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Evidence of pre-mating reproductive isolation in two populations of the Vlei rat *Otomys irroratus*: experiments of intra- and interpopulation male-female encounters

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Abstract

Studied was the male-female interaction of representatives of two allopatric *Otomys irroratus* populations (Kamberg and Karkloof) in 40 intrapopulation and 34 interpopulation encounters. The study aimed to: (1) establish the existence of population-specific courtship behaviour during intrapopulation encounters; and (2) ascertain whether or not differences in courtship behaviour rendered males and females incompatible during interpopulation encounters. During intrapopulation encounters, Kamberg males and females were less aggressive and more amicable than their Karkloof counterparts, and Karkloof males performed more sexual acts than Kamberg males. These behavioural differences apparently reflect the social organization and mating behaviour of each population. Compared to intrapopulation pairings, interpopulation pairings displayed more exploratory behaviour, fewer sexual acts, more aggression, less amicability, and later development of essentially amicable interaction. It is possible that population-specific olfactory, visual and tactile cues resulted in highly aggressive interpopulation encounters. Aggression may function as a pre-mating reproductive isolating mechanism between the Kamberg and Karkloof populations should they meet in nature.

Introduction

Pre-mating reproductive isolating mechanisms prevent interbreeding between males and females of closely-related species. The most effective pre-mating isolating mechanisms are ethological ones (Dobzhansky et al. 1968), particularly courtship behaviour (Koepfer 1987). Species-specific courtship behaviour permit conspecific males and females to recognize only one another as potential mates, thereby ensuring positive assortative mating (Beiles et al. 1984).

Mutual recognition between conspecific mates during courtship is normally mediated by means of male-female signals and responses, which may be referred to as the specific-mate-recognition system (i. e. SMRS; Paterson 1980, 1985). The signal-response chain comprises all behaviour patterns leading to copulation (McFarland 1987), and includes several modalities of communication (e. g. odour, postural changes; Carter and Brand 1986; Koepfer 1987; Dempster et al. 1992).

Previous experiments on mate recognition in two allopatric populations (from Kamberg and Karkloof) of the vlei rat *Otomys irroratus* has revealed preference for mates of the same population or their odours (Pillay et al. 1995). It was predicted that population-specific courtship behaviour, and olfactory cues in particular, influenced mate recognition in Kamberg and Karkloof *O. irroratus.*

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The present study aimed to investigate male-female interactions in intrapopulation encounters to establish the existence of population-specific courtship in Kamberg and Karkloof *O. irroratus*. An additional aim of the study was to ascertain whether or not differences in the courtship behaviour (i. e. differences in SMRSs) rendered representatives of these two populations behaviourally incompatible during interpopulation encounters. Such incompatibility would indicate the existence of pre-mating barriers to reproduction.

Material and methods

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

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

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

Prior to observations, an enclosure was divided into two parts with a wire mesh partition. A female in pro-oestrus, ascertained by means of vaginal smears, and a male were placed on either side of the partition at 18.00 h. Following a familiarization period of approximately 14 h (i. e. approximately 08.00 h the next day, when females usually displayed oestrus), the partition was removed and the first 20 min of interaction video-recorded. Testing coincided with the period of maximum diurnal activity, from 07.00 h to 09.00 h. Recordings were made under fluorescent white light, using a Hitachi KP-141 CCTV camera and a Hitachi VTL-30ED time-lapse video cassette recorder.

Pillay (1990) showed that male-female interactions in staged encounters involving three *O. irroratus* populations differed at both the intra- and interpopulation level even after two weeks of pairing. Consequently, additional video recordings of the interactions of each pair in the present study were made every two days from the day of pairing (designated Day 0), for 12 days. Recordings under incandescent red lights were conducted during the dark phase of the light cycle from 00.00 h to 01.00 h, which represented the period of maximum nocturnal activity.

All animals were sexually experienced. Members of a pair had never previously met in the laboratory. Each male was used in two encounters – one intrapopulation and one interpopulation encounter. Females were used only once.

