

# Cooperative Breeding in Groups of Synchronously Mating Females and Evolution of Large Testes to Avoid Sperm Depletion in African Striped Mice<sup>1</sup>

C. Schradin,<sup>2,3,4</sup> A.A. Kinahan,<sup>4</sup> and N. Pillay<sup>4</sup>

Zoological Institute,<sup>3</sup> University of Zurich, Zurich, Switzerland

School of Animal, Plant and Environmental Sciences,<sup>4</sup> University of the Witwatersrand, Johannesburg, South Africa

## ABSTRACT

Testis size has been related to the mating system of both vertebrates and invertebrates. These differences are regarded as adaptive responses to sperm competition in promiscuously mating species. However, not all variation in testis size can be explained by sperm competition. Here, we test the hypothesis that the evolution of large testes occurs when synchronously breeding females must be fertilized within a short period of time to avoid reproductive competition among the females. African striped mouse (*Rhabdomys pumilio*) males of a polygynous population with cooperative breeding and high risk of sperm depletion had testes and cauda epididymis twice as large as those of males of four different promiscuous populations with high risk of sperm competition. When paired with three females simultaneously in captivity, males of the polygynous population bred with three females within 8 days, leading to synchronous births in their harems, thereby potentially reducing the risk of infanticide. Males from the promiscuous population reproduced with only one or two females within 8 days, and births were not synchronous. We conclude that large testes are selected for in species with synchronously mating females, which is ultimately beneficial for the evolution of cooperative breeding.

behavior, communal breeding, reproductive skew, *Rhabdomys*, sperm, sperm competition, sperm depletion, testis

## INTRODUCTION

When females mate with more than one male simultaneously, the potential for sperm competition arises [1]. As a result, promiscuous species, such as the chimpanzee, have much larger testes than monogamous or polygynous species in which a single male mates with one or with several females successively, such as the gorilla [2–4]. Males of the promiscuous wild guinea pig *Galea musteloides* have much larger testes than males of the polygynous wild guinea pig *Cavia apera* [5–7]. Dung flies (*Scathophaga stercoraria*) kept under polyandrous circumstances for 10 generations evolved much larger testes than those kept monogamously [8]. Many studies demonstrate correlations between testis size and correlates of sperm competition, such as group size and mating system [3, 8–15] and show that males with larger testes sire

more offspring under conditions of sperm competition [16]. In addition, in several rodent species, males can adjust the size of their ejaculates depending on the risk of sperm competition—for example, as a consequence of perceived olfactory cues from other males [17, 18].

A large variation in testis size is found among species, which can be explained to some extent by sperm competition, but significant variation remains unexplained [3, 19, 20]. In addition to sperm competition, testis size might be influenced by sperm depletion. The sperm depletion hypothesis proposes that males of taxa that copulate often have relatively larger testes [21, 22]. Males must trade off between costly sperm production and investment in other life-history traits, such as parental care [10] or energy invested into searching for a mate. In mammals (but not in all other taxa [23]), the sperm production rate per gram parenchyma is constant (i.e., independent of testis size) such that larger testes produce more sperm and can provide a larger sperm reserve in the cauda epididymis [19, 24]. Thus, within a single species, males with larger testes can produce more sperm, giving them an advantage under conditions of sperm competition [24]. Additionally, males with larger testes also can ejaculate more often. In other words, theoretically, they can copulate with and inseminate more females, because they are less likely to experience sperm depletion [16].

In the present study, we compare testis size between males of a single species that experience a high degree of sperm competition or are at a high risk of sperm depletion. Striped mice (*Rhabdomys pumilio*; adult body mass, 30–80 g; diurnal [25]) show high intraspecific variation in their mating systems [26, 27]. In moist grasslands, characterized by a 6-mo breeding season and low population density, striped mice live solitarily [27]. Females defend territories against other females while males follow a roaming tactic, having home ranges twice as large as those of females. Male home ranges overlap with the territories of several females, and the territory of each female overlaps with those of several males. Males and females only associate for mating, and females can mate with several males, leading to a high risk of sperm competition [27–29]. Females from these grassland populations display synchronous estrus in captivity [30], and some indication of reproductive synchrony exists in nature [31]. In contrast, striped mice show a system of harem defense polygyny in the semidesert Succulent Karoo (Africa), characterized by a short, 3-mo breeding season and high population density [25]. Groups of up to four closely related, cooperatively breeding females (as indicated by genetic studies; Schradin and Lindholm, unpublished data) are defended by a single territorial, breeding male [32, 33]. Males and females of one group share one territory and have home ranges of similar size [27, 33]. In contrast to the roaming males from the grasslands, semidesert males invest in territorial defense [32, 33], in particular by patrolling their territorial boundaries [34] and aggressively repelling male intruders [32]. Thus, males of this polygynous population are thought to

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<sup>2</sup>Correspondence: C. Schradin, Zoological Institute, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland.  
FAX: 0041 44 6355490; e-mail: carsten.schradin@zool.uzh.ch

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experience lower levels of sperm competition. The females in their harems breed synchronously [33]; that is, males must be able to mate with up to four females synchronously.

