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Absence of reproductive suppression in young adult female striped mice living in their natal family

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Alternative reproductive tactics of males have been studied in many species, but few studies have focused on females. In many communally breeding mammals, females can be adult nonbreeding helpers, leave the group and breed solitarily, or be a breeder in their natal group, representing three alternative reproductive tactics. The reasons for delayed breeding are not well understood, but in many sociable species both male and female helpers are reproductively suppressed. Male helpers of communally breeding striped mice, *Rhabdomys pumilio*, have increased corticosterone levels and delayed sexual maturation compared with their singly housed brothers. In the present study, we tested whether similar effects occur in female striped mouse helpers. In the field, young adult females typically do not breed in their natal group, indicating that they might be reproductively suppressed. Seventeen sister pairs from 17 family groups were studied. One sister of each pair was kept in the family group, while the other was housed singly at 3 weeks of age. Sisters did not differ in either the age at which they reached puberty (at 6 weeks on average) or in their corticosterone and progesterone levels. However, in neutral encounter tests, singly housed sisters showed more amicable behaviours when presented with unfamiliar striped mice of both sexes. Their high sociable motivation might explain why most females remain philopatric under natural conditions. We conclude that philopatric female striped mice in monogamous family groups are not reproductively suppressed, but reproductive competition might occur in natural communal groups with multiple old breeding females, as observed under high population density.

In many animal species, individuals have the choice between alternative tactics, which are regarded to be the result of adaptive decision processes optimizing individual fitness (Dawkins, 1980; Gross, 1996). For individuals of social species, one such decision could be to choose between remaining philopatric as a helper or dispersing and starting independent breeding (Koenig & Dickinson, 2008). Such alternative reproductive tactics are more common in males than in females (Taborsky, Oliveira, & Brockmann, 2008), perhaps because male reproductive success generally varies more between individuals than does female reproductive success. However, many sociable mammal species have nonbreeding female helpers (Solomon & French, 1997), providing a good opportunity to study the female tactics of helping versus dispersing and solitary breeding. In communally breeding species, more than one female breeds per group, and philopatric nonbreeding female helpers might remain in these groups to start reproduction at a later stage (Hayes, 2000).

This absence of breeding in young adult females could be due to physiological reproductive suppression, defined as highly increased glucocorticoid levels induced by the presence of dominant breeders causing social stress (Creel, 2001; Reyer, Dittami, & Hall, 1986; Wingfield & Sapolsky, 2003). High corticosterone levels lead to suppression of progesterone secretion, thus inhibiting ovulation (Clarke, Miethe, & Bennett, 2001; Saltzman, Ahmed, Fahimi, Wittwer, & Wegner, 2006). Reproductive suppression of subordinate female helpers occurs in several communally breeding species (Brant, Schwab, Vandenbergh, Schaefer, & Solomon, 1998; Getz, Dluzen, & McDermott, 1983; Solomon, Brant, Callahan, & Steinly, 2001).

Alternative reproductive tactics are characterized by behavioural differences between tactics, especially in reproductive and social behaviour. When some individuals disperse while others

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remain philopatric, they follow two alternative tactics that are also characterized by differences in the social environment: living in the natal group, living solitarily, or emigrating into and living in a group of nonkin. Thus, it is not well understood whether behavioural differences between tactics are primarily due to motivational (internal) differences between individuals of the two different tactics, or alternatively simply a consequence of the external differences in social environment: individuals living in groups can show amicable behaviours towards group mates; solitary individuals cannot. Therefore, to understand differences in social behaviour between alternative tactics, it is important to study social behaviour under standardized conditions, for example using a neutral presentation arena.

In African striped mice, *Rhabdomys pumilio*, both sexes have three alternative reproductive tactics (Schradin, Lindholm, et al., 2012): (1) philopatric helper; (2) solitary breeder; or (3) communal breeder. The group-living tactics 1 and 3 occur when population density is very high, the solitary tactic when population density is very low and all tactics under intermediate population densities (Schradin, König, & Pillay, 2010). Under low population density, both males and females can leave their natal group in the season of their birth when they are 4–6 weeks old to start independent breeding (Schoepf & Schradin, 2012a). Dispersing young adults were more aggressive towards individuals of the same sex compared with philopatric individuals and more amicable towards individuals of the opposite sex (Schoepf & Schradin, 2012b). In sum, free-living young adult striped mice can either be group living or solitary living, making this species a prime model to test for reproductive suppression using an experimental approach (Schradin, Eder, & Müller, 2012; Schradin, Schneider, & Yuen, 2009). Philopatric males are known to be reproductively suppressed by the breeding male of the group (Schradin et al., 2009), showing high corticosterone but low testosterone levels, small testes and low, if any, sperm counts (Schradin, Eder, et al., 2012). However, we do not know whether philopatric females are also reproductively suppressed, which could explain why many of them do not breed as young philopatric adults.

In the present study, we set up 17 family groups to mimic the philopatric and the solitary female tactic. From each family, two same-litter sisters were used, one of which remained philopatric with the family, while the other was housed singly after reaching 3 weeks of age, the earliest age at which dispersal occurs (Schoepf & Schradin, 2012a). This experimental design has been used previously and successfully mimics the philopatric and solitary tactics in male striped mice, inducing increased testosterone and decreased corticosterone levels in singly housed males (Schradin et al., 2009), increased levels of stored arginine vasopressin in the brain (Schradin, William, Krackow, & Carter, 2013) and enhanced testes development (Schradin, Eder, et al., 2012).

The present study had three aims.

(1) We tested whether reproductive suppression occurs in philopatric females by investigating whether they show delayed onset of puberty (measured as age of first perforation of the vagina, indicating their readiness to mate), higher corticosterone levels and lower progesterone levels. Such reproductive suppression would explain why most young philopatric females do not breed in nature.

(2) We determined whether living alone or in the family group was associated with differences in response to unfamiliar conspecifics. If the solitary tactic were due to an internal motivation to avoid the company of conspecifics, we would expect solitary females to be more aggressive, less amicable and more explorative than philopatric females.