Analysis of video recordings made during the first 20 min of interaction entailed first encoding 18 male and 15 female behaviour patterns. Thereafter, all behavioural acts occurring in each 10-s interval of the 20 min observation period were recorded. Acts were identified when animals changed their behaviour (after Dempster et al. 1992).

The frequencies of all behaviour patterns of males and females of each pairing were calculated. Mean frequencies were obtained for each sex in respect of intrapopulation or interpopulation pairings.

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

Results obtained from diurnal and nocturnal recordings were treated separately because of the different time scales involved (i. e. 20 min and on 7 nights). Data from diurnal experiments were tested for significance using the Mann-Whitney U test (Sokal and Rohlf 1987); significant differences were accepted at $P < 0.05$. Data obtained from the nocturnal study illustrated trends that were sufficiently clear to obviate the need for statistical analysis.

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Results

Behaviour patterns identified in encounters are presented in table 1. All acts were displayed by both sexes, although follow-mounting and presenting were sex-specific activities (Tab. 1).

Table 1. Behaviour patterns identified in encounters. Those behaviour patterns that were combined to allow comparison between diurnal intrapopulation and interpopulation encounters are indicated as subsets A – D; see text.

Explore arena
Self-groom
Mark
Watch
Inactive
Naso-nasal contact
A) Agonistic
Aggressive approach
Chase
Upright sparring
Submissive flee
B) Amicable
Amicable approach
Groom invitation
Allogroom
Huddle
C) Follow-mount (males only)
Naso-anal contact
Follow
Attempted mount
Mount
D) Present (females only)
Move away
Dart
Present and lordosis

Interpopulation same-sex comparisons of the frequencies of behaviour patterns recorded in intrapopulation encounters revealed significant differences in respect of six male and four female acts. These acts represented mainly agonistic and amicable behaviour. Compared to their Karkloof counterparts, Kamberg males were involved in significantly less upright sparring ($\bar{x} \pm 2$ SE: 2.4 ± 1.4 vs 6.8 ± 2.0), and displayed significantly higher levels of amicable approach (19.8 ± 2.0 vs 14.9 ± 2.8), allogroom (4.5 ± 1.0 vs 1.4 ± 1.3), and huddle (14.2 ± 4.7 vs 5.3 ± 2.7) behaviour patterns. In contrast, Karkloof males performed significantly more following (10.5 ± 3.0 vs 6.6 ± 2.5) and attempted mounting (12.7 ± 2.0 vs 8.5 ± 1.3) acts than Kamberg males. Kamberg, compared to Karkloof, females displayed significantly less flee behaviour (4.3 ± 1.5 vs 7.5 ± 0.9), and were involved in significantly more grooming invitation (5.2 ± 2.4 vs 2.0 ± 1.2), allogrooming (6.0 ± 3.2 vs 1.4 ± 1.3) and huddling (14.2 ± 4.7 vs 5.3 ± 31.3) activities.

Mean frequencies of behaviour patterns recorded during diurnal interpopulation and intrapopulation encounters are compared in respect of males in figure 1 and females in figure 2. Preliminary examination of the data indicated a low frequency of some behavioural patterns during interpopulation encounters. These acts were therefore combined with other motivationally similar acts (see Tab. 1) in figures 1 and 2.

Both sexes spent a large proportion of time at opposite ends of the arena, and hence the frequency of inactive behaviour was higher than that of other behaviour patterns (Fig. 1, 2). Significantly higher levels of aggression and significantly lower levels of amicability were recorded in interpopulation than in intrapopulation encounters. There was a marked decrease in sexual activity during interpopulation encounters, as exemplified by fewer follow-mount acts by males and fewer presenting acts by females. Interestingly, exploratory behaviour by all animals was significantly lower during inter- than intrapopulation encounters (Fig. 1, 2).