In the current study, we tested the following hypothesis: If sperm competition is a more important selection pressure than sperm depletion in striped mice, males from the promiscuous population should have larger testes. In contrast, if the risk of sperm depletion is more important, males from the polygynous population should have larger testes. We demonstrated that males from the polygynous population had much larger testes, and we conducted an experiment to examine whether this could, in fact, result from sperm depletion by testing the following prediction: Males with larger testes should be more successful in fertilizing two or three females synchronously than males with smaller testes.

## MATERIALS AND METHODS

### Ethics

The present research adhered to the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*, the Association for the Study of Animal Behavior Guidelines for the Use of Animals in Research, and the legal requirements of South Africa (animal ethical clearance AESC 2003/106/3, 2006/19/2a, University of the Witwatersrand).

### Experiment 1: Testis Size

We measured testis mass and volume as well as cauda epididymis mass in males from four promiscuous and one polygynous populations. To date, only one striped mouse population has been reported to be group-living and polygynous (from Goegap Nature Reserve, Northern Cape Province, South Africa; 29.40°S, 17.53°E), which we used in the present study. Promiscuous populations were represented by three localities from South Africa—Pretoria (Gauteng Province; 25.40°S, 28.31°E), Suikerbosrand (Gauteng Province; 26.30°S, 28.15°E), and Kamberg (KwaZulu Natal Province; 29.23°S, 27.42°E)—and one population from Zimbabwe—Inyanga (18.12°S, 34.40°E). The approximate geographic distance between the localities from which the promiscuous populations were derived ranged from 80 to 1000 km. Striped mice from Goegap ( $n = 10$ ), Pretoria ( $n = 13$ ), and Suikerbosrand ( $n = 10$ ) were captive-bred ( $F_4$ ,  $F_3$ , and  $F_3$  generation, respectively) and kept under identical standardized captive conditions. To avoid any social influence on testis size, males were kept singly for at least 6 wk before measurements. Data from Kamberg ( $n = 10$ ) and Zimbabwe ( $n = 10$ ) represent field data collected by N. Pillay in 1993–1998.

Males were killed (overdosed with intramuscular injections of 200 mg of sodium pentobarbitone and 2% benzyl alcohol at a dosage of 1 ml/kg body mass). They were weighed to the nearest 0.1 g, and their head-body length was determined to the nearest 1 mm. Immediately thereafter, both testes were dissected out, and the combined testis mass (excluding the epididymis) and the paired cauda epididymis mass were recorded to the nearest 0.001 g. We determined testis volume by measuring the greatest length and width of each testis and applying the following ellipsoid formula:  $4/3\pi w^2l$ , where  $w$  = testis width and  $l$  = length. Linear measurements were taken with digital calipers to the nearest 0.01 mm. We were careful not to distort the testes during measurements, and linear measurements were determined from an average of three measurements per testis. Combined testis volume was determined by adding the volume of both testes.

### Experiment 2: Breeding Studies

*Study subjects.* Striped mice used in the present study were  $F_4$  generation individuals derived from Goegap Nature Reserve (designated polygynous striped mice) and  $F_3$  generation individuals from Pretoria (designated promiscuous striped mice). They were housed under partially controlled environmental conditions (light regime, 14L:10D; lights-on, 0500 h; temperature, 20–24°C; relative humidity, 30–60%). The floor of cages (for details, see below) was covered with a layer of wood shavings for bedding. One or more plastic nest boxes (27 × 20 × 17 cm) were provided, depending on the number of individuals housed together. Dry grass and paper towel were provided twice weekly for nesting material. One or more cardboard toilet roll/paper cups and twigs were provided weekly for enrichment. Subjects had access to water and Epol (Epol) mouse cubes ad libitum. The diet was supplemented with fresh fruit

or vegetables daily and approximately 5 g of seed at least twice a week per individual

