

THE BREEDING AND REPRODUCTIVE BIOLOGY OF  
THE VLEI RAT OTOMYS IRRORATUS

by

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## PREFACE

The experimental work described in this dissertation was carried out in the Department of Biology, University of Natal, Durban, from February 1989 to December 1990, under the supervision of Dr K. Willan and Professor J. Meester.

These studies represent original work by the author and have 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.

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Signed:  .....

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## ABSTRACT

The breeding and reproductive biology of the vlei rat Otomys irroratus representing three allopatric populations (Committee's Drift, Hogsback and Karkloof) have been studied in the laboratory. The study attempted to establish whether the three populations differed in respect of selected reproductive parameters, and whether the populations are reproductively isolated from one another.

The breeding biology of the Hogsback and Karkloof populations was similar while the Committee's Drift population differed from the other two in respect of its smaller litter size and increased interval between pairing and the production of the first litter. Pre-copulatory behaviour differed among populations, with a gradation of increasing intersexual aggression from Hogsback to Karkloof to Committee's Drift pairs. Postnatal growth and development patterns, as well as male reproductive morphology, were indistinguishable among the populations.

Attempts at interpopulation breeding were successful. However, some hybrids died before weaning, while those that survived beyond weaning were sterile, particularly those resulting from cross-matings of Hogsback animals with individuals representing the other populations. Interpopulation pairs displayed higher levels of agonistic

interaction than did the pure pairings. Growth and development and the reproductive morphology of male hybrids were indistinguishable from those of the parental populations.

Interpopulation reproductive variation in *O. irroratus* appears to be due to a combination of environmental and phylogenetic constraints. Both pre- and post-zygotic isolating mechanisms would impede gene flow between the populations should they meet in nature. All populations appear to be undergoing active speciation.

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## CHAPTER 1

### Introduction

#### 1.1 Background to the study

This study forms part of a research programme (the Speciation Programme) being undertaken in the Biology Department, University of Natal, on various aspects of the biology of the rodent subfamily Otomyinae. The particular emphasis of the programme is on chromosomal speciation (see Meester 1988). In the course of these studies, Contrafatto et al. (In press) and G-C. Contrafatto (Unpubl.) have shown that extensive karyotypic differences exist among populations of the vlei rat Otomys irroratus (Brants, 1827). Some populations display diploid numbers ranging from 28 to 30, with totally heterochromatic short arms on the first seven pairs of autosomes. In contrast, other populations have diploid numbers ranging from 24 to 32, with exclusively acrocentric karyotypes. It therefore seems that active speciation is occurring in O. irroratus at present, although there appears to be little identifiable genetic variation, as revealed by electrophoretic studies (Taylor et al. 1989; G. Campbell Unpubl.). Phenotypically, multivariate morphometric analysis has revealed minor interpopulation differences in skull morphology (Hoffmann 1990), but renal morphology is more or less unvarying between populations (Kearney 1990).

Many cases are known of closely related species which are phenotypically similar but differ markedly in chromosome morphology and/or number (inter alia Robbins & Baker 1978; Bickham & Baker 1980; Gordon 1984). On the basis of evidence from the literature, Meester (1988) proposed a model of speciation in which sibling species may arise following chromosomal rearrangements. Furthermore, it is known that individuals representing populations which are chromosomally different (cf. O. irroratus) may interbreed, but offspring resulting from such matings may be sterile and/or inviable because of chromosomal imbalances in the hybrids (inter alia Dobzhansky & Levene 1951; Dobzhansky et al. 1968; Capanna et al. 1985; Baker & Bickham 1986). These populations are then subject to post-zygotic isolation (see section 1.3).

Unlike earlier efforts to breed O. irroratus in captivity (inter alia Davis 1973; Willan 1982; Brown 1988), recent attempts have been highly successful (Meester 1988; Willan Unpubl.). This has allowed the study of the breeding and reproductive biology of O. irroratus in the laboratory, thereby permitting further investigation of evolutionary trends within this taxon. In particular, it has permitted interpopulation mating experiments to study the effects of chromosome imbalances on reproductive success. Accordingly, interpopulation breeding trials between animals representing O. irroratus populations that were karyotypically dissimilar were initiated.

Although a number of O. irroratus populations were available for inclusion in the study, for practical reasons only three were selected, representing two localities in the eastern Cape (Committee's Drift in the Fish River Valley, and Hogsback in the Amatole mountains) and one in Natal (Karkloof, near Howick). To have included a greater number of localities would have been desirable, but would have been impossible within the time available. These three populations were selected because (i) their diploid numbers (i.e. Committee's Drift = 28, Hogsback = 24, and Karkloof = 29 - 32) and chromosomal structure differ from one another, (ii) they exist as isolated demes in relation to one another, and (iii) the Hogsback and Karkloof localities appear environmentally similar to one another, while the Committee's Drift locality is markedly different from both of these localities (Table 1.1). It therefore seemed likely that study of the Committee's Drift, Hogsback and Karkloof populations would permit not only study of chromosomal speciation, but also assessment of possible mechanisms of allopatric speciation (see section 1.3). Moreover, because the environment selects for population attributes (e.g. behaviour, life histories; Hansson & Henttonen 1985), it was considered that these populations would also contribute to an understanding of adaptive variation of the breeding and reproductive biology of O. irroratus.

Table 1.1. Locality data of the Otomys irroratus populations represented in the study. Altitude (m) and rainfall (mm): nearest weather station; Weather Bureau (1984). Veld type: Acocks (1988).

Locality/ grid reference	Environmental parameters		
	Altitude	Mean annual rainfall	Veld type and number
Committee's Drift, Ciskei 33°10'S; 26°57'E	110	401	Karoo & Karroid valley bushveld; 23
Hogsback, Cape 32°33'S; 26°57'E	1450	1174	Highland sourveld; 44a
Karkloof, Natal, 29°17'S; 30°11'E	1440	906	Ngongoni veld of Natal mist-belt; 45

## 1.2 Approach to the study

The study set out to describe and compare the breeding performance and the pre-copulatory behaviour of intrapopulation and interpopulation pairings of individuals representing the Committee's Drift, Hogsback and Karkloof populations of O. irroratus. In addition, the breeding performance of crossbred offspring (i.e. the progeny of interpopulation pairs) was ascertained. Postnatal development studies were carried out to establish (i) whether population-specific growth and development patterns existed among purebred animals, and (ii) the growth and development patterns, as well as the viability, of crossbred young. In order to investigate the possibility of reproductive incompatibility between populations, various reproductive structures of purebred and crossbred males (i.e. glans penis, baculum, spermatozoa) were also studied.

Apart from setting out to document selected aspects of the breeding and reproductive biology of O. irroratus, the aims of the study were therefore to ascertain whether (i) the three populations differed in terms of their breeding and reproductive behaviour and biology, and (ii) reproductive isolating mechanisms would reduce or eliminate successful breeding between animals from different populations if environmental factors allowed reproduction to occur.

### 1.3 Speciation and reproductive isolation

Allopatric speciation is regarded by most evolutionary biologists as the major means by which sexually reproducing taxa form new species. The allopatric model posits the occurrence of speciation when barriers (e.g. geographical) fragment populations of formerly interbreeding organisms. After gene exchange ceases, genetic divergence occurs between isolated populations, either in response to selection for adaptations to local environmental differences, or merely as a result of accumulated mutations by genetic drift (Mayr 1969; Dobzhansky 1970). Differences in chromosomal structure and number may occur as a result of accumulated chromosome mutations when populations are separated (Meester 1988).

Should allopatric populations later become sympatric, genetic dissimilarities arising in allopatry may create barriers to interpopulation breeding. If divergence has proceeded to the extent where mating between males and females representing different populations does not occur, the populations are regarded as pre-zygotically isolated. When genetic divergence between populations is minor, and even in cases where the morphology of the chromosomes is different, interbreeding between members of the two populations may occur, but it is possible that either foetal death occurs or the crossbred offspring resulting from such matings are infertile and/or inviable. These populations are then defined as post-zygotically isolated (inter alia Mayr 1969; Dobzhansky 1970; Butlin 1987).

#### 1.4 General biology of O. irroratus

The biology of O. irroratus is well documented (inter alia Shortridge 1934; Roberts 1951; Davis 1972; De Graaff 1981; Smithers 1983), and has been extensively reviewed by Davis (1973), Willan (1982) and Brown (1988). Therefore, the biology of O. irroratus will not be repeated here, except to highlight features which are essential to the interpretation of data presented in this dissertation and to add new unpublished data to the general body of information on O. irroratus. In addition, major differences are outlined between the populations considered in the present study.

#### 1.4.1 Morphology

O. irroratus is a medium-sized, stockily built rodent, which has a shaggy pelage, blunt face, large, yellow, deeply-grooved incisors, and a short well-haired tail; the ears are rounded and well-haired (Roberts 1951; De Graaff 1981; Smithers 1983). These authors maintain that the coat colour of the vlei rat is essentially buffy-brown dorsally, with the throat, cheeks, sides and ventral parts paler. Of the populations considered in the present study, this description accurately describes Hogsback and Karkloof individuals, but animals from Committee's Drift have the pelage and tail more or less ashy-grey dorsally, the ventral parts dull white, and conspicuous bands of paler grey hairs above and below the eyes (Pers. obs.).

The following morphological features of O. irroratus are documented in the literature: Davis (1973) described the complex phallus and baculum; 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; Kearney 1990).

#### 1.4.2 Distribution

O. irroratus occurs widely on the southern savanna highveld, coastal montane and submontane grasslands, and in Cape Macchia (Davis 1974). It extends from the S.W. Cape through Natal, Lesotho and the Orange Free State to the Transvaal, and an isolated population exists in eastern Zimbabwe and the adjacent parts of Mozambique (De Graaff 1981; Smithers 1983; Figure 1.1). It is largely absent from the S.W. Arid and Namib biotic zones. In Figure 1.1, the Committee's Drift, Hogsback and Karkloof localities are shown.

O. irroratus inhabits 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; Smithers 1983). However, it is not restricted to such areas and may occur some distance from surface water on steep slopes in montane grasslands (Davis 1973) and Cape Macchia (Willan & Bigalke 1982). It is also known to inhabit areas where the rainfall is comparatively low and unpredictable, as at Committee's Drift (Table 1.1).

#### 1.4.3 Behaviour and habits

O. irroratus usually nests above ground under cover of dense vegetation, but in areas with poor cover it may



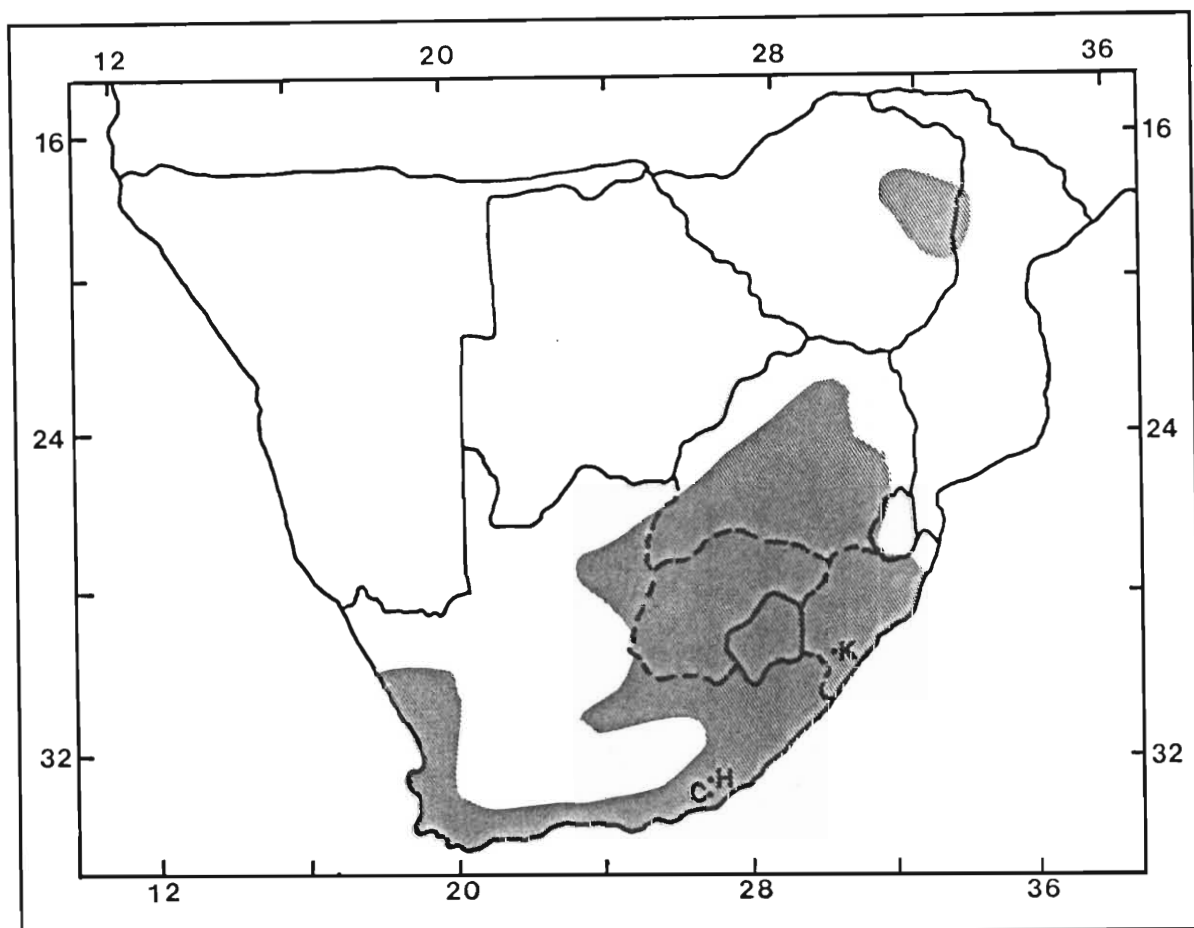


Figure 1.1. Southern African distribution of *O. irroratus* (after De Graaff 1981; Smithers 1983), indicating the position of the Committee's Drift (C), Hogsback (H) and Karkloof (K) localities.

utilize the abandoned burrows of other small mammals (Roberts 1951; De Graaff 1981; Smithers 1983); at Committee's Drift, individuals nested under piles of cut reeds (K. Willan, Pers. comm.). The species is mainly crepuscular, with some activity during both day and night (Davis 1972; Perrin 1981). The vlei rat is a strict herbivore (Davis 1973; Perrin & Curtis 1980), with a diet in nature consisting almost exclusively of grass leaves and stems, as well as herbs. O. irroratus exhibits a dispersed (asocial) social structure, incorporating temporal territoriality and adult isolation (Davis 1973; Willan 1982; Brown 1988). O. irroratus on the Transvaal highveld breeds mainly during the rainy season, and the mean litter size of animals here is 2.33 (Davis 1973; Davis & Meester 1981). In contrast, animals in the Fish River Valley (cf. Committee's Drift) are apparently reproductively active all year round, and mean litter size is 1.48 (Perrin 1980).

## CHAPTER 2

### Methods

#### 2.1 Introduction

Procedures which were specific to particular parts of the study are described in the relevant sections of the chapters dealing with these parts. In the present chapter, commonly recurring terms are defined, and procedures common to more than one chapter are outlined.

#### 2.2 Terminology

**MATING COMBINATION.** Any intrapopulation, interpopulation or backcross pairing; backcross pairings refer to mating combinations involving the progeny of interpopulation pairs. The terms "pure combination", "cross combination" and "backcross combination" distinguish between intrapopulation, interpopulation and backcross mating combinations, respectively. Mating combinations are in all cases denoted as male x female (e.g. a pairing of a Hogsback male and a Karkloof female is denoted as Hogsback x Karkloof); the reciprocal cross (i.e. a male from Karkloof paired with a female from Hogsback) is expressed as Karkloof x Hogsback. A cross pairing and its reciprocal cross pairing are collectively referred to as a "reciprocal cross combination". Offspring resulting from a cross pairing are

denoted in square brackets (e.g. animals resulting from a Hogsback x Karkloof cross pairing are denoted as [Hogsback x Karkloof]).

PUREBRED. Unless otherwise stated, the term "purebred" is used collectively to refer to wild caught animals and to offspring resulting from intrapopulation matings.

HYBRID or CROSSBRED. Offspring resulting from interpopulation matings.

INVIABLE. Animals, especially hybrids, were considered inviable if they were unable to survive to adulthood or if their growth and development was slower than that of other members of the population (Dobzhansky & Levene 1951; Mayr 1963).

ADULT or MATURE ANIMALS. Males were deemed to be adult or sexually mature when the testes had descended into the scrotal sac and were of full adult size (Measroch 1954). Females were considered sexually mature when the vaginal orifice opened (perforate; Measroch 1954).

### 2.3 Experimental animals

Animals used in this study were obtained from stocks livetrapped at the localities given in section 1.1 and held

by the Speciation Group, or were the captive born progeny (first to third generation) of parents from these localities.

### 2.3.1 Caging and maintenance

Four air-conditioned rooms in which the environment was partially controlled (20 - 25°C; 60 - 80% rH; and 15L : 9D light regime - fluorescent lighting) were employed in the study. Animals were toe-clipped for identification and held individually in Labotec holding cages 400x250x100 mm, or were paired in glass aquaria 900x300x400 mm. Each aquarium was provided with a single galvanized sheet iron nest box 150x150x150 mm, fitted with a removable lid. Coarse wood shavings were provided as litter, and animals used uneaten plant matter as nesting material. Cages and aquaria were washed and litter was replaced once each week.

Animals were provided with ad libitum coarse grass (mainly Panicum maximum) and other herbaceous plant material, fresh cabbage and carrot, Epol rabbit pellets and water.

### 2.4 Data analysis

Where possible, an attempt was made to apply statistical tests of significance to quantitative data. Kurtosis and

skewness coefficients were calculated initially to ascertain whether the spread of the data departed from normality; kurtosis and skewness coefficients approach a value of zero as the distribution of scores approaches a normal distribution (Sokal & Rohlf 1987). On the basis of the values of these coefficients, non-parametric tests were used in most cases, although parametric tests were employed when possible. Extensive use was made of the Mann-Whitney U test because it is a powerful and convenient test for comparing the means of two sample-sets (Siegel 1956; Sokal & Rohlf 1987); critical values of U were generated in respect of the larger sample when two samples were compared (Sokal & Rohlf 1987).

Single-tailed probabilities are given because, in all aspects of the study, the research hypotheses had direction (Sokal & Rohlf 1987).

## CHAPTER 3

### Breeding Study

#### 3.1 Introduction

On the basis of the concepts outlined in Chapter 1, breeding studies were initiated to ascertain whether (i) geographic variation of breeding patterns existed among the Committee's Drift, Hogsback and Karkloof populations, and (ii) these populations have diverged to the extent where they are reproductively isolated. In addition, it was expected that the breeding trials might reveal the broad nature of the isolating mechanisms, if any, between populations that were reproductively incompatible: lack of breeding success at the interpopulation level would indicate pre-zygotic reproductive isolation, while a decrease in litter size, fecundity and/or hybrid breeding success would indicate post-zygotic reproductive isolation (inter alia Mayr 1969; Dobzhansky 1970; Capanna et al. 1985; Butlin 1987).

In the present study, comparisons were made of breeding performance at both the intrapopulation and interpopulation levels. In addition, the fertility of crossbred progeny was ascertained and their breeding performance was compared with that of purebred animals.

### 3.2 Materials and Methods

Caging and maintenance, and the conditions under which breeding occurred, are described in section 2.3.1. The breeding performance of a total of 21 intrapopulation, 30 interpopulation and 90 backcross male/female pairs was ascertained. With the exception of progeny resulting from interpopulation matings (which were used in backcross breeding attempts), all animals used in breeding trials were known breeders.

Interpopulation breeding trials comprised reciprocal pairings of individuals representing different populations (see section 2.2). Backcross breeding trials involved matings between progeny resulting from interpopulation pairings with (i) individuals from the appropriate parent stocks (purebred animals; see section 2.2), and (ii) progeny resulting from the same cross combination category. In view of the complexity of the resulting variable matrix, the entire matrix for intrapopulation, reciprocal cross and backcross combinations is presented in Table 3.1. In this table, crossbred progeny used in backcross breeding are denoted in square brackets (e.g. [Hogsback x Karkloof]; see section 2.2). Where it was necessary to refer collectively to the five backcross trials involving animals resulting from the same cross combination category, the cross combination is denoted by "<" and ">" symbols (e.g. the five



Table 3.1. Variable matrix of breeding trials for the mating combinations indicated. Committee's = Committee's Drift. Mating combinations are given as male x female. For additional details, see text.

<u>Mating combinations</u>	<u>Replications</u>
<b>Intrapopulation</b>	
Committee's x Committee's	5
Hogsback x Hogsback	6
Karkloof x Karkloof	10
<b>Interpopulation</b>	
Committee's x Hogsback	5
Hogsback x Committee's	5
Committee's x Karkloof	5
Karkloof x Committee's	5
Hogsback x Karkloof	5
Karkloof x Hogsback	5
<b>Backcross</b>	
<Committee's x Hogsback>	
Committee's x [Committee's x Hogsback]	3
Hogsback x [Committee's x Hogsback]	3
[Committee's x Hogsback] x Committee's	3
[Committee's x Hogsback] x Hogsback	3
[Committee's x Hogsback] x [Committee's x Hogsback]	3
<Hogsback x Committee's>	
Committee's x [Hogsback x Committee's]	3
Hogsback x [Hogsback x Committee's]	3
[Hogsback x Committee's] x Hogsback	3
[Hogsback x Committee's] x Committee's	3
[Hogsback x Committee's] x [Hogsback x Committee's]	3
<Committee's x Karkloof>	
Committee's x [Committee's x Karkloof]	3
Karkloof x [Committee's x Karkloof]	3
[Committee's x Karkloof] x Committee's	3
[Committee's x Karkloof] x Karkloof	3
[Committee's x Karkloof] x [Committee's x Karkloof]	3
<Karkloof x Committee's>	
Committee's x [Karkloof x Committee's]	3
Karkloof x [Karkloof x Committee's]	3
[Karkloof x Committee's] x Committee's	3
[Karkloof x Committee's] x Karkloof	3
[Karkloof x Committee's] x [Karkloof x Committee's]	3

Table 3.1. Continued.