(3) We further predicted that solitary females would show higher levels of amicable behaviour towards males, as solitary females would be more ready to mate while philopatric females would defend their family territories even against strange males.

METHODS

Study Species

The African striped mouse is a communal breeder with nonbreeding helpers at the nest (Schradin, Lindholm, et al., 2012). Groups consist of one breeding male (immigrated from another group), up to four closely related communally breeding females and up to 25 adult young male and female philopatric individuals that act as helpers at the nest (Schradin, Lindholm, et al., 2012). Striped mice breed in spring (August/September to November/December) and most individuals born during the breeding season remain philopatric as young adults (>6 weeks old) in their natal group, where they stay for the entire dry season (December–April) and the cold wet winter (May–July). Some of the young adult philopatric individuals are able to reproduce successfully in the season of their birth by mating with partners from outside the extended family group (males: Schradin & Lindholm, 2011; females: Schradin, Schneider, & Lindholm, 2010), but the vast majority of young adults that remain as philopatric individuals do not reproduce until the next breeding season when they are 1 year old (Schradin, Schneider, & Lindholm, 2010). At this age, males disperse and attempt to immigrate into groups of communally breeding females, whereas females remain philopatric in their group and breed communally. In both sexes, solitary breeding also occurs as an alternative tactic, and individuals can leave their group as young as 4–6 weeks of age when free territories are available (Schoepf & Schradin, 2012a). Fewer than 1% of striped mice survive for a second breeding season. Thus, most females breed only during one breeding season when they are 1 year old, and produce two to three litters during this breeding season.

Sexual Maturity

We conducted an experiment to confirm whether a perforate vagina was indicative of sexual maturity in our study species. At the University of the Witwatersrand, South Africa, we assessed the mating behaviour of 32 young females that had a perforate vagina for the first time by pairing them individually with sexually mature and experienced males. These females were housed in their family groups before pairing, and vaginal smears were examined daily from the day they were first perforate for a maximum of 4 days or unless they displayed oestrus.

For the smears, we used the pipette lavage method, which was minimally stressful and did not cause vaginal trauma in any of the females. The female was restrained with a glove and a small plastic pipette with a rounded tip containing a few drops of isotonic saline was inserted approximately 5 mm into the vagina. The fluid was expelled and immediately sucked up. The cell contents in the saline were transferred onto a clean microscopic glass slide. The procedure was repeated three times to ensure adequate numbers of cells. The procedure from restraining to release of the female was about 90 s. The slides were air dried and stained with Crystal Violet stain. The cell composition of the smears was evaluated by light microscopy for the relative abundance of cornified epithelial cells, leucocytes and macrophages. Smears were obtained in the morning before 0900 hours and females in oestrus (superabundant cornified epithelial cells; Byers, Wiles, Dunn, & Taft, 2012) were placed in a neutral tank with a mature male for 30 min at 1100 hours, and the occurrence of lordosis (female), mounting and intromission was recorded.

Animals in Zurich

The colony consisted of animals originally trapped in 2002 in the Succulent Karoo (Goegap Nature Reserve) in South Africa. Animals were bred at the research station in Goegap under natural weather conditions and F10 descendants were exported to the University of Zurich, where a colony was established in October 2006.

Housing Conditions

Animals were kept at the University of Zurich under a 11.5:12.5 h light:dark cycle (LD 11.5:12.5 h) and a temperature of approximately 22 °C. Families were kept in two glass tanks 50 × 30 cm and 30 cm high, which were connected to one another with a flexible plastic tube. A second tube connected to one type 4 plastic cage 20 × 13 cm and 15 cm high where a water bottle was provided (Fig. 1). Single individuals were kept in one glass tank connected to two type 4 plastic cages (Fig. 1). All tanks and cages had 5 cm of wood shavings as bedding. The tanks additionally contained natural branches for environmental enrichment. Furthermore, each family and each singly housed mouse had one running wheel, which a pilot study showed reduces stereotypic behaviour.

Each family and each singly housed female had access to an extra enriched tank of 70 × 50 cm and 35 cm high for 1–2 days a week (Fig. 1). Connection was made by removing one type 4 cage and replacing it with another type 4 cage connected by a flexible tube both to the home tank and to the enriched tank. The enriched tank was provided with bedding, tubes and natural branches. Up to five families and single female mice had access to one enriched tank on different days, such that the mice experienced olfactory cues of unrelated/unfamiliar striped mice.

Wild rodents kept in captivity are prone to developing stereotypic behaviour (for striped mice see Jones, Mason, & Pillay, 2010), which is known to affect both social behaviour and physiology and brain structure (Würbel, 2001). Thus, all striped mice were kept under super-enriched conditions, which were successful in preventing the development of stereotypic behaviour. Females of 15 sister pairs were observed at the end of experiments (10 weeks old) for 15 min in their home cage to determine whether mice had developed stereotypic behaviour. To determine whether the experimental set-up was successful in avoiding stereotypic behaviours, we recorded whether mice showed flips, jumping in the corner or bar gnawing. Furthermore, the total time spent running in the wheel was measured. None of the 15 family housed females and none of the 15 singly housed females showed any stereotypic behaviours. Wheel running (measured in seconds/15 min) was observed in only five family housed and in two singly housed females and did not differ between the two female categories (59.8 ± 115.2 s versus 13.1 ± 45.4 s; Wilcoxon test: $T = 4$, $W = 13$, $P = 0.22$).

Mice were supplied with water ad libitum. Striped mice in the Succulent Karoo increase their body weight during spring and lose more than 10% of their weight during the following dry season (Schradin, Lindholm, et al., 2012). This might explain why they are prone to extreme obesity in captivity. To avoid obesity and as a form of enrichment, striped mice were not fed ad libitum but using the following schedule: a seed mix of 4.0 g per individual (guinea pig and hamster food, Haefliger AG, Herzogenbuchsee, Switzerland) in the morning; one piece (ca. 1.0 g) of fruit or vegetable per individual at noon; and two mealworms per individual in the afternoon. Striped mice do not compete with each other for food, and do not monopolize food (Schubert, Pillay, & Schradin, 2009); the growth rate was similar to that of captive mice fed ad libitum (Brooks, 1982) and faster than in mice in the field (C. Schradin, unpublished data). This procedure was approved by the Kantonale Veterinäramt in Switzerland, as it reduces obesity and obesity-related diseases such as diabetes (both are common in striped mice colonies fed ad libitum). The amount of food to give was known from many years of experience in South Africa.