*Relationship between testis size and reproductive output.* To assess the reproductive output of males, 14 adult males per population (Goegap and Pretoria) were subjected to an experimental and a control breeding treatment, which were conducted in random sequence. All males were kept singly for at least 8 wk before experiments. In the experimental treatment, a male was paired simultaneously with three sisters from his own population for 8 days. Female striped mice have synchronous estrus, the estrous cycle is 4 days long [30], and mating occurs during 1 day of the estrous cycle [35]. Thus, the 8 days of pairing was sufficient time for males to mate with all three females during one estrous cycle [30]. Striped mice were housed in glass tanks (46 × 30 × 32 cm). Groups were observed for approximately 15 min daily for signs of aggression and distress. After 8 days, females and the male were examined for evidence of damaging fights (e.g., wounds), the group was separated, and the females were housed individually in plastic holding cages (36 × 16 × 20 cm) until parturition. In the control, a male was paired with three sisters successively. These females were of similar ages to those in the experimental treatment, were housed individually in plastic holding cages (36 × 16 × 20 cm), and each was paired in turn (randomly determined based on the outcome of a random number generator) with the male of their own population for 8 days each. Males did not have a rest period between transfer to the next female. All males were sexually experienced, but all females were virgins and housed together from weaning (Day 21) until they were used in experiments (i.e., >100 days old). Males and females had not made prior contact and were unrelated.

In both treatments, we recorded the number of females that produced a litter and then calculated the frequency distribution of females that reproduced per population (i.e., the frequency at which all three, two, or only one female produced offspring; because at least one female per male per treatment reproduced, we did not include a zero-female category). We also recorded the litter size of all females that reproduced and the dates on which they gave birth to test whether births in replicates were synchronized. At the end of both experiments, testes and cauda epididymides were weighed and measured as described above.

*Testing for reproductive suppression.* An alternative explanation for reduced reproductive success of promiscuous males when paired with three females simultaneously (see *Results*) could be reproductive suppression within such female groups, either by olfactory cues or by social stress [36–39]. Whereas females from the polygynous population form cooperatively breeding groups in the field with no obvious reproductive skew [33], such groups are not observed in the promiscuous population. Therefore, the dominant female may be able to suppress reproduction in the other females of the promiscuous population only, which could explain why mainly only one of the three females bred.

We tested whether females of the promiscuous population from Pretoria reproductively suppressed each other by ascertaining the reproductive success of three breeding pairs housed in close proximity. For this, we used a circular apparatus (diameter, 1 m). The device was constructed with 3 mm of Plexiglas, was 45 cm high, and was covered with a wire-mesh lid. Internally, the device was divided into three equal-sized, pie-shaped segments with wire-mesh partitions. Each segment contained a breeding pair. The wire-mesh barrier prevented physical contact between the pairs, but females could see and smell their two neighboring sisters. We first kept three sisters together, then paired them individually for 8 days (1–2 estrous cycles) while still keeping them in close proximity, directly neighboring one another and separated by only a wire mesh. Thus, olfactory communication was maintained, and the experiment was short enough that the effects of social stress (during previous cohabitation) could still have been detected. A total of 10 replicates were run. Each replicate contained three sisters that were kept together until the start of the experiment such that they knew each other and a dominance hierarchy with reproductive suppression could already have been established before the start of the experiment. Each female was paired with an unrelated male, which had not made previous contact with one another or the females. Each replicate was run for 8 days, after which the females were housed individually. We recorded how many females in each replicate produced offspring. The apparatus was thoroughly washed and dried between replicates.

*Testing for importance of synchronized births for infanticide avoidance.* We kept groups of striped mice in our captive colony at a research station in South Africa under seminatural conditions, as described in detail elsewhere [40]. We collected data for polygynous groups consisting of two full sisters and one unrelated male. Polygynous groups were kept in two glass tanks (49 × 34 × 40 cm) connected by a tube. Altogether, we kept 40 polygynous groups from 2006 to 2008, and both females produced pups in 37 of these groups. We recorded birth dates to calculate the intervals between births of the two females in a group, and we noted any indications of infanticide (i.e., killed or partly eaten pups).

