | | Effect of parameter on habitat harshness |
	harsh	favourable	
Habitat/population characteristics			
Habitat stability	Lower	Greater	Increases DIM
Incidence of disease/ catastrophes	Greater	Less	"
Food supply			
Seasonality	Aseasonal	Seasonal	Lowers habitat stability and increases DIM
Predictability	Lower	Higher	"
Degree of seasonal fluctuation	Greater	Less	"
Abundance at any one time	Less	Greater	Increases energy requirements
Spatial distribution	Clumped	Even	"
Cover/nest sites			
Quality of cover from predators	Lower	Higher	Increases DIM via predation
Abundance of nest-sites	Less	Greater	"

uniform distribution of vegetation there. Rather, Karkloof

O. irroratus was associated with areas of high soil moisture,

which probably supported stands of nutritious plants

(Caldwell 1975). It is obvious that contrasting

population-specific habitat requirements directly reflect the

habitat characteristics, particularly food supply and cover

availability, which in turn are determined by the climate at

both localities.

The most important climatic factor affecting primary productivity is rainfall (Caldwell 1975). Therefore, the relatively high mean annual rainfall at Kamberg should have promoted better plant growth and maintained a more predictable food supply, but this was not the case. A complex of interrelated environmental factors probably accounts for the differences in the Kamberg and Karkloof habitats.

Kamberg falls within the sourveld and Karkloof within the 'Ngongoni mist belt grassland types defined by Acocks (1988). Generally, rainfall is higher and temperatures are lower in the sourveld than in the mist belt (van Oudtshoorn 1992), which was the case at Kamberg and Karkloof. As a result of the higher rainfall, the sourveld soil is constantly leached of nutrients, resulting in decreased soil pH and reduced fertility (van Oudtshoorn 1992). Geological studies (Humphrey 1983) conducted at the Kamberg Nature Reserve (± 5 km from study sites A & B) have found that the soil is indeed acidic and has reduced nutrient availability (Humphrey 1983). Moreover, the soil collected from Kamberg in the present study was dry and contained more clay than loam.

Leaching of plant nutrients adversely affects plant growth and reduces the palatability of plants to animals. This situation is exacerbated by the low mean annual temperature and severe frosts, which reduce nutrient uptake and limit plant growth (van Riper 1971). In response to these conditions, plants are adapted to store much of their nutrients in roots and stem bases, especially during

unfavourable conditions like winter (van Oudtshoorn 1992).
In sourveld areas like Kamberg, plants are therefore
nutrient-poor and food availability fluctuates seasonally.
In addition, plants probably flourish only in favourable
microhabitats, which results in their having a clumped
distribution.

Because of the lower rainfall, the soil in the mist belt
areas like Karkloof is less leached and probably more fertile
than that in the sourveld (Acocks 1988, van Oudtshoorn 1992).
Unfortunately, nothing is known about the soil composition at
Karkloof, and hence no comparisons can be made with that of
Kamberg soil, although Karkloof soil had a higher loam
content (Table 5). High soil fertility together with·the
high mean annual temperatures promotes nutrient uptake and
plant growth in the mist belt, and thus plants here may be
more nutritious and palatable than those in the sourveld
(Acocks 1988, van Oudtshoorn 1992). Mist is another
important factor affecting plant growth at Karkloof,
supplementing the water available to plants without
apparently leaching the soil. Hence, the mist belt has been
described as "... a misty country, which gives it an
agriculturally more favourable climate than the Highland
sourveld and makes it well suited for intense farming"
(Acocks 1988, p. 96).

It is evident from the preceding discussion that
locality-specific environmental conditions determined habitat
characteristics at Kamberg and Karkloof. The Kamberg habitat
differed from the Karkloof habitat in that the vegetation
(grass) was shorter, food availability was lower (i.e.

smaller proportion of green plants), and there was a greater degree of seasonal fluctuation in the availability of green plants. In addition, food and cover were patchily distributed at Kamberg, but were uniformly distributed at Karkloof. Two conclusions are possible here. First, it appears that the Karkloof population falls within the typically resource-stable category of O. irroratus described by Willan & Meester (1989), while the Kamberg population is resource-limited during some months, especially in winter. Second, according to the information outlined above, the Karkloof habitat is less harsh than the Kamberg one. However, many aspects in respect of density-dependent mortality and energy requirements for maintenance and reproduction are unknown, and only three sampling periods per locality were undertaken. Therefore, further research is needed to confirm the conclusions made here.

CHAPTER 8

SOCIAL STRUCTURE

INTRODUCTION

The spatio-temporal distribution of individuals in animal societies is determined by the social structure/system (Barash 1989) which, in turn, is determined by the inter-individual social behaviour of conspecifics (Crook 1965, Happold 1976, Barash 1989). Social systems are subject to intrinsic (genetic) and extrinsic (environmental) influences, and may vary in time and space (Crook 1965, Happold 1976, Crook et al. 1976, Standen & Foley 1989). Social systems are therefore capable of evolutionary change and are adaptive (Barash 1989), despite the fact that **individuals of a society usually act to maximise their personal and/or inclusive fitness (Hamilton 1964, Wilson 1975).**

Social systems tend towards being either dispersed or communal (Eisenberg 1967, Happold 1976), depending upon whether individuals favour interactions which cause mutual attraction (e.g. amicable behaviour) or mutual repulsion (e.g. agonistic behaviour) (Happold 1976). Dispersed systems are maintained by high levels of aggression between conspecifics, with amicable interaction restricted only to those periods when males and females meet for mating. In contrast, communal systems arise when interactions between conspecifics are predominantly amicable. Although many types of social systems exist between the two extremes, there

appears to be no accepted nomenclature for these intermediates (Happold 1976), possibly because social systems are characterized by many parameters, and classification based on fewer than all these parameters may fail to distinguish different systems (Happold 1976).

Although it is preferable to study social systems by means of field-based research (see Crook 1970, Barash 1974, Jarman 1974), most rodent species do not lend themselves to such study because of their secretive nature. Therefore, some studies of rodent social structure have relied on field-based studies (inter alia Madison 1980, FitzGerald & Madison 1983), while others have been based on laboratory studies exclusively (Mackintosh 1970, Shapiro & Dewsbury 1986) or in combination with research on free-living individuals (Happold 1976, Getz et al. 1981).

In the present study, data obtained in the field studies described in Chapter 7 were considered, and complementary captive studies undertaken, to ascertain the social structure of Kamberg and Karkloof Otomys irroratus. In spite of the suggestion by Happold (1976) that classification of social systems (i.e. along the continuum from dispersed to communal systems) should be based on all parameters potentially affecting a particular system, Wilson (1975) stated that classification based on all traits is a "bottomless pit" (p. 16). Hence, only those traits that were of direct relevance to the understanding of O. irroratus social structure (Davis 1973, Willan 1982), and which would permit elucidation of differences, if any, between the social systems of the Kamberg and Karkloof populations, were considered.

Of the many factors which characterize different social systems, spatial distribution of conspecifics, as revealed by the degree of territoriality and home range overlap, and mating strategy are regarded as being of fundamental importance (inter alia Happold 1976, Madison 1980, Rubenstein & Wrangham 1986, Kawata 1988), and were therefore ascertained in the present study. Four aspects were considered in this respect: (i) dispersion of free-living animals; (ii) sexual dimorphism in body size; (iii) nature of intrapopulation dyadic encounters; and (iv) behaviour of females in two-chamber choice tests. The rationale behind the choice of each aspect is provided below.

Dispersion

Trapping data (Chapter 7) were analyzed to provide information about the spatial relationship of free-ranging dyads of both populations. Two aspects were examined: (i) the number and level of intra- and intersexual associations (defined as the presumed dyadic interaction between animals in nature; Chapter 2); and (ii) the number of individuals of either sex who associated with a single member of the opposite sex; the abbreviation FOM was used when females associated with males, and MOF for males associating with females.

Sexual dimorphism in body size

Generally, the degree of sexual dimorphism in body mass reflects the mating system of a species (Dewsbury et al. 1980, Heske & Ostfeld 1990, Boonstra et al. 1993a). For

example, in polygamous species, males are much larger than females, while, in monogamous species, males and females are of equivalent size.

Data obtained from field studies (Chapter 7) suggested the existence of male-biased sexual dimorphism in body mass in Kamberg O. irroratus, but not in the Karkloof population. These observations were made on a small number of wild-caught individuals, however. This, together with the fact that mass varies with season, differential mortality, physical well-being, age, and nutrition (Ralls 1976), may have influenced the analysis. Therefore, intrapopulation comparisons of body mass and head-body length were made of known-aged captive-born males and females representing the Kamberg and Karkloof populations.

Intrapopulation dyadic encounters

Dyadic encounters were staged in order to examine elements of social interaction that cause attraction (e.g. amicable behaviour) and repulsion (e.g. aggressive behaviour). Such interactions are underlying determinants of the social system of species (Happold 1976).

Choice-chamber tests

It is known that some male rodents produce odours which are indicative of their social status (Jones & Newell 1973, Apps et al. 1988, Rozenveld & Rasmont 1991), and that females prefer the odour of dominants to that of subordinates (Huck et al. 1981), and usually mate with dominant males (Huck & Banks 1982). However, several studies have shown that,

depending on the mating strategy, females do not always prefer the dominant male when they are allowed to choose between dominant and subordinate males in choice-tests (Shapiro & Dewsbury 1986, Shapiro et al. 1986). Shapiro & Dewsbury (1986) showed that monogamously-mating females regularly preferred dominant males, while females of polygynous rodents failed to show a clear preference during tests, and it seems that behavioural mechanisms inherent in mate choice are key determinants of mating systems.

Based on these findings, female preference for same-population dominant versus subordinate males were investigated in the present study.

MATERIALS AND METHODS

Field studies

Dispersion of Kamberg and Karkloof O. irroratus was ascertained by estimating 'indices of association' (Willan 1982, Brown 1988), which express the number of associations occurring between free-living intrasexual and intersexual dyads (Willan 1982). These indices make maximum use of trapping data, and are especially useful when the number of captures is small, which was the case in the trapping study (Chapter 7). A dyad may be considered to be associated if they are caught at the same or adjoining trap stations (i.e. vertically, laterally or diagonally) within a set time period (Happold 1976). Willan (1982) used a time limit of 48 h, while I followed Brown (1988) and set the time period as the duration of the trapping session at 108 h, since home range

areas were unlikely to have shifted within this time (Happold 1976, Brown 1988).

Indices of association were calculated for adult dyads representing each of three sex combinations - male-male, female-female, male-female - for the summer and autumn trapping sessions. Indices were not calculated for the winter trapping session as sample sizes were very small (four and three adults at Kamberg and Karkloof respectively; Chapter 7).

