Pair bonding in monogamously and polygynously kept African striped mice, Rhabdomys pumilio

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Pair-bonding in monogamously and polygynously kept African striped mice (*Rhabdomys pumilio*)

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ABSTRACT

Pair-bonding, i.e. individuals showing a preference for a specific opposite sex individual, has been demonstrated in several socially monogamous species. However, social bonds also occur in non-monogamous species, but have received less attention. Currently, we do not know whether social bonds in monogamous pairs differ from social bonds in polygynous pairs. We studied the socially flexible African striped mouse (*Rhabdomys pumilio*) in the laboratory, conducting 3 hour partner preference tests typically used to measure pair bonds in socially monogamous prairie voles. In the field, striped mice typically live in polygynous groups, but socially monogamous pairs have also been observed. We compared social bonds between 12 monogamous pairs and 12 polygynous groups (1 male and 2 females). The social situation (monogamous versus polygynous) did not influence social bonds. Female striped mice showed a clear preference for their partner. While males spent more time in body contact with their partner they showed a sexual preference for strange females. Polygynous males did not show a preference for one of their two females. While significant preferences for partners were found in striped mice, social preference was less strong than those reported for socially monogamous prairie voles. In sum, our study supports that opposite sex social bonds do not only occur in

monogamous but also in species that can live in polygynous groups, but might be weaker in polygynous species.

INTRODUCTION

Socials bonds between two or more individuals are associated with direct benefits for the partners, such as an improved nest defence, a better resource gathering or an increased efficiency of territory use (Bercovitch, 1988; Cords, 2001; McFarland et al., 2015; Foerster et al., 2015; Kern & Radford, 2016). Socials bonds are generally described as a subset of affiliative and permanent social relationships between two or more conspecifics (Carter, 2005; Sachser, 2005). They are characterized by social proximity (Sachser, 2005) and amicable interactions, measured as a reduced physical distance between the partners (Carter & Porges, 2010). In mammals, social proximity and social engagement are common between mothers and their offspring, indicating that they are socially bonded (Nelson & Panksepp, 1996). Social bonds are also established between partners who cooperate to breed over extended periods of time (Carter et al., 1995). Socials bonds between two partners and bi-parental care often characterise socially monogamous species (Kleiman, 1977). Social monogamy is defined as a long term association between one adult male and one adult female covering at least one breeding season. About 10% of mammals are socially monogamous, and both males and females exhibit selective social behaviours, called "pair bonds" (Kleiman, 1977). Pair bonds are characterised by social preference and by the male and female remaining together after mating and often even during non-reproductive periods (Carter et al., 1995; Carter, 2005; Lukas & Clutton-Brock, 2013).

In mammals, pair bonds occurs in several species of primates, such as titi monkeys (*Callicebus cupreus*; Carp et al., 2015), owl monkeys (*Aotus azarai*; Fernandez-Duck & Huque, 2013) and marmosets (*Callithrix penicillata;* Smith et al., 2010). Pair bonds also occur in socially monogamous rodents such as California mice (*Peromyscus californicus*; Gubernick & Nordby, 1993) and prairies voles (*Microtus ochrogaster*; Carter et al., 1995; Blocker & Ophir, 2016). Prairies voles are the main study model for pair bonding. They show all characteristics

of monogamy: they live together, co-defend their shared territory and nest, and both sexes contribute to the care of the young (Johnson & Young, 2015). A recent study shows that even under ideal conditions for mating with multiple receptive females, male prairies voles prefer to establish a pair bond with one particular female (Blocker & Ophir, 2016). Pair bonds between two individuals are measured in partner preference tests (Carter & Keverne, 2002; Young & Wang, 2004; Devries et al., 1995). The criteria used to detect partner preference is the amount of time spent in physical contact by the focal individual with his partner compared to a stranger (Cho et al., 1999; Devries et al., 1995; Bales & Carter, 2003). While pair bonds occur between the mates of a pair, similar social bonds might also occur in other social organizations such as in polygynous groups.

In polygynous groups, individuals might develop social bonds with several non-related partners from the opposite sex (Parker et al. 2001; Karelina 2010). Prairie voles, which are considered monogamous, can also switch to a polygynous mating system under high population density (Getz et al., 1987). While the social system changes, social bonds between individuals still occur, even with multiple non-related partners of the opposite sex (Young, 1999). In another rodent species, the meadow vole (*Microtus pennsylvanicus*), females are territorial during summer but show high social tolerance to conspecifics during colder months in winter (McShea & Madison, 1984). In winter, meadow voles form stable groups composed of several females and at least one male (Madison et al., 1984). Voles form opposite-sex pair bonds in these groups (Parker et al., 2001). While social bonds can occur in polygynous groups, it has never been tested whether the strength of social relationships differs between polygynous groups and monogamous pairs. The comparison of social bonds between two species with a different social systems could be biased. However, within some species, individuals can show different forms of social organization, often depending on the environmental conditions and period. This is the case for several rodents' species such as prairies voles (Getz & Carter, 1996), house mice (*Mus musculus*, Latham & Mason, 2004) and African striped mice (*Rhabdomys*

79 *pumilio*, Schradin, 2013). These species are called socially flexible, as individuals can switch
80 their social tactics resulting in a change of the social system (Schradin, 2013). Consequently,
81 individuals of the same species can be polygynous while other individuals might be
82 monogamous. These socially flexible species are good candidates to investigate differences in
83 social relationships between monogamous and polygynous groups.