Experimental Procedure

In total, 17 families took part in the study. Families were kept together until offspring were 3 weeks old (D21) and weaned (juveniles). At this stage, only one male and one female offspring remained with the pair. These were the individuals of the family treatment. They remained with their parents and also experienced raising the next litter. To avoid crowding in family cages, all juveniles except one male and one female of the second litter were removed when they reached 3 weeks of age. No pair had a third litter within the study period of 10 weeks.

Of the juveniles that were removed at 3 weeks of age, one female was housed alone, as described above. We therefore used a paired data design by randomly assigning one sister to being family housed and the other sister being singly housed. Some of the other individuals were used for a similar study in males (Schradin et al., 2013) or kept in sibling groups.

The females from the family and single treatments were weighed once a week and their reproductive state determined until both females had a perforate vagina. For this, individuals were removed by hand from their tank, inspected visually and returned within 2 min. Female rodents, including female striped mice, are regarded as being sexually mature and ready to mate when their vagina is perforate (open; Brooks, 1982; this study), a definition we also use in field studies to determine sexual maturity and readiness to mate (Schoepf & Schradin, 2012a). When a singly housed female

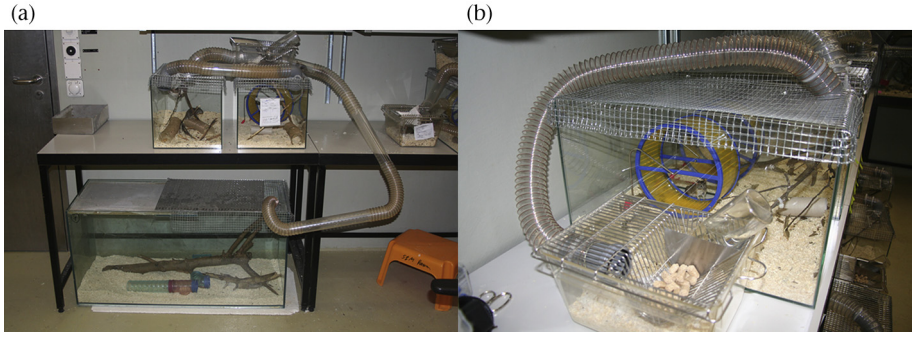

Figure 1. Experimental set-up. (a) A family set-up consisting of two glass tanks and a cage connected by flexible tubes. (b) A single individual set-up consisting of one glass tank and one cage. Running wheels were provided for each group/single individual, which were also allowed once a week to enter an enriched tank (top left). This highly enriched set-up was successful in preventing stereotypic behaviour.

showed a perforate vagina before her sister did, a blood sample was taken from each to compare their corticosterone levels. Blood samples were taken the day after the difference was found to avoid a stress response caused by handling during inspection. Samples were obtained within 3 min: the females were anaesthetized using methoxyflurane, as suggested by the ethical committee in Switzerland (1 ml on cotton wool in a 300 ml chamber, with wire mesh between the animal and the anaesthetic). Methoxyflurane is very similar to isoflurane but is always used without a vaporizer.

A blood sample of 100–300 μl (depending on body mass) was taken from a tongue vein (sublingual blood sampling; Heimann, Kasermann, Pfister, Roth, & Burki, 2009), the preferred method of the Swiss Veterinary Council for taking blood samples from mice. We never had any indication that mice were negatively influenced by blood sampling. We were careful not to take too much blood, which is also the reason why we only had enough serum for one hormone measurement (not both) for many individuals. The animals were returned to their home cage after 5 min, when they had recovered from the slight anaesthesia. Blood samples stood at room temperature for 1.5 h and were then centrifuged for 10 min. The resulting serum was pipetted and frozen in aliquots.

Behavioural Testing

Behavioural data were collected from 15 sister pairs. Females were housed in families or singly until they were 10 weeks old. We did two encounter tests per female on 2 different days, one with an unrelated female and another with an unrelated male. Both sisters were always tested on the same 2 days. For eight of the sister pairs, a same-sex test was done on the first day and an opposite-sex encounter test was done on the second day, and the reverse was done for the other seven sister pairs.

Social behaviours in rodents are scent dependent, and direct interactions (grooming, sitting in body contact) cannot be observed when separating the mice with a wire mesh (Schradin & Pillay, 2004). From a previous study with young adult males we expected very little aggression (Schradin, Lindholm, et al., 2012), and this was supported by our study (fewer than 50% of mice showed any aggression). After tests, animals were returned to their home cage and immediately checked. They were checked again a few hours later (at noon when experiments were done in the morning; at 1800 hours if experiments were done in the afternoon), and no indication of long-lasting effects (such as piloerection, sitting in one corner) were seen; instead mice were eating normally.

Stimulus animals in these tests were all adults housed in sibling groups. For all tests, we chose stimulus animals that weighed less than the test animal, because dominance is weight related in striped mice. Thus, the test animal was provided with the opportunity to dominate the stimulus subject, and in this way we tested for the motivation of the test and not the stimulus animal. For each sister pair, two different stimulus animals from the same sibling group (same cage) were used and chosen randomly. In half of the cases, the mouse from the family treatment was tested first, whereas in the other half the singly housed sister was tested first.