<u>Mating combinations</u>	<u>Replications</u>
Backcross	
<Hogsback x Karkloof>	
Hogsback x [Hogsback x Karkloof]	3
Karkloof x [Hogsback x Karkloof]	3
[Hogsback x Karkloof] x Hogsback	3
[Hogsback x Karkloof] x Karkloof	3
[Hogsback x Karkloof] x [Hogsback x Karkloof]	3
<Karkloof x Hogsback>	
Karkloof x [Karkloof x Hogsback]	3
Hogsback x [Karkloof x Hogsback]	3
[Karkloof x Hogsback] x Hogsback	3
[Karkloof x Hogsback] x Karkloof	3
[Karkloof x Hogsback] x [Karkloof x Hogsback]	3

backcross trials comprising the progeny resulting from the Committee's Drift x Hogsback cross combinations are indicated as the <Committee's x Hogsback> backcross combination.

All hybrids (i.e. crossbred animals) were sexually inexperienced when backcrossed, but only sexually mature animals (see section 2.2) were used. In backcross combinations involving progeny representing the same cross combination (e.g. [Hogsback x Karkloof] x [Hogsback x Karkloof]; Table 3.1), sibling pairings were not employed.

Each pair was held together for a total of 150 days or until the birth of the third litter, whichever occurred earlier. However, animals were separated if the female failed to produce a first litter within 80 days of being paired. Upon separation, males were transferred to holding

cages, while females and their unweaned young were retained in the aquaria. Males were used in further breeding trials, as necessary. Females were used in new matings only after the last litter was weaned (see below), or if they were not palpably pregnant.

Animals involved in breeding trials were inspected daily between 08h00 and 10h00, and their general condition was assessed. Animals that engaged in highly aggressive interaction were separated to prevent damaging fights; this was necessary on only two occasions. The date of birth of litters was noted when the neonates were first observed. As all births occurred at night, an uncertainty of up to approximately 14 h existed regarding the time of birth. Subsequent to parturition, the cage bedding was examined carefully for evidence of dead neonates. Post-mortem examination was confined to the external body surface of neonates. Surviving young were allowed to remain with the parents to an age of 20 - 30 days, and thereafter were transferred to holding cages (see section 2.3.1).

The following were recorded for successful breeding pairs: interval to the first litter (i.e. interval between pairing and the production of the first litter); interlitter interval (i.e. interval between successive litters); number of litters produced; litter size; primary sex ratio (i.e. sex ratio at birth); secondary sex ratio (i.e. sex

ratio at weaning); and pre-weaning infant mortality. Unavoidably, the sex ratios of a small number of litters resulting from intrapopulation matings were not recorded, as indicated in section 3.3. Intervals of greater than 60 days between pairing and the first litter, or between litters, were excluded from the data set because they probably represented a period of sustained anoestrus.

The term fecundity conventionally refers to the product of mean litter size and the number of litters born per annum (Willan & Meester 1989). In this study, however, fecundity is defined as the product of mean litter size and the number of litters born per 150 days; to avoid confusion, the term is hereafter used in quotation marks (i.e. "fecundity").

With the exception of pre-weaning mortality frequencies and primary sex ratios, mean values and standard errors were calculated for all parameters (above) for each mating combination. Kurtosis and skewness coefficients (section 2.4) showed that the distribution of values of litter size, interval to the first litter, interlitter interval and "fecundity" departed from normality. Differences in mean values of the above variables between different mating combinations were therefore tested for significance using the non-parametric Mann-Whitney U test (Sokal & Rohlf 1987). Statistical comparisons were made within the categories of intrapopulation matings, interpopulation crosses and

backcrosses. In addition, comparisons were made between intrapopulation matings and the relevant interpopulation crosses and backcrosses.

In order to ascertain levels of similarity among intrapopulation, interpopulation and backcross combinations, multivariate analysis (principal components and cluster analyses; NTSYS-pc; Rohlf 1988) of the following five variables was conducted: number of successful matings expressed as a percentage of total matings attempted; mean litter size; mean interval to the first litter; pre-weaning mortality; and mean "fecundity". Variables which were expressed as percentages were arcsine transformed to preserve the independence of the variance from the mean (Sokal & Rohlf 1987). Only successful mating combinations were considered because zero values recorded for breeding parameters of unsuccessful combinations might have biased the results of the multivariate analyses (Jolliffe 1986).

Principal components analysis was carried out on the correlation matrix derived from the above variables. As different scales were used in respect of the original variables (e.g. arcsine transformations, counts, time intervals), all variables were standardized to give a mean of zero and standard deviation of one. Cluster analysis using the unweighted pair group method with averages (UPGMA; Sneath & Sokal 1973) was performed on the matrix of average taxonomic distances among mating combinations.

### 3.3 Results

Data in respect of intrapopulation, interpopulation and backcross breeding are summarized in Table 3.2; results of the statistical analysis of these data are provided in Table 3.3. There were no significant differences between the reciprocal cross combinations for any of the parameters tested (see Appendix 1), and data for each reciprocal cross combination were thus pooled. Similarly, data for the five backcross trials per backcross combination were combined (e.g. data for the <Karkloof x Hogsback> backcross combination represents pooled values of the five representative backcross trials; see Appendix 1).

All intrapopulation matings were successful (Table 3.2) while only 83.3% of interpopulation cross pairings produced offspring. Backcross breeding showed severely reduced success, with only 23.3% of the pairings producing young. Furthermore, the <Committee's Drift x Hogsback>, <Hogsback x Committee's Drift> and <Karkloof x Hogsback> backcross combinations were entirely unsuccessful (Table 3.2).

The Committee's Drift population had the lowest mean litter size among the pure populations (Table 3.2), and this value differed significantly from the mean litter size of the Karkloof population (Table 3.3). In contrast, mean litter size did not differ significantly within either the

Table 3.2. Reproductive data in respect of the mating combinations indicated. Data for the interpopulation and backcross combinations represent pooled values for reciprocal cross combinations and backcross trials, respectively (see text). Committee's = Committee's Drift; M = male; F = female; n = sample size. 2 S.E. given in brackets.

Mating combinations	Matings		Litter size				Primary sex ratio M : F	Interval between:				Pre-weaning mortality			"Fecundity"		
	attempts	successes	n	$\bar{X}$	range	mode		pairing & first litter	litters		Total young	Number died	%	n	$\bar{X}$		
								n	$\bar{X}$	(days)	n	$\bar{X}$	(days)				
Intrapopulation																	
Committee's Drift	5	5	18	2.06 (0.30)	1-3	2	16 : 21	5	56.20 (3.48)	12	48.42 (1.40)	37	2	5.4	5	9 (4.47)	
Hogsback	6	6	18	2.28 (0.89)	1-4	3	16 : 188	3	48.67 (2.68)	11	47.55 (1.24)	38	3	7.9	6	11 (5.30)	
Karkloof	10	10	24	2.50 (0.26)	1-3	3	32 : 208	7	50.29 (2.22)	11	47.09 (1.30)	60	3	5.0	10	11 (4.91)	
Totals	21	21	60	2.26 (0.10)	1-4	3	64 : 59	15	51.93 (2.24)	34	47.71 (0.76)	135	8	5.9	21	11 (3.75)	
Interpopulation																	
Committee's x Hogsback	10	7	18	2.23 (0.22)	1-3	2	18 : 20	6	59.43 (5.30)	10	46.79 (1.30)	40	9	22.5	7	10 (2.62)	
Committee's x Karkloof	10	9	26	2.27 (0.28)	1-3	2	28 : 31	9	57.56 (3.92)	14	45.57 (1.50)	59	25	42.4	9	14 (3.61)	
Hogsback x Karkloof	10	9	19	2.26 (0.40)	1-4	2	21 : 26	9	51.34 (2.14)	9	46.56 (1.46)	47	3	6.4	9	12 (3.92)	
Totals	30	25	63	2.25 (0.18)	1-4	2	67 : 77	24	56.13 (2.50)	33	47.45 (0.84)	146	37	25.3	25	12 (2.05)	
Backcross																	
<Committee's x Hogsback>	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<Hogsback x Committee's>	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<Committee's x Karkloof>	15	10	18	1.06 (0.12)	1-2	1	10 : 9	7	56.43 (2.50)	5	47.20 (2.92)	19	7	36.8	10	2 (0.59)	
<Karkloof x Committee's>	15	8	15	1.00	-	1	5 : 10	3	56.00 (3.10)	5	47.80 (1.86)	15	3	20.0	8	2 (0.25)	
<Hogsback x Karkloof>	15	3	5	1.20 (0.40)	1-2	1	4 : 2	2	57.50 (5.00)	2	45.00 (4.00)	6	1	16.7	3	2 (1.33)	
<Karkloof x Hogsback>	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Totals	90	21	38	1.05 (0.08)	1-2	1	19 : 21	12	56.50 (1.64)	12	47.08 (1.52)	40	10	25.0	21	2 (0.34)	

8 = available data.

Table 3.3. Statistical comparison (Mann-Whitney U test) of mean values of the reproductive parameters of the mating combinations indicated. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof; s = Mann-Whitney statistic (U used where  $n_1$  &  $n_2 \leq 20$ , and z used where  $n_1$  or  $n_2 > 20$ ). P given where the level of significance of U or z was less than 5%. Sample sizes ( $n_1$  &  $n_2$ ) as in Table 3.2.

Mating combinations compared	Mean litter size		Mean interval between pairing & first litter litters				Mean "fecundity"	
	s	P	s	P	s	P	s	P
<b>Intrapopulation</b>								
Committee's Drift vs Hogsback	187		15	< 0.05	89.5		16.5	
Committee's Drift vs Karkloof	295	< 0.05	31.5	< 0.025	93		28	
Hogsback vs Karkloof	249		12.5		64.5		33.5	
<b>Interpopulation</b>								
Comm x Hogs vs Comm x Kar	271		37		81.5		46.5	
Comm x Hogs vs Hogs x Kar	200		55.5	< 0.01	46		37	
Comm x Kar vs Hogs x Kar	262.5		64	= 0.025	72		48.5	
<b>Backcross</b>								
<Comm x Kar> vs <Kar x Comm>	150		11		14		40	
<Comm x Kar> vs <Hogs x Kar>	54.5		8.5		7		19.5	
<Kar x Comm> vs <Hogs x Kar>	45		4		8		16.5	
<b>Intrapopulation &amp; interpopulation</b>								
Committee's Drift vs Comm x Hogs	168.5		22.5		89		17.5	
Committee's Drift vs Comm x Kar	275.5		25		115		33.5	
Hogsback vs Comm x Hogs	183.5		21	< 0.005	67.5		28	
Hogsback vs Hogs x Kar	173		19		60		37.5	
Karkloof vs Comm x Kar	367		53.5	< 0.025	77		55.5	
Karkloof vs Hogs x Kar	267		36.5		56		52.5	
<b>Intrapopulation &amp; backcross</b>								
Committee's Drift vs <Comm x Kar>	306.5	<0.001	19		39		45.5	< 0.01
Committee's Drift vs <Kar x Comm>	247.5	<0.001	8		36		36.5	< 0.01
Hogsback vs <Hogs x Kar>	75	<0.025	6		16.5		12	
Karkloof vs <Comm x Kar>	432	<0.001	45.5	< 0.005	27.5		90.5	< 0.001
Karkloof vs <Kar x Comm>	345	<0.001	20	= 0.025	32		73	< 0.005
Karkloof vs <Hogs x Kar>	87	<0.001	13.5		16		26.5	< 0.05



interpopulation cross or backcross categories. With the exception of the Committee's Drift pure pairs, all statistics describing litter size (i.e. mean, range and mode) separated well into three broad subsets, decreasing from intrapopulation to interpopulation to backcross pairings (Table 3.2). In addition, the difference in mean litter size between all backcross combinations and the appropriate pure populations were significant (Table 3.3).

The primary sex ratio (sex ratio at birth) of litters resulting from all mating combinations never differed significantly from unity. However, the secondary sex ratio (i.e. sex ratio at weaning) of 10 : 24 of the Committee's Drift x Karkloof crosses showed that significantly more females than males survived beyond weaning ( $\chi^2 = 5.76$ ;  $p < 0.025$ ). No significant difference in the secondary sex ratio was evident in litters resulting from other mating combinations.

Committee's Drift pure pairs took significantly longer to produce the first litter than did other pure pairs. Among the cross combinations, pairings involving Hogsback and Karkloof individuals produced the first litter significantly sooner than did the other cross pairings (Table 3.3). The interval between pairing and the birth of the first litter showed a general increase from pure to cross to backcross pairings, but only two of each of the cross and backcross

pairings differed significantly from the relevant pure pairings (Table 3.3). Although differences in mean values of the interval to the first litter between the <Hogsback x Karkloof> backcross combination and both Hogsback and Karkloof pure pairings were large, the computed critical values of U were not significant. This may be attributable to the small sample sizes of the backcross combinations (Table 3.2). Mean interlitter intervals were similar among all mating combinations (Table 3.2), and there were no significant differences in respect of this data set (Table 3.3).

Frequencies of pre-weaning mortality increased considerably from purebred offspring to crossbred and backcross progeny, and the highest mortalities were recorded for the [Committee's Drift x Karkloof] crossbred offspring and progeny resulting from the <Committee's Drift x Karkloof> backcross pairings (i.e. 42.4% and 36.8%, respectively; Table 3.2). The [Hogsback x Karkloof] crosses once again proved the exception, with mortalities lower than those of any of the other crosses or backcrosses (Table 3.2). None of the young that died during the study were wounded, and infanticide was excluded as a cause of death. Because post-mortem examination was restricted to the external body surface of neonates, any pathological causes of mortality were undetected.

"Fecundity" (i.e. the number of young produced per 150 days) was similar in the pure and cross combination categories. It is of interest that while the Committee's Drift x Karkloof pairs had the highest "fecundity", they also had the highest pre-weaning mortality of young (Table 3.2). The "fecundity" of backcross pairings was low, and mean values differed significantly in almost all cases from means for the pure pairings (Table 3.3). Although mean "fecundity" of the Hogsback pure pairs was considerably greater than that of the <Hogsback x Karkloof> backcross pairs, the critical value of U was not significant, possibly because of the small sample sizes for each mating combination (Table 3.2).

Eigenvector loadings for the first three principal components of the principal components analysis are given in Table 3.4. Whereas the first two principal components accounted for 93.2% of the total variance (i.e. 75.1% and 18.1%, respectively), the third component accounted for only an additional 5.9% of the variance, and the scattergrams of the first and third, as well as the second and third, principal components revealed no biologically meaningful trends within the data tested. Consequently, only the first two principal components were considered in the analysis.

Table 3.4. Eigenvector matrix of a five-variable principal components analysis of intrapopulation, interpopulation and backcross breeding involving animals from Committee's Drift, Hogsback and Karkloof.

Variables	Principal components		
	I	II	III
Number of successful matings %	0.891	-0.430	-0.124
Number of attempts			
Mean litter size	0.683	0.455	-0.284
Mean interval to the first litter	-0.870	0.670	0.160
Pre-weaning mortality	-0.959	-0.229	-0.080
Mean "fecundity"	0.903	0.113	0.406

High eigenvector loadings represent variables that contribute strongly towards a particular principal component (Pimentel 1979), and variation in the first principal component is therefore largely explained by an inverse relationship between pre-weaning mortality and mean "fecundity" (i.e. eigenvector values of -0.959 and 0.903, respectively; Table 3.4). The inverse relationship between the percentage of successful matings and the mean interval to the first litter were largely responsible for the variation in the second principal component (i.e. eigenvector loadings of -0.430 and 0.670, respectively; Table 3.4). It would therefore appear that while variation in the first principal component was due to post-zygotic factors (pre-weaning mortality and mean "fecundity"), variation in the second principal component may be explained by pre-zygotic factors (percentage successful matings and the interval to the first litter).

The scattergram of the first and second components for each successful mating combination is given in Figure 3.1, and generally confirms the above interpretation of the data presented in Table 3.2. The first principal component separated the mating combinations into three subsets mainly on the basis of post-zygotic factors (above). The first subset consisted of the <Hogsback x Karkloof> backcross combination, while the second subset revealed a grouping of Committee's Drift x Hogsback and Committee's Drift x Karkloof cross combinations, and <Committee's Drift x Hogsback> and <Committee's Drift x Karkloof> backcross combinations. The third subset was slightly removed from the two other groups, comprising all pure combinations plus the Hogsback x Karkloof cross combination. Backcross, cross and pure combinations separated from top to bottom in the second principal component, but overlap between the <Committee's Drift x Karkloof> backcross combination and all cross combinations is evident; these groups separated according to pre-zygotic components (above).

The position of the <Hogsback x Karkloof> backcross combination as an outlier (Jolliffe 1986) in the second principal component is associated with the extremely low breeding success of this backcross combination (i.e. 20%; Table 3.2). Removal of the outlier from the data set had no substantial influence on the overall spread of the

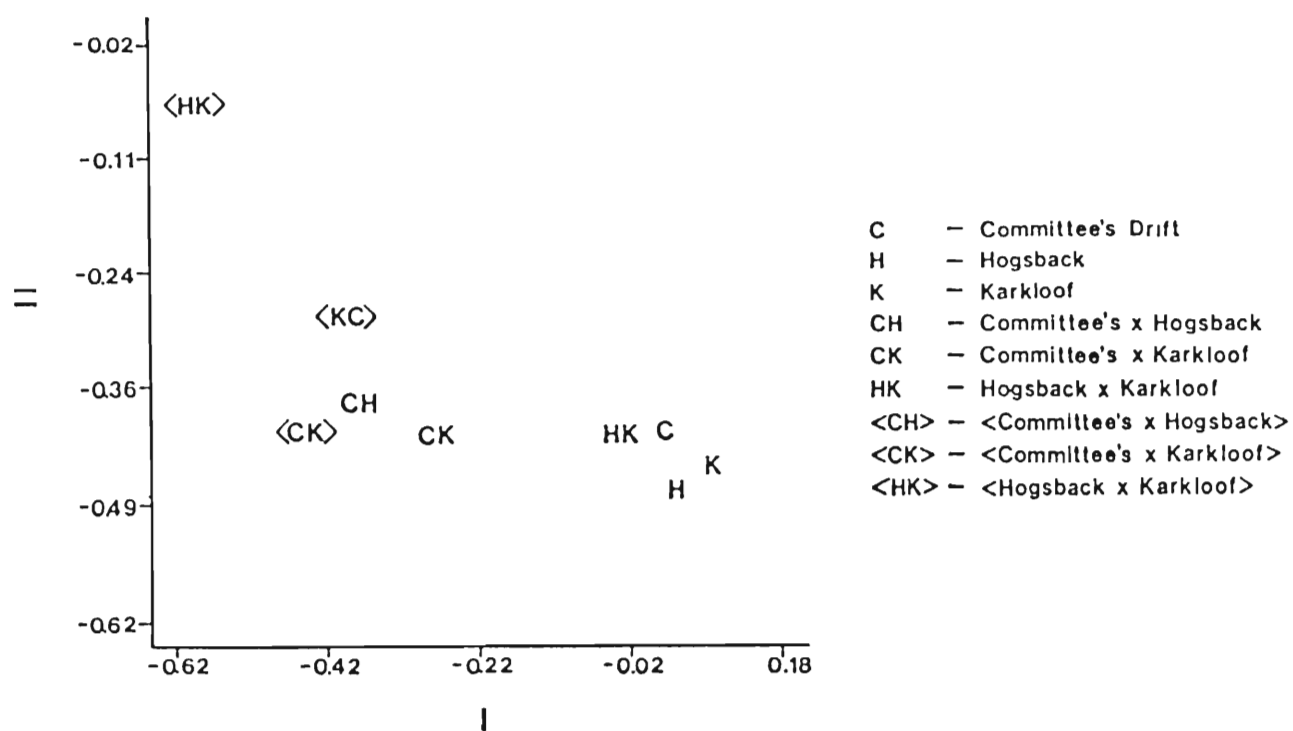


Figure 3.1. Scattergram of the first two components (I and II) of a five-variable principal components analysis of the breeding performance of the mating combinations indicated. Committee's = Committee's Drift.

scattergram, and similar groupings were maintained among the mating combinations (Figure 3.2). Therefore, following Jolliffe (1986), the <Hogsback x Karkloof> backcross combination was retained in the principal components analysis.

The cluster analysis based on the average taxonomic distance between successful mating combinations is illustrated in Figure 3.3. The coefficient of cophenetic correlation was 0.932, indicating a good correlation between the phenogram and the original matrix (Sneath & Sokal 1973). The mating combinations in the phenogram are divided into two major clusters at a distance of 0.52. The first cluster comprises the three pure combinations, together with the Hogsback x Karkloof cross combination. The second cluster consists of cross and backcross combinations, and no clear separation between these combinations within the cluster is evident.