Because they were materially similar, trapping data obtained from the two sites at each locality (Chapter 7) were combined. Each index was calculated using all data obtained during the relevant trapping session, as follows:

$$\text{Index of association} = \frac{a \times 1000}{h \times c}$$

where a = total number of dyadic associations, h = mean number of hours between associations, and c = total captures of the dyads concerned during a trapping session.

The values obtained for the indices of association were not amenable to statistical analysis (Sokal & Rohlf 1987).

FOM and MOF were established by simply counting the number of individuals of the opposite sex with which individual adult males and females were associated (i.e. caught at the same or adjoining trap stations). Mean values were calculated for each sex of both populations for the summer and autumn trapping sessions; data for each trap-site (i.e. A & B at Kamberg and C & D at Karkloof; Chapter 7) per locality were considered separately.

Captive studies

Details of the maintenance of animals in captivity and the environmental conditions under which the present study was conducted are described in Chapter 2.

Sexual dimorphism in body size

Animals used in this study were captive-born F1 and F2 individuals, which had been separated from the parents at 30 days of age and held individually in Labotec cages 40 x 25 x 10 cm. Their body mass and head-body length were measured over a period of approximately eight months. A maximum of seven recordings per litter were made, coinciding with the day of birth (Day 0), weaning (Day 14), sexual maturity of females (Day 49) and males (Day 70), plus values at 120, 240 and 320 days of age. In all cases, asymptotic head-body length was recorded at approximately Day 240, and thus measurements on Day 320 are not considered here. All data in respect of body mass were used.

Preliminary examination of the data set indicated that, as a result of variation in litter size, there was considerable variation in mass and measurements among litters which could have biased the analysis. In order to reduce this bias, only data from litters comprising two individuals, one of each sex, were used; the modal litter size was 2 individuals for both Kamberg and Karkloof populations.

Analysis of the data set was based on the matched pair method (Dewsbury et al. 1980, Breed 1983), in which comparisons are made of the mass and head-body length of the

male and female in each litter. When one member of a matched
pair had died or, in the case of females, was used in
breeding experiments (Chapter 3), data obtained from that
pair were not considered in further analyses. Two-tailed
paired t-tests (Zar 1984) were used to test the null
hypothesis that there were no differences in body mass and
head-body length between the sexes.

Intrapopulation dyadic encounters

The interaction of each of 64 Kamberg and 78 Karkloof
intrapopulation dyads (Table 1) was studied in intra- and
intersexual neutral arena encounters. The apparatus and
methods employed in this study are described elsewhere
(Chapter 5). However, several methods were unique to this
study, and are briefly outlined below.

Prior to observations, an enclosure was divided into two
parts with a wire mesh partition. Members of a dyad were
placed on either side of the partition. Following a
familiarization period of approximately 20 min, the partition
was removed and the first 20 min of interaction
video-recorded. Testing was undertaken during the light
phase of the light cycle, between 07h00 and 09h00.
Recordings were made under fluorescent white light using a
Hitachi KP-141 CCTV camera and a Hitachi VTL-30ED time-lapse
video cassette recorder.

All animals were sexually experienced, either
wild-caught or captive-born individuals. Each animal was
used in two encounters - one intrasexual and one intersexual.
Dyads had previously never met in the laboratory. All

Table 1. Number of intra- and intersexual dyadic encounters involving representatives of the Kamberg and Karkloof populations.

Dyadic encounter	Kamberg	Karkloof
Male-male	26	28
Female-female	15	25
Male-female	23	25

females were in met-oestrus, as confirmed by vaginal smears. During intrasexual dyadic encounters, only individuals weighing within 20 g of each other were used.

The one-zero time-sampling method with 10 s time intervals (Altman 1974) was used to record frequencies of several behaviour patterns, recognized as non-contact (exploratory and maintenance), agonistic (aggressive approach, chasing, upright sparring, submissive flee), fights (agonistic behaviour resulting in fights), amicable (amicable approach, allogrooming, huddling) and sexual (follow-mount and present-lordosis), following the definitions of Happold (1976) and Willan (1982). In other small mammal studies, data obtained for each dyad were usually combined when there was absolute reciprocity between interacting individuals (see Happold 1976, Willan 1982). Likewise, Mann-Whitney U tests indicated that in 93 % of dyads there were no significant differences in the frequencies of behaviour patterns displayed by interacting animals in encounters in the present study. Accordingly, data for each dyad were combined.

The frequencies of all behaviour patterns described above were calculated for each dyad, and mean frequencies for

intrasexual and intersexual dyads were calculated. Initial examination of the data indicated that frequencies of sexual behaviour were very low, and this behaviour is therefore not considered here. Data were tested for significance using the Mann-Whitney U test (one-tailed; Sokal & Rohlf 1987).

The method of Colvin (1973) was used to identify the dominant and subordinate individuals in each dyad. An animal was classified as dominant if, in comparison to its opponent, it exhibited more exploratory activity and displayed a higher number of aggressive and approach acts (Colvin 1973). These criteria for assessing dominance were appropriate because they took into account the context in which the behaviour occurred. This is crucial, since dominance is not readily defined in the literature (inter alia Colvin 1973, McFarland 1987), and aggression may reflect defence or dominance, and, in addition to aggression, other factors like exploratory behaviour must be considered when assessing dominance (Cranford & Derting 1983). In encounters where the outcome was unclear, neither animal was considered dominant and a tie was declared. Some of the Kamberg male-male and Karkloof female-female dyads were separated because of damaging fights. In this case, the above criteria for assessing dominance could not be used, and the animal which initiated the attack, and which was invariably unharmed during the fight, was declared the dominant individual.

Choice-chamber tests

The apparatus and methods employed were identical to those used in whole animal choice tests in Chapter 3;

detailed descriptions of the choice-chamber and its use are available in that chapter. However, the present study differed from the whole animal choice test in various ways.

Three days after their encounter, a dominant and subordinate male were used as stimulus animals in the choice tests; males representing dyads which had been declared ties were not used in these tests. The test animal, which was a female in oestrus was scored for the total time spent in the compartments adjacent to each stimulus male. Unlike whole animal choice tests, an acclimation period of 2 h preceded tests, and tests lasted 1 h, undertaken during the light phase of the light period.

Females were wild-caught individuals which had produced at least one litter in captivity, and had previously never met either the dominant or the subordinate male in the laboratory. All males and females were used only once as stimulus and test animals respectively.

One-tailed paired t-tests (Zar 1984) were used to test the hypothesis that females preferred dominant rather than subordinate males.

RESULTS

Field studies

Indices of association between sex combinations representing both the Kamberg and Karkloof populations are given in Table 2. With the exception of site A at Kamberg in autumn when two adult males were captured, only one adult male was caught at each Kamberg site during any particular trapping session (see Chapter 7). Hence, no values for

Table 2. Indices of association of free-living adult Kamberg and Karkloof <u>O. irroratus</u> during summer and autumn.

Season/	Sex combination		
Locality	Male-male	Female-Female	Male-female
Summer			
Kamberg	- [1]	27.49	49.04
Karkloof	32.50	17.23	67.31
Autumn			
Kamberg	0	26.53	47.22
Karkloof	26.30	17.16	42.80

1 = Only one adult male caught per trapline; see text.

male-male association are available (Table 2). At both localities and during both seasons, the level of intersexual association was higher than that of intrasexual association. The association between females was seasonally more or less unvarying in both populations, while male-male (Karkloof) and male-female (both populations) associations decreased from summer to autumn. There was no association between the two adult males at site A at Kamberg in autumn, and a value of zero was recorded. Generally, associations between Kamberg females were higher than those between Karkloof females. In summer, intersexual associations were higher in Karkloof than in Kamberg, while the opposite was true in autumn.

The number of females overlapping per male (FOM) and the number of males overlapping per female (MOF) are shown in Table 3. All Kamberg females at each trap-site overlapped with the single adult male present at that site. Even during autumn when two adult males were present at site A, females

Table 3. Mean number of females overlapping per male (FOM) and of males overlapping per female (MOF) for adult individuals at the Kamberg and Karkloof trap-sites during the seasons indicated. 2 SE given in brackets. n = sample size.

Season/		Females			Males		
Locality	Site	n	FOM		n	MOF	
Summer							
Kamberg	A	7	1.0	(0.0)	1	7.0	(0.0)
	B	6	1.0	(0.0)	1	6.0	(0.0)
Karkloof	C	7	2.1	(0.5)	8	2.4	(0.7)
	D	7	2.2	(0.4)	5	2.3	(0.6)
Autumn							
Kamberg	A	5	1.0	(0.0)	2	- [1]	
	B	4	1.0	(0.0)	1	4.0	(0.0)
Karkloof	C	9	1.4	(0.3)	7	1.7	(0.4)
	D	5	1.4	(0.5)	5	1.6	(0.5)

1 - no values given since each male overlapped with three or two females exclusively; see text.

shared their home range with only one male, and it appeared that each Kamberg male had exclusive access to three and two females respectively.

Karkloof males and females overlapped with an average of more than two individuals of the opposite sex during the breeding season (summer). The mean number of overlapping individuals was lower in autumn than in summer.

Captive studies

Sexual dimorphism in body size

Mean body mass of captive-born males and females representing the Kamberg and Karkloof populations is presented in Table 4. In the Kamberg population, males were

Table 4. Mean body mass (g) of captive-born males and females representing the Kamberg and Karkloof populations, at the ages indicated. n = number of litters. t = paired t-test values. 2 SE given in brackets.

Age (days)		Kamberg				Karkloof		
	n	Males	Females	t	n	Males	Females	t
0	21	21.39 (1.06)	17.87 (0.83)	5.03***	37	18.02 (0.52)	17.17 (0.50)	2.15*
14	21	52.56 (1.68)	50.32 (1.94)	2.04 ns	37	42.31 (1.14)	41.39 (1.09)	1.33 ns
49	19	115.35 (3.59)	99.67 (2.54)	15.66***	33	92.75 (1.98)	90.75 (2.04)	1.48 ns
70	19	149.10 (4.56	110.84 (4.37)	10.73***	30	111.91 (3.85)	110.87 (3.74)	0.77 ns
120	16	163.92 (7.60	119.43 (4.81)	10.19***	26	129.03 (3.37	124.97 (4.04)	1.41 ns
240	14	278.80 (5.33)	197.89 (6.46)	22.72***	24	156.66 (4.88)	150.69 (5.03)	2.09*
320	10	295.77 (7.95)	215.25 (4.41)	16.43***	22	219.51 (12.40)	200.88 (8.25)	2.20*

* = p < 0.05, *** = p < 0.001

significantly heavier than females at all times, except at weaning when males and females were of equivalent size. Karkloof males also weighed more than same-population females at all times, with significant differences recorded only at birth and Days 240 and 320. However, the degree of intersexual difference in mass during these two periods was not as pronounced as in the Kamberg population (5 % vs 0.1 % significance level; Karkloof vs Kamberg).