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

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For every pairing, levels of agonistic interaction were highest soon after animals were paired, and none of the pairs immediately displayed high levels of amicability (i. e. Day 0; Fig. 3). It is evident in all cases that levels of agonistic interaction decreased during encounters, with a corresponding increase in levels of amicability. The most important fea-

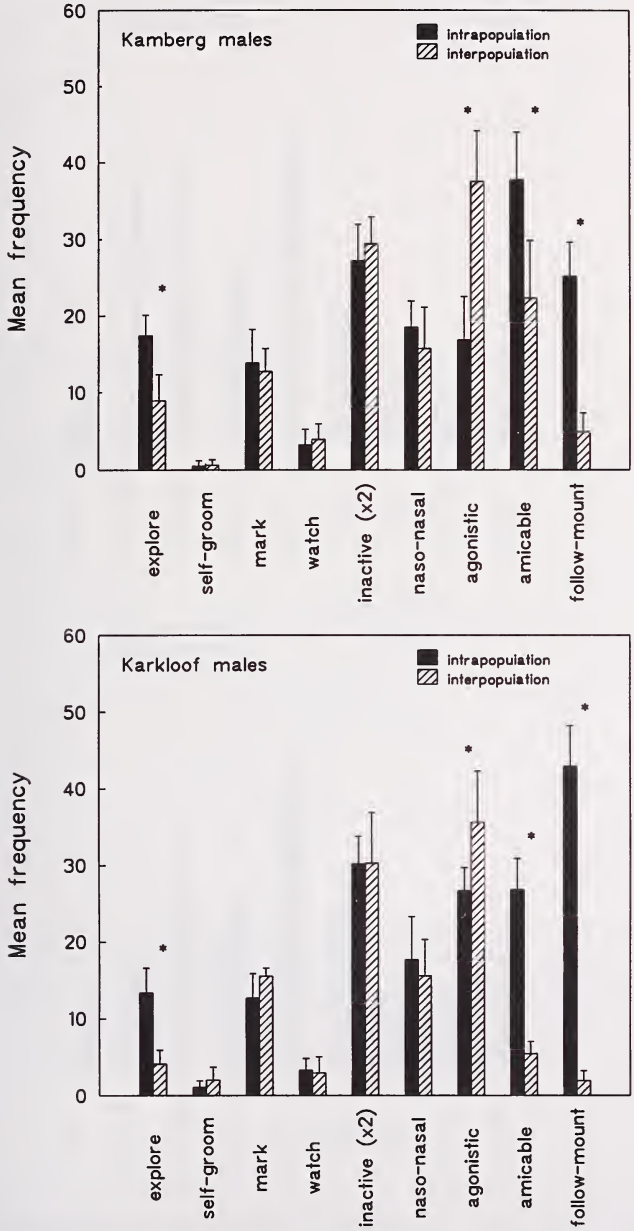

Fig. 1. Comparison of mean frequencies of behaviour patterns of males during the first 20 min of intrapopulation and interpopulation encounters. Inactive (×2) = twice the frequency value indicated. Error bars = 2 SE of the mean. * = values which differed at the 5% significance level, Mann-Whitney U test.

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ture illustrated in figure 3 is the variation in the time taken to the point of intersection of the curves representing agonistic and amicable interaction. Kamberg pairs displayed equal levels of amicable and agonistic interaction sooner than any other pairing (i.e. before Day 2), while the Karkloof pairs reached this stage just before Day 3. The curves re-