Two important features are apparent in the cluster analysis (Figure 3.3). First, the Hogsback and Karkloof pure combinations grouped together, while the Committee's Drift pure combination is slightly removed from these two combinations at a distance of 0.08. Second, the Hogsback x Karkloof cross combination grouped with the pure combinations, while the <Hogsback x Karkloof> backcross combination is greatly removed from these two mating

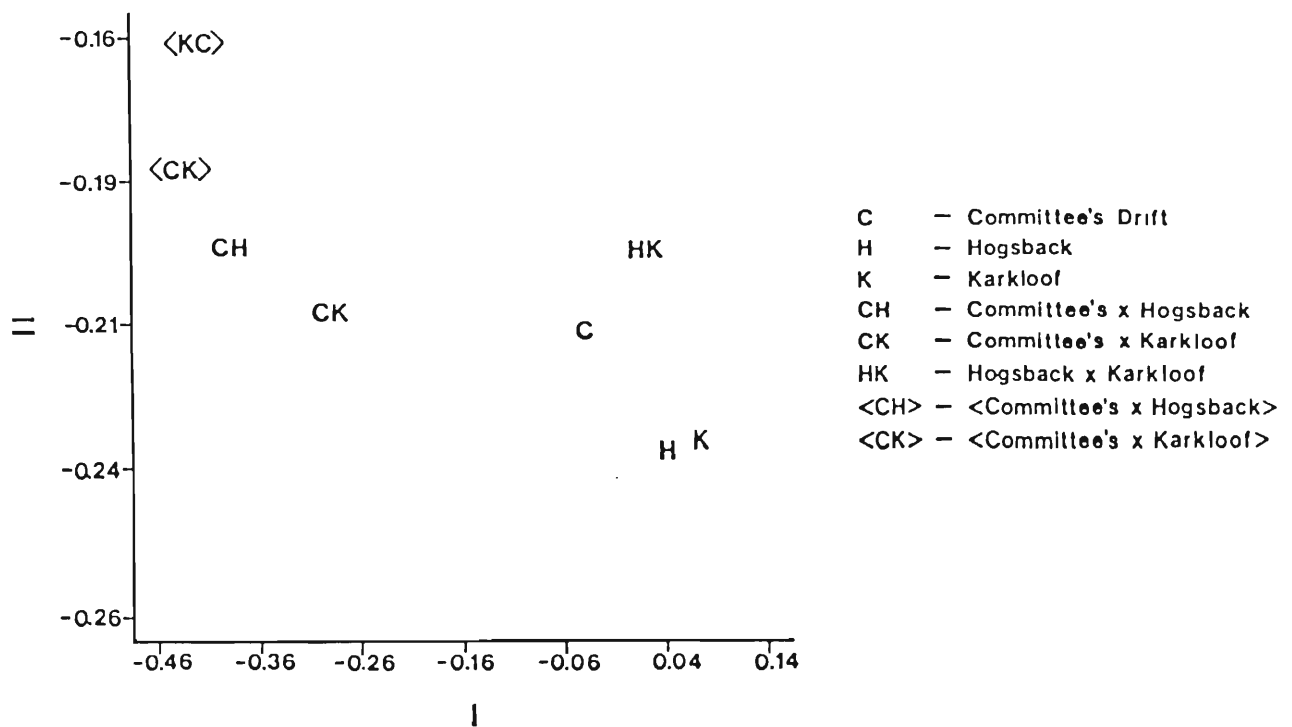


Figure 3.2. Scattergram of the first two components (I and II) of the principal components analysis of the breeding performance of the mating combinations indicated in Figure 3.1 excluding the <Hogsback x Karkloof> backcross combination. Committee's = Committee's Drift.



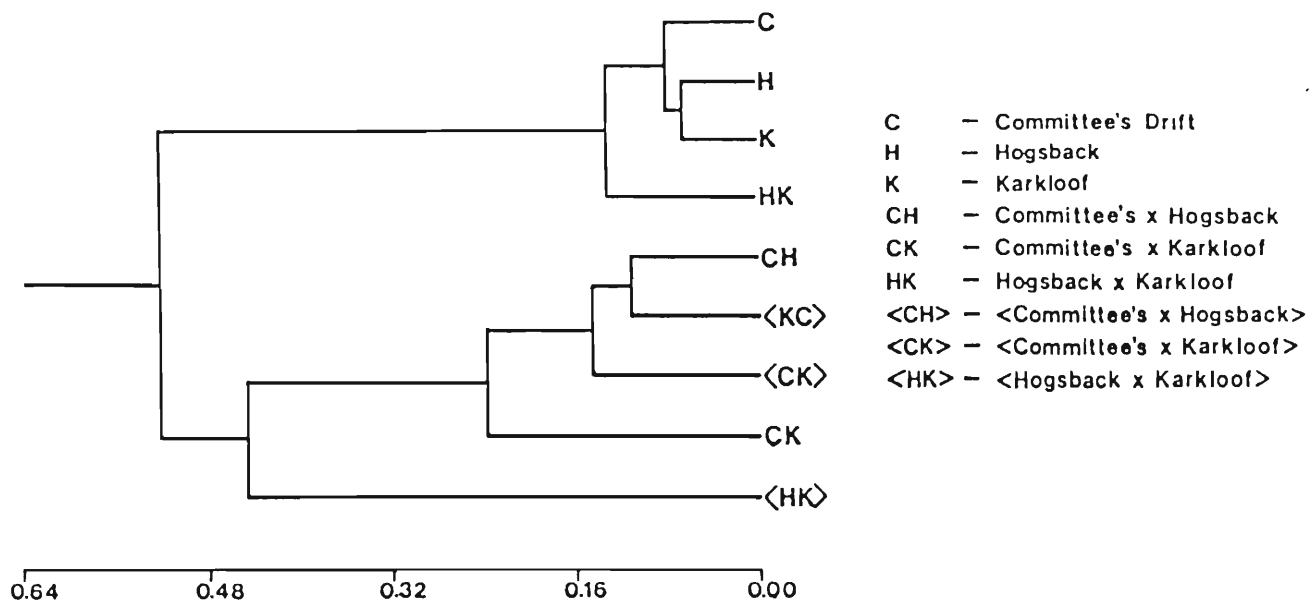


Figure 3.3. Distance phenogram of a five-variable cluster analysis (UPGMA) of the breeding performance of the mating combinations indicated. Cophenetic correlation coefficient = 0.932. Committee's = Committee's Drift.

combinations at a distance of 0.52, and is separated from all other combinations at a distance of approximately 0.44. Both these features reflect the breeding performances of these mating combinations, as outlined above.

### 3.3.1 Synopsis

In view of the complexity of the data set presented, a brief overview of the salient features of the results given in section 3.3 is presented below.

**Intrapopulation matings.** All pure pairings produced young, and the Hogsback and Karkloof populations displayed similar breeding patterns. Committee's Drift pairings displayed the lowest mean litter size and took longer to produce the first litter than did the other pure pairings.

**Interpopulation matings.** The breeding performance of the Committee's Drift x Hogsback cross combinations was similar to that of the respective pure pairings, but pre-weaning mortality was higher in the crosses. The Committee's Drift x Karkloof cross pairings, which were the most "fecund" of all cross pairings, also exhibited the highest pre-weaning mortality, especially with regard to males. In both the Committee's Drift x Hogsback and Committee's Drift x Karkloof cross combinations, the interval to the first litter was greater than in the

Hogsback and Karkloof pure pairings. Although the breeding success of the Hogsback x Karkloof cross combination was lower than that of the other cross pairings, their breeding performance was similar to that of the relevant pure pairings.

Backcrosses. All backcross breeding attempts involving progeny resulting from cross pairings which included Hogsback animals were markedly impaired, with only the <Hogsback x Karkloof> backcross pairings producing young. Progeny resulting from Committee's Drift x Karkloof cross pairings were more successful during backcross breeding trials, although the breeding performance of these backcross pairings was diminished. Successful backcross combinations performed significantly less well than pure pairings in respect of almost all parameters tested.

### 3.4 Discussion

On the basis of the parameters considered, the breeding performance of the Hogsback and Karkloof pure populations was similar, while that of the Committee's Drift pure population differed in some important respects from the others. In particular, the lower mean litter size of Committee's Drift females ( $\bar{X} = 2.06$ ) relative to Hogsback ( $\bar{X} = 2.28$ ) and Karkloof ( $\bar{X} = 2.50$ ) may possibly be explained in terms of environmental parameters. In contrast to the

precipitation regime at Hogsback (1174 mm p.a.) and Karkloof (906 mm p.a.), rainfall in the Committee's Drift area is low (401 mm p.a.; see section 1.1) and unpredictable (Perrin 1980). Thus, the food supply of a specialist herbivore such as O. irroratus (Davis 1973; Perrin & Curtis 1980) must also be unpredictable at Committee's Drift; field observations indicate that this is the case (K. Willan Pers. comm.). Therefore, compared to the Hogsback and Karkloof populations, which clearly fall into the typically mesophilic and resource-stable category of O. irroratus described by Willan & Meester (1989), the Committee's Drift population is most likely to be intermittently resource-limited. Committee's Drift O. irroratus appear to be reproductively adapted to the relatively harsh environment in two ways: they breed all year round (Perrin 1980), which does not occur in other populations (see Willan & Meester 1989); and they have a reduced litter size. The smaller litter size of the Committee's Drift population appears to be highly adaptive. This is because the unpredictable food supply, together with the inability of O. irroratus to rear many young (i.e. only two pairs of nipples are present; De Graaff 1981), inhibits the 'boom and bust' reproductive strategy which is usually associated with animals in unpredictable environments. Furthermore, a smaller litter would place lower energetic demands on the mother (see Millar 1977; McClure 1987; Millar 1987), so that the foetuses would be more likely to survive poor maternal

feeding conditions than would a larger litter. Consequently, the reduced litter size of Committee's Drift females may, in effect, represent an increase in their reproductive potential and inclusive fitness.

Although the number of cross combinations producing young was generally lower in comparison with the intrapopulation matings, sample sizes were too small to draw definite conclusions concerning reduced fertility at this level. The higher "fecundity" of Committee's Drift x Karkloof interpopulation pairings relative to the intrapopulation matings indicates that reproductive output increases when animals from Committee's Drift and Karkloof are mated. Although occurrences of increased fitness as a result of cross-mating are documented for other species, the reasons for this phenomenon are not clear (Godfrey 1958; Baker et al. 1983; Patton & Sherwood 1983).

The high pre-weaning mortality of [Committee's Drift x Hogsback] and [Committee's Drift x Karkloof] crossbred young suggests that at least some of these hybrids were inviable (see section 2.2). Higher pre-weaning mortality was probably due to differences in the configuration of the genes and/or chromosomes of the crossbred offspring, as reported for species of Drosophila (Dobzhansky & Levene 1951; Dobzhansky et al. 1968; Patton et al. 1980). Co-adapted gene or chromosome complexes which may become

disrupted during hybridization can lead to biochemical, physiological and behavioural breakdown in the hybrids, usually resulting in their death (Dobzhansky & Levene 1951). Harper (1981) maintained that potentially maladaptive behavioural responses prior to weaning may cause the mother to reject offspring. This may also have contributed to the high pre-weaning mortality of crossbred young in the present study.

The breeding performance of the Committee's Drift x Karkloof cross combination was intriguing for two reasons. First, these crosses possessed the highest "fecundity", but on the other hand, had the highest pre-weaning mortality. This dualism suggests that while certain gene combinations increase reproductive capacity, other gene combinations cause a reduction of fitness. Second, a significant departure from parity of the secondary sex ratio (10 : 24) was evident in litters resulting from these cross pairings. Trivers (1974) suggests that, under certain stressful circumstances (which are usually environmentally determined), siblings that potentially reduce the inclusive fitness of the parent may be abandoned or cannibalized. In such situations, moreover, it is usually male offspring, which tend to be larger than females and thus make greater metabolic demands on the mother, that are sacrificed. However, the conditions under which animals were kept during the present study were apparently optimal for breeding (see

section 2.3.1), while no differences in pre-weaning postnatal growth were observed between the sexes of the progeny of Committee's Drift x Karkloof cross pairings (see chapter 5). Unless other undetected stress factors were operative in the study (e.g. psychological stress; Harper 1981), it seems more likely that, in the present study, increased male mortality was due to sex-linked genetic (Weir 1955) and/or chromosomal (Sturtevant & Dobzhansky 1936; Hanks 1965) factors.

Taking into account data for O. irroratus representing several different localities, the period between pairing and the production of the first litter is usually less than 52 days ( $n > 150$  litters; K. Willan Unpubl.). Given that the gestation period of the species is about 40 days (Davis & Meester 1981; Willan & Meester 1989), it appears that animals are normally involved in courtship during the first 12 days after pairing (i.e. the pre-copulatory phase; Burley 1980). It is therefore tempting to speculate that prolonged intervals to the first litter of the Committee's Drift pure pairings, as well as the Committee's Drift x Hogsback and Committee's Drift x Karkloof cross combinations, may have been the result of delayed recognition by either or both sexes of the courtship behaviour of the other individual during the pre-copulatory phase. This issue is pursued in Chapter 4.

Whereas the interval to the first litter was protracted in the case of some of the above-mentioned mating combinations, interlitter intervals for these combinations remained more or less constant. This suggests that although initial recognition between members of a pair was delayed, they formed essentially amicable associations (Willan 1982; Brown 1988) after the courtship period, and that mating usually occurred during the first postpartum oestrous.

The severely impaired backcross breeding success of progeny resulting from cross pairings involving Hogsback animals may be explained by chromosomal imbalances in these hybrids. Contrafatto et al. (In press) have shown that a tandem fusion exists between chromosomes seven and 12 of the Hogsback karyotype. It is known that cross pairings in which one of the parents has a tandem fusion produce offspring that show reduced fertility (Moritz 1986). Moritz demonstrated that, in certain circumstances (depending on the relationship between the centromere position and chiasma formation) following a tandem fusion, only 25% of the gametes produced by hybrids are normal. Without supporting evidence that gamete viability of hybrids was reduced in the present study, it is uncertain whether chromosomal rearrangements or other factors were responsible for the lack of backcross breeding success. The literature indicates that other factors, such as modification of the reproductive anatomy (e.g. glans penis morphology; inter



alia Mayr 1969; Gordon 1984) and/or aberrant mating behaviour (inter alia Spieth 1958; Dobzhansky et al. 1968; Ahearn 1980; Koepfer 1987) of hybrids, may also inhibit backcross breeding.

Attempts at backcross breeding involving progeny resulting from the Committee's Drift x Karkloof cross pairings (i.e. <Committee's Drift x Karkloof> and <Karkloof x Committee's Drift> backcross combinations) were far more successful than the other backcross pairings. However, it is apparent that overall reproductive fitness of <Committee's Drift x Karkloof> and <Karkloof x Committee's Drift> backcross combinations was reduced, as they had lower litter sizes, increased intervals to the first litter, increased pre-weaning mortality of young and reduced "fecundity". Reduced fitness of the <Committee's Drift x Karkloof> and <Karkloof x Committee's Drift> backcross combinations presumably occurred for similar reasons to those invoked above to explain reduction of fitness of the Committee's Drift x Karkloof and Committee's Drift x Hogsback cross combinations.

On the basis of their breeding performance, it would appear that the Hogsback and Karkloof O. irroratus populations are genetically closer than either is to the Committee's Drift population. This conclusion is supported by the fact that the breeding performance of the Hogsback x

Karkloof cross pairings was similar to that of the pure pairings (Figures 3.1 and 3.3). In contrast, the breeding performance of the Committee's Drift x Hogsback and Committee's Drift x Karkloof cross pairings differed from the remaining pure and cross pairings.

Backcross breeding attempts involving [Committee's Drift x Hogsback], [Hogsback x Committee's Drift] and [Hogsback x Karkloof] crossbred animals were entirely unsuccessful, suggesting that these animals were sterile. Although the [Karkloof x Hogsback], [Committee's Drift x Karkloof] and [Karkloof x Committee's Drift] crossbred animals produced young during backcross trials, their success was limited, indicating that some of these hybrids were also sterile.

The ability to cross-breed in captivity need not imply reproductive continuity between free-living populations (Gordon 1984). Equally, the inability to breed in the laboratory is not indicative of reproductive isolation under natural conditions (Rubinoff & Rubinoff 1971). The results obtained in this study suggest, however, that differences in the genetic and/or chromosomal composition of the Committee's Drift, Hogsback and Karkloof O. irroratus populations (see section 1.1) results in reduced reproductive success among them. The presence of the tandem fusion in the Hogsback karyotype is particularly

significant, because crossbred progeny resulting from cross-matings involving Hogsback animals were sterile. This suggests that the tandem fusion is potentially important in the breakdown of reproduction, and that Hogsback *O. irroratus* may be an incipient sibling species, as defined by Meester (1988).

## CHAPTER 4.

### Pre-copulatory Behaviour

#### 4.1 Introduction

The term "pre-copulatory behaviour" (rather than "courtship behaviour") is used here because courtship usually refers to the interaction of pairs immediately prior to copulation (Tinbergen 1954; Lovecky et al. 1979). Pre-copulatory behaviour, in contrast, refers to all social interaction prior to mating, and may include courtship behaviour (Bekoff & Diamond 1976; Burley 1980).

The need for mutual recognition as potential mates by males and females of the same species is essential to the maintenance of species continuity; it is equally important that individuals recognize members of other closely related species as non-mates. Recognition may be achieved during courtship. The courtship behaviour of animals of the same species therefore depends upon male/female communication systems composed of species-specific signals and responses, which has been referred to as the specific-mate-recognition system (SMRS; Paterson 1978, 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 pre-zygotic barriers, preventing mating between animals of different populations; such species are then sexually/behaviourally isolated (Mayr 1969; Dobzhansky 1970). The biological function of behavioural isolation between allopatric populations is uncertain, because selection does not operate for recognition/isolation in allopatry (Paterson 1980, 1985). Allopatric populations may, nevertheless, be behaviourally isolated should they become syntopic. This is because modification of the courtship behaviour of allopatric populations may occur (i) as a response to local environmental differences (Paterson 1980, 1985; Verrel 1988), (ii) because of random genetic effects (Rubinoff & Rubinoff 1971), or (iii) as a result of the pleiotropic effects of genes (Muller 1939; Dobzhansky et al. 1968). In addition, Butlin (1987) suggests that selection may enhance (by reinforcement) or replace (by reproductive character displacement) previously developed post-zygotic differences.

In the present study, the interval to the first litter (i.e. interval between pairing and the production of the first litter) was longer in the cross combinations than in the appropriate pure pairings; this was most evident in the results of cross combinations incorporating Committee's Drift animals (see section 3.3). On the basis of these results, it was hypothesized that the observed differences

were due to underlying disparities in population-specific SMRSs. A series of observational studies was therefore undertaken to ascertain whether behavioural differences of animals from the populations under investigation rendered them behaviourally incompatible during cross pairing. If this were so, it would be taken to indicate a measure of pre-zygotic isolation among populations.

#### 4.2 Materials and methods

The pre-copulatory behaviour of 12 intrapopulation and 24 interpopulation male/female pairs (Table 4.1) was studied in neutral arena encounters, defined as a period of time during which a single pair was studied in an observation cage (inter alia Eisenberg 1963, 1967; Happold 1976; Burley 1980; Willan 1982). Direct and video recorded observations were undertaken of the interaction of each pair.

The environmental conditions in the observation room in which the study was conducted are described in section 2.3.1. Observations of "nocturnal" activity were made under incandescent red light. Observation cages 900x900x600 mm consisted of four glass-fronted asbestos enclosures. To facilitate direct observation and video recording of the subjects, enclosures were furnished only with coarse wood shavings. Between encounters, enclosures were washed with water and a 50% ethyl alcohol solution to remove odours of

Table 4.1. Number of male/female neutral arena encounters involving representatives of the Committee's Drift (Committee's), Hogsback and Karkloof populations.

Pairings	Mating combinations		Replications
Intrapopulation	Committee's	x Committee's	4
	Hogsback	x Hogsback	4
	Karkloof	x Karkloof	4
Interpopulation	Committee's	x Hogsback	4
	Hogsback	x Committee's	4
	Committee's	x Karkloof	4
	Karkloof	x Committee's	4
	Hogsback	x Karkloof	4
	Karkloof	x Hogsback	4

the previous occupants. Food and water were provided as in the case of the breeding colony (section 2.3.1). To permit identification of animals during observation, females were marked on the nape of the neck with a spot of white enamel paint (Humbrol).

Video recordings were conducted using a Hitachi KP - 141 CCTV camera unit fitted with an 8 mm F 1.3 wide angle lens. The camera was mounted on tracks on a gantry at a height of 2.1 m, at which position an entire enclosure could be filmed. All four enclosures were aligned under the gantry; using a pulley system, the camera unit could be moved to a selected position over any one of the enclosures. Video recordings were made using a Hitachi VTL - 30ED time-lapse video cassette recorder (1.5 mm tape), and a Hitachi

VM - 1720E video monitor was used to analyze the recordings. No facilities were available for audio recording.

#### 4.2.1 Experimental animals

Subjects were obtained from the breeding colony described in section 2.3. Prior to observations, members of a pair had never met one another in the laboratory. Males were used for a maximum of three encounters, each time in combination with a female from a different population. Females were used only once. To avoid unnecessary disturbance of animals during trials, no attempt was made to follow the oestrous cycle of females used in encounters.

#### 4.2.2 Experimental procedure

Prior to commencing formal observations, a pilot study was undertaken to (i) permit ready recognition of the elements of Otomys irroratus social behaviour described by Davis (1972, 1973) and Willan (1982), and (ii) ascertain periods of greatest activity, to facilitate selection of the most suitable observation periods. During the preliminary study, the interaction and activity of two pairs per locality was observed in enclosures on a more or less ad libitum basis for a total of approximately 40 h per pair.



Each encounter lasted 12 days. Direct observations were conducted for the first hour after pairing (08h30 - 09h30), and for one hour daily (at the same time) for four more days. The 08h30 - 09h30 period occurred at the beginning of the daylight phase of the light cycle (see section 2.3.1), and was the period of maximum diurnal activity. Observation was not continued beyond five days because of the marked decline in diurnal social interaction after this time, as noted by Willan (1982). In contrast, video recordings during the dark phase (00h00 - 01h00; the period of maximum "nocturnal" activity) showed that interaction was at a sufficiently high level to warrant continued sampling for 12 days. Because four encounters (one in each enclosure) were conducted simultaneously and the events in only one enclosure could be filmed at any time (see section 4.2), trials were arranged such that video recordings of each pair were made every fourth day from the day of pairing until the end of the encounter (i.e. day 12; above). This level of "nocturnal" sampling was adequate for making realistic comparisons between mating combinations (see below). Consequently, direct observation and video recording of each encounter jointly accounted for 9 h per pair.

Patterns of social behaviour were classified as agonistic, amicable or sexual, following the definitions of Happold (1976) and Delany & Happold (1979). When difficulty was experienced in separating behavioural patterns, they

were usually classified according to their apparent intent (Delgado 1966). However, some behaviour patterns could not be classified as representing any of the above-mentioned behavioural categories and were classified as "other" behaviour.

The one-zero (1/0) time-sampling method (inter alia Altman 1974) was employed in the study, using 10-second time intervals. This entailed scoring the occurrence or non-occurrence (rather than the absolute frequency) of different behavioural activities during successive 10-second periods directly on data-sheets. Although the 1/0 method has been criticized on the grounds of possibly biasing data in favour of behaviours of short duration (see Altman 1974; Dunbar 1976; Simpson & Simpson 1977), its use has been fully justified in earlier studies on the social behaviour of O. irroratus (Willan 1982; Brown 1988).

The percentage of all scores for agonistic, amicable, sexual and "other" behaviour was calculated for each pair, and mean percentages of the various categories of interaction were calculated. Results obtained from direct and video recorded observations were treated separately because of the different time scales involved (i.e. five days and 12 days, respectively). Data for the direct observations departed from normality (kurtosis and skewness coefficients; see section 2.4), and were thus tested for

significance using the Mann-Whitney U test (Sokal & Rohlf 1987). Data obtained from the video study illustrated trends that were sufficiently clear to obviate the need for statistical analysis.