All tests were performed in a neutral presentation arena made of wood, 80 × 40 cm and 60 cm high. At the beginning of tests, a partition in the middle divided the arena into two compartments: we placed the stimulus mouse on one side of the partition and the test mouse on the other. After an acclimation period of 5 min, the partition was removed and we scored the behaviour of the test mouse (focal animal sampling) for 15 min, which was found in previous studies (Schradin, Schneider, & Lindholm, 2010; Schradin et al., 2013) to be long enough to observe encounters also in shy individuals. We recorded the frequency of aggressive behaviours (fight, bite, chasing) as well as grooming of the stimulus mouse by the test mouse. We used the same experimental procedure in previous studies (Schradin, Schneider, & Lindholm, 2010; Schradin et al., 2013), and experiments would have been terminated when damaging fights occurred in which one mouse tried to bite another one (termination criterion). However, in the present study, biting was never observed. The total time spent in body contact with each other was also measured. For nine sister pairs, a blood sample was collected and processed as described above for hormone measurements 2 days after the last encounter.

Hormone Assays

Blood samples were analysed in the enzyme immunoassay (EIA) laboratory of the Zoological Institute, University of Zurich. Commercial kits from IBL International (Hamburg, Germany) were used. Procedures were as stated in the kit manuals. However, owing to high corticosterone levels typical of this species, samples were diluted 1:99. All measurements were well within the standard curve of the assay. Serial dilution of striped mouse sample pools paralleled the standard curve and the slopes were not different. Intra- and interassay variability was determined with pools from striped mice that had low and medium-high values. Eight measurements were done for intra-assay and five for interassay variability. Intra-assay variability was 8.3 and 22.3% for the medium and low pool, respectively. Interassay variability was 6.4 and 2.3%. Progesterone values were measured in one single assay. Intra-assay variability for two pools (8 and 10 samples) was 4% and 5.0%. We did not have sufficient amounts to measure both corticosterone and progesterone for all samples, and in these cases we focused on corticosterone, resulting in lower sample sizes for progesterone.

Ethical Note

The research adhered to the ASAB/ABS Guidelines for the Use of Animals in Research. We provided animals with environmental enrichment (as described above). The welfare of the animals was monitored by checking them three times a day visually during feeding, and by behavioural observations demonstrating the absence of stereotypic behaviours. The experimental procedures used did not have any obvious negative effects on the welfare of the striped mice. Singly kept females represented the solitary kept tactic observed in nature, and by providing females access to a tank which on other days was also used by different striped mice, females were not isolated olfactorily from conspecifics. Thus, our highly enriched conditions mimicked the situation of solitary females in the field. At the end of the experiment, all experimental animals were euthanized (family and singly housed females) by anaesthetizing them with methoxyflurane and euthanizing them by cervical dislocation followed by decapitation. The rest of the family group remained in the breeding stock of the colony. Animal ethical clearance was provided by the Kantonale Veterinärmt of the Kanton Zürich in Switzerland (ethical clearance number 91/2006) and the University of the Witwatersrand (AESC number: 2012/13/2B).

Statistical Analyses

Data were analysed using Instat 3.05 (www.graphpad.com). A paired data design was followed using paired *t* tests. Behavioural data were often not normally distributed and thus analysed using the nonparametric Wilcoxon signed-ranks test to test between sister pairs. All tests were two tailed. Data are presented as mean ± SD.

RESULTS

Perforate Vagina, Sexual Maturity and Readiness to Mate

Of the 32 young philopatric females tested for mating behaviour when their vagina was perforate, four were in oestrus on the day they were first perforate (day 0), 10 on day 1, 15 on day 2 and 3 on day 3. A total of 25 females displayed lordosis (78%) and 20 of these mated (63% of all females, 80% of those showing lordosis). The day of first oestrus (day 0–3) did not affect whether or not lordosis ($\chi^2_3 = 2.55$, $P = 0.47$) or mating ($\chi^2_3 = 0.36$, $P = 0.95$) occurred. Females that mated did not differ from those that did not mate in age (30.25 ± 4.96 days versus 31.42 ± 3.61 days; $t_{30} = 0.10$, $P = 0.92$) and body mass (37.01 ± 5.47 g versus 39.42 ± 4.78 g; $t_{30} = 0.31$, $P = 0.76$). Births were recorded in 13 females.

Comparison of Attainment of Sexual Maturity

Singly housed females did not differ from family housed females in either the age at which they were first observed to have a perforate vagina (5.8 ± 1.5 weeks versus 6.2 ± 1.7 weeks; $N = 17$ sister pairs, paired $t_{16} = 0.79$, $P = 0.44$) or in the body mass at which they first were observed to have a perforate vagina (31.0 ± 6.8 g versus 29.7 ± 5.5 g; $N = 17$ sister pairs, paired $t_{16} = 0.81$, $P = 0.43$).

Comparison of Hormone Levels

To test the hypothesis that high corticosterone levels delay the onset of female reproductive maturity, we compared corticosterone levels between family housed females that showed a perforate vagina later than their singly housed sisters. In doing so, we increased the power to detect the predicted difference, by excluding sister pairs with no differences in timing of perforate vagina. The same experimental design was used to test whether hormonal differences explain the timing of sexual maturity in brothers housed in a group and alone (Schradin et al., 2009). Sister pairs, like the brother pairs previously, that did not differ in the timing of maturity were excluded for ethical reasons to avoid blood sampling when no difference was expected. In contrast to our expectation, we found no difference in age of sexual maturity (see above), which we did not know before we determined our sample criteria. We analysed the hormones of those sisters from which we collected blood samples to separate the effects of hormonal influence and statistical noise.

Singly housed females that had a perforate vagina before their family housed sisters did not differ in corticosterone levels from their sisters at this stage (age on average 6 weeks; 1039 ± 610 ng/ml versus 912 ± 596 ng/ml; $N = 6$ sister pairs, paired $t_5 = 0.56$, $P = 0.60$). Singly housed females did not differ from family housed females in corticosterone levels at 10 weeks of age (732 ± 401 ng/ml versus 781 ± 331 ng/ml; $N = 10$ sister pairs, paired $t_9 = 0.34$, $P = 0.74$) when experiments were terminated.