84 The African striped mouse, a diurnal murid rodent, has been described as a socially flexible
85 species (Schradin et al., 2010; Schradin et al., 2012). Striped mice can live in polygynous
86 groups, in pairs, or solitarily (Schradin et al., 2010; Schradin et al., 2012; for pair living:
87 Schradin, unpubl. data). Females often remain philopatric and breed in their natal group, such
88 that breeding females of one group are closely related, typically sisters, half-sisters, or cousins
89 (Schradin & Pillay, 2004; Schradin et al. 2010; genetic comparison: Schradin & Lindholm,
90 unpubl. data). Individuals of one group show amicable behaviour towards each other, such as
91 grooming and sitting in body contact, but they are aggressively territorial towards individuals
92 from other groups (Schradin & Pillay, 2004). However, whether partners of striped mice groups
93 form affiliative bonds is so far unknown. A recent study indicating individual recognition in
94 striped mice makes it likely that social bonds occur (Brunner et al., 2016). The first aim of this
95 study was therefore to determine whether captive male and female striped mice from
96 polygynous and monogamous groups form selective opposite-sex partner preferences, and
97 whether the strength of social bonds differ between monogamously and polygynously kept
98 groups. We then compared our results to results from prairie voles that used similar
99 methodology, comparing the percentage of time spent in physical contact by the focal individual
100 with their partner in contrast to an unfamiliar individual (Blocker & Ophir, 2016). Our third
101 aim was to determine whether males from polygynous groups show preference toward one
102 particular female or if they bond equally with both females.

MATERIAL AND METHODS

Study animals and housing conditions

Striped mice were kept and bred at the animal facility of the IPHC-DEPE. The founders of this colony originated from a captive colony kept at the Succulent Karoo Research Station in South Africa, which arose from individuals trapped in 2003 and in 2016 in the Goegap Nature Reserve where a long term study about this specie is done. All animals used in the study were born at the IPHC-DEPE.

Breeding pairs were housed in two tanks (each 40 x 30 x 30 cm) connected by a tube. Tanks were provided with litter of crushed corn cobs and water was available ad libitum. To avoid obesity (leading to infertility and diabetes in this species) and thus to increase welfare of animals, animals were not fed ad libitum. In the mornings animals were fed with a mix of cereals, fruits and seeds ("Nager Krokant", ZG Raiffeisen) with approximately 4.25 gram/adult. In the afternoon one pinch (less than 2 grams) of food supplementation, consisting of a mix of seeds for tropical finches, was given for 4 adults. Juveniles were considered as half an adult for food quantity. With this diet, animals grew fast and reached a body mass typical for the heaviest individuals in the field during the moist season with highest food availability. To enhance the welfare of individuals, as enrichments, a shelter (10cm x 8cm x 12cm long) was provided in one of the two tanks, and in the second tank one wooden tunnel (20cm long) was provided, with 6 holes (3.5cm diameter) per tunnel (7.0cm diameter). Furthermore, the two tanks functioned to reduce stress during cleaning, as only one tank per week was cleaned, keeping one familiar tank for individuals at all times, and individuals were not handled during cleaning. The tanks were maintained in a room under 11:13 hour light-dark cycle. The light was turned on at 8 a.m. and off at 7 p.m., starting at 10% light at 8 a.m. increasing to 100% light at 12 p.m and decreasing slowly until 6 p.m., mimicking natural lighting conditions during the breeding season. The ambient temperature was maintained at 18°C during the night and rose to 25°C

during the day. Study animals were separated from their parents at an age of 21-30 days (weaning occurs at day 16) and housed in same-sex siblings groups (from two to six individuals). Sibling groups were kept in two connected tanks under the same conditions as described above.

Striped mice were paired when fully adult (90-120 days old). For the pairs, one male and one female were removed from two sibling groups and housed together in two cages connected by a tube, in the same conditions as previously mentioned. The female and the male were unrelated and had approximately the same age (99 days +/- 12 days). For the polygynous groups, one male was housed with two females that were unrelated to the male, but which were full sisters of the same age (originated from the same litter) and kept together in one sibling group. In the field, breeding females of a group are closely related and can be full sisters. The male and the two females had approximately the same age (97days +/- 14 days).