#### 4.3 Results

The following patterns of agonistic behaviour, as described by Davis (1972) and Willan (1982), were observed in all encounters: mutual avoidance; aggressive approaches; chase sequences; defensive threats; upright sparring and fighting; vocalizations; and tail shivering. Agonistic interaction was highly ritualized, thus reducing the incidence of damaging fights (Willan 1982). Amicable behaviour was dominated by huddling and allogrooming. Behaviour classified as "other" included mainly contact behaviour (i.e. investigatory behaviour, generally lacking overtly attracting or repelling elements; Willan 1982).

Sexual behaviour was observed (video recorded) in one Hogsback and one Karkloof pure pairing, and in a single Karkloof x Hogsback cross pairing. The small sample size prevents comparison of sexual interaction between mating combinations, but as *O. irroratus* copulatory behaviour has not been previously described, a summary of the basic motor patterns of the behaviour is provided below.

Like most other rodents (Dewsbury 1975), the male initiated sexual contact, which was mainly naso-nasal. During the initial stages, the female was aggressive, displaying upright sparring and defensive threats. Extensive chase sequences ensued, lasting for 30 - 120 s. When chases ceased, the female permitted naso-anal contact of some 5 - 10 s. This was followed by the female assuming a lordotic position, allowing the male to mount from the rear. The duration of mounting ranged from 4 - 10 s, and was accompanied by the male grasping the flanks of the female with his fore-feet, and clutching the nape of her neck with his teeth. Although rapid pelvic thrusts were observed, it is not known whether intromission and ejaculation were achieved. Following dismounting, both male and female autogroomed the genital region. O. irroratus sexual behaviour could not be classified using Dewsbury's (1972) classification of patterns of mammalian copulatory behaviour because of a lack of information concerning, among other features, the number of intromissions and ejaculations. Therefore, detailed comparison of O. irroratus copulatory behaviour with that of other rodent species is not possible.

Mean percentages of agonistic and amicable interaction of intrapopulation and interpopulation pairings for the first five days of encounters (direct encounters) are provided in Table 4.2, and the results of statistical analysis of these

data in Table 4.3. There were no significant differences between the results for reciprocal cross combinations (see Appendix 2), and data were therefore pooled.

Table 4.2. Mean percentage of agonistic and amicable behaviour recorded by direct observation of the mating combinations indicated. Data for the interpopulation combinations represent pooled values for reciprocal cross pairings; see text. Committee's = Committee's Drift; n = number of observation periods during which social interaction was observed. 2 S.E. given in brackets.

Mating combinations	n	$\bar{X}$ % interaction	
		Agonistic	Amicable
Intrapopulation			
Committee's Drift	18	46.4 (16.83)	47.0 (8.86)
Hogsback	20	26.8 (7.38)	61.6 (4.67)
Karkloof	16	37.4 (9.76)	52.9 (9.60)
Interpopulation			
Committee's x Hogsback	32	55.3 (4.64)	29.9 (5.50)
Committee's x Karkloof	36	59.0 (5.43)	24.1 (4.67)
Hogsback x Karkloof	37	38.3 (5.34)	45.8 (6.87)

There was a gradation in agonistic interaction within the pure pairings: Committee's Drift > Karkloof > Hogsback. The opposite trend was evident in respect of amicable interaction (Table 4.2). Levels of agonistic interaction of the Committee's Drift pure pairs were significantly higher than those of the Hogsback pure pairs (Table 4.3). In addition, Committee's Drift pure pairs displayed almost equal levels of agonistic and amicable interactions during the five days of direct observation, while Hogsback and

Karkloof pairs displayed far more amicability than aggression during this period (Table 4.2).

Table 4.3. Statistical comparison (Mann-Whitney U test) of mean percentage agonistic and amicable behaviour of the mating combinations indicated. Comm/Committee's = Committee's Drift; Hogs = Hogsback; Kar = Karkloof; s = Mann-Whitney statistic (U used where  $n_1$  &  $n_2 \leq 20$ , and z used where  $n_1$  or  $n_2 > 20$ ). P given where the level of significance of U or z was less than 5%. Sample sizes ( $n_1$  &  $n_2$ ) as in Table 4.2.

Mating combinations compared	Interaction			
	Agonistic		Amicable	
	s	P	s	P
Intrapopulation				
Committee's vs Hogsback	194.5	< 0.05	161	
Committee's vs Karkloof	167		153	
Hogsback vs Karkloof	186		137	
Interpopulation				
Comm x Hogs vs Comm x Kar	485		490	
Comm x Hogs vs Hogs x Kar	702.5	< 0.001	643	< 0.01
Comm x Kar vs Hogs x Kar	703	< 0.001	643	< 0.01
Intrapopulation & interpopulation				
Committee's vs Comm x Hogs	273		324	< 0.025
Committee's vs Comm x Kar	285		344	< 0.01
Hogsback vs Comm x Hogs	374.5	< 0.001	368	= 0.001
Hogsback vs Hogs x Kar	317		298.5	
Karkloof vs Comm x Kar	359	< 0.05	363	= 0.001
Karkloof vs Hogs x Kar	270.5		265.5	

Interpopulation encounters involving Committee's Drift animals revealed almost twice as much aggression as amicability (Table 4.2). Furthermore, mean values of

agonistic and amicable interaction for both the Committee's Drift x Hogsback and Committee's Drift x Karkloof cross combinations differed significantly from the equivalent values for pure pairings other than the Committee's Drift pairs, as well as from the the Hogsback x Karkloof cross pairings (Table 4.3). Like the Hogsback and Karkloof pure pairs, the Hogsback x Karkloof cross combination displayed higher levels of amicable than of agonistic interaction, but this cross combination had the ratio of agonistic to amicable interaction higher than the relevant pure pairings.

Mean percentages of video recorded interaction devoted to agonistic and amicable behaviour are plotted against time in Figure 4.1. Because statistically indistinguishable results were obtained for reciprocal cross pairings (see Appendix 2), data were once again pooled. For every mating combination, percentages of agonistic interaction were highest soon after animals were paired, and none of the pairs immediately displayed amicable interaction (i.e. Day 1; Figure 4.1). It is evident in all cases that levels of agonistic interaction decreased during encounters, while there was a corresponding increase in levels of amicability. The most important feature illustrated in Figure 4.1 is the variation in the time taken to the point of intersection of the curves representing agonistic and amicable interaction. Hogsback pure pairs displayed equal levels of amicable and agonistic interaction sooner than any other pairing

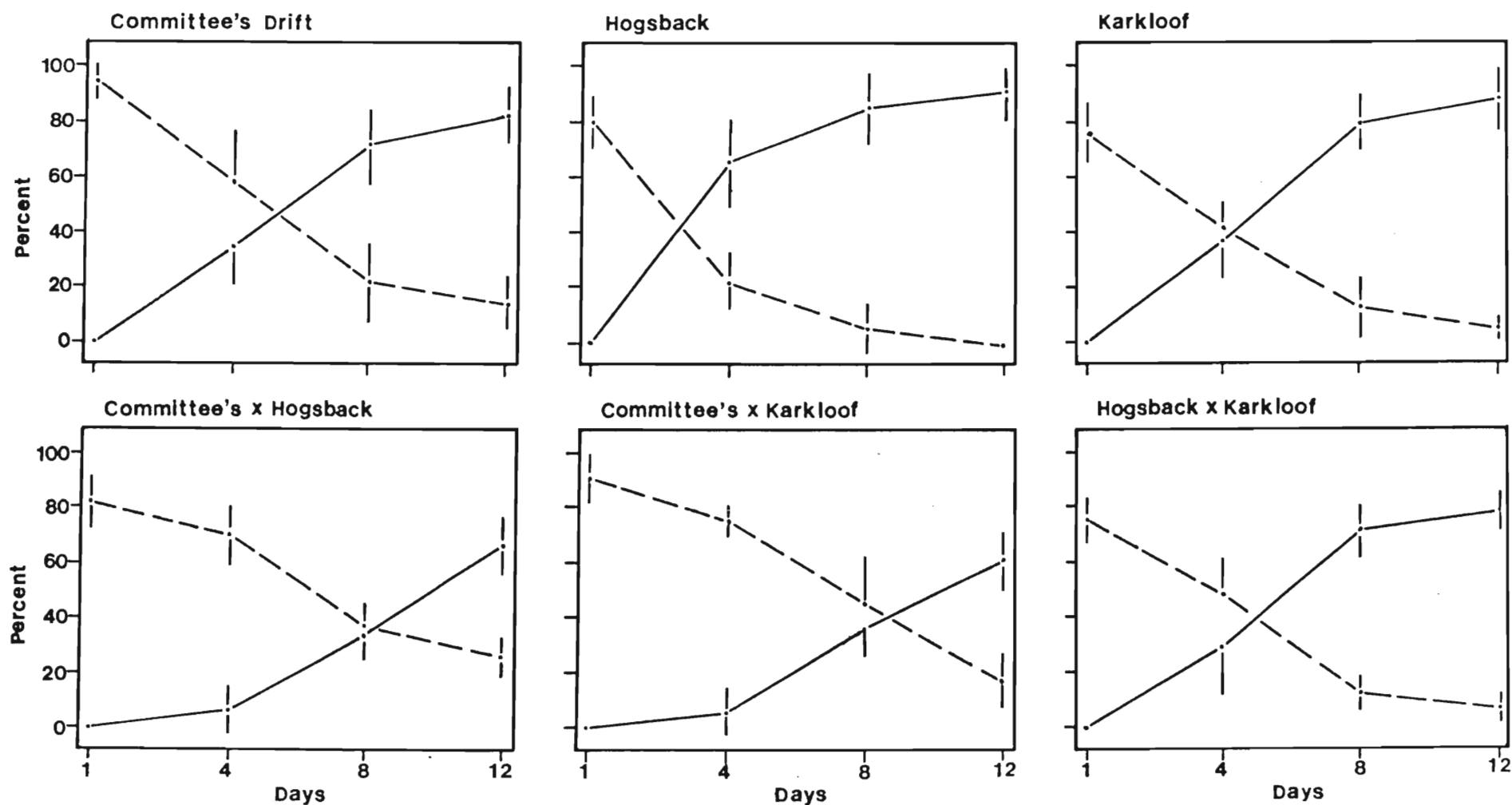


Figure 4.1. Mean percentage video recorded agonistic (---) and amicable (—) interaction for the mating combinations indicated. Data in respect of interpopulation combinations represent pooled values for reciprocal cross pairings. Vertical bars = 2 S.E. above and/or below the mean. Sample size = four each for pure pairings and eight each for cross pairings. Committee's = Committee's Drift.



(i.e. day 3), while the Committee's Drift and Karkloof pure pairs respectively reached this stage at about days 5 and 4. Among the cross combinations, the Hogsback x Karkloof pairs displayed equivalent levels of aggression and amicability at about day 5, and in this respect were similar to the pure pairings. In contrast, the curves of agonistic and amicable interaction of the Committee's Drift x Hogsback and Committee's Drift x Karkloof cross pairings intersected after day 8 (Figure 4.1).

It is also of interest that by day 12 only Hogsback pure pairings no longer displayed any aggression, and that the Committee's Drift x Hogsback and Committee's Drift x Karkloof cross combinations displayed higher levels of agonistic interaction than any other mating combination (Figure 4.1).

#### 4.4 Discussion

##### 4.4.1 O. irroratus mating behaviour

Eisenberg (1963) and other authors have noted that realistic interpretation of laboratory observations on animal behaviour requires understanding of the field biology of the species being studied. In the present study, therefore, interpretation of the pre-copulatory behaviour of O. irroratus necessitates first outlining some relevant

aspects of the social organization of this taxon, as set out in the following paragraph.

Studies at Transvaal highveld (Davis 1972, 1973), 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. 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, and competition within a hierarchical framework occurs among males for mating opportunities.

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 & Happold 1979; White & Fleming 1987), thereby enabling animals to maintain their territories. It follows that agonistic interaction between the sexes would 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 (Parker 1974; Hickman 1982).

On the basis of these concepts, the following interpretation of the results of encounters staged in the present study (Figure 4.1) appears logical. When a male O. irroratus enters a female's territory for the first time (e.g. at the start of the breeding season), the female would regard him as an intruder, resulting in agonistic confrontation between the pair; this situation is exemplified by the initially highly aggressive interaction between pairs on day 1 of all encounters (Figure 4.1). The aggressive responses of free-living pairs would tend to diminish with time, as in the laboratory, with amicable elements eventually superseding aggression. In the field, the development of a male-female social relationship which was conducive to mating would be expected to take longer than in the laboratory.

In comparison with other pairs investigated in this study, it is evident that those which displayed higher

levels of aggression than amicability during the first five days of encounters attained primarily amicable relationships later than others, while maintaining higher levels of aggression to the end of encounters. These pairs therefore required a longer period to overcome aggressive tendencies (Parker 1974; Hickman 1982), suggesting that recognition of the other individual as a potential mate by either or both the male and female was delayed. Possible explanations of this conclusion as regards intrapopulation and interpopulation pairings are provided in sections 4.4.2 and 4.4.3, respectively.

#### 4.4.2 Intrapopulation pairings

In terms of their pre-copulatory behaviour, all pure pairings differed from one another. These differences may be understood as an adaptive response to population-specific ecological circumstances, particularly in respect of the carrying capacity of the habitat. In areas of high carrying capacity and, hence, high population density, females would tend to meet males (i.e. potential mates) comparatively frequently, and selection would be predicted to favour reduced attractiveness of males to females. Males would also occur at high density in such a population, necessitating intense competition for receptive females. Under these circumstances, the most successful males would be those which were most able to rapidly subdue the

aggressive responses of the female (Parker 1974; Hickman 1982), thereby ensuring copulation. Hence, the pre-copulatory behaviour of asocial rodent species may be less aggressive, and the courtship period may be shorter, in areas of higher population density than in areas of lower density. This has been shown in bank voles Clethrionomys glareolus, which display less aggressive and less prolonged courtship at high than at lower population densities (Alder et al. 1981).

The population density of O. irroratus at Hogsback may exceed 80 individuals per hectare during the breeding season (Brown 1988), while visible O. irroratus signs suggest that densities are somewhat lower at Karkloof and extremely low at Committee's Drift (K. Willan, Pers. comm.). Differences in population density may have selected for the contrasting pre-copulatory behaviour observed in the laboratory, with levels of intersexual aggression, as well as the time taken to attain essentially amicable interaction, increasing from Hogsback to Karkloof to Committee's Drift pairs. The observed disparity in time taken to achieve mating (i.e. least in Hogsback and greatest in Committee's Drift pairs) therefore appears to reflect ecological dissimilarities among the populations studied.

#### 4.4.3 Interpopulation pairings

Failure to recognize specific olfactory, auditory and/or visual cues may result in high levels of aggression during encounters between individuals representing different rodent populations, and may lead to delayed recognition between potential mates (inter alia Bauer 1956; Godfrey 1958; Scott 1966; Alder et al. 1981; Nevo 1982).

In terms of their levels of intersexual aggression and the time taken for pairs to attain equivalent levels of aggression and amicability in the present study, the Hogsback x Karkloof pairings were marginally different from the appropriate pure populations. In contrast, Committee's Drift x Hogsback and Committee's Drift x Karkloof cross combinations were distinct from all other mating combinations. The increased aggression of these cross combinations may be directly attributable to contrasting interpopulation pre-copulatory behaviour, and, in particular, differences in the modes of communication of the Committee's Drift, relative to the Hogsback and Karkloof populations.

The importance of odour in the social interaction of O. irroratus is uncertain, and it has been suggested that auditory and visual stimuli may be more important than olfaction in this taxon (Kingdon 1974; Willan 1982).

Communication was not investigated in detail in the present study, and only limited observations were made: no differences were detected in the vocalizations of males and females from the different populations during cross-matings, while postural changes (i.e. visual cues) were similar for all subjects during encounters. Differences doubtless existed which were too subtle for detection, however, and it is therefore impossible to reach more than the general conclusion that olfactory, auditory and visual cues, either singly or in combination, provided the necessary stimuli to render animals from the different populations behaviourally incompatible during interpopulation encounters.

#### 4.4.4 Evolutionary implications

As in the breeding study (chapter 3), the Hogsback and Karkloof populations appear genetically closer in respect of their pre-copulatory behaviour than either was to the Committee's Drift population. These differences and/or similarities are also reflected in encounters of interpopulation pairings which included Committee's Drift animals; the pre-copulatory behaviour of the Committee's Drift x Hogsback and Committee's Drift x Karkloof cross combinations was markedly different from all other mating combinations. It is also noteworthy that, despite the similarity of the pre-copulatory behaviour of the Hogsback and Karkloof pure populations, encounters involving

Hogsback x Karkloof cross pairings differed from the pure pairings. There is no doubt that a measure of pre-zygotic isolation has arisen among the Committee's Drift, Hogsback and Karkloof populations, which would almost certainly lead to reduced mating success should these populations meet under natural conditions. Clearly pre-zygotic isolation is incomplete, but this is usually the case among allopatric populations of a species (Rubinoff & Rubinoff 1971).



## CHAPTER 5

### Postnatal Development

#### 5.1 Introduction

Knowledge of species-specific patterns of postnatal development may be useful in analyzing adaptive variation and evolutionary trends among taxa. For example, ontogenetic trends may be determined by climatic and habitat conditions (Layne 1968; Lackey 1978), while developmental data have been used to deduce phylogenetic relationships among species (Creighton & Strauss 1986). Moreover, gene/chromosome imbalances in crossbred offspring may cause impairment of their growth and development (Dobzhansky & Levene 1951; Dobzhansky et al. 1968). Such imbalance may adversely affect the young when they are older, potentially rendering the hybrids inviable (Godfrey 1958; Lovecky et al. 1979).

The Hogsback and Karkloof environments have many climatic and vegetational similarities, while the Committee's Drift area differs in several important respects from both of these (see section 1.1). The work of Layne (1968) and Lackey (1978) therefore suggested that the postnatal development of Hogsback and Karkloof young would be similar, while that of Committee's Drift young would differ from the other two. The present postnatal

development study was undertaken to test this hypothesis, and to ascertain whether the growth and development of those crossbred offspring which survived beyond weaning was impaired, suggesting that they were inviable (see section 2.2).

The postnatal physical and behavioural development of young O. irroratus has been well documented (Davis & Meester 1981). For this reason, no attempt is made to describe in detail the development of young born during the present study, and only information pertinent to the above objectives is given.

## 5.2 Materials and Methods

Caging and maintenance of animals are described in section 2.3.1.

The physical and behavioural development of a total of 24 purebred and 47 crossbred young resulting from matings comprising individuals representing the Committee's Drift, Hogsback and Karkloof populations was studied.

Litters were examined between 08h00 and 10h00 every second day for the first two weeks of life, and at weekly intervals thereafter to 14 weeks of age. The following standard linear measurements were taken: head-body and tail

length to the nearest millimetre, and hind foot and ear length to the nearest 0.5 mm. The mass of individuals comprising each litter was recorded to the nearest 0.1 g, and mean values were calculated for litters comprising more than one neonate. 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; eruption of the incisors; and the onset of weaning.

The reproductive status of males was assessed from the degree of testicular development: animals were deemed to be sexually mature when the testes had descended into the scrotal sac and were of full adult size (see section 2.2). Females were considered reproductively mature when the vagina became perforate (see section 2.2).

The ontogeny of maintenance and social behaviour was investigated every second day for the first two weeks of life, primarily by observing young in the breeding enclosure (aquarium). Observations were made of the following maintenance behaviour patterns of neonates: co-ordinated quadrupedal locomotion; eating solid food; and termination of nipple-clinging. Observations were also made of the development of patterns of amicable and agonistic behaviour among littermates, and between young and their parents. In

both categories, social interaction and related vocalization were noted. Experimental analysis of behaviour was carried out in a 290x130x130 mm holding cage or on the surface of the laboratory bench, as detailed in Table 5.1.

Table 5.1. Experimental analysis of behavioural development (after Brooks 1972).

Functional units	Typical positive response
Cliff drop aversion	Young moving away from the edge of the laboratory bench.
Grasp reflex	Young grasping a blunt instrument with the fore- and/or hind feet.
Negative geotaxis	Young turning and moving up a slope of 45°.
Righting	Young righting themselves when placed on the back.

In order to ascertain whether differences in growth rates of progeny resulting from the different mating combinations were statistically significant, the mean values at five biologically significant ages (below) of all linear measurements (i.e. head-body, tail, hind foot and ear lengths) and mass were subjected to  $\chi^2$  contingency analysis (Siegel 1956). For each variable, a 9 x 5 contingency table was constructed, where 9 = the number of mating combinations (i.e. three pure and six cross combinations) and 5 = measurements at birth (day 0), weaning (two weeks), sexual maturity of females (seven weeks) and males (10 weeks), plus values at the termination of the study (14 weeks). Values at the modal age at weaning and sexual maturity were used in these analyses.

### 5.3 Results

The timing of the onset or termination of the physical growth and behavioural developmental parameters considered here is given in Table 5.2. Except as regards age at sexual maturity, the development of males and females resulting from the same mating combination was not materially different, and no distinction is made between the sexes.

The developmental phenomena observed in the present study were closely similar to those described for O. irroratus from the Transvaal highveld (Davis & Meester 1981). Moreover, the development of young resulting from all mating combinations investigated in the present study was indistinguishable (Table 5.2).

Young resembled "miniature adults" (Davis & Meester 1981, p. 108) at birth, and they were fully furred, although the pelage was finer, fluffier and paler than that of the adults. In most cases, the eyes were open on day 0, and there were weak responses to sound and smell; neonates which did not have these senses functional at birth attained them by day 2 (Table 5.2). In addition, the incisors were in all cases erupted at birth and projected about 1 mm through the gumline, enabling young to nipple-cling (Davis 1973).

Table 5.2. Timing of the onset or termination (nipple-clinging) of postnatal developmental parameters for progeny resulting from the mating combinations indicated. With the exception of sexual maturity which is expressed in weeks, all other values represent days after birth. Committee's = Committee's Drift; M = male; F = female; n = number of young studied.