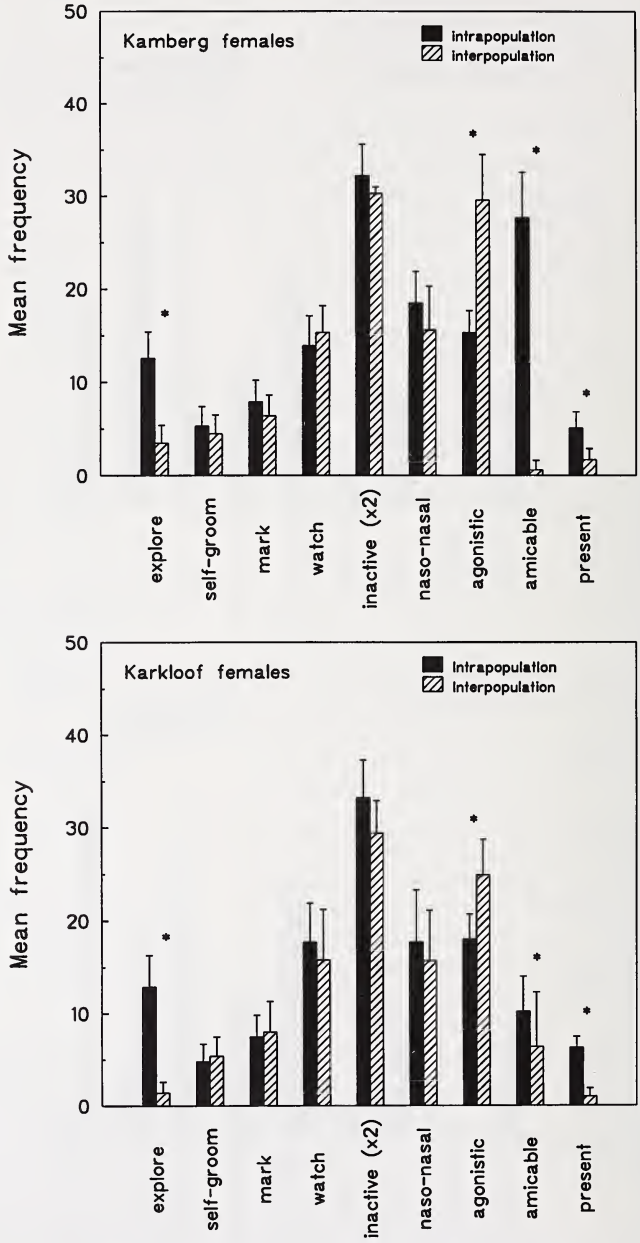

Fig. 2. Comparison of mean frequencies of behaviour patterns of females during the first 20 min of intrapopulation and interpopulation encounters. Inactive (×2) = twice the frequency value indicated. Error bars = 2 SE of the mean. * = values which differed at the 5% significance level, Mann-Whitney U test.

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presenting agonistic and amicable interaction of the interpopulation pairings intersected at approximately Day 6, and by Day 12 higher levels of agonistic interaction were recorded in interpopulation than in intrapopulation encounters (Fig. 3).