Experiment 1: Partner preference test

In total, individuals from twelve pairs (N= 24 individuals) and twelve trios (N= 24) were tested (12 females from trios were not tested). Our aim was to study social bonds independent of sexual motivation. After pairing, striped mice typically mate within 3-7 days and then females are not receptive anymore. Therefore, tests started seven days after pairing. This duration would have been sufficient, as it was longer than in previous studies on rodents (prairie voles, Carter et al., 1995; Wang & Aragona, 2004; Blocker & Ophir, 2016; and meadow voles, Parker et al., 2001), to allow the formation of social bonds between individuals. Receptiveness of females was then determined in retrospect from pregnancy and parturition. In total 19 females were pregnant during the preference test and 5 were not, of which only one had a perforated vagina potentially allowing for mating. These 5 females behaved the same as the other females with

152 no obvious differences. One female was excluded from the study because she did not move
153 through the tunnels of the three cages (figure. 1).
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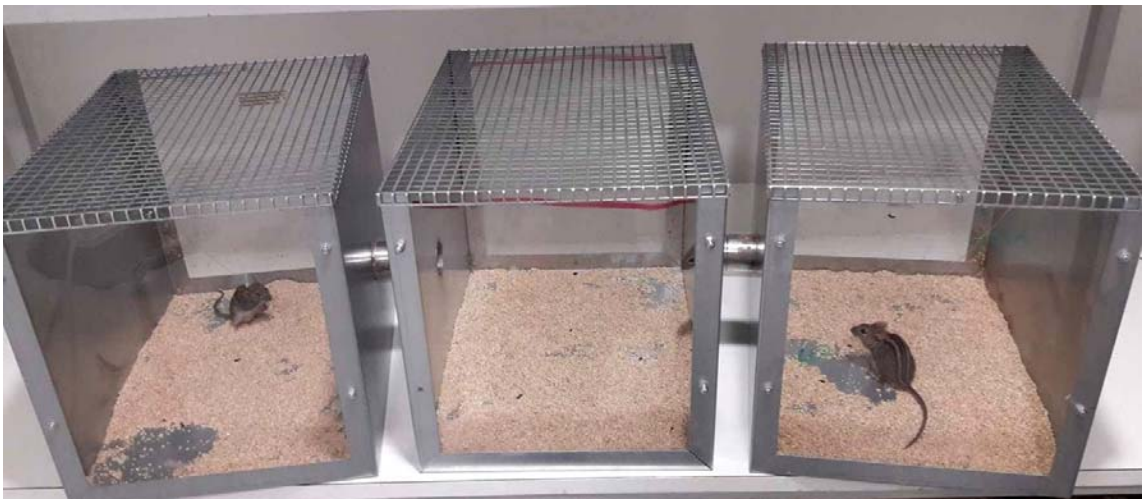


155

156 **Figure 1**: Setup used for preference test as well as polygynous tests, composed of three tanks
157 connected with two tubes. The mice at the two sides were tethered with a collar to which a
158 fishing line was attached to the lids. Here the line was taut and the mice could move until the
159 middle of their respective tanks but not further. The focal individual (in the right tube
160 connecting two tanks) was free to move through the entire device.
161

162 During the partner preference tests, a focal individual was presented with two mice from
163 the opposite sex, one being his partner and the other being a stranger (i.e. control individual),
164 and the interactions with both were recorded. Partner preference tests were running during 3h
165 the mornings between 9h30 to 12h30. Each test was video recorded with a camcorder (SONY,
166 HDR-CX405). In total, 48 individuals were tested for the partner preference tests, including 24
167 males and 24 females coming from 12 trios and 12 pairs. The partner preference tests were
168 conducted in a three tank apparatus (each tank 30 x 30 x 40 cm) with a central tank being
169 connected to the other two tanks by two tubes (figure 1). All mice used were weighted before

the experiment, and their reproductive status was noted. All males were scrotal. For females, it was recorded whether the vagina was closed or perforated. Pregnant females, as well as females with a closed vagina, were not sexually receptive. Two striped mice of the same sex with similar body mass ($mean_{control}$ = 46.6 +/- 5.9; $mean_{partner}$ = 46.3 +/- 5.4; Paired t test: t_{44}=0.24, p>0.05) and reproductive status were used for the partner preference test. One striped mouse was the partner of the focal mouse, with which it had cohabited during at least one week, and the second was a stranger, coming from another pair. The partner, as well as the stranger, were tethered by a fishing line (diameter of 0.4 mm) connected to a collar made of a cable-tie (2.5 x 98 mm). Each of them was placed in one of the two opposite tanks of the device, one on the left tank, one on the right (figure 1). For half of the experiments, the partners were one the right, and for the other half on the left. The line was attached to the lids of the tanks and was long enough to allow individuals to move normally in the cage, but too short for them to reach the connecting tube. After tethering the mice in the tanks, 20min acclimation was given to allow them to get used to the collars. After this period, the focal individual was released in the middle tank and it was free to move through all three tanks. Among the 48 tests realized, 22 experiments were shorter than 3h (mean 2h 14min $\pm$ 8min, range 1h 23min – 2 h 48 min) because the mice chewed through the cable and got free. Data from shorter videotaping did not differ from data obtained from videotaping for 3 hours (see Fig. 1 in electronic supplement). As data were analysed as percentage of total time (see below), these experiments could still be used. These cases were recognised quickly as experiments were monitored from outside via a video screen; mice never fought and never got injured. Two tests could not be analysed, one because the focal mouse did not jump through the tubes to move freely through the device, and the second because of a SD card problem leading to data loss.