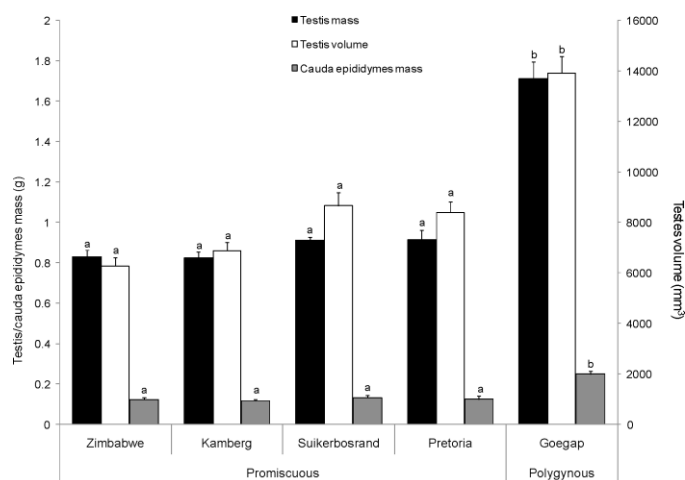


FIG. 1. Mean  $\pm$  SEM of testis mass and volume and cauda epididymis mass of male striped mice (*Rhabdomys pumilio*) originating from four promiscuous populations and one polygynous population. For each variable, different lowercase letters indicate significant differences.

### Statistics

Analyses were performed using Statistica 7.1 (StatSoft, Inc.). All data sets were tested for normality (Shapiro-Wilk test and homogeneity of variances) before statistical analyses. All tests were two-tailed, and  $\alpha = 0.05$ . Values are given as the mean  $\pm$  SEM throughout.

A general linear model (GLM) with a multivariate design was used to compare testis mass and volume as well as cauda epididymis mass among males of the five populations. Body mass and body length were included as continuous predictors (covariates) in the analysis to account for their influence on testis/cauda epididymis mass and testis volume. We included both body mass and body length, because they were not correlated (for the breeding experiment:  $r = 0.22$ ,  $n = 27$ ; Pearson correlation,  $P = 0.26$ ). Also, we expected testis mass and cauda epididymis mass to scale with body mass and testis volume with body length.

A generalized linear model (GLZ) with an ordinal multinomial error structure and a probit link function was used to analyze the frequency distribution of females reproducing by population and treatment (i.e., three, two, and one female). In these analyses, significance was determined using Wald statistics, and estimate coefficients ( $\beta$ ) were used to assess the strength of each independent factor on the dependent variable when the Wald statistic was significant.

Total numbers of offspring sired by males of both populations were compared using a GLM with a repeated-measures design, in which treatment was the repeated-measures variable. We also included the residuals of testis mass and volume as continuous predictors (covariates) in the model. Tukey post hoc tests were used to identify specific differences. We also regressed testis mass and cauda epididymis mass against body mass and testis volume against head-body length and plotted the residuals of these values for males used in the breeding experiments.

The difference in time taken for the three females in each triad to produce offspring was compared between polygynous and promiscuous females using a Student *t*-test. Fisher exact tests were used to analyze the proportion of females that produced offspring on the same day (i.e., a test for synchrony) and the proportion of pairs that reproduced in the test for reproductive suppression.

The interbirth interval between the two females of polygynous groups was compared between groups with and without infanticide using a Student *t*-test.

## RESULTS

### Experiment 1: Testis Size

Comparisons of testis mass and volume and cauda epididymis mass revealed that males from the polygynous population had significantly larger testes than males from the promiscuous populations ( $F_{12,116} = 7.90$ ,  $P < 0.001$ ) (Fig. 1), and no difference was found among males of the promiscuous populations (Tukey post hoc tests). No influence of body mass ( $F_{3,44} = 1.13$ ,  $P = 0.347$ ) and body length ( $F_{3,44} = 0.48$ ,  $P =$