		Physical development								Maintenance behaviour				Social behaviour		Nipple-
Mating combinations	n	Eyes	Hearing	Olfaction	Weaning	Sexual maturity		Coordinated locomotion	Solid food	Cliff drop aversion	Grasp reflex	Negative geotaxis	Righting	Amicable	Agonistic	clinging
		open				M	F									
trapopulation																
Committee's Drift	10	0-2	0-2	0-2	8-14	7-10	5-9	0-4	4-12	0	0	0	0	0	4-6	8-10
Hogsback	6	0-2	0-2	0-2	8-14	6-11	6-8	0-4	4-14	0	0	0	0	0	4-6	8-12
Karkloof	8	0-2	0-2	0-2	6-14	7-10	5-7	0-4	6-12	0	0	0	0	0	6-8	6-10
interpopulation																
Committee's x Hogsback	8	0-2	0-2	0-2	6-14	7-10	5-9	0-4	4-12	0	0	0	0	0	4-6	6-12
Hogsback x Committee's	7	0-2	0-2	0-2	8-14	6-10	5-7	0-4	4-10	0	0	0	0	0	6-8	8-12
Committee's x Karkloof	9	0-2	0-2	0-2	8-14	8-10	5-9	0-4	6-10	0	0	0	0	0	4-8	8-14
Karkloof x Committee's	6	0-2	0-2	0-2	6-14	6-12	6-7	0-4	4-12	0	0	0	0	0	4-8	6-14
Hogsback x Karkloof	9	0-2	0-2	0-2	8-16	5-11	6-8	0-4	6-14	0	0	0	0	0	6-8	8-16
Karkloof x Hogsback	8	0-2	0-2	0-2	8-14	6-9	5-9	0-4	6-12	0	0	0	0	0	4-8	8-14

Neonates were born with all experimentally ascertained motor responses fully developed (Table 5.2). Locomotor abilities were poorly developed at birth, but a few young from each mating combination were capable of crawling on the laboratory bench; all neonates were fully mobile by day 4. Strong social bonds, exemplified by huddling, were present on day 0.

Feeding on solid food was first observed on day 4, coinciding with the onset of weaning; neonates first displayed agonistic behaviour patterns at this time (Table 5.2). Weaning was usually complete by day 14, when nipple-clinging had ceased and it was no longer possible to express milk from the mother's nipples.

Sexual maturity was usually attained earlier in females than males (Table 5.2).

The physical growth of purebred and crossbred progeny, exemplified by an increase in body mass, is illustrated in Figures 5.1 and 5.2, respectively. Body mass was selected for this purpose as it was considered to provide a good measure of the condition of developing young, as well as permitting crude assessment of the viability of crossbred young. Data in respect of head-body, tail, hind foot and ear lengths are provided in Appendix 3. Growth rates of males and females resulting from the same mating combination

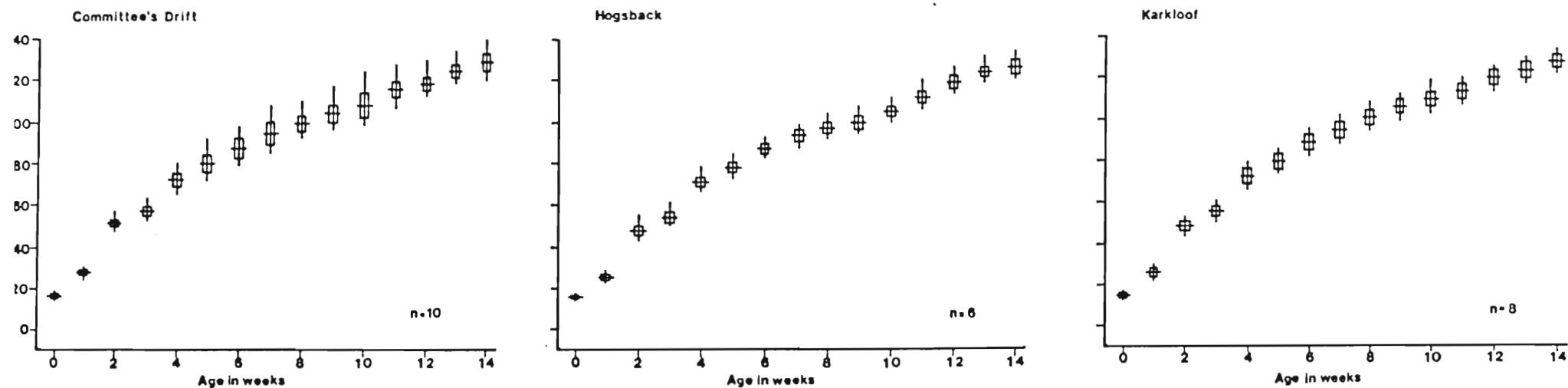


Figure 5.1. Mass increase of young representing the *O. irroratus* populations indicated. Mean indicated by horizontal lines, two standard errors of the mean by enclosed rectangles, and total sample variation by vertical lines. Sample size (n; number of young) indicated in the body of the figure.



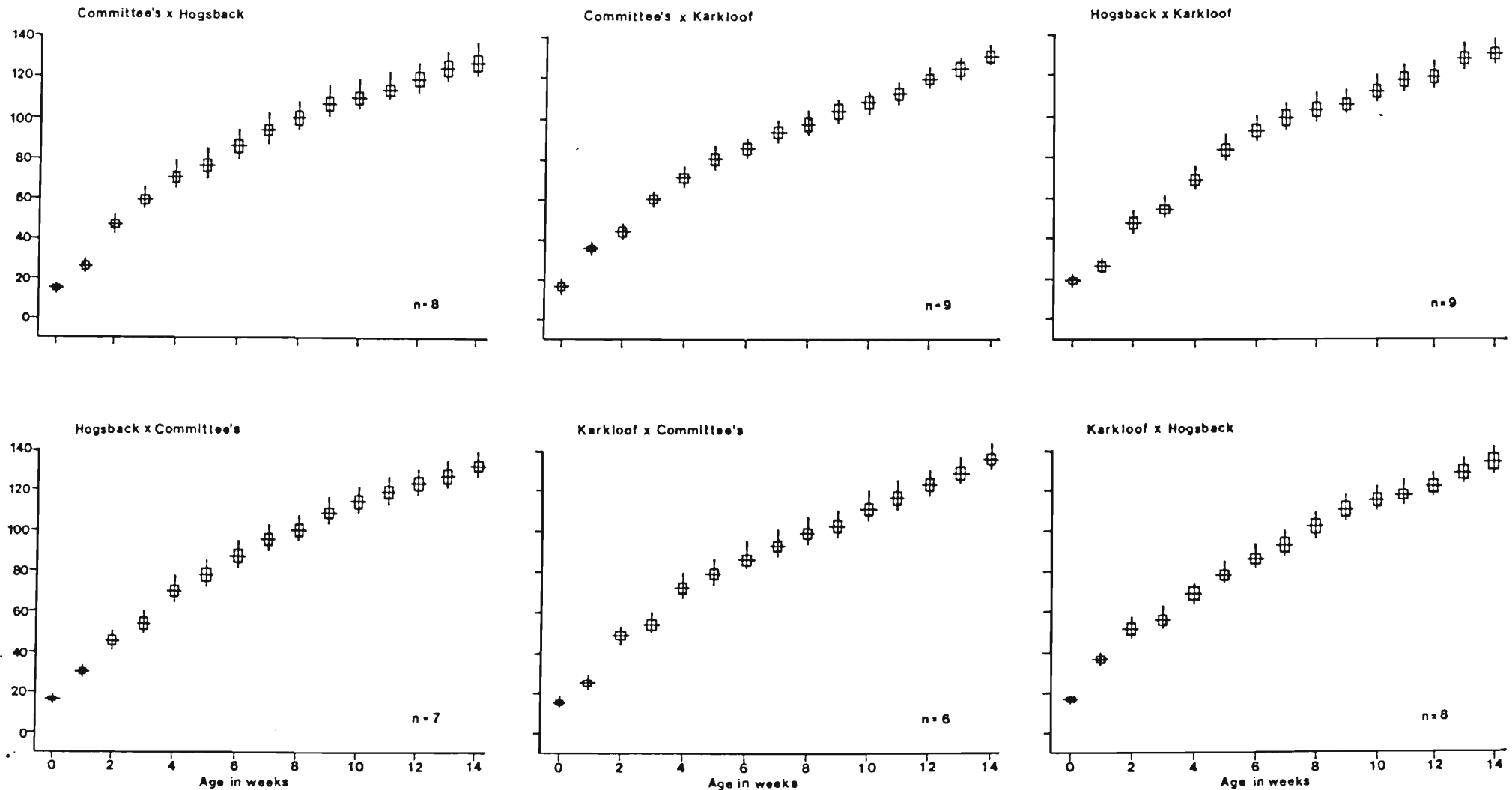


Figure 5.2. Mass increase of young resulting from the cross pairings indicated. Mean indicated by horizontal lines, two standard errors of the mean by enclosed rectangles, and total sample variation by vertical lines. Sample size (n: number of young) indicated in the body of the figure.

were not materially different, and data were therefore combined. Both purebred (Figure 5.1) and crossbred (Figure 5.2) progeny had similar rates of body mass increase. These rates, as well as those for the four categories of linear measurements (i.e. head-body, hind foot, ear and tail lengths) were statistically indistinguishable for the nine mating combinations considered (Table 5.3).

Table 5.3. Results of  $\chi^2$  contingency analysis of age- and combination-specific values of the parameters indicated. For additional details, see text.  
df = degrees of freedom.

Parameters	Statistics		
	df	$\chi^2$	P
Head-body length	32	5.69	> 0.995
Tail length	32	3.12	> 0.995
Hind foot length	32	0.73	> 0.995
Ear length	32	0.51	> 0.995
Mass	32	1.76	> 0.995

#### 5.4 Discussion

It is evident that young O. irroratus are highly precocial, grow rapidly, and wean and attain sexual maturity comparatively early. The adaptive significance of these features is comprehensively discussed by Davis & Meester (1981), and these issues are not pursued here.

On the basis of local environmental conditions, it was hypothesized that the postnatal development of Hogsback and Karkloof progeny would prove similar, while Committee's Drift young would differ from the other two (section 5.1). The results obtained in the present study did not support this hypothesis, however. Although similarity in the rates of growth and development of closely related taxa adapted to contrasting environments has been reported for other rodent species (Layne 1968; Lackey 1978; Creighton & Strauss 1986), a generally applicable explanation of this phenomenon is lacking. Lackey (1978) maintained that the similar rate of postnatal development of young white-footed mice Peromyscus leucopus occurring in dissimilar environments was due to plesiomorphic physiological constraints. In addition, following an intensive review of the literature on the growth and development of cricetine rodents, Creighton & Strauss (1986) suggested that phylogenetic constraints are more significant in determining developmental patterns than are environmental effects. The present results indicate that such constraints may also function in respect of the postnatal development of O. irroratus.

The growth and development of crossbred O. irroratus appeared to be indistinguishable from that of the purebred populations, although differences may have occurred which were too subtle for detection. It would therefore appear that the gene/chromosome sequences controlling growth and

development of hybrid offspring were not deleteriously affected (see section 5.1), and that the crossbred young examined in this study were fully viable.

## CHAPTER 6

### Morphology of Male Reproductive Structures

#### 6.1 Introduction

Recognition between potential mates during courtship (section 4.1) may be achieved by a signal-response chain involving several modes of communication, referred to as the specific-mate-recognition system (SMRS; Paterson 1978, 1985). Paterson maintained that components of the fertilization system (e.g. the morphology of the reproductive anatomy, especially male reproductive structures) may also form part of the SMRS. This implies that, should mating be attempted between members of closely related species, differences of the male reproductive structures, if any, may prevent interbreeding. Such species are then, by definition, pre-zygotically isolated (Mayr 1969; Dobzhansky 1970).

Although differences in the morphology of penile (i.e. glans penis and baculum) and spermatozoan structures are apparent among many closely related species, the functional significance of these variations is as yet unclear. It has been suggested that mismatch of these and the appropriate female structures may function mechanically to prevent successful interspecific mating. For example, differences in the size and structure of the glans penis and

baculum may, during copulation, either prevent intromission or inhibit the transfer of sperm (Gordon 1984). Similarly, variation in spermatozoan morphology (e.g. acrosome and perforatorium) may impede the penetration of the cumulus oophorus and/or zona pellucida of the oocyte, thereby preventing fertilization (Austin & Bishop 1958b; Visser & Robinson 1987).

It is well documented that speciation may be accompanied by changes in male reproductive structures, which may or may not prevent mating between individuals representing different populations (inter alia Mayr 1963; Dice 1968). These changes may be brought about by random genetic drift (Breed & Yong 1986) or the pleiotropic effects of genes (Muller 1939; Mayr 1963). On the basis of these concepts, an attempt is made in the present study to describe and compare the penile and spermatozoan structures of purebred and crossbred males.

## 6.2 Materials and Methods

The morphology of the glans penis, baculum and spermatozoa of purebred males representing the Committee's Drift, Hogsback and Karkloof populations, and of crossbred males resulting from cross pairings involving the three populations, was studied. Animals used in the study were fully adult (see section 2.2), being at least 150 days old.

Material was obtained from a total of 20 purebred and 30 crossbred subjects which were sacrificed either specifically for this study, or for other purposes (see Meester 1988). All sacrificed individuals were prepared as study specimens, and will be lodged in the mammal collections of the Durban Natural Science Museum.

Anatomical terminology used here follows that of Burt (1936) and Austin & Bishop (1958a) in respect of glans penis and baculum morphology, and of Elder & Hsu (1981) and Gordon (1984) in respect of spermatozoan morphology.

#### 6.2.1 Glans penis and baculum morphology

The glandes penes of freshly sacrificed animals were everted from the prepuce and excised at the base. Thereafter, the four structures illustrated in Figure 6.1A & B were immediately measured using a digital caliper.

Phalli were stored in 70% alcohol for 3 - 5 days, and were cleared in 4% KOH for 5 - 7 days at room temperature. The glandes were then dissected away to expose the bacula (Lidicker 1968); the cartilaginous distal bacula were not retained. The remaining osseous proximal bacula (hereafter referred to as the bacula) were stained with Alizarin red, and the six structures illustrated in Figure 6.1C & D were measured using an optical micrometer.

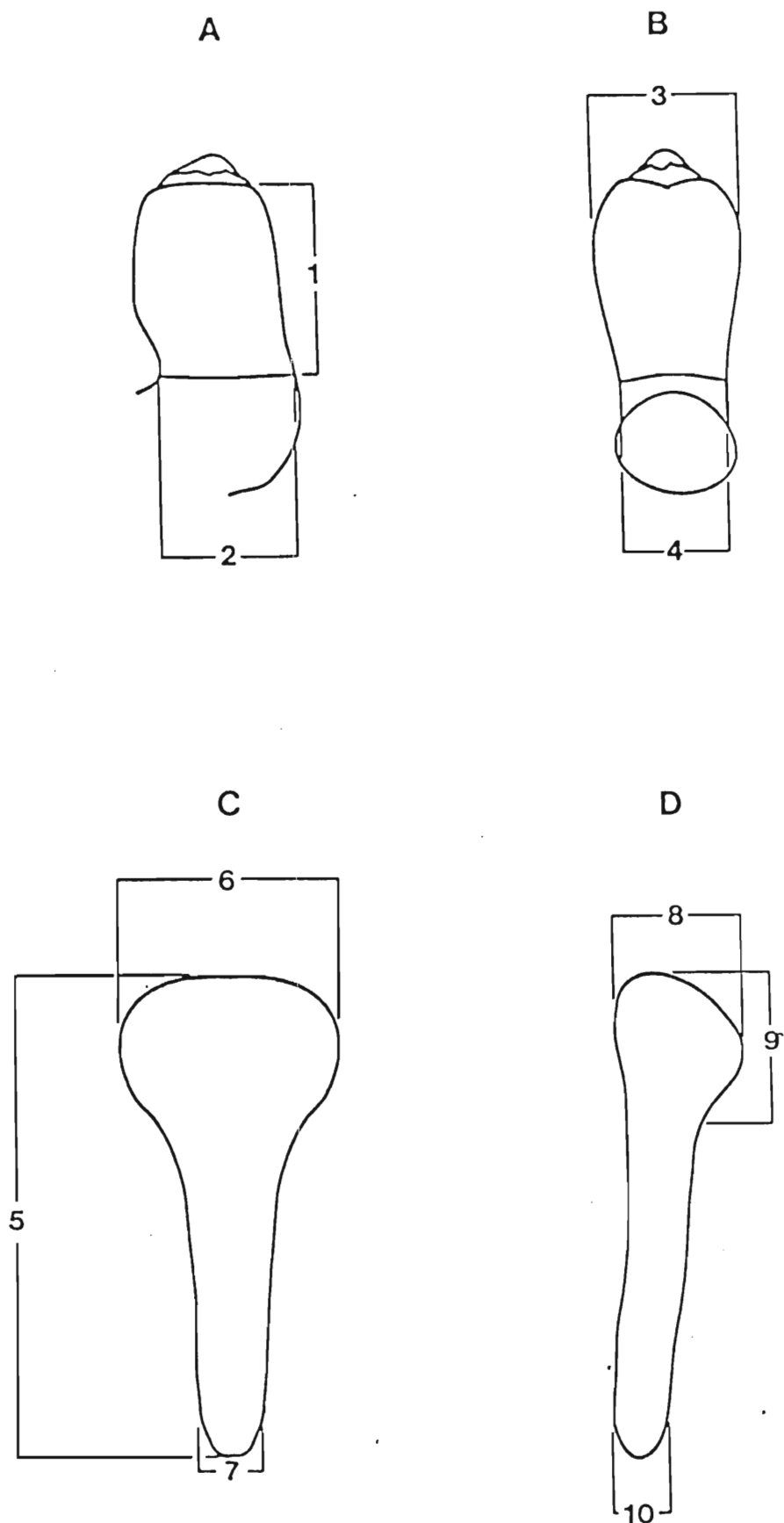


Figure 6.1. Diagrammatic representation of the structures of the glans penis (A, B) and baculum (C, D) examined. 1 = greatest glans length; 2 = lateral glans width; 3 = glans tip width; 4 = ventral glans length; 5 = greatest baculum length; 6 = greatest base width; 7 = distal shaft width; 8 = lateral base width; 9 = base height; 10 = lateral distal shaft width.



### 6.2.2 Spermatozoan morphology

Spermatozoa were obtained from the cauda epididymides. The preparation and staining of slides followed the techniques outlined by Elder & Hsu (1981). Spermatozoa were expressed into 1 ml of Hank's balanced salt solution, and were fixed with three drops of 3% formalin. The suspension was smeared onto microscope slides and air dried. Thereafter, the slides were sequentially washed for 2 min in one change each of 70% and 90% alcohol, soaked in borate buffer ( $0.1 \text{ M NaSO}_4 + 0.005 \text{ M Na}_2\text{BO}_7$ ) for 20 min, and flooded with filtered 50% aqueous silver nitrate containing 0.03% formalin. The slides were covered with coverslips and incubated in a moist chamber ( $\text{rH} = 85\%$ ) at  $60^\circ\text{C}$  for 1 - 1.5 h. The silver-stained spermatozoa were examined and photographed employing bright field optics using a Zeiss 2730 photomicroscope. The five structures illustrated in Figure 6.2A & B were measured using an optical micrometer, for a sample of 15 intact spermatozoa from each animal.

### 6.2.3 Data analysis

Mean values of the appropriate variables for purebred and crossbred subjects were compared using the t-test (Sokal & Rohlf 1987), as kurtosis and skewness coefficients indicated that the distributions followed normal trends (section 2.4). Statistical comparison of all parameters was made within

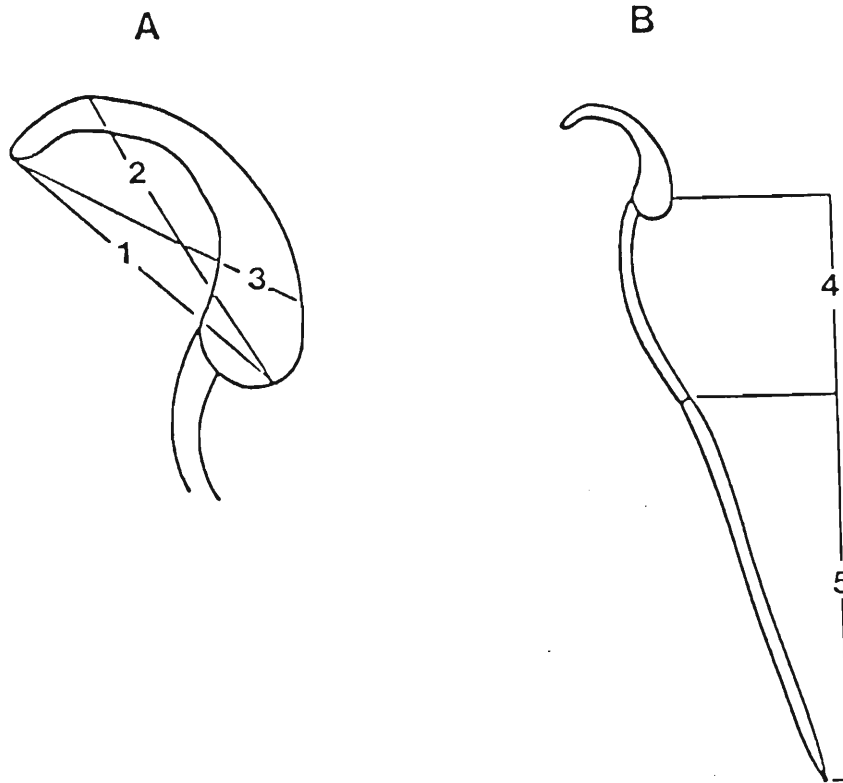


Figure 6.2. Diagrammatic representation of the structures of the spermatozoan head (A) and tail (B) examined.  
1 = perforatorium length; 2 = head length; 3 = greatest head width; 4 = mid-tail length; 5 = principal-tail length.

purebred and crossbred categories, and between purebred and the relevant crossbred categories.

### 6.3 Results

#### 6.3.1 Glans penis and baculum morphology

The morphology of the glans penis (Figure 6.3) and baculum (Figure 6.4) was similar in purebred and crossbred animals. In all cases, the phallus was cylindrical and approximately twice as long as wide. The distal surface was essentially featureless, while the penis was slightly indented at the basal prepuce junction (lateral view), and at the mid-section (lateral and ventral views). Two ventral grooves extended from the midsection of the indentation to the terminal crater. Because the terminal crater of the penis was not examined, only the papilla was evident (Figure 6.3).