All males from pairs and trios, as well as all females from pairs and one designated female per trio, were included in a maximum of three tests: once as the focal, once as the partner, and once as the control individual. Eight out of 48 mice were tested only twice (as the focal and

196 the partner). The mice were chosen to have randomized tests (between control, partner and
197 focus) to allow independent statistical analysis between the different tests. Between each
198 experiment, the tanks were cleaned with water, dried, and clean litter was provided.
199

200 *Experiment 2: Male preference test in polygynous groups*

201 The goal of this experiment was to determine whether polygynously mated males show a
202 preference for one of their two females. Polygynous preference tests were done in the same
203 room as experiment 1 and under the same conditions in the three tank apparatus, with both
204 females being tethered and the male moving freely in the apparatus. The tests were conducted
205 in the afternoons between 2 p.m. to 6 p.m. during 30min per trio and each trio was tested eight
206 times. For 4 trios only 6 videos were recorded because one female gave birth. Two trios had to
207 be excluded from the analysis, one because a female gave birth before the sixth test and the
208 second one because one mouse died of unknown reasons in the home tank (not during
209 experiment). Only one test per day per trio was done. Each day, the side where each female was
210 tethered was alternated compared to the previous day, one day to the left and the next one to
211 the right.

212

213 *Video analysis*

214 Videos were analysed with the software BORIS (version 3.48; Friard & Gamba, 2016). Four
215 categories of behaviours were distinguished: 1. sexual behaviours (mounting, sniffing genitals
216 and lordosis), 2. sociopositive behaviours (grooming and sitting in body contact), 3. aggressive
217 behaviours (standing upright, charge), and 4. presence, i.e. the presence of the focal mouse in
218 the middle tank or in the same tank as another mouse but without interacting with it. Aggressive

behaviour was very rare and biting never occurred (see results) and no individual was injured. Sexual behaviours and sociopositive behaviours were in some cases analysed together as "social contact". All behaviours were recorded as states (duration) and they were all exclusive, i.e. the start of one behaviour stopped the previous one. A first set of modifiers, "Partner", "Control" and "Alone", was added to record in which tank the focal mouse was for each expressed behaviour. For the polygynous test, the modifiers were "Left" or "Right" instead of Partner/Control. We also recorded whether the focal mouse initiated or received the behaviour. When one mouse initiated a behaviour, we also noticed if the other mouse tried to avoid this behaviour.

Welfare note

We adhered to the ASAB/ABS Guidelines for the Use of Animals in Research. All experiments conducted received ethical clearance from the CREMEAS Committee (reference n° 2016121213346905). Mice were checked in their home cages twice a day in the morning and afternoon for their well-being. The welfare of animals was improved by using highly enriched cages, and by only changing one cage per week, such that animals had not to be handled for cleaning and always had one familiar cage. After tethering, mice which were left alone in the experimental room for a 20minute acclimation phase. During this entire period they were watched from outside on a monitor via a webcam to ensure that they were not in distress. After experiments, individuals were either euthanized or remained as breeding stock in the colony.

Statistical analysis

All analyses were made in Rstudio using R (version 3.1.1). Data are presented as mean ± SD. To test for partner preference, a Linear Mixed-Effects Model (LMM) was used with the percentage of time the focal individual spent in social interactions with the partner or the stranger as dependant variable. The tested model was: social contact ~ sex*group*stimulus + whatfirst + (1|ID). "Social contact" was the proportion of time spent by the focal mouse in social contact with a conspecific (socio-positive and sexual behaviours combined), either with the partner or with the stranger, such that each individual had two data points. ID of the focal individual was thus added as a random factor. "Sex" was the sex of the focal individual. "Category" represented whether the focal mouse came from a monogamous pair or a polygynous group. "Stimulus" was the role of the conspecific tested, "Partner" or "Control". As individuals were often used in two tests, as stimulus or as focal individual, which could influence their behaviour, we included this as the categircal factor "what first" into the model. Tukey Contrasts were used as post-hoc tests. To test whether males and females spent the same amount of time in the three tanks of the apparatus, a second Linear Mixed-Effects Model (LMM) was used. The tested model was: time ~ sex * stimulus + (1|ID). Here, "stimulus" represented the tank where the focal was present ("Partner", "Control", or "Middle"). Therefore, one Kruskall-Wallis test per sex was used to test for a difference in the time spent in the different tanks. To compare the results between sexes, Conover tests were used. In order to see which component of social contact (sexual, grooming, and body contact) could influence the choice of individuals, three additional LMMs were run. The tested models were for each component: Grooming ~ sex*stimulus*group + (1| ID), Body Contact~ sex*stimulus*group + (1| ID), and Sexual ~ sex*stimulus*group + (1| ID). To test for a preference of the males for one of female in the polygynous groups, we used paired t test per trio. For this, each female of each trio received randomly the status "female A" or "female B". Therefore, for each trio, we compared the proportion of time the focal male spent in physical contact with female A *versus*

female B. In order to correct for multiple comparisons, sign tests were used where each trio contributed one data point (categorical: significant preference yes or no).