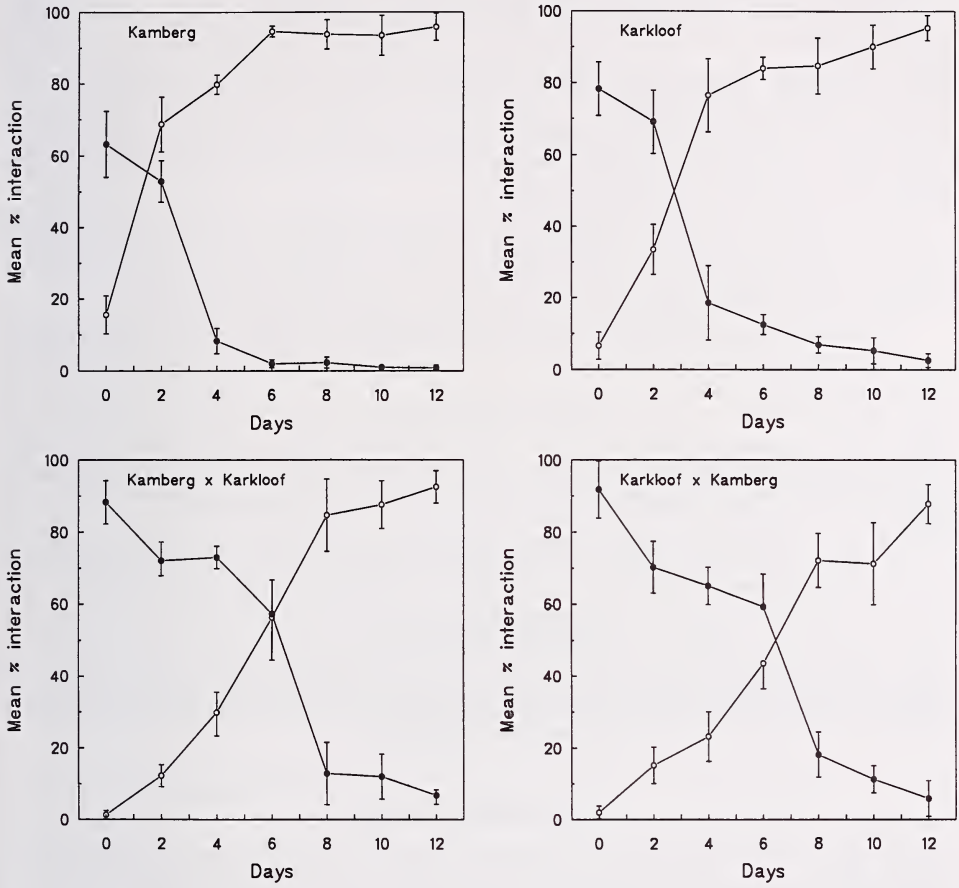

Fig. 3. Mean percentage nocturnal agonistic (filled circles) and amicable (open circles) interaction for the intrapopulation and interpopulation pairings indicated. Error bars = 2 SE above and/or below the mean. Sample size = 20 each for the intrapopulation pairings. Sample size for Kamberg × Karkloof and Karkloof × Kamberg pairs = respectively 18 and 16 on Day 0, and 9 and 12 from Day 2 to 12.

Discussion

The results obtained in this study demonstrate the existence of population-specific courtship behaviour. In addition, it appears that recognition as potential mates by members of the Karkloof, Kamberg × Karkloof and Karkloof × Kamberg pairs was comparatively delayed. Possible explanations of this conclusion as regards intrapopulation and interpopulation encounters are provided below.

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Intrapopulation pairings

The differences in the interactions of Kamberg and Karkloof intrapopulation pairs may have been due to underlying disparities in population-specific social organization and mating behaviour.

The social organization of the Karkloof population (Pillay 1993) appears to be similar to that of other *O. irroratus* populations (Davis 1973; Willan 1982; Brown 1988). Breeding females are intrasexually aggressive and appear to be intrasexually highly territorial, while males have intrasexually overlapping home ranges and have dominance hierarchies. The home ranges of males overlap those of females. In contrast, Kamberg males are intrasexually highly aggressive, and they appear to defend their territories from other males (Pillay 1993). There is a high degree of social tolerance among Kamberg females, whose territories may overlap (Pillay 1993).

On the basis of the social organization of both populations, it has been hypothesized that the mating systems of Kamberg and Karkloof populations are polygynous and promiscuous respectively (Pillay 1993): Kamberg males apparently have exclusive access to several females, while Karkloof males compete for access to receptive females.

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

In polygynous species, males maintain intrasexually exclusive territories which they would have previously established by means of male-male competition (Shapiro and Dewsbury 1986). In order to ensure mating with a genetically-fit male, females simply mate with the male within whose territory they occur (Emlen 1976; Shapiro and Dewsbury 1986). Therefore, these females may be less discriminating during mate choice than promiscuously-mating females, potentially resulting in reduced aggression and higher amicability during the courtship of polygynous species.

Interpopulation pairings

The results of the interpopulation encounters support the prediction that both Kamberg and Karkloof individuals discriminate between mates from the same and the other population. Increased sexual activity and reduced aggression in intra- relative to interspecific encounters were apparently indicative of mate recognition in *Gerbillurus* species (Dempster et al. 1992).