For progesterone, only samples from eight females of four of the six sister pairs were available for the period when females differed in timing of showing a perforate vagina. All females had measurable progesterone levels (singly housed: 7.0 ± 4.6 ng/ml; family housed: 2.2 ± 1.2 ng/ml), but no statistical comparisons were possible. Singly housed females did not differ from family housed females in progesterone levels at 10 weeks of age (4.8 ± 3.0 ng/ml versus 9.9 ± 20.6 ng/ml; $N = 8$ sister pairs, paired $t_7 = 0.73$, $P = 0.49$) at the end of experiments.

Social Interactions with other Females at 10 Weeks of Age

Singly housed females groomed the stimulus females significantly more often than family housed females (5.7 ± 5.4 times/300 s versus 0.1 ± 0.4 times/300 s; Wilcoxon signed-ranks test: $T = 0$, $W = -120$, $N = 15$ sister pairs, $P < 0.0001$; Fig. 2a), they spent significantly more time in body contact with them (314.7 ± 186.4 s/300 s versus 55.0 ± 148.091 s/300 s; $T = 6.5$, $W = -107$, $N = 15$ sister pairs, $P = 0.001$; Fig. 2b), and they showed significantly less aggression towards them (0.3 ± 0.7 times/300 s versus 2.0 ± 2.6 times/300 s; $T = 0$, $W = 45$, $N = 15$ sister pairs, $P = 0.004$; Fig. 2c). Aggression was rare: in six sister pairs, none of the females showed aggression, and only two of the singly housed and nine of the family housed females showed some aggression (chasing; Fig. 2).

Social Interactions with Males at 10 Weeks of Age

Singly housed females groomed the stimulus males significantly more often than did family housed females (7.3 ± 7.4 times/300 s versus 0.6 ± 1.1 times/300 s; $T = 1$, $W = -76$, $N = 15$ sister pairs, $P = 0.001$; Fig. 2d), they spent significantly more time in body contact with them (280.3 ± 220.4 s/300 s versus 135.1 ± 173.3 s/300 s; $T = 17$, $W = -71$, $N = 15$ sister pairs, $P = 0.02$; Fig. 2e), and they showed significantly less aggression towards them (0.5 ± 0.8 times/300 s versus 2.3 ± 2.8 times/300 s; $T = -5$, $W = 45$, $N = 15$ sister pairs, $P = 0.02$; Fig. 2f). No mating or attempts of mating were observed.

Response Towards Stimulus Males Versus Stimulus Females

Family housed females did not groom the stimulus males significantly more often than the stimulus females ($T = 6$, $W = -16$, $N = 15$ sister pairs, $P = 0.22$), nor did they differ in their aggression levels towards the two sexes ($T = -32.5$, $W = 1$, $N = 15$ sister pairs, $P = 0.97$). However, they spent significantly more time in body contact with males than with females ($T = 18$, $W = -69$, $N = 15$ sister pairs, $P = 0.03$).

Singly housed females did not groom the stimulus males more often than the stimulus females ($T = 31$, $W = -43$, $N = 15$ sister pairs, $P = 0.19$), they did not spend significantly more time in body contact with them ($T = -38$, $W = 44$, $N = 15$ sister pairs, $P = 0.23$), nor did they show less aggression towards them ($T = 13$, $W = -10$, $N = 15$ sister pairs, $P = 0.55$).

DISCUSSION

Physiological reproductive suppression of subordinate females occurs in many cooperatively and communally breeding mammals (Abbott, 1984; Brant et al., 1998; Clark & Galef, 2001; Getz et al., 1983; Moehlman & Hofer, 1997; Saltzman et al., 2006; Savage, Ziegler, & Snowdon, 1988; Solomon et al., 2001), but we found no evidence for this in the communally breeding striped mouse. Thus, there must be other proximate and ultimate reasons for why young females often do not breed, which could include stress induced by high population density, inbreeding avoidance, and energy allocation trade-offs between growth/survival and reproduction. Behaviourally, we found significant differences between philopatric and solitary female striped mice, with singly housed females being more amicable and less aggressive to strangers than their family housed sisters.

Both family housed females and their singly housed sisters had a perforate vagina at approximately 6 weeks of age. In an additional experiment, we were able to demonstrate that newly perforate females were sexually mature, showed lordosis and were ready to mate, supporting the assumption of previous studies that a