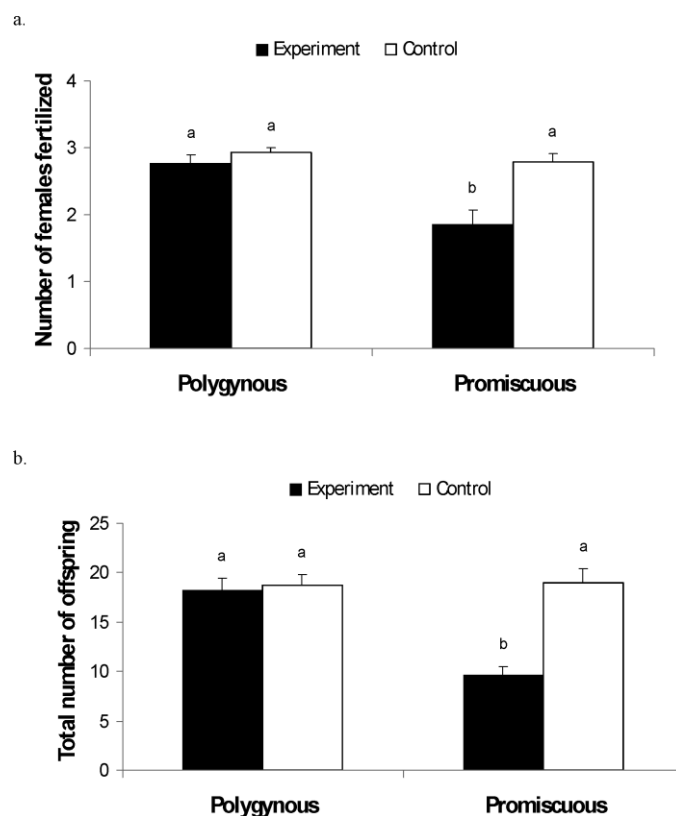


FIG. 2. a) Number of females that a male fertilized (mean  $\pm$  SEM) when paired with three sisters simultaneously or successively. Only polygynous males were successful in fertilizing three females synchronously, whereas promiscuous males only succeeded in fertilizing three females when they were paired with them in succession, not simultaneously. b) The reproductive output of males of a polygynous and promiscuous population of striped mice (*Rhabdomys pumilio*). Number (mean  $\pm$  SEM) of offspring produced is shown. Bars with different symbols are significantly different. Filled bars = experiment (a male was paired with a group of three females for 8 days); open bars = control (a male was paired with three single females and spent 8 days with each female successively).

0.700) was observed on testis mass and volume and epididymis mass. Males from the polygynous population had 2.0-fold heavier testes ( $1.71 \pm 0.18$  vs.  $0.87 \pm 0.03$  g) and 1.8-fold larger testis volume ( $13924 \pm 1412$  vs.  $7555 \pm 407$  mm<sup>3</sup>) than promiscuous males. Polygynous males also had 2.0-fold heavier cauda epididymis mass than promiscuous males ( $0.25 \pm 0.01$  vs.  $0.125 \pm 0.01$  g).

### Experiment 2: Breeding Studies

**Reproductive success and sperm depletion.** We did not observe any aggression in female triads of both the polygynous and promiscuous populations during the 8 days of cohabitation, and no individuals from any populations had wounds indicating aggression. Most (11 of 14) of the males from the polygynous population were able to fertilize all three females within 8 days, and the remaining three males fertilized two females (Fig. 2a). This was in contrast to the males of the promiscuous population: None managed to fertilize all three females, only six males managed to fertilize two females, and the remaining eight males fertilized only one female each (Fig. 2a). However, when males paired with three females in succession (8 days with each female), males from the promiscuous population were as successful in fertilizing three females as males from the polygynous population had been

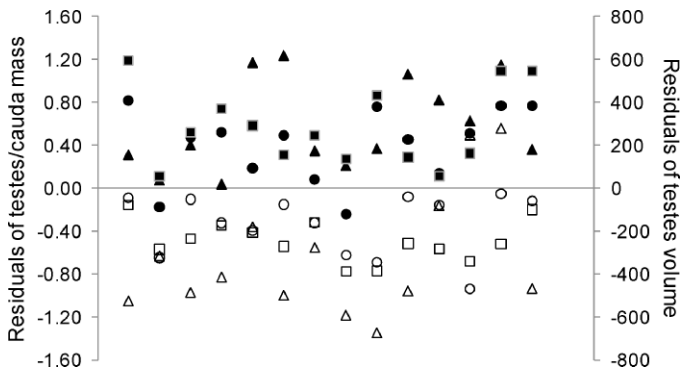


FIG. 3. Comparison of the testis mass (circles), volume (squares), and cauda epididymis mass (triangles) between males of a polygynous (closed markers) and a promiscuous (open markers) population of striped mice (*Rhabdomys pumilio*). The values represent residual values derived from the regression of testis mass and cauda epididymis mass against body mass and of testis volume against head-body length. Cauda epididymis mass values were multiplied by 10 for illustrative purposes.

when paired with three females simultaneously for 8 days (Fig. 2a).

A generalized log-linear model confirmed that population (Wald  $\chi^2_1 = 6.12$ ,  $P = 0.013$ ), treatment (Wald  $\chi^2_1 = 6.82$ ,  $P = 0.009$ ), and population  $\times$  treatment (Wald  $\chi^2_1 = 4.10$ ,  $P = 0.043$ ) were all significant predictors of the frequency distribution of reproducing females (one, two, or three reproducing females). In particular, males from the polygynous population had greater success in fertilizing females than males of the promiscuous population ( $\beta = 0.71$ , SEM = 0.28,  $P = 0.010$ ), males in successive matings had greater success than those in simultaneous pairings ( $\beta = 0.76$ , SEM = 0.58,  $P = 0.003$ ), and males from the promiscuous population paired with three females simultaneously had the lowest success ( $\beta = 0.52$ , SEM = 0.29,  $P = 0.047$ ).