In terms of head-body length, Kamberg males were always significantly larger than their female counterparts, while significant differences were recorded between Karkloof males and females only on Day 240 (Table 5). As with body mass,

Table 5. Mean head-body length (mm) of captive-born males and females representing the Kamberg and Karkloof populations, at the ages indicated. Sample size as in Table 4. t = paired t-test values. 2 SE given in brackets.

| Age (days) | Kamberg | | | Karkloof | | |
	Males	Females	t	Males	Females	t
0	88.33 (1.68)	81.14 (1.84)	4.83***	82.04 (1.30)	79.14 (1.63)	0.75 ns
14	149.67 (2.57)	141.48 (3.65)	3.69**	109.45 (3.99)	110.50 (3.92)	-0.45 ns
49	161.42 (3.29)	151.32 (3.38)	11.05***	160.62 (3.04)	157.49 (2.99)	1.79 ns
70	189.84 (3.85)	171.74 (3.60)	6.39***	179.42 (2.27)	177.72 (2.83)	1.18 ns
120	200.75 (4.10)	179.63 (2.93)	6.48***	187.42 (3.48)	185.35 (2.41)	1.44 ns
240	214.79 (3.58)	190.36 (3.68)	6.89***	198.62 (2.59)	192.62 (3.59)	2.21*

* = p < 0.05, ** = p < 0.01, *** = p < 0.001

the degree of sexual dimorphism in head-body length was more pronounced in the Kamberg than in the Karkloof population, as indicated by the level of significance of paired t-test values.

Intrapopulation dyadic encounters

Mean frequencies of behaviour patterns recorded during Kamberg and Karkloof intrapopulation encounters are given in Tables 6 and 7 respectively. Included in both tables are the results of pair-wise intrapopulation comparisons (Mann-Whitney U test) of the behaviour recorded in all dyadic encounters.

Table 6. Mean ± 2 SE frequencies of the behaviour patterns recorded in same- and opposite-sex Kamberg dyadic encounters, and statistical comparisons (Mann-Whitney U test) of the behaviour of each dyad. Sample sizes as in Table 1, except for the number of male-male encounters which was 12 encounters (see text). M = male, F = female.

Behaviour patterns	Dyadic encounters			Statistical comparisons		
	Male-male	Female-female	Male-female	MM-FF	MM-MF	FF-MF
Non-contact	22.67±8.30	37.55±6.31	37.70±5.63	83***	75.5*	56.5 ns
Aggressive	54.67±7.80	20.90±3.20	18.80±5.74	99***	90***	58.5 ns
Fights	11.44±4.87	3.20±0.76	0.20±0.26	91***	84**	73 ns
Amicable	0.33±0.67	12.09±3.00	26.20±5.74	89***	90***	109.5***

* = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001

Table 7. Mean ± 2 SE frequencies of the behaviour patterns recorded in same- and opposite-sex Karkloof dyadic encounters, and statistical comparisons (Mann-Whitney U test) of the behaviour of each dyad. Sample sizes as in Table 1, except for the number of female-female encounters which was 21 encounters (see text). M = male, F = female.

Behaviour patterns	Dyadic encounters			Statistical comparisons		
	Male-male	Female-female	Male-female	MM-FF	MM-MF	FF-MF
Non-contact	40.06±3.82	27.00±5.58	44.91±7.11	183***	129 ns	115.5***
Aggressive	33.72±6.53	46.00±9.62	32.27±6.79	155*	103.5 ns	101*
Fights	1.78±0.96	7.08±2.60	1.55±0.98	186***	101 ns	115.5***
Amicable	0.94±0.77	0.83±0.73	13.64±2.48	110 ns	198***	132***

* = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001

Encounters between Kamberg males resulted in significantly lower levels of non-contact and amicable behaviour and significantly higher frequencies of aggressive and fighting behaviour than the other two Kamberg encounters (i.e. female-female and male-female; Table 6). As a result of damaging fights, 14 of the original 26 male-male encounters had to be terminated shortly after the start of tests, and the data presented in Table 6 represent the behaviour of the remaining 12 dyads.

Compared to other Karkloof encounters, female-female encounters favoured significantly more aggressive and fighting behaviour which resulted in the termination of four

female-female encounters due to damaging fights (Table 7). Karkloof intrasexual dyads were involved in significantly less amicable behaviour than the intersexual dyads (Table 7).

Most dyads developed dominant-subordinate relations, except for one Kamberg and two Karkloof encounters which were declared ties. The larger individual was always dominant over the smaller one during intrasexual encounters, and males were dominant over females.

Choice-chamber tests

Results of female preference for dominant versus subordinate males are summarized in Figure 1. Both Kamberg and Karkloof females preferred the dominant male, but this preference was statistically significant only in respect of Karkloof females ($t_{24} = 3.25$, $p < 0.005$).

DISCUSSION

In order to facilitate understanding of the social structure of the Kamberg and Karkloof populations, information from previous studies of the social system of O. irroratus is of value. Such information also allows comparison of the results of the present study with those of the others.

Studies at Transvaal highveld (Davis 1972, 1973), other Natal Midlands (Willan 1982) and Eastern Cape (Brown 1988) localities have shown that O. irroratus has a dispersed (asocial) social system, incorporating elements of hierarchical ranking, territorial defence of a core area of the home range, and temporal territoriality - territories
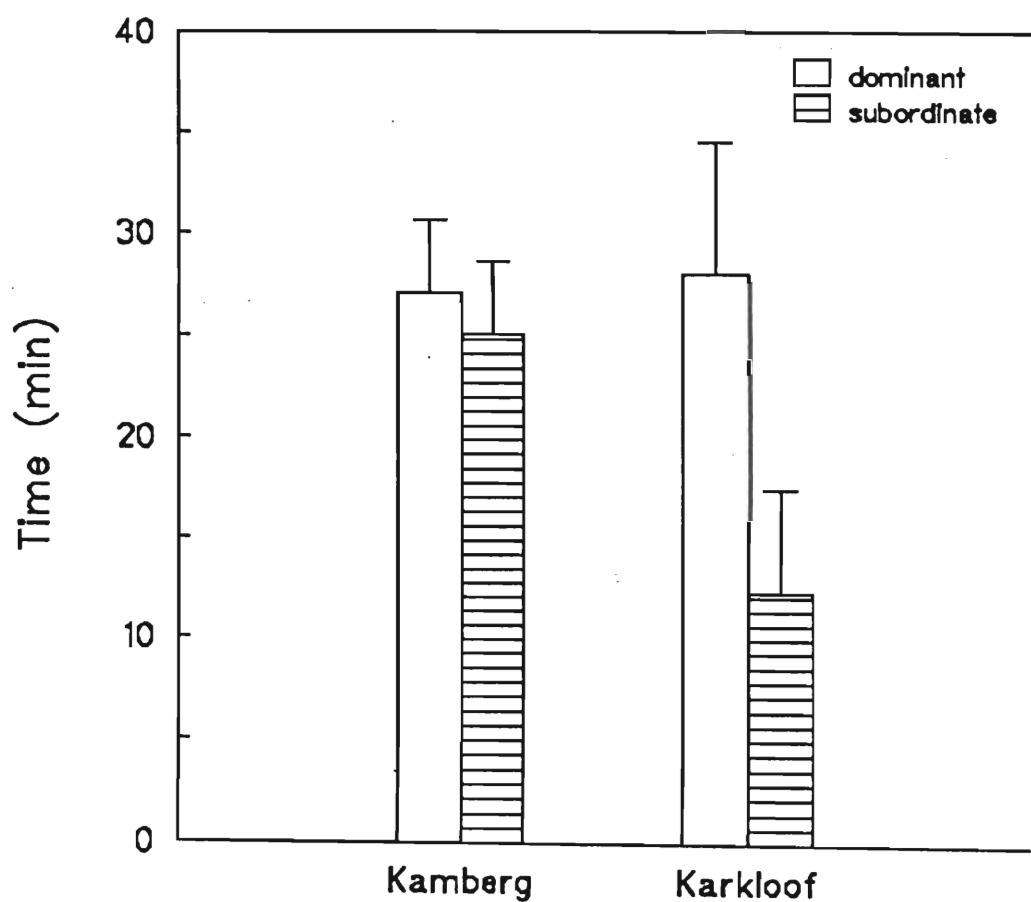
Figure 1. Mean number of minutes spent in chambers adjacent to same-population dominant and subordinate males by the females indicated. n = 24 Kamberg and 25 Karkloof trials. Vertical bars = 2 SE of the mean.

which overlap in space but not in time. Breeding females are intrasexually more aggressive than males, and are intrasexually highly territorial. This enables females to provide their young, which disperse only 11 - 12 m from the maternal nest (Davis 1973, Brown 1988), with an area in which to establish a home range (Willan 1982). In contrast, there is extensive intrasexual home range overlap among males, among which dominance hierarchies exist. The home ranges of **reproductively active males overlap those of females** (Davis 1973).

Dispersion

In terms of its spatial relationships, the Karkloof population appeared to be similar to other O. irroratus populations, while the Kamberg population differed from the **others in some important respects, as set out below.**

Indices of association have been used to infer the degree of home range overlap in other O. irroratus populations (Willan 1982, Brown 1988). Consequently, the extensive intra- and intersexual association among individuals at the Karkloof study sites suggests that these individuals may have a high degree of home range overlap (Willan 1982, Brown 1988). However, it is not known whether there was temporal home range overlap. Intrasexual dyads engaged in high levels of agonistic/repelling behaviour, exhibiting virtually no amicable/attracting behaviour. This suggests that there is mutual avoidance among conspecifics (Happold 1976). It is possible, therefore, that temporal intrasexual associations in the Karkloof population are rare

in nature and that this population displays temporal
territoriality. In fact, no two animals of the same sex were
ever caught at a particular trap station at the same time in
the trapping study described in Chapter 7, or in previous <u>ad
libitum</u> trapping (removal trapping on traplines in 1991,
1992; Pers. obs.).

The low levels of association between Karkloof females,
compared with those between Karkloof males, hints at the
possibility that females are more territorial than males
(Davis 1973, Willan 1982, Brown 1988). This observation is
supported by high levels of agonistic and fighting behaviour,
including four instances of damaging fights, in female
intrasexual dyads. Such behaviour would allow Karkloof
females to maintain their territories, as reported in other
highly territorial species (Eisenberg 1967, Madison 1980).

On the basis that damaging fights were rare during
dyadic encounters, Willan (1982) maintained that aggression
is ritualized in <u>O. irroratus</u>. Observations of Karkloof
encounters appear to confirm Willan's observations, as most
of these dyads did not engage in damaging fights. Overall
ritualization of aggression appeared to be better developed
in the Karkloof population than in the Kamberg population; 14
Kamberg male-male encounters were terminated because of
fighting, and Kamberg female-female encounters engaged in
very low levels of aggression, so that there was no need for
ritualized agonism.