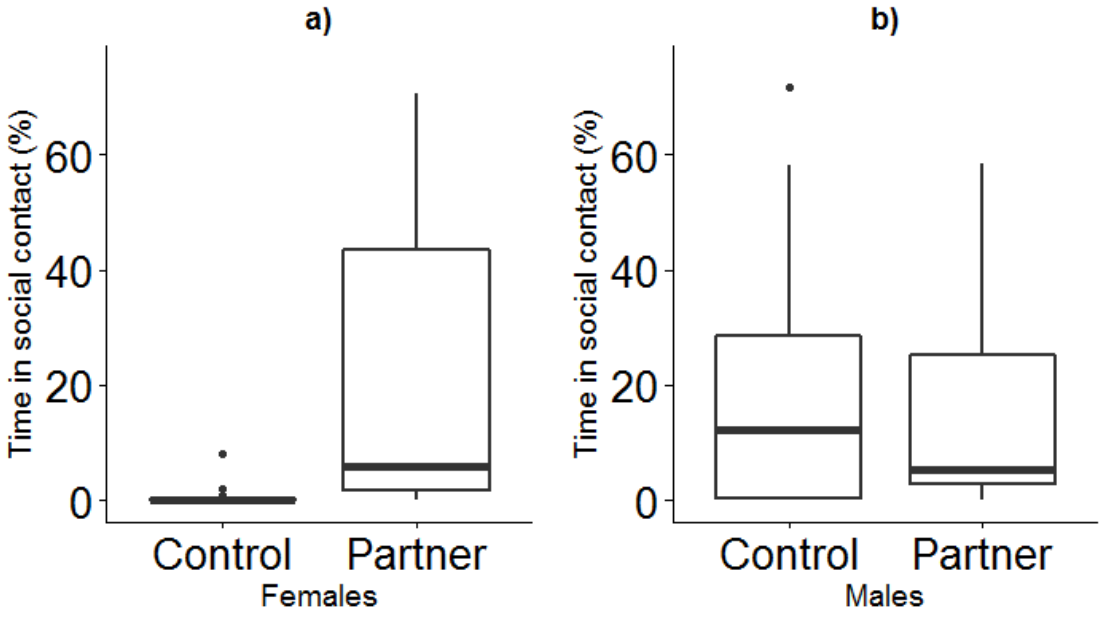
Results

Table 1: Results of the LMM investigating which variables relate to the time spent in social contact by the focal mice. The four variables tested are the sex of focal individual (male or female), whether they were kept in monogamous or polygynous "group", and the stimulus (control or partner). Significant variables are indicated in bold.

Model 1 : Social contact ~ sex*stimulus*group + whatfirst + (1|ID)			
Variable	Estimate (Chisq)	df	p value
sex	2.48	1	0.11
stimulus	**10.87**	1	**0.0009**
group	0.006	1	0.93
whatfirst	0.07	1	0.78
sex*stimulus	**4.16**	1	**0.04**
sex*group	0.7	1	0.4
stimulus*group	0.86	1	0.35
sex*stimulus*group	0.1	1	0.74

Experiment 1: Partner preference test

Stimulus and the interaction between sex and stimulus were significantly related to the time focal mice spent in social contact with other mice, while there was no significant difference in preference between individuals coming from monogamous or polygynous groups, nor was there an effect whether an individual was first tested as stimulus or focal individual (whatfirst) (table 1). Females spent significantly more time with their partner than the control, while there was no significant difference for males (Multiple Comparison of Means: Tukey contrasts; Figure 2).



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286 **Figure 2:** Percentage of time spent in social contact (+/- Standard deviation) by the focal
287 individual with the control and the partner individual for a) females (N=23) and b) males
288 (N=23). The bold line represents the median.