The greater success of polygynous males in fertilizing several females simultaneously led to a greater reproductive output for these males (Fig. 2b). The number of offspring sired by males was influenced by population ( $F_{1,23} = 12.89$ ,  $P = 0.016$ ) and treatment (repeated-measures variable;  $F_{1,23} = 89.88$ ,  $P < 0.001$ ) and population  $\times$  treatment ( $F_{1,23} = 11.20$ ,  $P = 0.003$ ). In addition, testis mass ( $F_{1,23} = 11.19$ ,  $P = 0.003$ ), testis volume ( $F_{1,23} = 20.81$ ,  $P < 0.001$ ), and cauda epididymis mass ( $F_{1,23} = 4.36$ ,  $P = 0.048$ ) were positive predictors of the number of offspring sired. Tukey post hoc tests showed that males of the promiscuous population sired significantly fewer offspring when paired with three females simultaneously ( $P < 0.001$ ) compared to males in the other population  $\times$  treatment combinations (Fig. 2b).

**Synchrony of births.** Births in females from the polygynous population were synchronized, but not in females of the promiscuous population. The mean difference in time to parturition from the first to the last female (second or third, depending on whether all three or just two females gave birth) was  $2.6 \pm 0.4$  days for the polygynous population but  $5.0 \pm 0.3$  days for the promiscuous population ( $t_{18} = 3.363$ ,  $P = 0.004$ ). Because a maximum of two females of the promiscuous population gave birth in experimental treatments (compared with two or all three females in the polygynous population), we also compared the interval between the first and second birth between populations: The mean difference was significantly smaller for the polygynous than for the promiscuous population ( $1.1 \pm 0.3$  vs.  $5.0 \pm 0.3$  days;  $t_{18} = 7.553$ ,  $P < 0.001$ ). Furthermore, in six of the 14 replicates from the polygynous population, two females gave birth on the

same day, and in two more replicates, all three females gave birth on the same day, whereas synchronous births never occurred in the promiscuous population (Fisher exact test:  $P = 0.002$ ).

**Testis size in the breeding experiment.** Males from the polygynous population had significantly larger testes than males from the promiscuous population ( $F_{2,23} = 39.55$ ,  $P < 0.001$ ) (Fig. 3); again, no influence of body mass ( $F_{2,23} = 2.53$ ,  $P = 0.101$ ) or body length ( $F_{2,23} = 0.598$ ,  $P = 0.558$ ) was observed. The testes of polygynous males were 1.8-fold heavier ( $1.69 \pm 0.11$  vs.  $0.96 \pm 0.13$  g) and 1.7-fold larger ( $14\,100 \pm 564$  vs.  $8\,209 \pm 224$  mm<sup>3</sup>) than those of promiscuous males. In addition, the polygynous males had 1.9-fold heavier cauda epididymides than promiscuous males ( $0.25 \pm 0.16$  vs.  $0.135 \pm 0.02$  g).

**Testing for reproductive suppression.** In the test for reproductive suppression, all three females produced offspring in seven replicates (70%), and two females reproduced in the remaining three replicates. From our breeding experiment (see above), it was expected that only one female would reproduce in 57% of the replicates and two females in 43%. Therefore, significantly more females than expected bred (Fisher exact test:  $P = 0.01$ ).

**Testing for importance of synchronized births for infanticide avoidance.** No infanticide was observed in 20 polygynous groups with an interbirth interval of  $3.7 \pm 4.1$  days between females. In contrast, evidence of infanticide was found in 17 polygynous groups with an interbirth interval of  $8.9 \pm 8.2$  days between females. The interbirth interval was a significant predictor of the occurrence of infanticide and was longer in polygynous groups in which infanticide occurred ( $t_{35} = 2.52$ ,  $P = 0.017$ ).

## DISCUSSION

Promiscuous striped mouse males from four populations in which receptive females mate with several males, potentially resulting in sperm competition, have testes only half as large as those of males from a polygynous population in which a single male defends a harem of two to four females. Allometric relationships between testis size and body mass in mammals [20] predict a testis mass of approximately 1.0 g for striped mice, which is found in the grassland populations, but testes are 1.7-fold heavier than expected in the polygynous Succulent Karoo population. Preliminary data indicate that testes of free-living males from the polygynous population are more than 2% of body mass, probably because free-living males have less body fat than captive males (Schradin, unpublished data). We also demonstrated that polygynous males had 2-fold heavier cauda epididymides and are able to fertilize three females within a short period of time (whereas promiscuous males can only fertilize one or two females) and that this outcome is related to testis size.