Compared to their Karkloof counterparts, Kamberg females
showed a greater degree of intrasexual association. In
addition, encounters between Kamberg females were more

amicable than any other intrasexual encounter. Hence, Kamberg females appeared to be more tolerant of one another, and it is possible that free-living Kamberg females are intrasexually less territorial than Karkloof females. Two females were often captured simultaneously at the same trap-station (four occasions in summer and six occasions in autumn and winter) during capture-mark-recapture trapping in 1992 and 1993 (Chapter 7), further supporting the conclusion that Kamberg females are intrasexually more tolerant. On several occasions two females were caught at the same burrow during removal trapping at Kamberg in autumn 1987 (K. Willan Unpubl. data), and in autumn 1991 and autumn 1992 (Pers. obs.), possibly providing evidence of nest cohabitation by females.

Apart from high levels of amicability, Kamberg females also displayed aggressive behaviour during female-female encounters. This implies that Kamberg females are at least partially territorial, although see Chapter 9 (Socio-ecology) for an alternative interpretation.

The presence of only one adult male at each of the Kamberg trap-sites during summer 1992 (Chapter 7), and lack of intrasexual association between the two males at study site A in autumn (zero index of association), suggest that males are highly territorial. As the data upon which this conclusion is based represent trapping studies conducted during only two periods (i.e. summer and autumn), the observation that Kamberg males are highly territorial remains inconclusive. However, encounters between Kamberg males revealed high levels of agonistic interaction and fighting

behaviour, to a degree that necessitated separation of several of these dyads because of damaging fights. High levels of aggression and fighting to the death can occur between males of territorial species (Wilson 1975), and selection is likely to favour males occupying discrete, non-overlapping home ranges (Madison 1980), which may be the case among Kamberg males.

In both populations, intersexual associations, which were more common than intrasexual associations, were seemingly permitted by a greater tendency towards amicable behaviour intersexually than intrasexually during dyadic encounters. Such a situation clearly promotes mating. Support for this conclusion is provided by levels of intersexual association which were higher in summer than in autumn at Karkloof, and were of similar magnitude in both these seasons at Kamberg, corresponding with periods of reproductive activity of individuals of both populations; Kamberg adults were sexually active in summer and autumn and Karkloof adults only in summer (Chapter 7).

Higher levels of intersexual association were observed at Karkloof than at Kamberg. This was probably due to the presence of equal numbers of males and females at the Karkloof trap-sites, resulting in more associations between the sexes.

Mating strategy

The mating system of O. irroratus from different localities is reported to be promiscuous (Willan 1982, Brown 1988): the home ranges of reproductively active males overlap

those of females, and males compete within a hierarchical framework for mating opportunities. It is essential to define the concept of a promiscuous mating strategy because this term is often confused in the literature. Promiscuity occurs when females practice simultaneous polyandry and males practice serial polygyny (Boonstra et al. 1993b). This definition suggests that random matings occur between any number of males and females, and that mate selection does not occur. However, field studies of several promiscuous vole species revealed that male-male competition occurs for mating opportunities with receptive females, with females usually selecting dominant male/s as mates and attacking or seriously injuring subordinate males which solicit them (Madison 1980, Heske & Ostfeld 1990).

Two factors indicate that Karkloof O. irroratus also mates promiscuously. First, adult males and females associated with an average of more than two individuals of the opposite sex during the breeding season. This appears to indicate that the home ranges of males and females overlapped with more than two individuals. Similar relationships between home range overlap of males and females and a promiscuous mating system have been found in other rodent species (Madison 1980, Kawata 1988). Second, indices of male-male associations were higher in summer (i.e. the breeding season) than in autumn, indicating that males probably compete for mating opportunities. It appears that the dominance system among males would afford larger males, which are usually dominants, priority of access to females (Brown 1988).

Only one adult male was caught at each Kamberg trap-site, and each male associated with all females at that site (FOM and MOF values, Table 3), suggesting that the Kamberg population, unlike any other O. irroratus population studied to date, has a polygynous mating system. More support for this suggestion is perhaps provided by the fact that the two adult males present at site A in autumn never associated with one another (zero index of association). This suggests that the home range of each male did not overlap with that of the other, and that they each seemed to have exclusive access to more than one female.

Once again, the above predictions about mating strategy of the Karkloof and Kamberg populations are based on trapping studies during only three sampling sessions, and may not reflect the situation in nature. When the results of field studies are considered together with those of the laboratory studies, however, additional evidence of polygyny in Kamberg O. irroratus and of promiscuity in the Karkloof population is found, as provided below. Moreover, the absence of nest co-habitation between breeding pairs of both populations, as well as a lack of parental behaviour by males, indicates that neither population is monogamous (Thomas & Birney 1979).

Sexual dimorphism occurs when one sex competes for access to the other sex, usually for mating (Darwin 1871). In mammals, where the most common mating system is polygyny (Kleiman 1977, Clutton-Brock & Harvey 1978), male-biased sexual dimorphism in body size usually occurs as a result of inter-male competition. It has been predicted that intense selection occurs among males when there is greater potential

for monopolizing more than one mate, resulting in a polygynous mating strategy (Emlen & Oring 1977, Ostfeld 1990, Birkhead & Møller 1992). Theoretically, knowledge of the mating system should allow prediction of the degree of sexual dimorphism, and vice versa. Recent attempts to relate the degree of sexual dimorphism to the mating system in North American microtines revealed the following trends in respect of both head-body length (Heske & Ostfeld 1990) and body mass (Boonstra et al. 1993a): (i) in polygynous mating systems, where one male has exclusive access to several females, dimorphism was greatest; (ii) in promiscuous mating systems, in which no one male has exclusive access to a single female, dimorphism was slight or non-existent; and (iii) in monogamous mating systems (i.e. pair-bonding), males and females were of equivalent size.

Except for body mass at weaning, Kamberg males were always significantly larger than females, i.e. heavier and with greater head-body length. In contrast, Karkloof males were significantly larger than same-population females only at birth and at the end of testing (Days 240 & 320). Of greater significance was the higher degree of sexual dimorphism in the Kamberg than in the Karkloof population. These observations corroborated the results of trapping studies which showed that during the breeding season, male-biased sexual dimorphism in body mass was evident in the Kamberg population, but not in the Karkloof population (Chapter 7).

It is assumed here that male-biased sexual dimorphism is the result of male-male competition for access to receptive

females, but sexual dimorphism may also occur for other reasons (Ralls 1976). For example, selection may favour divergent body sizes between the sexes when there is intraspecific competition for limited resources (i.e. niche partitioning). However, the interaction of intra- and intrasexual dyads, as well as the results of female preference in choice-chamber tests, support the hypothesis that the degree of sexual dimorphism observed in the Kamberg and Karkloof populations reflects the extent to which sexual selection is operating on the males of both populations.

Intrasexual encounters

Among species which mate promiscuously, intrasexual social interaction is characterized by more pronounced aggressive behaviour between females than between males (Cranford & Derting 1983, Ostfeld 1990), so that intrasexual territoriality is better developed among females than among males. Females have spatio-temporally non-overlapping home ranges, while males usually have intrasexually overlapping home ranges (Madison 1980, Kawata 1988, Bonaventura et al. 1992). Such a situation rules out the possibility of a polygynous mating system, since it is unlikely for one male to monopolize matings with several females (Emlen & Oring 1977, Boonstra et al. 1993b).

Males which mate polygynously are intrasexually highly aggressive and highly territorial (Ostfeld 1986, 1990, Boonstra et al. 1993b), while females are more tolerant of one another and less territorial (Trivers 1976, Carothers 1984, Ostfeld 1986, Boonstra et al. 1993b). Increased

tolerance among females usually permits spatio-temporal clumping of this sex, which makes it possible for a single male to monopolize matings with several females (Emlen & Oring 1977, Ostfeld 1990).

Karkloof females were intrasexually more aggressive than males, and intrasexual associations were rare among females and extensive among males. Kamberg males were, in contrast, intrasexually highly aggressive, and possibly occupy discrete territories, while Kamberg females participated in amicable activity during female-female dyadic encounters, and appeared to have intrasexually overlapping home ranges, as suggested by indices of intrasexual association.

Intersexual encounters and choice tests

Karkloof intersexual dyads displayed significantly lower levels of amicability and significantly higher levels of aggression than Kamberg intersexual dyads. Similar results were obtained in a study of intrapopulation courtship involving Kamberg and Karkloof O. irroratus (Chapter 5). In choice-chamber tests, Karkloof females significantly preferred dominant males, while Kamberg females were less discriminating. This lack of preference by Kamberg females appears inconsistent with the potential reproductive benefits accrued by mating with dominant males (see Halliday 1983).

In promiscuously-mating species, receptive females would be solicited by several males, and selection would be predicted to favour reduced attractiveness of males to females (Madison 1980, Alder et al. 1981). Hence, females may respond aggressively to male solicitation, since

aggression may be used as a mechanism for mate choice (Ferkin 1987). Hence, the courtship of promiscuous species is complex and involves high levels of aggression. In addition, females would improve their inclusive fitness if they mated with dominant males, and selection is likely to favour female discrimination between males of differing social status, possibly on the basis of olfactory or other cues (Shapiro & Dewsbury 1986, Apps et al. 1988).

In polygynous species, where males maintain intrasexually exclusive territories, males would have previously established their territories on the basis of male-male competition. In order to assure mating with a male which is functionally dominant, females mate with the male in whose territory they occur (Emlen 1976, Shapiro & Dewsbury 1986). This also means that, during choice-tests, polygynously-mating females may not be as discriminating of males of differing social status as promiscuously-mating females (Shapiro & Dewsbury 1986), resulting in reduced aggression and higher amicability during the courtship of polygynous species (Alder 1981).

Social organization

Before attempting to analyze the social organization of Kamberg and Karkloof O. irroratus, it must be recognized that the dispersion and mating strategy of Kamberg and Karkloof O. irroratus were explained by post hoc 'best fit' models, based mainly on laboratory studies. Therefore, the conclusions reached here remain tentative, until additional field studies are undertaken.

Karkloof females were intrasexually highly aggressive and their lack of intrasexual association suggests that they may be intrasexually highly territorial. Similar findings were reported in other O. irroratus populations (Davis 1973, Willan 1982, Brown 1988). Karkloof males displayed high levels of intrasexual and intersexual association, and it was suggested that mating here is promiscuous. It therefore appears that, like other O. irroratus populations (Davis 1973, Willan 1982, Brown 1988), the Karkloof population exhibits a dispersed social system, incorporating temporal territoriality.