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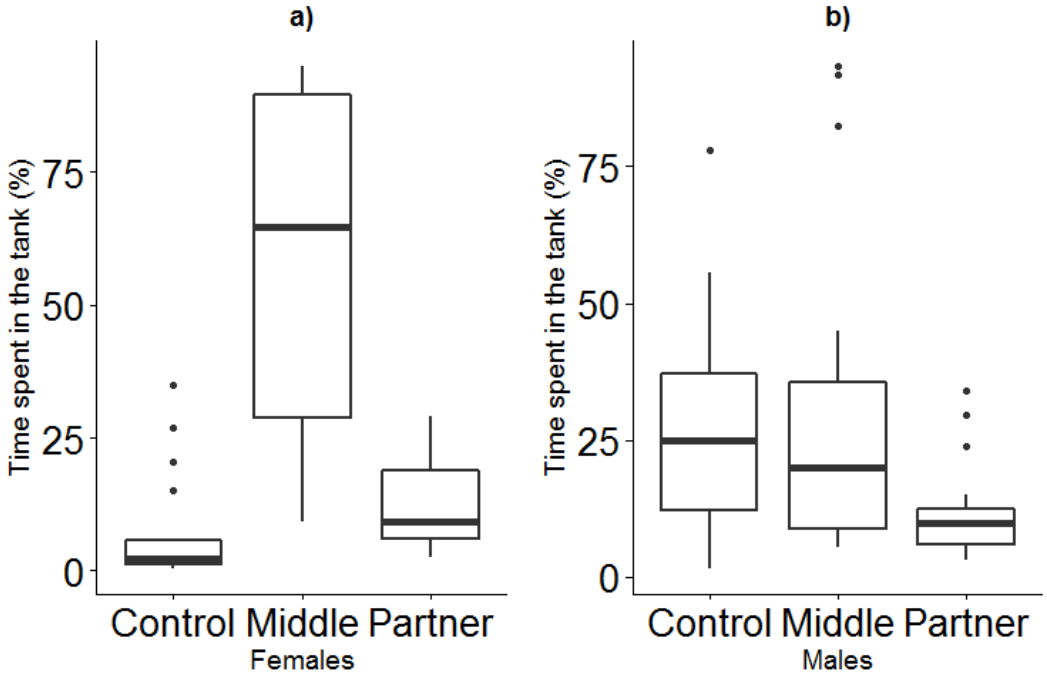

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291 **Figure 3:** Percentage of time the focal individual spent in each tank (+/- Standard deviation) of
292 the three-chamber apparatus without interacting with the other mice for (a) females (N=23) and
293 (b) males (N=23). The bold lines are the median.

The time spent in the three different compartments (middle/partner/control) was significantly influenced by the interaction between sex and tank (LMM, $p<0.001$). The time spent in the three different tanks differed both for females (Kruskal Wallis $Chisq^2= 41.20$. $P<0.001$) and for males (Kruskal Wallis $Chisq^2 = 10.87$, $P<0.01$). Females spent more time alone in the middle tank than with their partner, and they spent more time in their partner's tank than in the control's tank (Conover test, $P_{Middle\text{-}Control} <0.001$, $P_{Middle\text{-}Partner} <0.001$, $P_{Partner\text{-}Control} <0.001$, Figure 3a). Males spent significantly less time in the tank with the partner, with no difference in time spent in the control and the middle tank (Conover test, $P_{Middle\text{-}Control} =0.49$, $P_{Middle\text{-}Partner} <0.01$, $P_{Partner\text{-}Control} <0.01$ Figure 3b).

Table 2: Results of the three LMMs investigating which variables influence the time spent by the focal mice with the two stimulus "control" and "partner" in A) grooming interaction B) in body contact, and C) sexual interaction. The variables tested are the sex of focal individual "males" or "females", their group "monogamous" or "polygynous" and their identity "ID" added as a random factor. Significant variables are in bold.

A) **Model 3 : Grooming ~ sex*stimulus*group + (1| ID)**

Variable	Estimate (Chisq)	df	p value
sex	**6.15**	**1**	**0.013**
stimulus	**3.96**	**1**	**0.046**
group	1.2	1	0.27
sex*stimulus	2.54	1	0.11
sex*group	0.46	1	0.49
stimulus*group	0.13	1	0.71
sex*stimulus*group	0.04	1	0.82

B) **Model 4 : Body contact ~ sex*stimulus*group + (1| ID)**

Variable	Estimate (Chisq)	df	p value
sex	0.93	1	0.33
stimulus	**11.42**	**1**	**0.0007**
group	0	1	0.99

sex*stimulus	3.24	1	0.07
sex*group	0.53	1	0.46
stimulus*group	1.05	1	0.3
sex*stimulus*group	0.1	1	0.74

C) **Model 5 : Sexual ~ sex*stimulus*group + (1| ID)**

Variable	Estimate (Chisq)	df	p value
sex	**5.27**	**1**	**0.021**
stimulus	0.14	1	0.7
group	0.013	1	0.9
sex*stimulus	**5.1**	**1**	**0.023**
sex*group	0.43	1	0.5
stimulus*group	0.043	1	0.83
sex*stimulus*group	0.0046	1	0.94

There was a sex difference concerning the time spent grooming the stimulus individual (LMM sex difference: P=0.04; stimulus difference P=0.01; Table 2A). Males spent significantly more time grooming control individuals compared to females but males and females did not differ in the amount of time spent grooming their partner (Figure 4 a,b). Time spent in body contact differed according to the stimulus animal (Table 2C) but not between sexes. Striped mice from both sexes spent significantly more time in body contact with their partner than with the control (Figure 5). Model 5 found a significant interaction between sex and stimulus for sexual behaviour (LMM, P=0.023, Table 2B). Mice from both sexes spent a similar amount of time in sexual interactions with their partner (Figure 4d) but males spent more time in sexual interactions with strangers than females (Figure 4c).