Dorsally, the baculum appeared club-shaped, with the base forming the head of the "club". The shaft was narrower than the base, with a blunt terminal end. The baculum was roughly spatulate in lateral view (Figure 6.4).

Descriptive statistics of glans penis measurements of purebred and crossbred animals are presented in Table 6.1, and the results of statistical comparisons in Table 6.2.

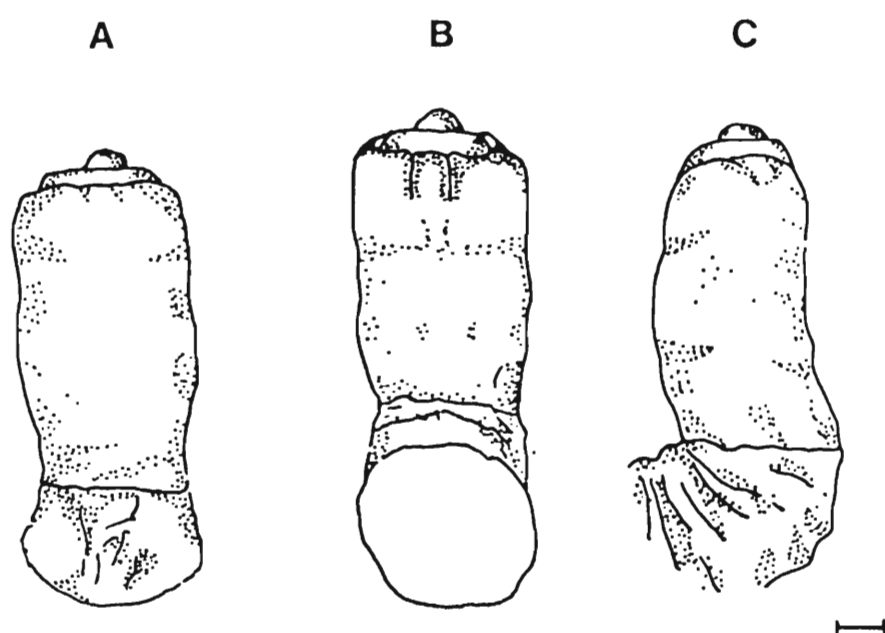


Figure 6.3. Glans penis of *O. irroratus* in dorsal (A), ventral (B) and lateral (C) view. Scale line represents 1 mm.

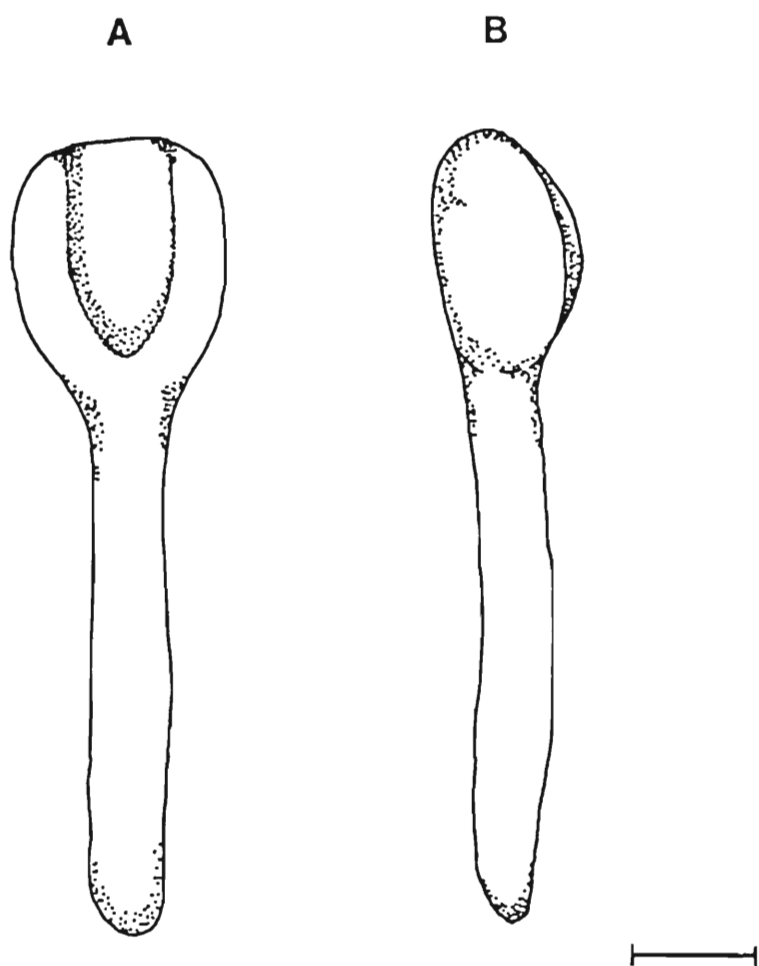


Figure 6.4. Baculum of O. irroratus in dorsal (A) and lateral (B) view. Scale line represents 1 mm.

Table 6.1. Data in respect of glans penis structures (mm) of the subjects indicated. Data for crossbred animals represent pooled values for progeny of reciprocal cross combinations (see text). Committee's = Committee's Drift; n = sample size. 2 S.E. given in brackets.

Subjects	n	Greatest length		Lateral width		Tip width		Ventral width	
		$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range
Purebred Animals									
Committee's Drift	5	7.65 (0.23)	7.31-7.93	3.72 (0.13)	3.53-3.86	4.57 (0.33)	4.13-4.97	3.62 (0.35)	3.17-4.13
Hogsback	5	7.52 (0.40)	6.81-7.93	3.90 (0.22)	3.63-4.27	4.61 (0.32)	4.03-4.92	4.00 (0.22)	3.73-4.30
Karkloof	5	7.60 (0.10)	7.38-7.79	3.76 (0.15)	3.66-3.97	4.96 (0.23)	4.72-5.25	3.70 (0.16)	3.52-3.91
Crossbred Animals									
[Committee's x Hogsback]	7	7.54 (0.12)	7.33-7.76	3.68 (0.10)	3.59-3.91	4.70 (0.09)	4.54-4.89	3.63 (0.07)	3.51-3.72
[Committee's x Karkloof]	8	7.63 (0.09)	7.50-7.84	3.76 (0.12)	3.62-3.96	4.76 (0.16)	4.41-5.18	3.85 (0.20)	3.46-4.37
[Hogsback x Karkloof]	8	7.51 (0.16)	7.28-7.78	3.83 (0.10)	3.56-4.01	4.69 (0.09)	4.61-4.89	3.70 (0.04)	3.62-3.77

Table 6.2. Calculated t values for differences between mean measurements for glans penis structures of the subjects indicated. With the exception of values indicated by an ‡, no significant differences were evident. Comm/Committee's = Committee's Drift; Hogs = Hogsback; Kar = Karkloof; df = degrees of freedom.

Subjects compared	df	Greatest length	Lateral width	Tip width	Ventral width
Purebred Animals					
Committee's vs Hogsback	8	0.56	1.44	0.17	0.86
Committee's vs Karkloof	7	0.40	0.41	1.92	0.42
Hogsback vs Karkloof	7	0.39	1.38	1.77	2.23
Crossbred Animals					
[Comm x Hogs] vs [Comm x Kar]	13	1.22	1.02	0.65	2.12
[Comm x Hogs] vs [Hogs x Kar]	13	0.31	2.08	0.16	1.82
[Comm x Kar] vs [Hogs x Kar]	14	1.33	0.89	0.43	1.48
Purebred & Crossbred Animals					
Committee's vs [Comm x Hogs]	10	0.84	0.49	0.75	0.05
Committee's vs [Comm x Kar]	11	0.16	0.44	1.01	1.15
Hogsback vs [Comm x Hogs]	9	0.10	1.85	0.54	3.29‡
Hogsback vs [Hogs x Kar]	11	0.05	0.58	0.48	2.74‡
Karkloof vs [Comm x Kar]	10	0.46	0.00	1.42	1.18
Karkloof vs [Hogs x Kar]	10	0.98	0.76	2.17	0.00

‡ =  $P < 0.05$

Statistics describing baculum structures appear in Tables 6.3 and 6.4. There were no significant differences between the progeny of the reciprocal cross combinations in respect of any of the parameters measured (see Appendix 4), and data were therefore pooled. Measurements of all parameters were similar for all purebred and crossbred males studied, and considerable interpopulation overlap in the ranges of most structures was evident (Tables 6.1 and 6.3).

The [Committee's Drift x Hogsback] and [Hogsback x Karkloof] crossbred individuals differed significantly from the Hogsback population in terms of the ventral glans width (Table 6.2). No significant differences existed in any other penile structures either within or between the purebred or crossbred categories (Tables 6.2 and 6.4).

#### 6.3.2 Spermatozoan morphology

A photomicrograph of an O. irroratus spermatozoan, representing the Karkloof population, appears as Plate 6.1. As in the morphology of penile structures, no differences were apparent in the morphology and staining patterns of the spermatozoa of the animals studied. In all cases, the spermatozoan head had a fairly broad base which tapered to a single hook. The tail typically consisted of a shorter mid-tail and a longer principal-tail piece.



Table 6.3. Data in respect of baculum structures (mm) of the subjects indicated. Committee's = Committee's Drift. Data for crossbred animals represent pooled values for progeny of reciprocal cross combinations (see text). Sample sizes as in Table 6.1. 2 S.E. given in brackets.

Subjects	Greatest length		Greatest base width		Distal shaft width		Lateral base width		Base height		Lateral distal shaft width	
	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range
Purebred Animals												
Committee's Drift	6.46 (0.12)	6.29-6.58	1.81 (0.12)	1.62-2.04	0.63 (0.06)	0.57-0.73	0.89 (0.20)	0.72-1.01	2.24 (0.19)	2.00-2.69	0.66 (0.11)	0.53-0.85
Hogsback	6.57 (0.11)	6.50-6.78	1.78 (0.25)	1.57-2.25	0.71 (0.11)	0.56-0.89	0.86 (0.16)	0.61-1.10	2.25 (0.17)	2.12-2.63	0.69 (0.08)	0.60-0.85
Karkloof	6.59 (0.09)	6.49-6.72	1.72 (0.08)	1.63-1.83	0.59 (0.08)	0.51-0.70	1.10 (0.12)	0.94-1.23	2.38 (0.05)	2.33-2.42	0.56 (0.09)	0.50-0.69
Crossbred Animals												
Committee's x Hogsback]	6.51 (0.13)	6.36-6.72	1.73 (0.06)	1.61-1.83	0.63 (0.03)	0.58-0.68	1.03 (0.17)	0.63-1.27	2.31 (0.11)	2.05-2.55	0.66 (0.07)	0.58-0.81
Committee's x Karkloof]	6.49 (0.25)	5.85-7.04	1.72 (0.37)	1.47-1.87	0.67 (0.06)	0.51-0.76	1.04 (0.12)	0.75-1.31	2.21 (0.16)	1.74-2.49	0.61 (0.06)	0.41-0.71
Hogsback x Karkloof]	6.51 (0.18)	6.38-6.91	1.67 (0.06)	1.58-1.80	0.61 (0.02)	0.57-0.66	1.07 (0.11)	0.90-1.26	2.33 (0.09)	2.10-2.51	0.60 (0.06)	0.56-0.63

Table 6.4. Calculated t values for differences between mean measurements for baculum structures of the subjects indicated. No significant differences existed, so P is not given. Degrees of freedom as in Table 6.2. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof.

Subjects compared	Greatest length	Greatest base width	Distal shaft width	Lateral base width	Base height	Lateral distal shaft width
Purebred Animals						
Committee's Drift vs Hogsback	1.40	0.22	0.65	0.24	0.08	0.45
Committee's Drift vs Karkloof	1.76	1.27	0.60	1.82	1.46	1.44
Hogsback vs Karkloof	0.29	0.46	1.81	2.11	1.49	2.15
Crossbred Animals						
[Comm x Hogs] vs [Comm x Kar]	0.14	0.48	1.25	0.10	1.02	1.10
[Comm x Hogs] vs [Hogs x Kar]	0.00	0.93	1.12	0.30	0.28	1.40
[Comm x Kar] vs [Hogs x Kar]	0.13	0.27	1.98	0.40	1.29	0.24
Purebred & Crossbred Animals						
Committee's Drift vs [Comm x Hogs]	0.58	1.22	0.00	1.08	0.64	0.00
Committee's Drift vs [Comm x Kar]	0.22	0.47	1.23	1.29	0.24	0.82
Hogsback vs [Comm x Hogs]	0.72	0.39	1.46	1.47	0.59	0.58
Hogsback vs [Hogs x Kar]	0.57	0.85	1.86	2.17	0.77	1.86
Karkloof vs [Comm x Kar]	0.77	0.00	1.63	0.70	2.05	0.91
Karkloof vs [Hogs x Kar]	0.78	1.00	0.48	0.37	0.78	0.73

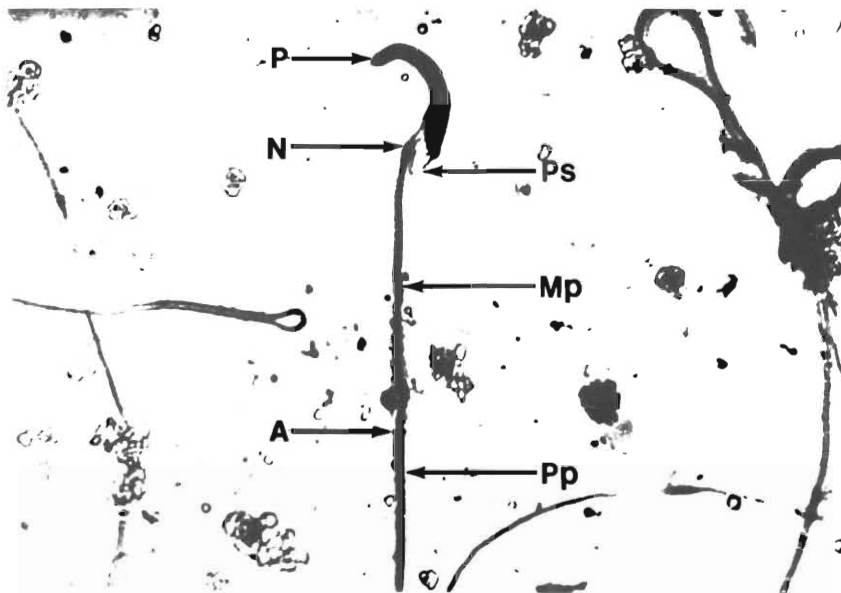


Plate 6.1. Photomicrograph of the spermatozoan of *O. irroratus* (Karkloof population). A = annulus; Mp = mid-tail piece; N = neck; P = perforatorium; Pp = principal-tail piece; Ps = postacrosomal sheath. Magnification = X 1300.

Differential staining of spermatozoa revealed that the perforatorium of O. irroratus is argentophilic, while the postacrosomal sheath is silver negative; the acrosome could not be distinguished in the spermatozoa examined. The neck of the tail below the postacrosomal sheath is inserted ventrally, and is silver positive. The mid- and principal-tail pieces, together with the annulus (which separates the two tail pieces), also stained positive with silver nitrate (Plate 6.1).

Descriptive statistics of spermatozoan head and tail measurements of purebred and crossbred animals are presented in Table 6.5, and the results of the appropriate t-tests in Table 6.6. There were no significant differences between the progeny of the reciprocal cross combinations in respect of any of the parameters examined (see Appendix 4), and the data were pooled.

Mean measurements of both head and tail structures were similar among all animals studied, and extensive overlap of the ranges of all values was apparent (Table 6.5). No significant differences were apparent among the males in respect of the spermatozoan structures measured (Table 6.6).

Table 6.5. Data in respect of spermatozoa head and tail structures ( $10^{-2}$  mm) of the subjects indicated. Data for crossbred animals represent pooled values for progeny of reciprocal cross combinations (see text). Committee's = Committee's Drift; n = number of animals studied. 2 S.E. given in brackets.

Subjects	n	Perforatorium length		Head length		Greatest head length		Mid-tail length		Principal-tail length	
		X	range	X	range	X	range	X	range	X	range
Purebred Animals											
Committee's Drift	5	1.16 (0.01)	1.15-1.18	1.23 (0.04)	1.15-1.25	1.37 (0.03)	1.32-1.41	2.59 (0.09)	2.43-2.72	9.89 (0.27)	9.68-10.41
Hogsback	5	1.13 (0.02)	1.09-1.16	1.18 (0.03)	1.14-1.26	1.33 (0.04)	1.30-1.36	2.59 (0.19)	2.52-2.65	10.10 (0.21)	9.69-10.31
Karkloof	4	1.15 (0.03)	1.11-1.18	1.20 (0.07)	1.11-1.28	1.32 (0.07)	1.21-1.37	2.54 (0.11)	2.42-2.89	9.93 (0.20)	9.69-10.16
Crossbred Animals											
[Committee's x Hogsback]	8	1.13 (0.04)	1.07-1.19	1.21 (0.03)	1.13-1.25	1.30 (0.06)	1.23-1.37	2.63 (0.07)	2.50-2.75	9.85 (0.20)	9.58-10.31
[Committee's x Karkloof]	7	1.13 (0.07)	1.10-1.17	1.22 (0.02)	1.11-1.25	1.35 (0.02)	1.32-1.41	2.63 (0.12)	2.52-2.94	9.90 (0.15)	9.71-10.33
[Hogsback x Karkloof]	8	1.11 (0.03)	1.06-1.14	1.20 (0.04)	1.18-1.27	1.30 (0.07)	1.26-1.36	2.60 (0.07)	2.46-2.78	9.96 (0.17)	9.73-10.27

Table 6.6. Calculated t values for differences between mean measurements for spermatozoan head and tail structures of the subjects indicated. No significant differences existed, so P is not given. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof; df = degrees of freedom.

Subjects compared	df	Perforatorium length	Head length	Greatest head length	Mid- tail length	Principal tail length
<b>Purebred Animals</b>						
Committee's Drift vs Hogsback	7	2.31	2.32	1.68	0.00	1.25
Committee's Drift vs Karkloof	7	0.63	0.74	1.25	0.68	0.24
Hogsback vs Karkloof	6	1.05	0.52	0.24	0.44	1.18
<b>Crossbred Animals</b>						
[Comm x Hogs] vs [Comm x Kar]	13	0.00	0.60	1.59	0.21	0.40
[Comm x Hogs] vs [Hogs x Kar]	14	0.83	0.46	0.00	0.64	0.84
[Comm x Kar] vs [Hogs x Kar]	13	1.19	1.03	1.34	0.89	0.54
<b>Purebred &amp; Crossbred Animals</b>						
Committee's Drift vs [Comm x Hogs]	11	1.43	0.91	2.13	0.68	0.24
Committee's Drift vs [Comm x Kar]	10	0.92	0.51	1.05	0.99	0.66
Hogsback vs [Comm x Hogs]	10	0.00	1.60	0.87	0.28	1.74
Hogsback vs [Hogs x Kar]	10	1.12	0.94	0.75	0.10	1.05
Karkloof vs [Comm x Kar]	9	0.56	0.53	0.77	1.07	0.24
Karkloof vs [Hogs x Kar]	10	2.01	0.00	0.39	0.91	0.23

#### 6.4 Discussion

Davis (1973) showed that the glans penis and baculum of O. irroratus on the Transvaal highveld are characteristic of the complex phallus type described by Hooper & Musser (1964). With the exception of the baculum, no attempt was made in the present study to examine the internal structure of the phallus, so that the phalli could neither be compared with those of animals examined by Davis nor evaluated using the classification of Hooper & Musser. At the level of resolution employed by Davis and in the present study, the bacula of O. irroratus representing different populations appear to be indistinguishable.

At the electron microscope level, O. irroratus spermatozoa are similar to those of the Murinae, particularly in the presence of a lateral acrosomal lip which is absent in the spermatozoa of the Cricetinae (Bernard et al. 1990). The acrosome, as well as other spermatozoan structures (e.g. number of mitochondria), was not detected at light microscope level in the present study, however. Therefore, no comparisons could be made with the work of Bernard et al., and no conclusions could be reached as to the phylogenetic affinities of O. irroratus.

#### 6.4.1 Comparison of male reproductive structures

Lack of significant differences in the glans penis, baculum and spermatozoan morphology in the present study were entirely predictable in view of the interpopulation reproductive compatibility of males and females representing the Committee's Drift, Hogsback and Karkloof populations (Chapter 3). Moreover, penile and spermatozoan structures are not subject to major adaptive variation (Breed & Yong 1986). A number of cases have been reported where homogeneity of male reproductive structures exists even among quite distantly related species. Interspecific similarities of the glans penis (Dice 1968) and baculum (Best & Schnell 1974) are apparent in many rodent taxa, while significant differences in spermatozoan morphology may be absent even at the interfamilial level among marsupials (Hughes 1965).

Progeny resulting from cross pairings involving Hogsback individuals (i.e. [Committee's Drift x Hogsback] and [Hogsback x Karkloof] hybrids) displayed a significantly smaller ventral glans width than purebred Hogsback animals (Table 6.2); breeding attempts involving these hybrid offspring were markedly impaired, and they were considered infertile (see section 3.4). Although it is tempting to speculate that such differences in the glans penis in some way affected the breeding performance of these hybrids, two



factors indicate this may not be the case. First, significant variation was apparent in respect of only one structure, and second, hybrid females resulting from cross-matings comprising Hogsback animals were also sterile (see section 3.3).

#### 6.4.2 Evolutionary implications

The evidence presented here indicates that population-specific penile and spermatozoan disparities do not exist among the populations studied, and it follows that such factors cannot have influenced breeding performance (see chapter 3). It is also apparent that, except for differences in the glans penis of some hybrids, reproductive structures did not contribute to the lack of backcross breeding success of crossbred males. Consequently, the male reproductive structures examined in this study probably served neither as pre-zygotic isolating mechanisms during cross breeding, nor as post-zygotic isolating mechanisms during backcross breeding.

## CHAPTER 7

### Conclusion

#### 7.1 Geographic variation

On the basis of breeding performance, the Hogsback and Karkloof populations were similar, while the Committee's Drift populations differed from the other two in several important respects (chapter 3). The Committee's Drift population is separated from the Hogsback population by only 130 km, however, while the Hogsback and Karkloof populations occur approximately 500 km apart. On the basis of linear distance between populations, it might be expected that geographic separation, and hence genetic divergence, between the Committee's Drift and Hogsback populations occurred later than that between the Hogsback and Karkloof populations. These observations may be interpreted in two ways, as presented below.