Kamberg males were intrasexually highly aggressive and males did not associate with one another. The home ranges of Kamberg females appeared to overlap extensively with those of other females, as suggested by indices of association. Moreover, encounters between females were more amicable than those between males. It also appeared that pairs of females shared nests in nature. As a result of these differences in territorial behaviour of each sex, males may have exclusive access to several females, and mating may be polygynous. On the basis of the views of Eisenberg (1967) and Happold (1976), it appears that Kamberg O. irroratus has a communal social organization, since females displayed a degree of mutual attraction. Lack of tolerance and the apparent territoriality among males is not typical of species having truly communal social systems, however (Happold 1976, Ostfeld 1986, Barash 1989), and I therefore consider the social organization of the Kamberg population to be partially communal.

An aspect of social systems not considered here was juvenile and sub-adult dispersal. Previous studies have shown that dispersal is an important determinant of the social organization of rodents (inter alia Willan 1982, Barash 1989, Wolff 1989), and the dispersal of young Kamberg and Karkloof O. irroratus needs to be investigated.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

INTRODUCTION

The present study, concerned with selected aspects of the biology of two chromosomally-distinct allopatric populations of the vlei rat <u>Otomys</u> <u>irroratus</u>, from Kamberg and Karkloof, has aimed to investigate two important processes in evolution: speciation and adaptive variation. Speciation was studied by ascertaining the existence of reproductive isolating mechanisms which would potentially reduce or eliminate successful breeding between Kamberg and Karkloof individuals should they meet in nature. Adaptive variation was studied by establishing whether or not the populations differed in respect of their socio-ecological characteristics. For convenience, the discussion is divided into three sections, arranged as follows: socio-ecology; speciation and reproductive isolation; and evolutionary considerations.

SOCIO-ECOLOGY

Socio-ecology, defined as the study of social structure in relation to ecology (Crook 1970), is concerned with the adaptive significance of social systems. Of the many factors affecting social structure, environmental parameters have received much attention (<u>inter</u> <u>alia</u> Crook 1970, Happold 1976), possibly because the relationships between the environment and social organization are often very obvious.

An attempt is made here to interpret the social phenomena of the Kamberg and Karkloof populations in terms of population-specific habitat characteristics and other aspects of the ecology of these populations. In addition, possible genetic determinants of social organization are considered.

Of the many methods employed to interpret social structure, the inductive approach has been favoured by several authors (see Barash 1989). This approach involves a logical process in which conclusions are derived from more information than is provided by the available data (inter alia Willan 1982, Barash 1989). I have also adopted the inductive approach, but have paid attention to those social attributes that appear to be most distinctly adaptive.

A summary of the habitat characteristics of the Kamberg and Karkloof study sites, as well as the social structure of O. irroratus occurring at these localities, is given below.

Kamberg. Comparatively harsh habitat, in which food (grass) and vegetative cover are limiting and patchily distributed. Also, food availability fluctuates seasonally. Social system is partially communal. Females are tolerant of one another and display a high level of intrasexual association, while male-male interaction is highly aggressive. Males appear to be highly territorial, as suggested by indices of intrasexual association. Moreover, males may have exclusive access to a group of females, resulting in a polygynous mating system.

Karkloof. Habitat less harsh. Food and cover are less prone to seasonal variation, and are uniformly distributed. Social system is dispersed. Generally, adults occur in close

spatial association with one another and probably have temporal territoriality. Females are intrasexually highly aggressive and have lower indices of association than males. Aggression among males is ritualized, probably permitting them to associate with one another in nature, suggesting that they may have spatially overlapping home ranges. Hence, males may compete for access to receptive females, resulting in promiscuous matings.

The most noticeable difference between the social structures of the two populations was the degree of gender-specific association and levels of intrasexual tolerance, which are probably the underlying determinants of the spatial distribution and mating strategy of members of both populations. What are the probable causes of population-specific female and male social structure? The following discussion will attempt to answer this question.

Female social structure

Because of the high energetic demands of pregnancy and lactation, the ease of acquisition of food strongly influences the level of territoriality of female mammals (Millar 1977, Clutton-Brock & Harvey 1978, McClure 1987). Ostfeld (1985, 1990) predicted that female rodents relying on relatively sparse, patchy, and slowly renewed food would be expected to defend territories with food patches, and would be intrasexually territorial. In contrast, when food is abundant, widespread and rapidly renewed, females would be less territorial. Ostfeld based his predictions on

cost-benefit or optimality models, stating that, when resources are abundant and uniformly distributed in space, the costs of patrolling a territory against invasion from intruders are high, favouring reduced territoriality; the opposite is predicted when resources are clumped.

Contrary to Ostfeld's hypotheses, Kamberg females were apparently intrasexually less territorial, as suggested by the indices of association and the interaction during dyadic encounters, despite food (grass) being sparse, patchy and seasonally variable at Kamberg. Karkloof females appeared to be intrasexually aggressive and perhaps intrasexually highly territorial, notwithstanding the seasonally more abundant and uniform distribution of food at Karkloof. This apparent contradiction of Ostfeld's theory relates mainly to the shortcoming of most socio-ecological investigations in viewing energy requirements (food) as the most important determinant of the spatial distribution of females (Wilson 1975, Ostfeld 1985, 1990). A host of factors, like nest-sites (Willan 1982), may have determined the intrasexual interaction of Kamberg and Karkloof females. Moreover, O. irroratus is adapted to a poor quality diet (De Graaff 1981, Brown 1988), implying that food quality is not a critical factor in the social organization of O. irroratus, at least at Kamberg (Chapter 7).

The lack of overt aggressive behaviour by Kamberg females may be explained by either or both of two factors. First, as cover is sparse and temperatures are low at Kamberg, females might have derived thermoregulatory benefits (e.g. by social huddling; Willan 1990) from being

intrasexually more tolerant, particularly if they nested communally (Millar & Derrickson 1992), which was the case with some females (see Chapters 7 and 8). Second, lack of cover increases the risk of predation, and communality may be adaptive in facilitating predator avoidance (Wilson 1975). However, neither olfactory (Pers. obs.) nor acoustic (Chapter 6) warning signals have been detected in Kamberg O. irroratus.

In previous socio-ecological studies on O. irroratus from the Natal Midlands and Eastern Cape, it was found that the habitats preferred by O. irroratus are often of limited size (Willan 1982) and population densities are high during the breeding season (Brown 1988). Both Willan (1982) and Brown (1988) claimed that aggressive behaviour and lack of associations between females are forms of a territorial defence mechanism by females, relating mainly to providing young, which disperse only 11 - 12 m from the maternal nest, with an area in which to establish a home range. Clearly, such a strategy is adaptive, since it increases a female's inclusive fitness.

In terms of levels of intrasexual association and the degree of intrasexual aggression, the Karkloof population appeared to be similar to other O. irroratus populations. Furthermore, the habitat of Karkloof O. irroratus appears to be similar to that of other O. irroratus (Davis 1973, Willan 1982, Brown 1988), with prime areas of habitat restricted to a few areas along water-courses, linked by clearings in the Karkloof forest (Chapter 7). The aggressive behaviour of Karkloof females may, therefore, allow these females to

maintain their territories, and functions to provide dispersing young with adequate space and resources for establishing a home range.

An alternative explanation for the aggressive behaviour and lack of intrasexual association may relate to predation. In habitats where cover is dense, as at Karkloof, animals rely on crypsis as a predator-avoidance mechanism (Wilson 1975). A solitary habit no doubt improves crypsis, so that the absence of communal behaviour of Karkloof O. irroratus may facilitate better predator-avoidance.

Male social structure

In most mammals, male investment in reproduction ends once copulation is achieved, and very few males participate in parental duties (Millar 1977, Clutton-Brock & Harvey 1978). This, together with the comparatively low energetic demands of sperm production (McClure 1987), implies that the limiting 'resource' for males may be the availability of receptive females, i.e. copulations (Ostfeld 1990). Therefore, territoriality in male rodents is usually less responsive to the distribution of food/cover and more responsive to the distribution of mates. Copulations occur as discrete and widely scattered events in space and time, and thus probably require different strategies of acquisition than do food and cover, resources which are more or less continuously available (Ostfeld 1985).

Differences in the indices of intrasexual association and in the degree of intrasexual tolerance between Kamberg males and between Karkloof males suggest the existence of

population-specific territoriality among males. These
differences closely conform to the predictions made by the
environmental potential for polygyny (EPP) model (Emlen &
Oring 1977). This model postulates that, when females are
intrasexually non-territorial, males would be intrasexually
territorial, to monopolize matings with a group of females,
and mating is polygynous (Emlen & Oring 1977, Ims 1990b,
Ostfeld 1990). Conversely, when females are uniformly
distributed, usually as a result of intrasexual
territoriality, males cannot monopolize females by
territorial defence, and would employ a combination of
contest and scramble competition for access to receptive
females, promoting promiscuous matings (Ims 1990b, Ostfeld
1990). In this case, males would have intrasexually
overlapping home ranges.

Kamberg females apparently had high indices of
intrasexual association, while Karkloof females appeared to
display the opposite trend. Population-specific indices of
association of females may have been responsible for the
difference in the levels of tolerance and indices of
association of males of the two populations; Kamberg males
were intrasexually aggressive, displaying low levels of
intrasexual association. In contrast, comparatively high
levels of association occurred among Karkloof males.

Several authors have challenged the biological validity
of the EPP model (see Ims 1990a, Boonstra et al. 1993b). For
example, Ims (1990a) found that in captive red-backed voles
Clethrionomys rufocanus, males were on average more
successful at locating receptive females which were spatially

clumped than those which were spatially dispersed, despite the promiscuous mating system of this species. Ims warned that male territoriality based on the EPP model should be interpreted with caution. Without relevant information, the findings of Ims and other workers cannot be tested, so it is most feasible for the moment to assume that the contrasting levels of association and intrasexual tolerance between Kamberg and Karkloof males conforms to the EPP model, although alternative explanations deserve consideration.

Theoretical considerations

The adaptive significance of the social systems is discussed above in terms of the fitness of individuals - the level at which natural selection traditionally operates (Trivers 1985). The evolution of social systems may be subject to various other forces of natural selection, however, including kin selection (Hamilton 1964, Trivers 1985) and r- and K-selection (MacArthur & Wilson 1967, Pianka 1970).

Kin selection theory considers the gene as the unit of selection, and postulates that selection favours individuals adopting strategies which increase their inclusive fitness. Kin selection may have influenced the social systems of Kamberg and Karkloof O. irroratus in different ways, as set out below.

Ostfeld et al. (1988) found that clumping of a group of related female meadow voles Microtus pennsylvanicus increased the overall reproductive output of the group, thereby increasing the inclusive fitness of each female in that

group. It is therefore tempting to speculate that kin selection may also be operating in the Kamberg population, in which females were intrasexually more tolerant, and sometimes shared nests. The familial relationship of Kamberg females at each trap-site sampled (Chapter 7) is unknown, however, as is the dispersal behaviour of Kamberg sub-adult females; kin selection theory predicts that females would be natally philopatric (Boonstra et al. 1987).