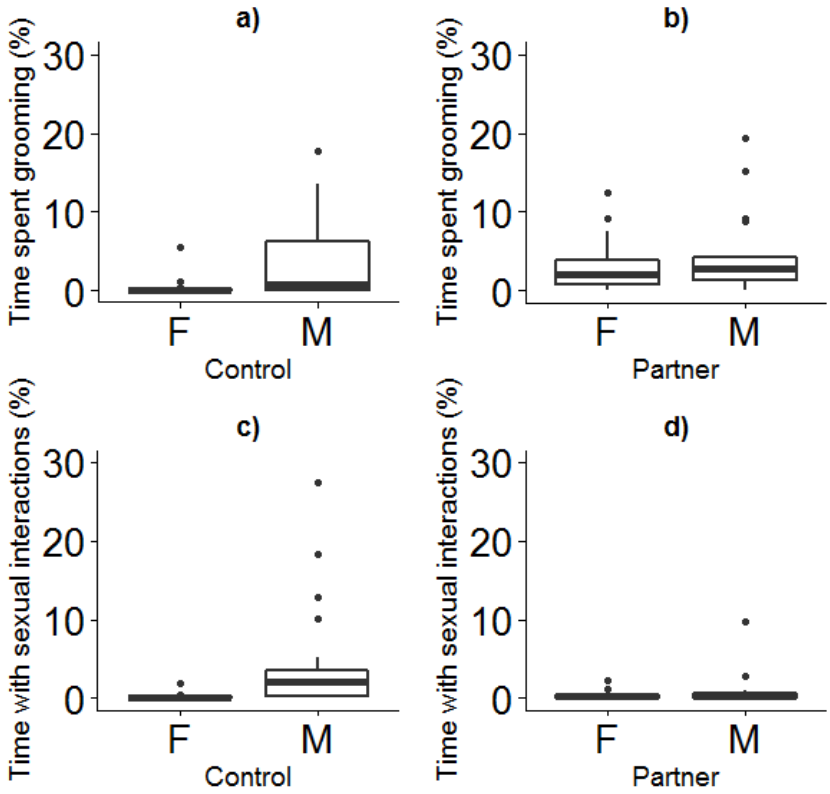

Figure 4: Percentage of time spent by the focal mice in grooming interactions with a control (a) or with its partner (b) for both sexes (F: females. M: males, each N=23). Percentage of time spent by the focal mice in sexual interaction with a control (c) or with its partner (d) for both sexes (F: females. M: males, each N=23). The median is represented by the bold horizontal line.

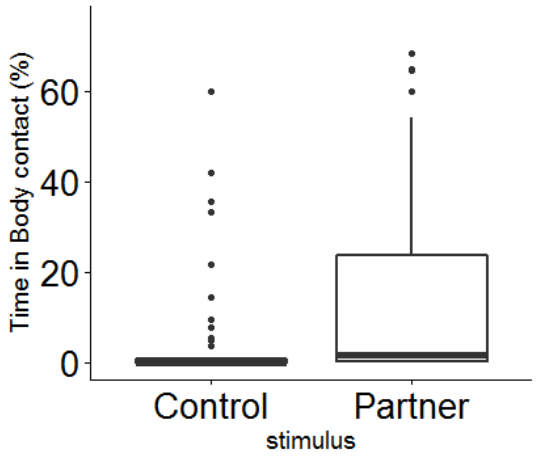

Figure 5: Percentage of time spent by the focal mice from both sexes in body contact with a control or with their partner (N=46). The median is represented by the bold horizontal line.

Comparison with prairies vole published data

Males did not show a partner preference (see above) and were not analysed here. Females spent on average 22.62% +/- 26.7; range [0.14; 70.47] of their time with their partner. Only 3 out of 23 female striped mice reached the criteria of 66% reported for female prairie voles (Devries 1995). Females striped mice spent significantly less time in social contact with their partner compared to an expectation based on data reported from female prairie voles (one sample t test with $\mu=0.66$, $t_{22}=-7.73$. $p<0.001$).

Experiment 2: Male preference in polygynous groups

Only two out of ten males showed a significant preference for one particular female (Trio 4, $t_7=-4.34$; $p<0.01$; and Trio 6, $t_7=-4.61$; $p<0.01$). After correction for multiple comparisons, these differences did not remain significant. Male striped mice did not significantly prefer one of their females over their other female (sign test, $p>0.1$).

DISCUSSION

Understanding social bonds between sex partners is important to understand the variety of social systems we observe. However, so far the focus on studying social bonds was only on one specific social system, social monogamy. This is not sufficient to understand social evolution. Here we studied social bonds in a socially flexible species that can live in pairs, but typically lives in polygynous groups. We found that female striped mice form selective partner preferences, independently whether they lived in a monogamous or a polygynous group. In contrast, males did not show social preferences. Males showed a sexual preference for a strange female over their partner female. Interestingly, males and females did not differ in the amount

of social interactions with their partner, but males interacted more than females with the unfamiliar individual. Thus, the preference of males for an unfamiliar individual might not indicate that they are less bonded to their female partner than females are bonded to their male partner. There was no difference between monogamously and polygynously kept striped mice regarding their preference for familiar versus strange partners. Finally, there was little evidence that polygynous males prefer one female over the other. Our study shows that social bonds similar to pair bonds can occur in polygynous species, though these bonds seem to be less strong.