The variation in testis and cauda epididymis size is not a plastic response to prevailing social and ecological conditions, because the promiscuous males have small testes even though they occur over a wide geographic distance and the pattern is observed both in captivity and in the wild. More importantly, our breeding experiments were conducted under standard laboratory conditions, which were identical for captive-born males from both populations. Thus, a plastic response is unlikely; instead, we propose that the variation in testis size is an evolutionary response.

Sperm competition can occur in the promiscuous population, because females could mate with several roaming males [27–29]. Sperm competition also can occur in the polygynous

population, in which roaming males with a sneaking tactic have been reported [41]. Analyses from the polygynous population indicate that roamers sire only 5% of the pups, that neighboring breeding males can sire 10% of the pups of a group, and that multiple paternities within litters are possible (Schradin and Lindholm, unpublished data). Thus, the larger testes also could be a response to sperm competition for polygynous males. However, although genetic studies on multiple paternities indicating sperm competition in the promiscuous population are lacking, behavioral observations for two different promiscuous populations demonstrate that females mate with several males, which would lead to intense sperm competition [31, 42]. Also, the present study shows that large testes in polygynous males function to achieve synchronous fertilization of females within a single communal group. Therefore, large testes could be a response to both sperm depletion and sperm competition [16], although the large interpopulation difference in testis size can be better explained by sperm depletion.

Sexual selection theory maintains that males have a greater potential than females to produce many offspring because of their production of more gametes [43]. However, although an egg is fertilized by a single sperm, ejaculates with few sperm might not lead to a successful fertilization event. The World Health Organization regards human males with a sperm count of less than 20 million sperm/ml as subfertile (oligozoospermia [44]), and studies demonstrate that reduced fertility in humans occurs at a sperm count of less than 40 million sperm/ml [45]. Thus, millions of sperm might be needed for a successful fertilization event. Similarly, we assume that a striped mouse male cannot simply divide up its sperm for several females but, instead, might be sperm depleted after mating with a single female if he cannot produce extra ejaculations with sufficient sperm. Studies measuring sperm numbers in the ejaculates of males from both populations would be interesting.

Males cannot simply evolve larger testes to avoid sperm depletion and be successful in sperm competition, because sperm production is costly [22]. Males have to trade off their investment in testis size with other life-history traits, such as growth, survival, parental care, and gaining access to fertile females [10, 46]. In this regard, males of the striped mouse populations differ markedly. The polygynous population from the Succulent Karoo semidesert has a short, 3-mo breeding season [25], and the promiscuous grassland populations have a 6- to 7-mo breeding season [28, 29]. Assuming that the total reproductive investment is similar for both populations, polygynous males have only half the time to make the equivalent reproductive investments as promiscuous males do. Striped mice in the Succulent Karoo have access to more food resources during the breeding season than those in grassland habitats [26], providing the necessary energy resources for larger testes. Finally, the home ranges of the polygynous males (0.13 ha) are much smaller than those of promiscuous males (1.25 ha), but males of both populations have, on average, access to the same number of breeding females (between two and three females [27]). Thus, promiscuous males have to cover an area 10-fold larger than polygynous males to gain access to the same number of females. The extra energy spent on mate searching in a food-restricted environment might account for the comparatively smaller testes in males of promiscuous populations. Dissimilar net trade-offs between investment in testis size and other energy-demanding life-history traits might help to explain alternative evolutionary outcomes of testis size.

Smaller testis size means less sperm per ejaculation and, thus, reduced chances of fertilization under conditions of sperm competition, which together with the smaller cauda epididymis,

could lead to fewer total numbers of ejaculations within a short period of time. To minimize sperm depletion, males that mate more often need larger testes, as is the case for soay sheep, in which males with larger testes copulate more often and sire more offspring, independent of their dominance status [16]. In contrast, this prediction was not supported in birds initially [19], but the importance of sperm depletion might have been masked by sperm competition, because both involve multiple matings [19]. Furthermore, estimates of testis size were rather unreliable, leading to a low resolution [47]. A later and more comprehensive analysis found evidence that sperm depletion might significantly influence testis size in birds [10].