If kin-selection was an underlying determinant of sociability of Kamberg females, aggressive interaction during female intrasexual dyadic encounters is unexpected (Chapter 8), because such behaviour occurs among unrelated individuals (inter alia Barash 1989, Willan 1990). On this basis, it was proposed that Kamberg females are partially territorial (Chapter 8). All females used in the encounters were wild-caught, however, captured ad libitum at different sites at Kamberg and were probably unrelated; aggression is predictable between such individuals. Clearly, this hypothesis needs further testing.

Kin selection may have caused ritualization of aggression and temporal territoriality in the Karkloof population, as was suggested in other O. irroratus populations (Willan 1982, Brown 1988). Both ritualized aggression and temporal territoriality are adaptive in the sense that they reduce the possibility of damaging conflict between neighbours, who are often closely related as a result of the short juvenile dispersal distance in O. irroratus (Willan 1982).

Despite its simple dualism (Stearns 1976), the theory of r- and K-selection (MacArthur & Wilson 1967), as developed by Pianka (1970), has been used to explain many life-history phenomena. The theory predicts that, in unstable (more harsh) habitats, species are comparatively r-selected, while, in stable (less harsh) habitats, species are comparatively K-selected; no organism is completely r- or K-selected but may be placed along a continuum between the two extremes (Pianka 1970). The forces of r- and K-selection affect several species characteristics (reviewed in Willan & Meester 1989), including sociability: communal and dispersed social systems are favoured by r- and K-strategists respectively.

In terms of habitat harshness, r-K theory predicts that Kamberg *O. irroratus* is more r-selected than the Karkloof population. Except for the partially communal social system of Kamberg *O. irroratus*, however, all variables considered indicated that this population may have more K-attributes than its Karkloof counterpart. For example, Kamberg *O. irroratus* had a smaller litter size (Chapter 3) and a longer breeding season than Karkloof *O. irroratus* (Chapter 7). Some authors (e.g. Willan 1982, Brown 1988) are of the opinion that differences in the levels of sociability are sufficient grounds to warrant r- and K-classification, so that the partially communal Kamberg population would be more r-selected than the dispersed (asocial) Karkloof population. Nonetheless, I consider such conclusions premature until additional studies are undertaken. Furthermore, the social systems of the Kamberg and Karkloof populations should be compared in terms of other life history models (e.g.

bet-hedging; Stearns 1976), but this is clearly beyond the scope of this study.

Comparison of the socio-ecological features of Kamberg and Karkloof _O. irroratus_ demonstrated a positive relationship between the levels of sociability and habitat harshness, as was reported in other rodents (Nel 1975, Happold 1976, Brown 1988, Barash 1989). These findings support the Willan (1982) prediction that _O. irroratus_ displays different degrees of sociability across its range. Overall habitat harshness may be affected by several habitat characteristics (see Table 8 in Chapter 7), although seasonal availability and abundance of food and cover appear to be of particular significance. The availability of cover seemed to be the primary determinant of the level of sociability of the Kamberg and Karkloof populations: sparse and patchy cover **evidently selected for a partially communal social system in** Kamberg _O. irroratus_ females, while abundant and uniform cover selected for a dispersed social system in Karkloof _O. irroratus_ females.

The social structure of a species/population is subject to intrinsic (genetic) and extrinsic (environmental) evolutionary forces. The results of the present study suggest that both contrasting habitats and genetic constraints (e.g. kin selection) may work in concert to influence the social organization of the populations under consideration.

SPECIATION AND REPRODUCTIVE ISOLATION

In this section, possible pre-mating and post-zygotic barriers to interpopulation breeding between Kamberg and Karkloof O. irroratus are discussed. In addition, speculations are presented about the probable outcome of the contact between Kamberg and Karkloof O. irroratus in nature.

In whole animal choice tests (Chapter 4), individuals preferred same-population mates, suggesting the existence of mate recognition. Olfactory choice-tests (Chapter 4) and courtship behaviour studies (Chapter 5) provided evidence that mate recognition was achieved by means of contrasting olfactory, tactile and visual communicatory cues between Kamberg and Karkloof individuals, which suggest the existence of unique specific-mate-recognition-systems (Paterson 1985) in both populations. These differences in sensory stimuli **may act as behavioural isolating mechanisms, preventing** mating between individuals of the Kamberg and Karkloof populations, should they become sympatric.

Differences in tactile and visual communication apparently reflect population-specific courtship behaviour, which in turn was determined by the contrasting social organization of both populations. Lack of intrasexual tolerance, together with a low degree of intrasexual association, suggests that Karkloof females are territorial. This possibly selected for promiscuous matings in Karkloof O. irroratus, which resulted in complex, highly aggressive courtship. In Kamberg O. irroratus, male monopoly of a group of females resulted in polygynous matings and comparatively amicable courtship.

Unlike tactile and visual communication, there seemed to be no detectable relationship between differences in olfactory communication and population-specific courtship. However, differences in olfactory communication, as in the case of other communicatory cues, may have arisen in allopatry as a response to other environmental pressures (e.g. predation; territorial advertisement, Menzies et al. 1992), because of random genetic effects (Rubinoff & Rubinoff 1971), or as a result of the pleiotropic effects of genes (Dobzhansky et al. 1968).

Olfactory choice tests provided evidence that individuals preferred same- rather than different-population mates, but it does not necessarily follow that positive odour discrimination equals mate recognition. Instead, test **animals may have preferred the odour of same-population** individuals, regardless of their sex, so that response to odour in choice-tests may have been population- rather than mate-directed. Nevertheless, positive response to the odour of individuals of the same population clearly improves the likelihood of positive assortative mating.

Spectrographic analysis of acoustic signals produced by Kamberg and Karkloof subjects failed to reveal any evidence of population-specific auditory communication, nor whether acoustic signals function as a pre-mating reproductive isolating mechanism. Otomys irroratus acoustic signals are associated with aggressive interaction which, at least in Kamberg males and in all Karkloof individuals, may explain the lack of intrasexual associations, and possibly the existence of intrasexual territoriality. Predictably,

acoustic signals produced in this context are not subject to adaptive variation, as would perhaps have been the case if they were associated with courtship behaviour (Mayr 1963, Heth & Nevo 1981, Paterson 1985) and been part of the specific-mate-recognition-system (Paterson 1985).

A greater number of acoustic signals were produced during interpopulation than intrapopulation pairings, implying that acoustic signals may complement interpopulation differences in olfactory, visual and tactile cues in promoting aggression. As reported in populations of other rodent species (see Nevo et al. 1986, Corti et al. 1989), aggression may function as a pre-mating barrier to reduce breeding success between Kamberg and Karkloof individuals.

In both the breeding (Chapter 3) and courtship behaviour studies (Chapter 5), about 50 % of Kamberg x Karkloof interpopulation pairings were separated because of damaging fights. Such fights occurred in Karkloof x Kamberg pairings during only courtship studies and only in 25 % of cases. In all these pairings, males attacked and wounded females, but only Karkloof females (eight out of the 19 attacked) died as a result of attacks by Kamberg males. This suggests that ethological barriers to reproduction are better developed in Kamberg x Karkloof than in Karkloof x Kamberg pairings, and that pre-mating reproductive isolation may be partially uni-directional, as was reported between strains of Drosophila silvestris (Ahearn 1980). This observation is significant, indicating that interbreeding between free-living Karkloof males and Kamberg females would be more likely than the reciprocal situation.

In spite of potential pre-mating barriers to interbreeding, about 48 % of interpopulation pairs produced offspring. Similar findings have been reported in other studies (inter alia Rubinoff & Rubinoff 1971, Dobzhansky et al. 1968, Chang & Ayala 1989). Interbreeding may have occurred because the confined space of captivity permits animals to overcome pre-mating isolating mechanisms that exist between free-living populations (Spieth 1958), or because complete behavioural isolation has not yet evolved (Dobzhansky et al. 1968, Cobb & Jallon 1990).

If interpopulation mating occurs when the populations under investigation are sympatric, some females will produce young. However, 26 - 38 % of hybrids died before weaning, with about 70 % of these being killed by the mother, possibly due to maladaptive behaviour of hybrid young (Chapter 3). Moreover, those hybrids surviving to adulthood displayed, on average, depressed growth rates, mainly in respect of body mass. Probably the most significant consequence of interpopulation breeding was that only one out of 57 hybrids produced young. Therefore, should interbreeding occur in nature, the populations would be post-zygotically isolated.

The production of inviable and/or sterile hybrids is energetically wasteful, and represents a reduction of the reproductive potential and inclusive fitness of animals which mate with individuals representing other populations. Baker & Bickham (1980) suggested that viable but sterile hybrids may compete for food, space and reproductive opportunities with other individuals which are capable of reproducing. In addition, the slower growth rate of hybrids could reduce

their reproductive success, especially in males, in which larger body size increases the ability to compete for mating opportunities. Thus, the evolutionary and ecological implications of hybrid inviability/sterility are significant, and selection may thus operate against hybrids, either by enhancing (by reinforcement) existing pre-mating differences (inter alia Dobzhansky et al. 1968, Baker & Bickham 1980, Solginac 1981, Capanna et al. 1985, Butlin 1987), or perhaps more correctly by replacing (by reproductive character displacement) post-zygotic barriers with pre-mating ones (Butlin 1987).

EVOLUTIONARY CONSIDERATIONS

Two aspects are discussed in this section. First, the probable evolutionary and geographic divergence between the Kamberg and Karkloof populations is considered, and speculations are presented as to which of the two populations is more likely to be ancestral. Second, socio-ecological explanations are provided for the relative levels of heterozygosity found in the two populations, as revealed by mtDNA and allozyme studies.

Evolutionary and geographic divergence

Palaeontological (Pocock 1976) and biochemical (Taylor et al. 1989) evidence indicates that the subfamily Otomyinae is divided into two lineages derived from a Pliocene ancestor, Prototomys. One lineage includes O. irroratus and the Angoni vlei rat O. angoniensis, and the other, the ice rat O. sloggetti and bush Karoo rat O. unisulcatus. On

mainly palaeoclimatic grounds, Taylor et al. (1989)
postulated that Prototomys was mesically adapted. This
hypothesis was supported by a complex of interrelated
ecological and behavioural factors, including habitat
characteristics, nesting habits, maternal behaviour and
relative vulnerability to predation of young of Otomys spp.
studied to date (Willan 1990, Pillay et al. 1993).

Nipple-clinging is present in all otomyines (Pillay et
al. 1993), although it is most adaptive in those species
which nest on the surface and under dense vegetative cover
(Willan 1990), which is typical of O. irroratus and
O. angoniensis (De Graaff 1981, Skinner & Smithers 1990);
nipple-clinging reduces the risk of predation on unweaned
young of surface-nesting species. The presence of
nipple-clinging in O. sloggetti and O. unisulcatus is
unexpected, because both species occupy sparsely vegetated
habitats and have protected nest-sites (burrows or lodges;
Willan 1990, Brown & Willan 1991). Willan (1990) suggested
that nipple-clinging is a plesiomorphic otomyine character,
and speculated that it would have been unlikely for
nipple-clinging to evolve in an ancestral otomyine in which
the nest was adequately protected against predators. It is
probable therefore that Prototomys was adapted to high cover
density, nested on the surface, and had nipple-clinging
young.