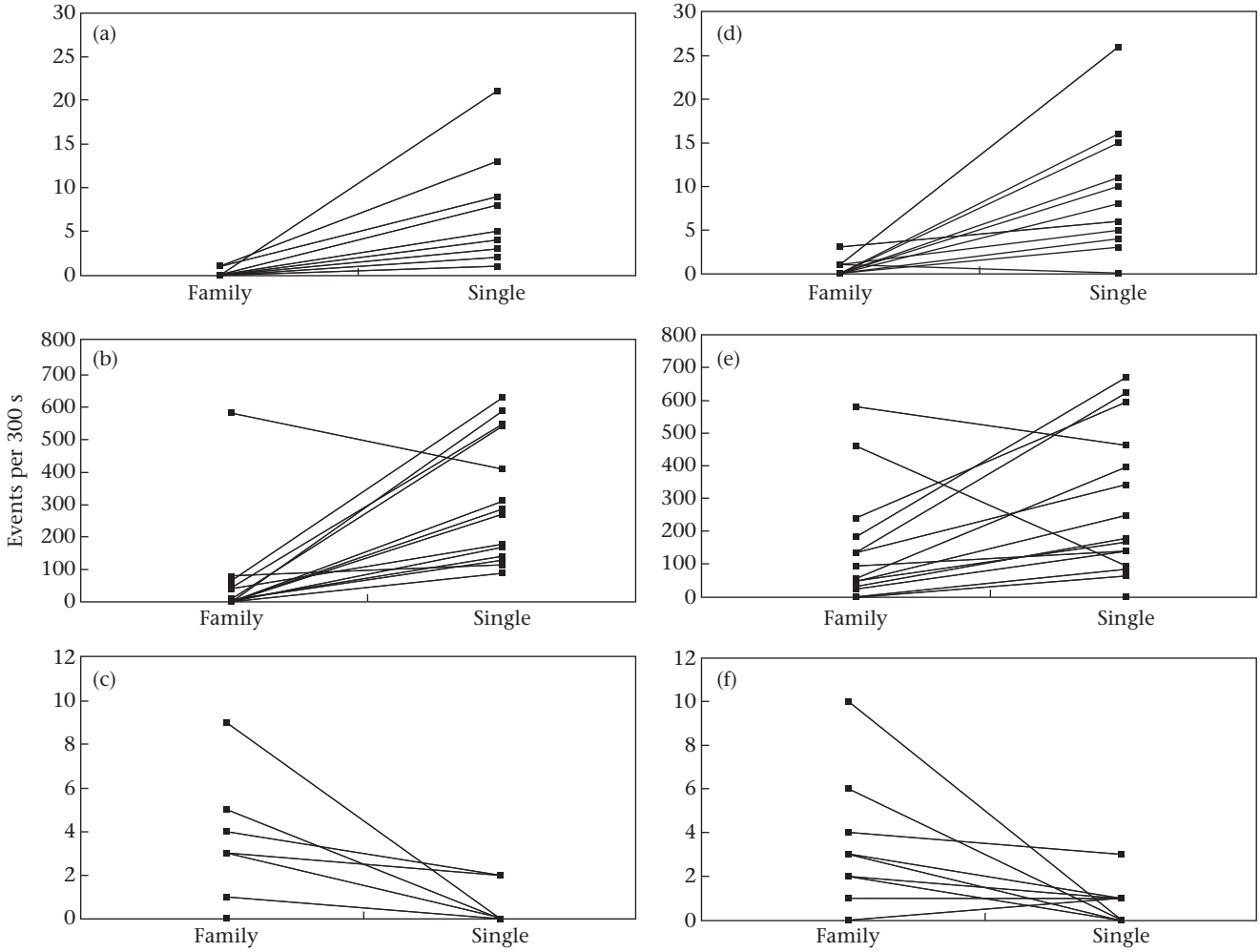

Figure 2. Behavioural differences between family housed females and their singly housed sisters. The data of each sister pair are connected by a line. (a–c) Behaviours shown towards an unfamiliar female in a neutral presentation arena. (d–f) The same behaviours shown towards strange males in a neutral presentation arena. (a, d) Grooming. (b, e) Sitting in body contact. (c, f) Aggression. All comparisons were significant ($P \leq 0.02$).

perforate vagina is an indication of sexual maturity (Perrin & Johnson, 1999; Willan, 1982). Importantly, the females in our study were young adult philopatric individuals living in their family group and they readily mated when in oestrus, even though in their family they would not reproduce because of inbreeding avoidance (Pillay, 2002). In the field, young females reproduce when population density is low, and they do so with males outside their family group (Schradin, Schneider, & Lindholm, 2010).

In accordance with our morphological and behavioural data, our progesterone data indicate that females were physiologically ready to reproduce, as all females had clearly measurable progesterone levels. This was also represented in the vaginal smears, indicating oestrus in young females living in their natal family group. For the closely related sister species *Rhabdomys dilectus* (previously regarded as the same species, *R. pumilio*; Rambau & Robinson, 2003), females have been reported to reach sexual maturity at an earliest age of 5 weeks in the field (Pillay, 2002) and 6 weeks in captivity (Brooks, 1982); this fits the results of our present study, in which females were 5–6 weeks old when the vagina was first perforate. We found no differences in corticosterone or progesterone levels between philopatric and singly housed females, indicating that the two classes did not differ morphologically or physiologically in the age when they reached sexual maturity.

In conclusion, there was no evidence for reproductive suppression of philopatric females. Using the same experimental approach, multiple studies have shown that male philopatric striped mice are sexually suppressed (Schradin, Eder, et al., 2012; Schradin et al., 2009; Schradin et al., 2013). Thus, we do not believe that the experimental set-up can explain why no reproductive suppression was observed in females. Sex differences in the absence/presence of reproductive suppression indicate a more egalitarian social structure for females than for males, which is reflected in the field by each group having only one breeding male but up to four breeding females (Schradin, Lindholm, et al., 2012). Support for this egalitarian relationship between females was provided in another study, which did not show competition for food between sisters (Schubert et al., 2009).

In the field, the percentage of young adult females that breed in the season of their birth varies from year to year, probably as a function of population density. In years with very high population density, almost no young adult female reproduces (Schradin, Lindholm, et al., 2012); in years with intermediate population density 28% of young adult females reproduce; and in years with low population density 90% of young adult females breed (Schradin, Schneider, & Lindholm, 2010). Our study cannot rule out the possibility of reproductive suppression under high population densities. Mice were kept in family groups with only one breeding

female, which was the mother of the philopatric female, representing low population density in the field. Under high population density, two to four older females breed communally in a group. It is possible that the presence of several breeding females, especially aunts, which are less closely related to young philopatric females than their mothers, induces sexual suppression. Communal groups are characterized by competition between the breeding females (Schradin, König, et al., 2010), and it is possible that the older breeders will reproductively suppress young females.

High population density is likely to increase the frequency of intergroup territorial encounters, which are typically lost by smaller individuals such as young adult females. Thus, the absence of reproduction in young adult females could be the result of territorial aggression inducing stress or increased within-group competition, as the number of breeding females per group correlates with population density (Schradin, König, et al., 2010). Another reason could be behavioural suppression and female–female aggression within communal groups, which has been reported repeatedly (Schradin, König, et al., 2010; Schubert et al., 2009). A third reason for free-living philopatric females not breeding might be inbreeding avoidance. In captivity, females do not breed with a familiar breeding male of their group regardless of genetic relatedness (Pillay, 2002), and in the field, young adult females avoid mating with the breeding male of their group and seek copulations with males outside their natal group (Schradin, Schneider, & Lindholm, 2010). A fourth possibility is a trade-off in energy allocation. It might be more beneficial for young females to invest in growth and survival than in reproduction. Thus, a combination of aggression experienced by both females from neighbouring groups and older females within the group together with inbreeding avoidance and resource allocation might explain why many young adult striped mouse females do not breed in their natal group, but these factors need future examination.