The somewhat reduced exploratory behaviour observed in interpopulation pairings may have been the result of pairs engaging in increased agonistic interaction. Similarly, male-female interactions involving *Microtus pennsylvanicus* and *M. pinetorum* (Cranford and Derting 1983) indicated that, unlike intraspecific pairs, interspecific pairs favoured aggressive and contact-orientated behaviour over exploratory activity.

Failure to recognize specific auditory, olfactory, tactile and/or visual cues may result in high levels of aggression during interspecific encounters of closely-related rodent species, and may lead to delayed recognition between potential mates (Bauer 1956; Godfrey 1958; Scott 1966; Koepfer 1987). Consequently, differences in behaviour between intra- and interpopulation Kamberg and Karkloof pairings, as exemplified by increased aggression during interpopulation pairings, may be directly attributable to contrasting courtship behaviour, and, in particular, population-specific modes of communication.

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Kamberg and Karkloof individuals preferred odours of same-population mates in olfactory discrimination experiments (Pillay et al. 1995), demonstrating that olfactory cues are important in mate recognition. In the present study, the importance of olfactory cues in courtship was indicated by high frequencies of naso-nasal and naso-anal contact in all encounters.

Except for inactive behaviour and possibly exploratory behaviour, all other behaviour patterns observed in encounters constituted tactile and visual signals (Davis 1972; Willan 1982; Pillay 1990). Hence, tactile and visual communication appear to be important in the courtship behaviour of both populations.

As in previous studies of mate recognition involving Kamberg and Karkloof populations (Pillay 1993; Pillay et al. 1995), the results of the present study indicate the existence of population-specific courtship behaviour. Differences in courtship behaviour (i. e. an important SMRS) are clearly reflected in interpopulation encounters where underlying differences in population-specific olfactory, tactile and visual cues apparently resulted in high levels of agonistic interaction during interpopulation encounters. The role of aggression as a pre-mating reproductive isolating mechanism has been identified in chromosomal races of *Spalax ehrenbergi* (Nevo et al. 1986) and in populations of *Mus musculus* (Corti et al. 1989). Aggression may function also as a pre-mating barrier to breeding between Kamberg and Karkloof individuals should the populations meet in nature.

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Zusammenfassung

Hinweise für einen Isolationsmechanismus der Reproduktion in der Vorpaarungszeit bei zwei Populationen der Ohrenratte Otomys irroratus: Experimente zu Begegnungen der Geschlechter innerhalb und zwischen Populationen

Untersucht wurden Interaktionen zwischen Männchen und Weibchen von Individuen aus zwei Populationen der allopatrischen Ohrenratte *Otomys irroratus* (Kamberg- und Karkloof-Population). 40 Zusammentreffen von Individuen gleicher Population und 34 von Angehörigen verschiedener Populationen konnten durchgeführt werden. Während der Zusammentreffen von Tieren gleicher Population waren Kamberg-Männchen und -Weibchen friedlicher und weniger aggressiv als die Karkloof-Tiere, und Karkloof-Männchen zeigten ein stärkeres Sexualverhalten als die Kamberg-Männchen. Diese unterschiedlichen Verhaltensweisen spiegeln möglicherweise eigene Sozialstrukturen und Besonderheiten im Paarungsverhalten der jeweiligen Population wider.

Verglichen mit Paarungen von Individuen gleicher Populationen zeigten die Tiere bei Paarungen von Angehörigen verschiedener Populationen verstärktes Erkundungsverhalten sowie geringeres Sexual-, stärkeres Aggressions- und weniger Demutsverhalten und gleichzeitig eine verzögerte Entwicklung von Demutsverhalten. Es ist möglich, daß populationsspezifische olfaktorische, visuelle und taktile Besonderheiten zu diesen aggressiven Begegnungen zwischen den Populationen führten. Im Falle, daß sich Individuen der Kamberg- und der Karkloof-Populationen in freier Wildbahn begegnen, könnten sich in der Vorpaarungszeit die Aggressionen als Isolationsmechanismen auswirken.

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