In terms of its ecology, nesting habits and maternal
behaviour, Karkloof O. irroratus appears to be analogous to
Prototomys. The Karkloof habitat was densely vegetated, and
free-living O. irroratus nested on the surface (Chapter 7),

while captive-born Karkloof young were rarely detached from their mothers' nipples before weaning (Pillay et al. 1993). In contrast, cover was sparse at Kamberg, and O. irroratus here nested in burrows or in well-constructed enclosed nests (Chapter 7).

Observations of maternal behaviour in 25 Kamberg and 33 Karkloof females (N. Pillay Unpubl. data) indicated that Kamberg pups were often (in 63 % of cases) found in the nest-box, detached from the mother's nipples, while she spent periods of up to 50 min outside the nest-box; no Karkloof young were observed detached from the mother's nipples prior to weaning. Extrapolation of these data to the field situation suggests that Kamberg females might leave their unweaned young unattended in the safety of the enclosed nest/burrow during foraging or post-partum mating.

In view of the fact that environmental factors largely dictate species-specific life history phenomena (Pianka 1970; Stearns 1976), it is not unreasonable to assume that Karkloof O. irroratus would have retained attributes that were established in the ancestral form. If this is so, it follows that the nesting habits and maternal behaviour of the Kamberg population, compared to those of the Karkloof population, demonstrate secondary adaptation in response to the sparse, patchy cover at the Kamberg locality.

Further support for the hypothesis that Kamberg O. irroratus was derived from a Karkloof-type ancestor is provided by evidence from chromosomal morphology. A noticeable karyotypic difference between the Kamberg and Karkloof populations was the absence of chromosome pairs

seven and 12 and the presence of a pair of large acrocentric autosomes in the Kamberg karyotype (Chapter 1; Contrafatto et al. 1992b). G-banding suggested that the large acrocentric pair may have been derived from the geographically more widespread unfused seven and 12 pair (Contrafatto 1992a, 1992b). The tandem fusion is probably an autapomorphic character (Contrafatto Pers. comm.), suggesting that the Kamberg population was derived from a Karkloof-type ancestor.

Raubenheimer (In prep.) estimated the percentage sequence divergence between the mtDNA of the Kamberg and Karkloof populations to be 2.6 %. Since muroid mtDNA evidently evolves at an average rate of 7.1 % per million years (She et al. 1990), it appears that the Kamberg population was derived from a Karkloof-type ancestor about $3 \times 10^5 - 4 \times 10^5$ years ago, during the mid-Pleistocene. During this epoch, the vegetation of southern Africa was greatly influenced by variation in rainfall associated with alternating dry and wet phases, and the preferred habitat of ancestral O. irroratus, which was apparently mesic grasslands with dense cover, was periodically far more extensive than at present (Cooke 1962). Therefore, range expansion during wet periods, and contraction during dry periods, of ancestral O. irroratus probably occurred on several occasions. Geographic separation, and subsequent genetic and chromosomal divergence, of various southern African O. irroratus populations apparently occurred as a series of founder events during drier phases (Taylor et al. 1992; Chapter 1). Based on the palaeoclimate of southern Africa, it is probable that

the Kamberg population arose from a Karkloof-type ancestor as a result of population fragmentation induced during a mid-Pleistocene dry period.

Socio-ecology, mtDNA and allozyme electrophoresis

Allozyme electrophoresis of 12 southern African O. irroratus populations revealed greater, but not statistically significant, heterozygosity in the Kamberg population (H = 0.130) than in any other population, including the Karkloof population (H = 0.096) (Taylor et al. 1992). Mitochondrial DNA analysis corroborated the allozyme data, with 11 discrete mtDNA lineages in the Kamberg population, but only five in the Karkloof population (Raubenheimer In prep.).

High heterozygosity is characteristic of an outbreeding population which possesses the following socio-ecological characteristics: less harsh habitat, a high degree of aggression among conspecifics, and territoriality which promotes dispersal. Of the two populations studied, the socio-ecological features of Karkloof O. irroratus closely matched the above criteria for high heterozygosity; Kamberg O. irroratus apparently had a partially communal social structure, mainly as result of a lack of uniform cover. Although the dispersal behaviour of Kamberg young was not ascertained, the patchy cover suggests that dispersal may be limited, and probably occurs only in male offspring which are intrasexually aggressive and had did not associate intrasexually in nature. Two explanations are possible here.

Assuming that kin selection and natal philopatry is operating in Kamberg females, closely-related females probably occur in spatially isolated 'pockets' in various areas at Kamberg. At the same time, high levels of agonism between males suggest that sub-adult males may disperse from the natal site and, as adults, challenge dominant males for access to their groups of females. In her mtDNA study, Raubenheimer (In prep.) used adult animals trapped ad libitum at various Kamberg sites over a three-year period. Since mtDNA is maternally inherited, it is conceivable that females from a particular site represented lineages unique to that site. In addition, the mtDNA of adult males may have been representative of their own natal sites, which would have been different from those of the females they dominated. Kamberg O. irroratus used in electrophoretic studies (Taylor et al. 1992) were trapped in a similar fashion to that of Raubenheimer (In prep.), and it is possible that heterogeneity in the allozymes was the result of the partially communal social structure of the Kamberg population. Similar genetic heterogeneity as a result of female natal philopatry and male-biased dispersal has been demonstrated in several rodent species, including house mice (Selander 1970), yellow-bellied marmots Marmota flaviventris (Schwartz & Armitage 1980) and meadow voles Microtus pennsylvanicus (Plante et al. 1989).

Alternatively, genetic heterozygosity may be related to the degree of habitat harshness. It has long been recognized that complete genetic homogeneity resulting from natural selection is unlikely, and that genetic variation is usually

the norm (Mayr 1963). Several mechanisms which protect genetic variation in natural populations have been recognised (see Mayr 1963, p. 216), although mechanisms based on environmental diversity have received most attention. The climatic unpredictability or niche-width hypothesis predicts that populations in spatio-temporally comparatively harsh environments would demonstrate greater genetic variation (Nevo et al. 1989), since the fitness of phenotypes will vary in space and time, causing fluctuation in the selective value of genes (Mayr 1963). Such genes may therefore persist in a population, increasing genetic variation. The Kamberg habitat was harsher than the Karkloof habitat, and the higher degree of genetic heterogeneity of Kamberg O. irroratus is therefore predictable.

CONCLUSIONS

This study is part of a growing body of information on speciation, geographic variation and adaptation in southern African small mammals in general, and chromosomally dissimilar O. irroratus populations in particular.

Results of interpopulation matings, choice tests and courtship studies indicate that genetic divergence has occurred in allopatry to the extent that gene exchange between populations may be drastically reduced by a host of ethological factors should these populations become sympatric.

Apart from possible genetic differences which may have caused hybrid breakdown (Chapter 3), chromosomal differences between the populations (see Chapter 1) acted as a

post-zygotic isolating mechanism when individuals

representing the Kamberg and Karkloof populations interbred

in captivity. Karyotypic rearrangements, of which the tandem

fusion in the Kamberg population is particularly noteworthy

since it may have caused hybrid sterility, seem to be

accompanied by limited phenotypic change (Chapter 1), as

shown by a low genetic distance (Nei's I = 0.02) between the

Kamberg and Karkloof populations (Taylor et al. 1992).

Consequently, Kamberg O. irroratus, like the Hogsback

population which also has a tandem fusion (Pillay 1990;

Chapter 1), represents a chromosomally-determined incipient

sibling species, as predicted by Meester (1988).

Socio-ecological studies indicated that contrasting

environmental pressures, expressed in terms of relative

habitat harshness, influenced population-specific social

structure, and in particular the degree of sociability.

Despite reservations by some workers that explanations for

adaptive variation are tantamount to story-telling (see Gould

1982), adaptive processes are essential evolutionary forces

(Nevo 1991), as they ultimately provide the necessary

mechanisms for speciation. Therefore, ecological

differences, primarily those of climate and habitat, are

important determinants of adaptive speciation in the Kamberg

and Karkloof populations, as was found in karyotypically

unique populations of mole rats Spalax ehrenbergi (Nevo 1991).

The present study has provided evidence of both

adaptation and speciation in Kamberg and Karkloof

O. irroratus. Geographic separation between the populations

appeared to have originated as a founder event (Taylor et al.

1992), with a Karkloof-type ancestor as the precursor of extant Kamberg _O. irroratus_. The existence of population-specific social phenomena indicates that adaptation to local environmental circumstances has occurred in allopatry. The partially communal social organization of the Kamberg population is clearly an adaptation to the comparatively harsh Kamberg habitat, in which both cover and food were clumped and seasonally variable. The dispersed social system of the Karkloof population reflects the less harsh Karkloof habitat where resources were evenly distributed and seasonally less variable. Assuming that the habitat of the _O. irroratus_ ancestor was similar to that at Karkloof, it is likely that, in terms of its social organization, the Karkloof population is similar to the ancestor, since there is normally a correlation between the social system of species and environmental conditions (Crook 1970, Wilson 1975, Barash 1989). For example, studies of several _O. irroratus_ populations indicate that all have dispersed social systems and occupy habitats similar to that of the Karkloof population (Davis 1973, Willan 1982, Brown 1988). This implies that the increased sociability of Kamberg _O. irroratus_, at least among females, was derived from the dispersed social organization present in other _O. irroratus_ populations, supporting the hypothesis that communal social systems evolve from dispersed ones (Alexander 1974, Wilson 1975).

The presence of genetically-determined ethological differences is related mainly to differences between the courtship behaviour and social structure of the two

populations. Hence, pre-mating isolating mechanisms appear to have evolved with socio-ecological change in allopatry. The tandem fusion in the Kamberg population may have arisen as an incidental by-product of the founder effect (Contrafatto et al. 1992a, 1992b, Taylor et al. 1992). Consequently, the presence of both ethological and chromosomal differences suggest that pre-mating (ethological) and post-zygotic (chromosomal) isolating mechanisms arose independently in the Kamberg and Karkloof populations. The accumulation of effective pre-mating ethological barriers seems to have occurred gradually in allopatry, because, even after a few hundred thousand years, complete pre-mating reproductive isolation has still not been attained, but this is usually the case with allopatric populations (Dobzhansky et al. 1968).

Future studies

Like most other studies of speciation and socio-ecology, the present investigation has suggested many avenues for future research. Some of the more important avenues, which may provide information essential to the understanding of adaptive variation and/or evolutionary trends in the Kamberg and Karkloof populations, as well as in other O. irroratus populations, are mentioned below.