In our study, singly housed females were more amicable and less aggressive towards strangers of both sexes than their family housed sisters. By contrast, in the field, aggressive philopatric females were more likely to become solitary, and solitary striped mice (both sexes combined) were more aggressive towards same-sex individuals (Schoepf & Schradin, 2012b). Most (75%) of the females that became solitary in the field did also reproduce, but none of our singly housed females had an opportunity to breed. Aggressive behaviour of solitary females in the field might have been related to hormonal changes associated with pregnancy, which is known to increase aggression (Rosenblatt, Factor, & Mayer, 1994).

Singly housed females were more amicable and less aggressive towards males than the philopatric females were, and the same levels of tolerance were found for strange females. Ontogenetic changes in social behaviour can occur pre- and postdispersal. In solitary species, affiliative behaviour predispersal can be followed by intolerance of and aggression directed at conspecifics around dispersal and thereafter (Schoepf & Schradin, 2012b). Affiliative behaviour in group-living species is typically restricted to group members (Smith, Chung, & Blumstein, 2013), and aggression can occur at maturity because of competition for positions on a dominance hierarchy (Walters, 1980). Whereas xenophobia is common in group-living species, tolerance of strangers as an adaptive response to form new groups can occur (Ganem & Bennett, 2004; Moore, 1984). The high levels of amicable and low levels of aggressive behaviours in singly housed females might indicate a general tendency to sociality, which could explain why striped mice always choose to live in groups in periods without reproductive competition, as occurs during the nonbreeding season (Schoepf & Schradin, 2012a; Schradin, König, et al., 2010).

Our study indicates that living as a young adult in a family group has very different consequences for male and female striped mice. Whereas several previous studies demonstrated reproductive suppression in philopatric males living in family groups, this does not occur in female striped mice. The alternative explanations for why many young adult females do not breed, even though they might be physiologically capable of doing so, are territorial aggression, within-group competition between breeding females, inbreeding avoidance and energy allocation trade-offs between reproduction and growth/survival. Future studies will have to test these factors. Still, reproductive suppression might occur under high population density in communally breeding groups. Additionally, our study indicates that being housed alone induces behavioural changes in young adult females, which become more amicable and less aggressive.

In conclusion, our study demonstrates the high flexibility of social behaviour in striped mice, which can change their reproductive and social tactics depending on variable ecological conditions (Schradin, Lindholm, et al., 2012). The reason that females choose to disperse and breed solitarily instead of breeding communally is apparently not to avoid physiological reproductive suppression in family groups, as young females are not reproductively suppressed, although we cannot exclude the possibility that this occurs under high population density. Staying in the natal group as a young nonreproducing female can be regarded as a real alternative reproductive tactic, which avoids costs of reproductive competition within groups in the form of infanticide and female–female aggression Schradin, Lindholm et al. 2012. This tactic is predicted to lead to increased survival owing to the benefits of group living and ultimately increased lifetime reproductive success.

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References

Abbott, D. H. (1984). Behavioral and physiological suppression of fertility in subordinate marmoset monkeys. *American Journal of Primatology, 6*, 169–186.
Brant, C. L., Schwab, T. M., Vandenbergh, J. G., Schaefer, R. L., & Solomon, N. G. (1998). Behavioural suppression of female pine voles after replacement of the breeding male. *Animal Behaviour, 55*, 615–627.
Brooks, P. M. (1982). Aspects of the reproduction, growth and development of the four-striped mouse, *Rhabdomys pumilio* (Sparrman, 1784). *Mammalia, 46*, 53–64.
Byers, S. L., Wiles, M. V., Dunn, S. L., & Taft, R. A. (2012). Mouse estrous cycle identification tool and images. *PLoS One, 7*, e35538.
Clark, M. M., & Galef, B. C. (2001). Socially induced infertility: familial effects on reproductive development of female Mongolian gerbils. *Animal Behaviour, 62*, 897–903.
Clarke, F. M., Miethe, G. H., & Bennett, N. C. (2001). Reproductive suppression in female Damaraland mole–rats *Cryptomys damarensis:* dominant control or self–restraint?. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 268*(1470), 899–909.
Creel, S. (2001). Social dominance and stress hormones. *Trends in Ecology & Evolution, 16*, 491–497.
Dawkins, R. (1980). Good strategy or evolutionary stable strategy? In G. W. Barlow, & J. Silverberg (Eds.), *Sociobiology: Beyond nature/nurture* (pp. 331–367) Boulder, CO: Westview Press.
Ganem, G., & Bennett, N. C. (2004). Tolerance to unfamiliar conspecifics varies with social organization in female African mole-rats. *Physiology & Behavior, 82*, 555–562.
Getz, L. L., Dluzen, D., & McDermott, J. L. (1983). Suppression of reproductive maturation in male-stimulated virgin female *Microtus* by a female urinary chemosignal. *Behavioural Processes, 8*, 59–64.
Gross, M. R. (1996). Alternative reproductive strategies and tactics: diversity within the sexes. *Trends in Ecology & Evolution, 11*, 92–98.
Hayes, L. D. (2000). To nest communally or not to nest communally: a review of rodent communal nesting and nursing. *Animal Behaviour, 59*, 677–688.
Heimann, M., Kasermann, H. P., Pfister, R., Roth, D. R., & Burki, K. (2009). Blood collection from the sublingual vein in mice and hamsters: a suitable alternative