First, similar climatic and habitat conditions at Hogsback and Karkloof (see section 1.1) may have selected for the similar life-history characteristics of these populations, while environmental conditions at Committee's Drift may have selected for the contrasting attributes of the D. irroratus population at that locality. Similarly, geographic variation of the reproductive parameters among geographically isolated populations of the bank vole

Clethrionomys glareolus may be related to local differences of the environment (Hansson & Henttonen 1985).

Second, Prototomys, the Pliocene ancestor of the modern Otomyinae (Pocock 1976), is hypothesized on paleoclimatic and biochemical grounds to have been adapted to moist habitats (Taylor et al. 1989). Evidence also exists which suggests that O. irroratus, in relation to other extant Otomys spp., is ecologically similar to Prototomys (Willan In press). In view of the fact that environmental factors largely dictate species-specific life-history tactics (Pianka 1970; Stearns 1976), it is not unreasonable to assume that relatively mesophilic O. irroratus populations (e.g. Hogsback and Karkloof) would have retained attributes that were established in the ancestral form. If this is so, it follows that differences in the breeding biology of the Committee's Drift population, in relation to that of the two other populations, demonstrates secondary adaptation in response to the more xeric conditions at Committee's Drift locality (see section 1.1).

As in the case of breeding biology, differences in pre-copulatory behaviour may be explained by the ecological circumstances of each population (see chapter 4). Thus, whereas some characteristics of the ancestral form may have been retained by extant populations (i.e. the Hogsback and Karkloof populations), different ecological circumstances

may have determined other aspects of the reproductive biology (e.g. interaction of pairs during the pre-copulatory phase) of these populations.

Growth and development (chapter 5) and male reproductive morphology (chapter 6) were indistinguishable among individuals from all three populations. It would appear that phylogenetic constraints are more significant in determining the postnatal development of these populations than are environmental effects.

As in other studies on geographic variation (inter alia Lackey 1978; Smith 1979; Hansson 1985; Smith & Patton 1988), the conclusion emerging from the present study is that interpopulation variation in the breeding and reproductive biology of O. irroratus is complex and unpredictable. In the present context, unpredictability arises mainly from the following: a paucity of life history data in respect of each population; inadequate knowledge of the effects that the environment exerts on reproductive parameters; and an inability to estimate the role of the evolutionary history of each population in determining their current reproductive patterns.

## 7.2 Speciation and reproductive isolation

The pre-copulatory behaviour of interpopulation pairings suggests that behavioural isolating mechanisms may reduce reproductive compatibility among the populations, should they become sympatric under natural conditions (Table 7.1). These pre-zygotic barriers to reproduction were most obvious during pairings involving Committee's Drift animals and individuals representing the Hogsback and Karkloof populations.

Table 7.1. Possible reproductive isolating mechanisms among the Committee's Drift (Committee's), Hogsback and Karkloof populations.

Populations compared	Isolating mechanisms	
	pre-zygotic	post-zygotic
Committee's vs Hogsback	behavioural differences	hybrid mortality; hybrid sterility
Committee's vs Karkloof	behavioural differences	hybrid (mostly male) mortality; reduced hybrid breeding success
Hogsback vs Karkloof	minor behavioural differences	hybrid sterility

It is sometimes possible that allopatric populations differ in respect of their courtship behaviour, but that individuals representing these populations mate successfully if they later become sympatric or, as in the present study, during laboratory breeding tests. This phenomenon has been referred to as "mating error" (Rubinoff & Rubinoff 1971, p. 65). "Mating error" may occur because (i) confined spaces

(e.g. captivity) permit animals to overcome pre-zygotic isolating mechanisms that exist between free-living populations (Spieth 1958), or (ii) complete behavioural isolation has not evolved (Dobzhansky et al. 1968). Consequently, if cross-mating occurs when the populations under investigation are sympatric, then all females would produce young. However, many of the hybrids resulting from cross-matings involving animals of Committee's Drift origin died before weaning, particularly male offspring resulting from cross-matings between Committee's Drift and Karkloof individuals. Probably the most significant consequence of cross-matings involving individuals of any of the three populations is that most hybrids were sterile, especially those resulting from cross-pairings involving Hogsback animals. Moreover, those which were capable of producing young had markedly reduced breeding success. Therefore, if interbreeding did occur in nature, the Hogsback population would be post-zygotically isolated in relation to other two populations, and the Committee's Drift and Karkloof populations would be partly isolated from one another at the post-zygotic level (Table 7.1).

The production of hybrids which have low fitness (i.e. 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. Thus, selection is likely to subsequently favour the establishment of pre-zygotic barriers to reproduction (inter alia Dobzhansky et al. 1968; Baker & Bickham 1980; Solginac 1981; Capanna et al. 1985). Therefore, it is possible that existing pre-copulatory behavioural differences among the Committee's Drift, Hogsback and Karkloof populations would be reinforced in sympatry, so that the production of inviable and/or sterile young is prevented. 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; the evolutionary and ecological implications of this are significant, and selection may thus operate against hybrids, favouring the appearance of pre-zygotic barriers to interbreeding. Almost all hybrids that were born during the present study, and survived beyond weaning, appeared fully viable (postnatal development; chapter 5), but many were infertile.

It may be concluded from the study of O. irroratus interpopulation matings that genetic and/or chromosomal divergence has occurred in allopatry to the extent that gene exchange between populations might be drastically reduced should these populations become sympatric. Differences of the pre-copulatory behaviour of all three populations, which may be explained by contrasting climate/habitat conditions

(section 1.1), would certainly act as pre-zygotic barriers to reproduction between the Committee's Drift, Hogsback and Karkloof populations. Chromosomal differences among the populations (see section 1.1) acted as post-zygotic isolating mechanisms when individuals representing the three populations interbreed. These chromosomal differences are most obvious in the Hogsback population (i.e. the tandem fusion; section 3.4), and do not appear to be accompanied by obvious phenotypic disparities. Hence, it appears that the Hogsback population represents an incipient sibling species (Meester 1988; see chapter 1). In addition, the Committee's Drift and Karkloof populations may also be undergoing active speciation.

### 7.3 Recommendations for further study

The present study on the breeding and reproductive biology of O. irroratus suggests many avenues for future study. Some of the more important suggestions, which might provide information essential to the understanding of the adaptive variation and/or evolutionary trends in the O. irroratus, are outlined below.

Possible mechanisms of behavioural (i.e. pre-zygotic) isolation between populations were studied by staging encounters of interpopulation pairings. Because interpopulation breeding attempts between cross-paired males



and females were successful, it is unlikely that they represented populations that were behaviourally isolated. A more meaningful test for sexual isolation would be to offer a choice of mates from different populations to either the male or female to test for assortative mating. If behavioural isolation was complete, then there would be no cross-mating when the appropriate mate was present and mating was positively assortative (Blair & Howard 1944; Gordon 1947; Gordon 1984).

Behavioural incompatibility between males and females representing the different populations was probably due to population-specific communicatory differences, but details of communication in O. irroratus remain unknown. Detailed analyses should be undertaken, as follows:

olfactory communication - by means of chemical assays (e.g. gas-liquid chromatography; inter alia Jorgenson et al. 1978) of the urine or other bodily secretions which may function as olfactory cues, or by observing the reactions of animals to scents/odour of representatives of other populations (Godfrey 1958); auditory communication - by means of spectrographic analysis (e.g. sonagrams) of vocalizations recorded during encounters (Gordon 1984); and visual communication - by means of careful observational analysis of courtship rituals (Alder et al. 1981).

Study of the reproductive morphology of male O. irroratus at the light microscope level failed to reveal certain features that were detected using the electron microscope (Bernard et al. 1990). Therefore, if ultrastructural differences in male reproductive structures existed in the animals studied, they were undetected. Examination of reproductive structures at the electron microscope level should thus be undertaken. Other aspects that directly affect male fertility should be investigated. For example, Dice (1968) showed that spermatogenesis of hybrid Peromyscus leucopus males was impaired.

Individual variation was not considered in the present study, mainly because the aims of the study were to elucidate general trends with regard to adaptive variation and evolutionary divergence among populations. However, Keller (1968) has showed that individual voles Microtus spp. respond markedly differently to similar olfactory cues, and he stressed the importance of individual variation in the reproductive adaptations in this taxon. Therefore, the responses of individuals should be considered in future studies on the breeding and reproductive biology of O. irroratus.

The study of adaptive variation and evolutionary trends among the Committee's Drift, Hogsback and Karkloof populations in the present study has set the basis for

future research of other O. irroratus populations. Probably the most interesting study would be one of populations that occur long distances apart and/or populations occurring in environmentally dissimilar localities.

## SUMMARY

Selected aspects of the breeding and reproductive biology of three allopatric O. irroratus populations were studied in the laboratory. The localities represented were Committee's Drift and Hogsback (eastern Cape) and Karkloof (Natal). The primary objective of the study was to establish whether (i) the three populations differed in terms of breeding and reproductive parameters, and (ii) mechanisms existed whereby the populations were reproductively isolated from one another.

On the basis of these objectives, the breeding performance and the pre-copulatory behaviour of intrapopulation and interpopulation pairings of representatives of the above populations were investigated. In addition, the breeding performance of progeny of interpopulation pairs was ascertained by means of backcross breeding experiments. Postnatal development studies were carried out to establish whether population-specific growth and development patterns were discernible, and to investigate the growth and development patterns, as well as the viability, of crossbred young. To ascertain the possibility of reproductive incompatibility between populations, various reproductive structures of purebred and crossbred males (i.e. glans penis, baculum, spermatozoa) were also studied.

The breeding performance of the Hogsback and Karkloof populations was similar, while the Committee's Drift population differed significantly from the other two in terms of its smaller litter size and increased interval between pairing and the birth of the first litter. The observed patterns may be explained in terms of environmental conditions at the localities inhabited by each population: conditions at Hogsback and Karkloof are similar, while the significantly lower rainfall, and hence carrying capacity, at Committee's Drift distinguishes this locality from the others.

Attempts at interpopulation breeding reflected the reproductive variation observed among the pure pairings. The breeding performance of the Hogsback x Karkloof cross pairings was similar to that of the parental populations, while that of the Committee's Drift x Hogsback and Committee's Drift x Karkloof cross combinations was at least partially impaired. The backcross breeding success of progeny resulting from cross pairings involving Hogsback animals was severely impaired, while backcross breeding of the progeny of Committee's Drift x Karkloof cross pairings was more successful. Reduced reproductive fitness at both the cross and backcross levels is thought to reflect mainly genetic and chromosomal incompatibility, but behavioural factors, which could themselves be genetically determined, may also have contributed to hybrid inviability and/or sterility.

The pre-copulatory behaviour of intrapopulation and interpopulation pairings was studied in neutral arena encounters by means of direct and video recorded observations. Among the pure pairings, Hogsback pairs most rapidly, and Committee's Drift pairs least rapidly, developed amicable relationships, apparently reflecting the ecological circumstances of each of the three populations studied: the carrying capacity at Committee's Drift is thought to have selected for higher levels of intersexual aggression. In comparison with the pure pairings, all cross combinations displayed higher ratios of agonistic to amicable interaction and later development of essentially amicable relationships. These differences, which were more obvious in the Committee's Drift x Hogsback and Committee's Drift x Karkloof cross pairings than in the Hogsback x Karkloof cross pairings, may indicate impaired recognition of olfactory, auditory and/or visual cues between the three populations.

There were no major differences in the postnatal physical and behavioural development of purebred and crossbred young. Phylogenetic constraints therefore appear to have been more significant than environmental effects in determining the postnatal development of O. irroratus young from the three localities. Crossbred O. irroratus that survived beyond weaning were fully viable.

With the exception of ventral glans width, which was smaller in hybrid males resulting from cross-matings involving Hogsback animals, no significant differences were recorded for any penile or spermatozoan structures of purebred and crossbred animals examined. It thus appears that male reproductive structures cannot have influenced breeding success at the interpopulation and backcross levels.

In conclusion, geographical variation in the breeding and reproductive biology of O. irroratus appears to be due to environmental effects in some cases and phylogenetic constraints in others. Moreover, it was hypothesized that the Committee's Drift, Hogsback and Karkloof populations have diverged to the extent that, should they become sympatric under natural conditions, they would be partly pre-zygotically isolated from each other through behavioural means. If mating did occur, however, post-zygotic barriers (i.e. hybrid inviability and sterility) to mating, which appear to be chromosomally mediated, at least in case of the Hogsback population, would prevent genetic exchange between the populations. Hence, all populations appear to be undergoing active speciation.

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**APPENDIX 1**

Breeding data which are pooled in section 3.3 due to lack of statistical difference are presented in Table 9.1. The results of the relevant statistical comparisons appear in Table 9.2. Table 9.3 gives the results of the breeding trials for all backcross permutations.

Table 9.1. Reproductive data in respect of the cross combinations indicated. Committee's = Committee's Drift; M = male; F = female; n = sample size. 2 S.E. given in brackets.

Cross combinations	Matings		Litter size				Primary sex ratio M : F	Interval between:				Pre-weaning mortality			"Fecundity"	
	attempts	successes	n	X	range	mode		pairing & first litter		litters		Total young	Number died	%	n	X
								n	X (days)	n	X (days)					
Committee's x Hogsback	5	4	10	2.20 (0.40)	1-3	2	11 : 11	3	59.25 (4.20)	6	45.67 (0.71)	22	4	18.2	4	10 (2.46)
Hogsback x Committee's	5	3	8	2.25 (0.50)	1-3	2	7 : 9	3	59.67 (3.93)	4	48.00 (0.71)	18	5	27.8	3	10 (5.00)
Committee's x Karkloof	5	4	12	2.33 (0.46)	1-3	3	10 : 18	4	56.75 (2.25)	7	46.17 (1.70)	28	10	35.7	4	16 (7.61)
Karkloof x Committee's	5	5	14	2.21 (0.38)	1-3	2	18 : 13	5	58.20 (3.25)	8	45.13 (0.48)	31	15	48.4	5	13 (3.05)
Hogsback x Karkloof	5	4	9	2.23 (0.78)	1-3	2	8 : 15	4	52.75 (2.25)	4	46.80 (0.69)	23	1	4.3	5	11 (5.63)
Karkloof x Hogsback	5	5	10	2.30 (0.42)	1-4	3	13 : 11	5	51.40 (1.03)	4	46.25 (0.48)	24	2	8.3	4	14 (5.92)
Totals	30	25	63	2.25 (0.18)	1-4	2	67 : 77	24	56.13 (2.50)	33	47.45 (0.84)	146	37	25.3	25	12 (2.05)

Table 9.2. Statistical comparison (Mann-Whitney U test) of mean values of the reproductive data of the cross combinations indicated. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof; U = Mann-Whitney U statistic. Sample sizes (n1 & n2) as in Table 9.1.

Cross combinations compared	Mean litter size		Mean interval between:				Mean "fecundity"	
	Mean litter size		pairing & first litter		litters		Mean "fecundity"	
	s	P	s	P	s	P	s	P
Comm x Hogs vs Hogs x Comm	54	> 0.10	6	> 0.10	20	> 0.05	7	> 0.10
Comm x Kar vs Kar x Comm	93	> 0.10	11	> 0.10	26.5	> 0.10	14	> 0.10
Hogs x Kar vs Kar x Hogs	47.5	> 0.10	10	> 0.10	12.5	> 0.10	14	> 0.10

Table 9.3. Reproductive data in respect of the backcross combinations indicated. Comm/Committee's = Committee's Drift; Hogs = Hogsback; Kar = Karkloof. M = male; F = female; n = sample size. 2 S.E. given in brackets.

Backcross combinations	Matings		Litter size					Primary sex ratio M : F	Interval between					Pre-weaning mortality			"Fecundity"	
	attempts	successes	n	range mode					pairing & first litter			litters		Total young	Number died	%	n	X
				X					n	X (days)	n	X (days)						
<Committee's x Hogsback>																		
Comm x [Comm x Hogs]	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hogs x [Comm x Hogs]	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[Comm x Hogs] x Comm	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[Comm x Hogs] x Hogs	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[Comm x Hogs] x [Comm x Hogs]	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(1) Totals	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<Hogsback x Committee's>																		
Comm x [Hogs x Comm]	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hogs x [Hogs x Comm]	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[Hogs x Comm] x Comm	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[Hogs x Comm] x Hogs	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[Hogs x Comm] x [Hogs x Comm]	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(2) Totals	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<Committee's x Karkloof>																		
Comm x [Comm x Kar]	3	3	5	1.00	-	-	1	3 : 2	2	55.00 (0.00)	2	45.00 (4.00)	5	1	20.0	3	2 (0.67)	
Kar x [Comm x Kar]	3	3	4	1.25	-	1-2	1	3 : 2	2	57.00 (6.00)	1	48.00 (0.00)	5	3	60.0	3	2 (2.00)	
[Comm x Kar] x Comm	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
[Comm x Kar] x Kar	3	3	6	1.00	-	-	1	2 : 4	2	57.50 (9.00)	1	46.00 (0.00)	6	2	33.3	3	2 (0.00)	
[Comm x Kar] x [Comm x Kar]	3	1	3	1.00	-	-	1	2 : 1	1	56.00 (0.00)	1	52.00 (0.00)	3	1	33.3	1	3 (0.00)	
(3) Totals	15	10	18	1.06 (0.12)	1-2	1		10 : 9	7	56.43 (2.50)	5	47.20 (2.92)	19	7	36.8	10	2 (0.59)	
<Karkloof x Committee's>																		
Comm x [Kar x Comm]	3	2	3	1.00	-	-	1	2 : 1	1	58.00 (0.00)	1	51.00 (0.00)	3	0	0.0	2	2 (1.00)	
Kar x [Kar x Comm]	3	2	4	1.00	-	-	1	1 : 3	1	54.00 (0.00)	1	47.00 (0.00)	4	1	25.0	2	2 (0.00)	
[Kar x Comm] x Comm	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
[Kar x Comm] x Kar	3	3	6	1.00	-	-	1	2 : 4	1	56.00 (0.00)	2	48.00 (1.99)	6	2	33.3	3	2 (0.00)	
[Kar x Comm] x [Kar x Comm]	3	1	2	1.00	-	-	1	0 : 2	-	0.00 -	1	45.00 (0.00)	2	0	0.0	1	2 (0.00)	
(4) Totals	15	8	15	1.00	-	-	1	5 : 10	3	56.00 (3.10)	5	47.80 (1.86)	15	3	20.0	8	2 (0.25)	

Table 9.3. Continued.

Backcross combinations	Matings		Litter size					Primary sex ratio		Interval between:						Pre-weaning mortality			*Fecundity*	
	attempts	successes	n	X				M	F	pairing & first litter			litters			Total young	Number died	%	n	X
				range	mode	n	X			(days)	n	X	(days)							
<Hogsback x Karkloof>																				
Hogs x [Hogs x Kar]	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kar x [Hogs x Kar]	3	2	3	1.00	-	-	1	3 : 0	1	55.00	(0.00)	1	47.00	(0.00)	3	0	0.0	2	2 (1.99)	
[Hogs x Kar] x Hogs	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
[Hogs x Kar] x Kar	3	1	2	1.50	(1.00)	1-2	2	1 : 2	1	60.00	(0.00)	1	43.00	(0.00)	3	1	33.3	1	3 (0.00)	
[Hogs x Kar] x [Hogs x Kar]	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
(5) Totals	15	3	5	1.20	(0.40)	1-2	1	4 : 2	2	57.50	(5.00)	2	45.00	(4.00)	6	1	16.7	3	2 (1.33)	
<Karkloof x Hogsback>																				
Kar [Kar x Hogs]	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Hogs x [Kar x Hogs]	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
[Kar x Hogs] x Hogs	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
[Kar x Hogs] x Kar	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
[Kar x Hogs] x [Kar x Hogs]	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
(6) Totals	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Sum of Totals (1+2+3+4+5+6)	90	21	38	1.05	(0.08)	1-2	1	19 : 21	12	56.50	(1.64)	12	47.08	(1.52)	40	10	25.0	21	2 (0.34)	

**APPENDIX 2**

Data obtained from direct observations which are pooled in section 4.3 due to lack of statistical difference are presented in Table 10.1. The results of the relevant statistical comparisons appear in Table 10.2. Similarly, the results of video recorded interaction are given in Tables 10.3 and 10.4.

Table 10.1. Mean percentage of agonistic and amicable behaviour recorded by direct observation of the cross combinations indicated. Committee's = Committee's Drift; n = number of observation periods during which social interaction was observed. 2 S.E. given in brackets.

Cross combinations	n	$\bar{X}$ % interaction	
		Agonistic	Amicable
Committee's x Hogsback	16	52.9 (4.34)	30.3 (5.85)
Hogsback x Committee's	16	58.0 (3.56)	29.4 (7.16)
Committee's x Karkloof	19	54.5 (9.45)	26.2 (3.88)
Karkloof x Committee's	17	64.1 (5.19)	21.6 (7.03)
Hogsback x Karkloof	17	37.6 (6.34)	45.8 (8.48)
Karkloof x Hogsback	20	33.0 (8.96)	51.9 (4.78)



Table 10.2. Statistical comparison (Mann-Whitney U test) of mean percentage agonistic and amicable behaviour of the cross combinations indicated. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof; U = Mann-Whitney U statistic. Sample sizes (n1 & n2) as in Table 10.1.

Mating combinations compared	Interaction			
	Agonistic		Amicable	
	U	P	U	P
Comm x Hogs vs Hogs x Comm	158	> 0.10	154	> 0.10
Comm x Kar vs Kar x Comm	198.5	> 0.10	165.5	> 0.10
Hogs x Kar vs Kar x Hogs	187	> 0.10	179.5	> 0.10

Table 10.3. Mean percentage of agonistic and amicable behaviour video recorded every four days during encounters for the cross combinations indicated. Committee's = Committee's Drift; n = number of encounters. 2 S.E. given in brackets.