It has been assumed throughout the study that pre-mating isolating mechanisms are the result of the accumulation of genetic differences in allopatry, possibly because this offers, at present, the most parsimonious argument for speciation in O. irroratus. However, it is possible that

pre-mating barriers, although incomplete, were the result of reinforcement following secondary contact between the Kamberg and Karkloof populations.

Kamberg and Karkloof _O. irroratus_ may have established contact periodically during pre-historic and historic times, as a consequence of climatic variation in southern Africa (Cooke 1962). Certainly, the low genetic distance between the populations suggests that contact between the two populations could have occurred. Hence, the ethological differences could have arisen to prevent energetically wasteful matings between Kamberg and Karkloof individuals where and when they met.

Trapping studies in several areas between the Kamberg and Karkloof localities have recently been initiated, and it is anticipated that these studies may shed some light on the **above mentioned issue, either by identifying areas of** sympatry between Kamberg- and Karkloof-like _O. irroratus_, or by revealing possible zones of hybridization between the populations. Lack of areas of sympatry would strengthen the case for allopatric speciation. The presence of hybrid zones would indicate that speciation is incomplete, although it would be parsimonious to assume that speciation would have been allopatric, or at least peripatric (Mayr 1963).

Hybrids which are heterozygous for a tandem fusion are likely to produce unbalanced gametes, caused by malsegregation of meiotic multivalents (Moritz 1986). As far as I am aware, only one study has examined the meiotic cells of hybrids to confirm that a tandem fusion brings about unbalanced gametes in hybrids: Ryder et al. (1989) showed

that, because of a tandem fusion in the Y chromosome, male hybrid dik-dik (Madoqua kirki) produced a reduced number of spermatozoa. Similar cytological studies of gametes of hybrid O. irroratus are needed to ascertain whether or not the presence of the tandem fusion has brought about hybrid sterility. Furthermore, the role of the fusion in causing hybrid sterility was questioned in this study, mainly because fewer hybrids produced offspring than would have been predicted by chance (Chapter 3). Further studies are needed to interpret these results.

The social structure of Kamberg and Karkloof O. irroratus was respectively partially communal and dispersed. These predictions were based on trapping studies during only three seasons coupled with extensive laboratory studies. Intensive field studies are undoubtedly needed to verify these predictions. However, free-living rodents are difficult to study because of their cryptic nature, and trapping data usually do not provide precise information about many social phenomena. A more reliable alternative would be radiotelemetric tracking, which has been used extensively to provide information on patterns of movement, dispersal, habitat utilization and social organization in free-living small mammals (Madison 1980).

A number of studies have attempted to ascertain the mating strategy of rodent populations by using genetic analyses (e.g. DNA fingerprinting, allozyme electrophoresis; inter alia Ribble 1992, Boonstra et al. 1993b). Polymorphic genetic markers have been used to establish the familial relationships between males and juveniles at a particular

trap-site: litters of mixed paternity provided strong evidence for promiscuity, while polygyny was suspected when most young were related to the dominant male. Apart from mating systems, genetic analysis may be used to test theories of kin-selection, as was demonstrated in meadow vole populations (Sheridan & Tamarin 1986).

The information obtained from field studies seemed adequate in order to achieve the objectives of the present study, but clearly more detailed trapping and habitat assessment studies are required to test the hypotheses presented here. Field work was undertaken during three seasons, and during a time of severe drought in southern Africa. Habitat characteristics change both seasonally and annually, potentially influencing population characteristics. For example, as a result of variation in food availability and density of individuals, the territorial behaviour and mating systems of some meadow vole populations show seasonal plasticity, ranging from single female territories in summer to breeding female dyads in the following spring (Madison & McShea 1987).

Adaptive variation was studied by establishing the existence of differences in the socio-ecological attributes of the two populations, but other factors may be investigated, including physiological adaptation. Contrasting temperature regimes at Kamberg and the Karkloof forest, together with the larger body size of Kamberg animals, hint at the possibility of physiological differences between individuals of the two populations.

The study of adaptive variation and evolutionary trends in the Karkloof and Kamberg populations suggests the need for future research on other <u>O. irroratus</u> populations. Probably the most interesting study would be one of chromosomally-distinct sympatric populations.

SUMMARY

Selected aspects of the biology of two chromosomally-distinct, allopatric O. irroratus populations were studied. The populations, at Kamberg and Karkloof, differing in the presence of a tandem fusion between chromosomes seven and 12 in the Kamberg population, occur less than 50 km apart in the Natal Midlands. The study set out to investigate two important processes in evolution: speciation and adaptive variation. The study aimed to establish whether: (i) mechanisms existed whereby the Kamberg and Karkloof populations were reproductively isolated from one another; and (ii) the populations displayed contrasting socio-ecological characteristics.

Laboratory breeding trials and comparative studies of the postnatal development of progeny resulting from intrapopulation and interpopulation pairs provided evidence of post-zygotic barriers. In contrast to the 100 % breeding success of intrapopulation pairs, less than 50 % of interpopulation pairs produced young, and overall reproductive performance of these pairs was reduced. Furthermore, fitness of hybrids was dramatically impaired, as indicated by increased pre-weaning mortality and, on average, inhibited growth in respect of body mass. Only one out of 57 surviving hybrids produced young in backcross and/or hybrid-cross matings, indicating that almost all were sterile.

Of the many types of pre-mating reproductive isolating mechanisms, ethological ones are most important and were therefore given priority here. Six ethological factors were

studied, namely mate recognition, courtship behaviour, and various communicatory cues, comprising olfactory, tactile, visual and auditory stimuli.

In 'whole-animal' choice tests, male and female preferences for opposite sex individuals of the same and different population were tested. These tests indicated that individuals showed a significant preference for mates of their own population over those of the other population.

Choice-chamber tests of preference for odours belonging to same- and different-population mates were undertaken to test olfactory discrimination. These tests provided further evidence of mate recognition, and suggested that olfactory cues were important for mate recognition in both populations.

In order to ascertain whether or not differences in courtship behaviour between Kamberg and Karkloof O. irroratus rendered them behaviourally incompatible during interpopulation encounters, intra- and interpopulation male-female interactions were observed in neutral arena encounters. During intrapopulation encounters, Kamberg pairs were less aggressive and more amicable than Karkloof pairs, and Karkloof males performed more sexual acts than Kamberg males. These differences apparently reflected the mating strategy of the two populations. Compared to intrapopulation pairings, interpopulation pairings were characterized by more aggression, less amicability, and later development of essentially amicable interaction. Furthermore, fewer sexual acts were performed in inter- than in intrapopulation encounters. It was postulated that differences in responses to olfactory, visual and tactile cues between the two

populations resulted in highly aggressive interpopulation encounters.

Acoustic signals produced during intra- and interpopulation social interactions were spectrographically analyzed in order to describe the structure of recorded sounds, and to ascertain the behavioural conditions under which sounds were produced. In addition, the role of acoustic signals as pre-mating barriers to reproduction was investigated. Three major sound types were identified, all of which were of low frequency (i.e. < 24 kHz). These were chits, alarm squeals and tail-quivering; two distinct forms of each of chits and alarm squeals were distinguished. The production of low frequency sounds appeared to be an adaptation to the preferred grassland habitats of O. irroratus. All acoustic signals were associated with aggressive social interaction, promoting ritualized aggression in the taxon. No differences were found between the sounds produced by Kamberg and Karkloof O. irroratus. A greater number of acoustic signals were detected during inter- than intrapopulation pairings, however, and it was postulated that acoustic signals may complement other communicatory cues in causing increased aggression during interpopulation pairings; aggression is an important pre-mating barrier.

The field of socio-ecology is concerned with the relationship between social structure and ecology. The present study was concerned primarily with social phenomena which were investigated mainly by means of laboratory work.

Field work, which consisted of trapping and habitat
assessment studies, was carried out over three seasons, and
allowed comparison of the ecological requirements of the two
populations, which in turn facilitated analysis of
population-specific socio-ecology.

Food and cover were patchily distributed and their
availability fluctuated seasonally at Kamberg. At Karkloof,
these resources were uniformly distributed, and were equally
abundant in summer and autumn, but were less so in winter.
Such differences in resource availability reflected the
contrasting climatic profiles, mainly in terms of rainfall
and temperature, at the two localities. It was concluded
that the Kamberg habitat was harsher than the Karkloof
habitat.

Social structure was ascertained by means of behavioural
observation of the interactions of intrapopulation intra- and
intersexual dyadic encounters. In addition, sexual
dimorphism in body size (body mass and head-body length) and
mate choice behaviour of females were investigated. Trapping
data were used to calculate indices of association of
free-living dyads.

The results of these studies, together with information
available in the literature, permitted the following
descriptions of the social organization of the two
populations: Kamberg - females were tolerant of one another,
had comparatively high indices of intrasexual association,
and probably shared nests in nature. Males were
intrasexually highly aggressive and displayed low indices of
intrasexual association in nature. This suggests that

Kamberg males are highly territorial. In addition, male-male aggression, coupled with a high degree of male-biased sexual dimorphism, suggests that dominant males have exclusive access to a group of females, and mating is polygynous. The high degree of associations between females implies that the social system is partially communal. <u>Karkloof</u> - females were intrasexually highly aggressive, demonstrating few intrasexual associations in nature. Ritualized aggression among males seems to be an adaptation which may allow extensive overlap of their home ranges. These data, together with a lower degree of male-biased sexual dimorphism, imply that males do not have exclusive access to receptive females, and mating is therefore promiscuous. The results of choice tests indicate that females may preferentially mate with dominant males, however. Like that of other <u>O. irroratus</u> populations, the social system of the Karkloof population is probably dispersed (asocial), but may incorporate temporal territoriality.

The availability of cover appeared to be the underlying determinant of the degree of sociability of both populations, at least among the females. The social organization of males of both populations seemed to fall into the categories defined by the 'environmental potential for polygyny' model.

The existence of population-specific courtship and communicatory cues hints at the presence of unique specific-mate-recognition-systems in the Kamberg and Karkloof populations. It was speculated that should the Kamberg and Karkloof populations become sympatric under natural conditions, they would be isolated from one another other

through a host of behavioural factors. If mating did occur, however, chromosomally-mediated post-zygotic barriers to reproduction, such as hybrid inviability and sterility, would prevent genetic exchange between the populations.

Several conclusions are possible, at least two of which appear to be important. First, socio-ecological studies indicated that adaptation to local environmental circumstances has occurred in allopatry, and that the Kamberg and Karkloof populations are undergoing adaptive speciation. Second, pre-mating (ethological) and post-zygotic (chromosomal) isolating mechanisms seem to have evolved independently in the two populations. The presence of the tandem fusion in the Kamberg karyotype which, together with other genetically-determined factors, may have caused hybrid sterility, suggests that this population is a chromosomally-determined incipient sibling species.

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