to retrobulbar technique that provides large volumes and minimizes tissue damage. *Laboratory Animals, 43*, 255–260.
Jones, M. A., Mason, G., & Pillay, N. (2010). Early social experience influences the development of stereotypic behaviour in captive-born striped mice *Rhabdomys*. *Applied Animal Behaviour Science, 123*, 70–75.
Koenig, W. D., & Dickinson, J. L. (2008). Cooperative breeding as an alternative reproductive tactic. In R. F. Oliveira, M. Taborsky, & H. J. Brockmann (Eds.), *Alternative reproductive tactics* (pp. 132–174). Cambridge, U.K.: Cambridge University Press.
Moehlman, P. D., & Hofer, H. (1997). Cooperative breeding, reproductive suppression, and body mass in canids. In N. G. Solomon, & J. A. French (Eds.), *Cooperative breeding in mammals* (pp. 76–128). Cambridge, U.K.: Cambridge University Press.
Moore, J. (1984). Female transfer in primates. *International Journal of Primatology, 5*, 537–589.
Perrin, M. R., & Johnson, S. J. (1999). The effect of supplemental food and cover availability on a population of the striped mouse. *South African Journal of Wildlife Research, 29*, 15–18.
Pillay, N. (2002). Father-daughter recognition and inbreeding avoidance in the striped mouse, *Rhabdomys pumilio*. *Mammalian Biology, 67*, 212–218.
Rambau, R. V., & Robinson, T. J. (2003). Molecular genetics of *Rhabdomys pumilio* subspecies boundaries: mtDNA phylogeography and karyotypic analysis by fluorescence *in situ* hybridization (FISH). *Molecular Phylogenetics and Evolution, 28*, 564–575.
Reyer, H. U., Dittami, J. P., & Hall, M. R. (1986). Avian helpers at the nest: are they psychologically castrated? *Ethology, 71*, 216–228.
Rosenblatt, J. S., Factor, E. M., & Mayer, A. D. (1994). Relationship between maternal aggression and maternal care in the rat. *Aggressive Behavior, 20*, 243–255.
Saltzman, W., Ahmed, S., Fahimi, A., Wittwer, D. J., & Wegner, F. H. (2006). Social suppression of female reproductive maturation and infanticidal behavior in cooperatively breeding mongolian gerbils. *Hormones and Behavior, 49*, 527–537.
Savage, A., Ziegler, T. E., & Snowdon, C. T. (1988). Sociosexual development, pair bond formation, and mechanisms of fertility suppression in female cotton-top tamarins (*Saguinus oedipus oedipus*). *American Journal of Primatology, 14*, 345–359.
Schoepf, I., & Schradin, C. (2012a). Better off alone! Reproductive competition and ecological constraints determine sociality in the African striped mouse (*Rhabdomys pumilio*). *Journal of Animal Ecology, 81*, 649–656.
Schoepf, I., & Schradin, C. (2012b). Differences in social behaviour between group-living and solitary African striped mice, *Rhabdomys pumilio*. *Animal Behaviour, 84*, 1159–1167.
Schradin, C., Eder, S., & Müller, K. (2012). Differential investment into testes and sperm production in alternative male reproductive tactics of the African striped mouse (*Rhabdomys pumilio*). *Hormones and Behavior, 61*, 686–695.
Schradin, C., König, B., & Pillay, N. (2010). Reproductive competition favours solitary living while ecological constraints impose group-living in African striped mice. *Journal of Animal Ecology, 79*, 515–521.
Schradin, C., & Lindholm, A. K. (2011). Relative fitness of alternative male reproductive tactics in a mammal varies between years. *Journal of Animal Ecology, 80*, 908–917.
Schradin, C., Lindholm, A. K., Johannesen, J., Schoepf, I., Yuen, C., König, B., et al. (2012). Social flexibility and social evolution in mammals: a case study of the African striped mouse (*Rhabdomys pumilio*). *Molecular Ecology, 21*, 541–553.
Schradin, C., & Pillay, N. (2004). Prolactin levels in paternal striped mouse (*Rhabdomys pumilio*) fathers. *Physiology & Behavior, 81*, 43–50.
Schradin, C., Schneider, C., & Lindholm, A. K. (2010). The nasty neighbour in the striped mouse (*Rhabdomys pumilio*) steals paternity and elicits aggression. *Frontiers in Zoology, 7*, 19.
Schradin, C., Schneider, C., & Yuen, C. H. (2009). Age at puberty in male African striped mice: the impact of food, population density and the presence of the father. *Functional Ecology, 23*, 1004–1013.
Schradin, C., William, R., Krackow, S., & Carter, C. (2013). Staying put or leaving home: Endocrine, neuroendocrine and behavioral consequences in male African striped mice. *Hormones and Behavior, 63*, 136–143.
Schubert, M., Pillay, N., & Schradin, C. (2009). Parental and allo-parental care in a polygynous mammal. *Journal of Mammalogy, 90*, 724–731.
Smith, J. E., Chung, L. K., & Blumstein, D. T. (2013). Ontogeny and symmetry of social partner choice among free-living yellow-bellied marmots. *Animal Behaviour, 85*, 715–725.
Solomon, N. G., Brant, C. L., Callahan, P. A., & Steinly, B. A. (2001). Mechanisms of reproductive suppression in female pine voles (*Microtus pinetorum*). *Reproduction, 122*, 297–304.
Solomon, N. G., & French, J. A. (1997). *Cooperative breeding in mammals*. Cambridge, U.K.: Cambridge University Press.
Taborsky, M., Oliveira, R. F., & Brockmann, H. J. (2008). The evolution of alternative reproductive tactics: concepts and questions. In R. F. Oliveira, M. Taborsky, & H. J. Brockmann (Eds.), *Alternative reproductive tactics* (pp. 1–22). Cambridge, U.K.: Cambridge University Press.
Walters, J. (1980). Interventions and the development of dominance relationships in female baboons. *Folia Primatologica, 34*, 61–89.
Willan, B. R. (1982). *Social ecology of* Otomys irroratus, Rhabdomys pumilio *and* Mastomys natalensis (Unpublished doctoral dissertation). South Africa: University of Natal.
Wingfield, J. C., & Sapolsky, R. M. (2003). Reproduction and resistance to stress: when and how. *Journal of Neuroendocrinology, 15*, 711–724.
Würbel, H. (2001). Ideal homes? Housing effects on rodent brain and behaviour. *Trends in Neurosciences, 24*, 207–211.