Cross combinations	n	$\bar{X}$ % agonistic interaction				$\bar{X}$ % amicable interaction			
		Days				Days			
		1	4	8	12	1	4	8	12
Committee's x Hogsback	4	79.9 (7.50)	77.2 (13.50)	35.9 (8.60)	22.6 (5.22)	0.0 (0.00)	10.7 (7.30)	51.1 (10.91)	66.6 (6.72)
Hogsback x Committee's	4	86.1 (9.16)	64.4 (9.03)	39.1 (4.19)	30.9 (4.64)	0.0 (0.00)	15.1 (11.85)	55.6 (3.10)	58.3 (8.03)
Committee's x Karkloof	4	92.2 (8.86)	79.6 (3.23)	55.0 (10.91)	14.7 (10.67)	0.0 (0.00)	8.1 (12.15)	34.4 (8.68)	63.2 (5.30)
Karkloof x Committee's	4	88.4 (8.58)	73.2 (5.82)	38.8 (9.14)	22.3 (9.75)	0.0 (0.00)	2.8 (3.85)	41.7 (6.96)	62.5 (11.32)
Hogsback x Karkloof	4	80.6 (4.75)	52.9 (17.74)	13.1 (5.85)	3.3 (2.96)	0.0 (0.00)	25.2 (11.68)	69.8 (12.71)	82.3 (8.89)
Karkloof x Hogsback	4	72.4 (7.07)	43.8 (9.99)	12.1 (5.84)	3.5 (2.85)	0.0 (0.00)	33.9 (19.28)	80.4 (8.67)	77.1 (4.63)

Table 10.4. Statistical comparison (Mann-Whitney U test) of mean percentage agonistic and amicable behaviour video recorded every fourth day during encounters of the cross combinations indicated. No statistical comparisons are made of mean amicability on Day 1 because none of the pairs displayed amicable interaction at this time; see Table 10.3. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof; U = Mann-Whitney U statistic. Sample sizes (n1 & n2) as in Table 10.3.

Cross combinations compared	Agonistic interaction								Amicable interaction							
	Days								Days							
	1		4		8		12		4		8		12			
	U	P	U	P	U	P	U	P	U	P	U	P	U	P	U	P
Comm x Hogs vs Hogs x Comm	12	> 0.10	13	= 0.10	9	> 0.10	14	> 0.05	8.5	> 0.10	12	> 0.10	13	= 0.10		
Comm x Kar vs Kar x Comm	11	> 0.10	14	> 0.05	14	> 0.05	12	> 0.10	12	> 0.10	11	> 0.10	9	> 0.10		
Hogs x Kar vs Kar x Hogs	14	> 0.05	12	> 0.10	9	> 0.10	8	> 0.10	9	> 0.10	9	> 0.10	8.5	> 0.10		

**APPENDIX 3**

Head-body, tail, hind foot and ear measurements of purebred and crossbred are given in Tables 11.1 to 11.4, respectively.

Table 11.1. Mean head-body measurements from birth to 14 weeks of age of progeny resulting from the mating combinations indicated. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof; n = number of young studied. 2 S.E. given in brackets.

Mating combinations	n	Weeks														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Intrapopulation																
Committee's Drift	10	77 (0.98)	114 (1.25)	127 (0.96)	133 (0.87)	147 (0.92)	163 (0.89)	171 (0.89)	178 (0.84)	184 (0.85)	187 (0.83)	190 (0.78)	195 (0.68)	199 (0.65)	199 (0.64)	200 (0.70)
Hogsback	6	81 (0.68)	113 (0.75)	133 (0.73)	137 (0.68)	149 (0.72)	167 (0.75)	175 (0.78)	181 (0.74)	187 (0.74)	191 (0.89)	196 (0.78)	199 (0.84)	201 (0.82)	203 (0.84)	204 (0.78)
Karkloof	8	85 (0.69)	114 (0.68)	129 (0.64)	135 (0.64)	149 (0.75)	168 (0.79)	174 (0.75)	183 (0.75)	188 (0.68)	193 (0.67)	197 (0.67)	203 (0.66)	207 (0.68)	209 (0.68)	210 (0.62)
Interpopulation																
Comm x Hogs	8	83 (0.98)	112 (1.07)	127 (0.94)	134 (0.92)	148 (0.92)	167 (0.96)	173 (0.92)	177 (0.98)	184 (0.89)	189 (0.78)	190 (0.79)	196 (0.75)	199 (0.75)	201 (0.73)	205 (0.66)
Hogs x Comm	7	84 (0.67)	109 (0.69)	125 (0.76)	132 (0.67)	144 (0.64)	157 (0.68)	175 (0.86)	180 (0.76)	186 (0.75)	191 (0.74)	195 (0.72)	199 (0.73)	201 (0.72)	204 (0.68)	204 (0.68)
Comm x Kar	9	81 (0.68)	108 (0.98)	123 (0.87)	130 (0.85)	143 (0.82)	159 (0.84)	172 (0.72)	179 (0.77)	186 (0.67)	192 (0.68)	197 (0.67)	202 (0.68)	205 (0.67)	206 (0.69)	211 (0.72)
Kar x Comm	6	85 (0.87)	115 (0.86)	127 (0.76)	136 (0.68)	149 (0.67)	163 (0.86)	174 (0.74)	179 (0.76)	184 (0.66)	189 (0.62)	193 (0.60)	197 (0.60)	199 (0.58)	201 (0.58)	203 (0.56)
Hogs x Kar	9	83 (1.14)	102 (1.04)	127 (0.95)	142 (1.16)	153 (0.98)	164 (0.88)	174 (0.92)	180 (0.92)	183 (0.94)	190 (0.94)	195 (0.92)	197 (0.94)	200 (0.68)	203 (0.58)	206 (0.60)
Kar x Hogs	8	85 (0.85)	115 (0.78)	129 (0.75)	131 (0.87)	149 (0.84)	163 (0.75)	170 (0.78)	179 (0.67)	183 (0.68)	190 (0.67)	195 (0.68)	198 (0.67)	201 (0.64)	201 (0.66)	201 (0.65)

Table 11.2. Mean tail measurements from birth to 14 weeks of age of progeny resulting from the mating combinations indicated. Sample sizes as in Table 11.1. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof. 2 S.E. given in brackets.

Mating combinations	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Intrapopulation															
Committee's Drift	33 (0.92)	49 (0.87)	58 (0.92)	64 (0.86)	75 (1.01)	83 (0.89)	87 (0.91)	90 (0.98)	93 (1.29)	95 (0.73)	99 (0.73)	99 (0.77)	101 (0.77)	101 (0.77)	103 (0.73)
Hogsback	35 (0.67)	47 (0.56)	56 (0.76)	65 (0.88)	78 (0.87)	84 (0.67)	92 (0.67)	99 (0.56)	99 (0.68)	99 (0.56)	100 (0.98)	101 (0.87)	103 (0.97)	104 (0.67)	105 (0.76)
Karkloof	34 (0.89)	48 (0.76)	62 (1.09)	68 (0.67)	78 (0.78)	86 (0.88)	91 (0.56)	96 (0.76)	97 (0.67)	103 (0.98)	104 (0.78)	104 (0.78)	104 (0.78)	104 (0.78)	105 (0.76)
Interpopulation															
Comm x Hogs	33 (1.06)	46 (0.56)	58 (0.76)	71 (0.78)	83 (0.89)	91 (0.87)	95 (0.87)	96 (0.84)	98 (0.87)	102 (0.89)	103 (0.87)	103 (0.87)	103 (0.87)	104 (0.87)	104 (0.75)
Hogs x Comm	34 (0.78)	48 (0.67)	57 (0.89)	69 (0.63)	81 (0.67)	90 (0.66)	93 (0.98)	95 (0.87)	97 (0.67)	101 (0.76)	105 (0.78)	106 (0.82)	106 (0.82)	106 (0.89)	107 (0.84)
Comm x Kar	33 (0.89)	43 (0.65)	57 (0.89)	75 (0.86)	87 (0.78)	93 (0.67)	97 (0.67)	99 (0.78)	102 (0.69)	103 (0.68)	103 (0.59)	104 (0.75)	105 (0.78)	106 (0.68)	106 (0.65)
Kar x Comm	35 (0.78)	45 (0.68)	57 (0.78)	74 (0.86)	83 (0.78)	86 (0.68)	90 (0.78)	94 (0.96)	99 (0.67)	102 (0.66)	103 (0.68)	103 (0.68)	104 (0.89)	104 (0.67)	104 (0.64)
Hogs x Kar	37 (0.56)	46 (0.75)	54 (0.77)	68 (0.87)	81 (0.67)	87 (0.75)	93 (0.98)	98 (0.66)	99 (0.76)	99 (0.87)	101 (0.68)	101 (0.68)	103 (0.78)	103 (0.72)	104 (0.57)
Kar x Hogs	34 (0.78)	47 (0.88)	56 (0.76)	75 (0.45)	83 (0.89)	90 (0.59)	97 (0.87)	99 (0.67)	100 (0.84)	100 (0.86)	101 (0.84)	102 (0.68)	102 (0.68)	103 (0.74)	103 (0.68)

Table 11.3. Mean hind foot measurements from birth to 14 weeks of age of progeny resulting from the mating combinations indicated. Sample sizes as in Table 11.1. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof. 2 S.E. given in brackets.

Mating combinations	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Intrapopulation															
Committee's Drift	21 (0.66)	27 (0.67)	31 (0.67)	33 (0.87)	35 (0.78)	35 (0.78)	35 (0.78)	35 (0.78)	35 (0.78)	36 (0.87)	36 (0.87)	36 (0.87)	36 (0.87)	36 (0.87)	36 (0.87)
Hogsback	22 (0.87)	26 (0.87)	29 (0.68)	32 (0.64)	33 (0.64)	34 (0.67)	35 (0.72)	35 (0.72)	35 (0.72)	35 (0.72)	35 (0.72)	35 (0.72)	35 (0.72)	35 (0.72)	35 (0.72)
Karkloof	21 (0.72)	27 (0.68)	31 (0.74)	33 (0.72)	35 (0.68)	35 (0.66)	35 (0.66)	35 (0.66)	35 (0.66)	35 (0.66)	35 (0.66)	35 (0.66)	36 (0.68)	36 (0.68)	36 (0.68)
Interpopulation															
Comm x Hogs	22 (0.84)	25 (0.74)	27 (0.65)	28 (0.68)	32 (0.75)	33 (0.67)	34 (0.84)	34 (0.84)	34 (0.84)	34 (0.84)	35 (0.72)	35 (0.72)	35 (0.72)	35 (0.72)	35 (0.72)
Hogs x Comm	22 (0.87)	26 (0.86)	28 (0.67)	30 (0.72)	32 (0.78)	32 (0.78)	33 (0.64)	33 (0.64)	33 (0.64)	33 (0.64)	34 (0.68)	34 (0.68)	34 (0.68)	34 (0.68)	34 (0.68)
Comm x Kar	23 (0.68)	26 (0.78)	28 (0.87)	33 (0.75)	33 (0.75)	33 (0.75)	33 (0.75)	34 (0.64)	34 (0.64)	35 (0.68)	35 (0.68)	35 (0.68)	35 (0.68)	35 (0.68)	35 (0.68)
Kar x Comm	23 (0.85)	25 (0.67)	27 (0.74)	31 (0.68)	31 (0.68)	32 (0.64)	35 (0.63)	35 (0.63)	35 (0.63)	35 (0.63)	35 (0.63)	35 (0.63)	35 (0.63)	35 (0.63)	35 (0.63)
Hogs x Kar	23 (0.67)	26 (0.68)	28 (0.68)	30 (0.67)	30 (0.67)	31 (0.68)	33 (0.58)	34 (0.67)	35 (0.69)	35 (0.69)	35 (0.69)	35 (0.69)	35 (0.69)	35 (0.69)	35 (0.69)
Kar x Hogs	21 (0.75)	26 (0.68)	28 (0.72)	29 (0.74)	31 (0.72)	32 (0.67)	32 (0.67)	33 (0.72)	33 (0.72)	34 (0.68)	34 (0.68)	34 (0.68)	35 (0.68)	35 (0.68)	35 (0.68)

Table 11.4. Mean ear measurements from birth to 14 weeks of age of progeny resulting from the mating combinations indicated. Sample sizes as in Table 11.1. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof. 2 S.E. given in brackets.

Mating combinations	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Intrapopulation															
Committee's Drift	10 (0.68)	13 (0.68)	15 (0.92)	18 (0.87)	19 (0.84)	20 (0.89)	21 (0.89)	23 (0.84)	24 (0.82)	24 (0.82)	25 (0.72)	25 (0.72)	25 (0.72)	25 (0.72)	25 (0.72)
Hogsback	11 (0.72)	14 (0.68)	16 (0.68)	20 (0.65)	21 (0.65)	23 (0.68)	24 (0.67)	24 (0.67)	24 (0.67)	24 (0.67)	24 (0.67)	24 (0.67)	24 (0.67)	24 (0.67)	24 (0.67)
Karkloof	11 (0.68)	15 (0.76)	17 (0.67)	19 (0.63)	21 (0.63)	21 (0.63)	22 (0.58)	23 (0.75)	23 (0.75)	23 (0.75)	24 (0.65)	24 (0.65)	24 (0.65)	24 (0.65)	24 (0.65)
Interpopulation															
Comm x Hogs	12 (0.84)	14 (0.86)	17 (0.63)	21 (0.67)	23 (0.58)	24 (0.59)	24 (0.59)	24 (0.59)	24 (0.59)	25 (0.62)	25 (0.62)	25 (0.62)	25 (0.62)	25 (0.62)	25 (0.62)
Hogs x Comm	10 (0.89)	14 (0.63)	15 (0.60)	19 (0.64)	20 (0.67)	20 (0.67)	21 (0.64)	22 (0.72)	22 (0.72)	23 (0.67)	23 (0.67)	23 (0.67)	23 (0.67)	23 (0.67)	23 (0.67)
Comm x Kar	13 (0.66)	15 (0.68)	16 (0.72)	17 (0.62)	19 (0.63)	20 (0.68)	21 (0.67)	21 (0.67)	22 (0.69)	23 (0.72)	23 (0.72)	23 (0.72)	23 (0.72)	23 (0.72)	23 (0.72)
Kar x Comm	13 (0.68)	17 (0.62)	18 (0.67)	19 (0.68)	20 (0.67)	21 (0.72)	21 (0.72)	23 (0.68)	23 (0.68)	23 (0.68)	24 (0.75)	24 (0.75)	24 (0.75)	24 (0.75)	24 (0.75)
Hogs x Kar	11 (0.72)	13 (0.64)	15 (0.76)	16 (0.67)	17 (0.72)	19 (0.74)	21 (0.68)	22 (0.73)	22 (0.73)	23 (0.69)	23 (0.69)	23 (0.69)	24 (0.66)	24 (0.66)	24 (0.66)
Kar x Hogs	12 (0.78)	16 (0.67)	17 (0.64)	18 (0.63)	19 (0.75)	21 (0.70)	21 (0.70)	22 (0.68)	22 (0.68)	23 (0.59)	23 (0.59)	23 (0.59)	23 (0.59)	23 (0.59)	23 (0.59)



**APPENDIX 4**

Descriptive statistics and the results of statistical comparison (t-test) in respect of glans penis and baculum measurements of subjects resulting from reciprocal cross pairings are presented in Tables 12.1 to 12.4. Descriptive statistics in respect of spermatozoan head and tail measurements of subjects resulting from reciprocal cross pairings are given in Table 12.5, and the results of statistical comparison (t-test) in Table 12.6.

Table 12.1. Data in respect of glans penis structures (mm) of the crossbred subjects indicated. Committee's = Committee's Drift; n = sample size. 2 S.E. given in brackets.

Subjects	n	Greatest length		Lateral width		Tip width		Ventral width	
		$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range
[Committee's x Hogsback]	4	7.53 (0.15)	7.33-7.69	3.67 (0.05)	3.59-3.79	4.78 (0.09)	4.69-4.89	3.65 (0.08)	3.54-3.72
[Hogsback x Committee's]	3	7.55 (0.22)	7.38-7.76	3.80 (0.10)	3.72-3.91	4.59 (0.05)	4.54-4.62	3.61 (0.12)	3.51-3.71
[Committee's x Karkloof]	4	7.70 (0.12)	7.50-7.84	3.72 (0.23)	3.62-3.82	4.73 (0.11)	4.41-5.18	3.87 (0.06)	3.46-4.37
[Karkloof x Committee's]	4	7.57 (0.04)	7.52-7.61	3.91 (0.06)	3.82-3.96	4.80 (0.11)	4.69-4.89	3.82 (0.05)	3.56-3.95
[Hogsback x Karkloof]	5	7.39 (0.19)	7.28-7.51	3.79 (0.10)	3.56-4.01	4.69 (0.40)	4.61-4.81	3.69 (0.43)	3.62-3.74
[Karkloof x Hogsback]	3	7.64 (0.13)	7.41-7.78	3.85 (0.08)	3.72-3.96	4.69 (0.14)	4.62-4.89	3.70 (0.06)	3.62-3.77

Table 12.2. Calculated t values for differences between mean measurements for glans penis structures of the subjects indicated. No significant differences existed, so P is not given. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof; df = degrees of freedom.

Crossbred subjects compared	df	Greatest length	Lateral width	Tip width	Ventral width
[Comm x Hogs] vs [Hogs x Comm]	5	0.15	2.25	1.78	0.57
[Comm x Kar] vs [Kar x Comm]	6	2.08	1.60	0.90	1.28
[Hogs x Kar] vs [Kar x Hogs]	6	2.34	1.61	0.00	0.05

Table 12.3. Data in respect of baculum structures (mm) of the crossbred subjects indicated. Committee's = Committee's Drift. Sample sizes as in Table 12.1. 2 S.E. given in brackets.

Subjects	Greatest length		Greatest base width		Distal shaft width		Lateral base width		Base height		Lateral distal shaft width	
	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range
[Committee's x Hogsback]	6.51 (0.19)	6.48-6.72	1.77 (0.05)	1.71-1.83	0.63 (0.09)	0.60-0.68	1.05 (0.04)	0.63-1.27	2.32 (0.20)	2.05-2.55	0.69 (0.10)	0.59-0.81
[Hogsback x Committee's]	6.53 (0.21)	6.36-6.71	1.68 (0.10)	1.61-1.78	0.64 (0.06)	0.58-0.67	0.99 (0.04)	0.98-1.05	2.29 (0.02)	2.27-2.31	0.61 (0.04)	0.58-0.67
[Committee's x Karkloof]	6.30 (0.35)	5.85-6.67	1.61 (0.17)	1.47-1.84	0.68 (0.12)	0.51-0.76	0.99 (0.23)	0.75-1.31	2.15 (0.32)	1.74-2.49	0.57 (0.11)	0.41-0.66
[Karkloof x Committee's]	6.68 (0.26)	6.47-7.04	1.79 (0.06)	1.63-1.87	0.66 (0.04)	0.61-0.70	1.09 (0.10)	0.98-1.21	2.26 (0.10)	2.12-2.36	0.64 (0.05)	0.60-0.71
[Hogsback x Karkloof]	6.46 (0.06)	6.38-6.50	1.65 (0.05)	1.62-1.71	0.61 (0.03)	0.58-0.66	1.12 (0.08)	1.02-1.21	2.37 (0.08)	2.29-2.47	0.61 (0.02)	0.59-0.63
[Karkloof x Hogsback]	6.66 (0.21)	6.48-6.91	1.68 (0.10)	1.58-1.80	0.61 (0.06)	0.57-0.65	1.04 (0.01)	0.90-1.26	2.30 (0.02)	2.10-2.51	0.60 (0.04)	0.56-0.63

Table 12.4. Calculated t values for differences between mean measurements for baculum structures of the subjects indicated. No significant differences existed, so P is not given. Degrees of freedom as in Table 12.2. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof.

Crossbred subjects compared	Greatest length	Greatest base width	Distal shaft width	Lateral base width	Base height	Lateral distal shaft width
[Comm x Hogs] vs [Hogs x Comm]	0.14	1.59	0.18	2.22	0.29	1.30
[Comm x Kar] vs [Kar x Comm]	1.76	2.03	0.32	0.80	0.66	1.16
[Hogs x Kar] vs [Kar x Hogs]	1.85	0.53	0.00	1.97	1.67	0.51

Table 12.5. Data in respect of spermatozoan head and tail structures ( $10^{-2}$  mm) of the crossbred subjects indicated. Committee's = Committee's Drift; n = number of animals studied. 2 S.E. given in brackets.

Subjects	n	Perforatorium length		Head length		Greatest head length		Mid-tail length		Principal-tail length	
		$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range
[Committee's x Hogsback]	5	1.12 (0.06)	1.07-1.19	1.22 (0.03)	1.19-1.25	1.28 (0.04)	1.23-1.36	2.64 (0.03)	2.51-2.75	9.81 (0.09)	9.63-10.31
[Hogsback x Committee's]	3	1.15 (0.04)	1.12-1.18	1.19 (0.06)	1.13-1.23	1.31 (0.06)	1.26-1.37	2.61 (0.13)	2.50-2.72	9.89 (0.38)	9.58-10.23
[Committee's x Karkloof]	3	1.15 (0.02)	1.14-1.17	1.22 (0.04)	1.19-1.25	1.35 (0.03)	1.32-1.37	2.64 (0.08)	2.59-2.72	9.96 (0.25)	9.71-10.12
[Karkloof x Committee's]	4	1.12 (0.05)	1.10-1.15	1.22 (0.05)	1.11-1.23	1.35 (0.03)	1.32-1.41	2.61 (0.05)	2.52-2.94	10.01 (0.22)	9.79-10.33
[Hogsback x Karkloof]	4	1.10 (0.04)	1.06-1.14	1.23 (0.04)	1.19-1.27	1.29 (0.02)	1.27-1.33	2.57 (0.09)	2.46-2.67	9.87 (0.25)	9.73-10.13
[Karkloof x Hogsback]	4	1.13 (0.04)	1.07-1.17	1.21 (0.03)	1.18-1.24	1.31 (0.04)	1.26-1.36	2.63 (0.10)	2.58-2.78	10.05 (0.22)	9.77-10.27

Table 12.6. Calculated t values for differences between mean measurements for spermatozoan head and tail structures of the subjects indicated. No significant differences existed, so P is not given. Comm = Committee's Drift; Hogs = Hogsback; Kar = Karkloof; df = degrees of freedom.

Crossbred subjects compared	df	Perforatorium length	Head length	Greatest head length	Mid- tail length	Principal- tail length
[Comm x Hogs] vs [Hogs x Comm]	6	0.02	0.93	0.79	0.21	0.35
[Comm x Kar] vs [Kar x Comm]	5	1.12	0.00	0.00	0.64	0.31
[Hogs x Kar] vs [Kar x Hogs]	6	1.10	0.81	0.84	0.89	1.09