In addition to larger testes, polygynous males had heavier cauda epididymides, which is important for sperm maturation and storage in mammals, functioning as a sperm reserve [48, 49]. Whereas the epididymal transit time for striped mice is unknown, data from other rodents predict a period of approximately 1 wk [48, 49]. This means that during the 8 days of the mating experiments, most of the sperm used for inseminating females might have been derived from the epididymal reserve and not directly from the testis. The lower sperm production (smaller testes) and smaller sperm reserve (lighter cauda epididymides) apparently resulted in promiscuous striped mouse males being sperm depleted, because none of the 14 males was successful in fertilizing all three females within 8 days (two estrous cycles) whereas most of polygynous males (larger testes, heavier cauda epididymides) were successful in fertilizing all three females. An alternative explanation could be that the dominant female suppresses subordinate females in the promiscuous but not the polygynous population. However, it has been shown previously that reproductive output of subordinate females from a promiscuous population is not influenced by them living in close proximity to dominant females [50]. Similarly, in our controlled experiments, in which females were housed in close proximity, almost all promiscuous females were impregnated. In this experiment, olfactory communication was maintained, and the experiment was short enough that an effect of social stress (during previous cohabitation) could still have been detected. Furthermore, we did not observe any incidences of aggression between females of either population in any of our experiments. Thus, we have no indication of sexual suppression in striped mouse females, and in fact, promiscuous females synchronized their estrus in our colony [51]. We cannot totally exclude the possibility of sexual suppression, but the most parsimonious explanation of our results is that sperm depletion occurred in the promiscuous but not the polygynous population, leading to significant fitness costs for males and females.

Polygynous striped mouse males must fertilize several females within 1–2 days of their synchronous estrus [51], leading to synchronized births, as observed in the field [33]. In cooperatively breeding rodents, unsynchronized births yield the risk of infanticide by females that give birth later [52–55]. This also is the case in polygynous striped mice, as demonstrated by the strong relationship between interbirth intervals and the occurrence of infanticide in captive polygynous groups. Also, we found indication for infanticide in 46% of the polygynous groups, indicating that reproductive competition between females is common and costly such that avoiding this competition could be a strong selection pressure. Thus, synchronized births can be regarded as beneficial for the evolution of cooperative breeding in some rodent species. Synchronized fertilization also would be beneficial and could be achieved by all females mating with different males at the

same time or by all females mating with the same male in sequence.

Males will enhance their fitness if they mate with all females in a group over a short time period, which would result in a high selection pressure against sperm depletion and for large testes. In communally breeding rodents, such as the house mouse (*Mus domesticus*), sperm depletion can reduce a female's chances of being fertilized to such an extent that they compete for access to reproductive males [56]. Cooperative breeding in female rodents leads to benefits, but the risk of reproductive competition also exists [54, 55, 57–59]. Cooperatively breeding striped mice benefit from reduced costs of thermoregulation [60], improved pup development [40], and the defense of their nest by all group members [32]. Whereas relationships between females in communal nests typically are egalitarian, reproductive competition in the form of infanticide can occur as well [61]. In support, the interbirth interval between two females of polygynous groups was much longer when infanticide occurred than when females raised their pups cooperatively. This indicates that synchronized births are beneficial for cooperative breeding in striped mice. Care-giving mothers probably do not commit infanticide of unfamiliar conspecific pups in their nest, both because of their inability to differentiate between own and unfamiliar offspring before pups are 10 days of age [30] and because they are likely to be hormonally primed to show maternal care [62–64].

The risk that sperm depletion prevents synchronous fertilization and, thus, cooperative breeding might have been one of the main selection forces for large testes in males of the polygynous striped mouse population. This would predict larger-than-expected testis size in other species with a similar social and mating system, especially in other rodents. Data regarding testis size in rodents are abundant, and large variation exists, with some species having 10-fold smaller testes than expected but others more than 8-fold larger tested than expected [20]. In contrast, data concerning the natural mating and social system of rodents are scarce because of their small size and their often shy and nocturnal behavior (see, however, [65, 66]). Furthermore, testis size in the same species might differ dramatically between populations (present study; see also [20]) and, in some species, even within the same population between years with different ecological conditions [67]. Comparative studies of extensive but poor-quality testis data sets therefore are problematic [47]. Even multiple rodent populations of the same species might differ both in mating system and in testis size. Unfortunately, to date, reliable data concerning social organization and testis size of single rodent populations are rare (the present study being the exception). It has been shown previously that sperm depletion is costly [22, 56], but the present study is one of the first showing that these costs can lead to the evolution of larger testes.

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