Monogamously and polygynously kept striped mice did not differ in the social bonds they formed with their opposite sex partner(s). Whether striped mice lived in pairs or polygynous groups was determined by the experiment and did not represent a choice of the individuals. Therefore, the imposed mating system could explain the absence of differences obtained between monogamous and polygynous mice. Importantly, female striped mice showed a partner preference by spending twice as much time with their partner compared to the stranger, independent from the social situation (monogamous or polygynous) under which they were living. In contrast, male striped mice showed a sexual preference for control individuals, even though the females in our study were not sexually receptive. This might explain why sexual behaviour was shown very rarely by males and why females showed very little if any sexual interest in any male (Fig. 4d). Our experimental setup aimed at only measuring social preference and avoiding any sexual motivation and a different set of experiments using receptive females would have to be used to measure sexual preference. Importantly, in our study males did not show less interest (social contact, grooming, body contact) in their female partners than did females show for their male partners. Thus, the difference between the sexes in partner preference might not be due to differences in the strength of social bonding towards their partner. Instead, a higher sexual attractiveness for potential extra-group mates in males can

385 explain the results, and this would indicate an adaptive strategy, as male fitness is to one fifth
386 (21%) determined by extra-group paternity (Schradin et al., 2010).

387 Striped mice spent less time with their partners (22%) compared to what has been reported
388 for prairie voles (66%) by Devries et al (1995). Only three out of 23 female striped mice reached
389 the mean of 66% reported for prairie voles and male striped mice showed a sexual preference
390 for control individuals. While there have been differences between our study and studies on
391 prairie voles (our test apparatus did not allow mice to see the other two compartments, focal
392 individuals also acted as tethered partners, and duration of cohabitation before testing was
393 longer), these results indicate that these two species differ in social bonding.

394 Social bonds between opposite sex members do exist in polygynous species but might be
395 weaker than in some monogamous species. However, socially monogamous species without
396 pair-bonds also exist (Macroscellides, elephant shrews; Sauer 1973) and some socially
397 monogamous species prefer a stranger over their own partner (marmosets *Callithrix penicillata;*
398 Smith et al., 2010), while other polygynous species have also been reported to form pair-bonds
399 (meadow voles, Parker et al. 2001). Partner preference tests might fail to demonstrate a
400 preference even in species with pair bonds. For example in socially monogamous California
401 mice, males do not show a preference for their partner under normal conditions, but do so if
402 they are stressed (Kowalcyk et al. 2018). Additionally, social preferences can also exists
403 between familiar same sex individuals, as reported for prairie voles (but not laboratory mice;
404 Beery et al. 2018), meadow voles (Parker & Lee 2003), and titi monkeys (*Callicebus moloch*;
405 Carp et al. 2016) using partner preference tests in captivity, and also for non-reproductive adult
406 striped mice observed in the field (Brunner et al. 2016). This variety offers an important source
407 to study the mechanisms underlying social-bonding, independent of the social system of the
408 species. Research measuring the fitness consequences of variation in pair-bond strength is then
409 needed to explain the variation in bonding pattern over different forms of social organisation.

Prairie voles are typically monogamous (DeVries et al., 1995; Getz & Carter, 1996), while free-living striped mice generally form polygynous groups of one male and up to four breeding females (Schradin & Pillay, 2004). It has recently been demonstrated that even in the presence of two receptive females, male prairie voles prefer to establish a pair bond with one particular female and do not try to mate with the other (Blocker & Ophir, 2016). In prairie voles, when the male from a pair dies, eighty percent of the females do not form a new bond with another male (Pizzuto & Getz, 1998). Whether selective social bonds exist in polygynous species is so far unknown. Striped mice have recently been shown to be capable of individual recognition (Brunner et al., 2016) and polygynous males could thus have a favourite female partner. Instead, polygynous male striped mice seemed to bond equally with their two females in our study. Communal groups of striped mice consist of closely related breeding females such as sisters, half-sisters, and cousins (Schradin et al. 2010; Schradin & Lindholm, unpublished genetic data). In our study, females were full siblings from the same litter, i.e. of the same age and body mass. Thus, their fecundity and reproductive value for the male might have been equal, which – from an ultimate point of view - could explain why males did not show a preference towards one particular female. Under natural conditions, where females differ in body mass (Hill et al., 2015) and thus probably fecundity, and are genetically more diverse (Schradin et al. 2010), male preference for a particular female might be adaptive and might occur.

In conclusion, we found indication for pair bonding in the form of proximity between partners in socially flexibly African striped mice. Female striped mice showed a stronger partner preference while males had a sexual preference for strangers. The imposed mating system (i.e. polygyny or monogamy) had no impact on individual's preference. Thus, socials bonds between mating partners occur in monogamous and in polygynous species but they seem to be less strong in polygynous species. To understand the evolution of sociality it will be important to study social bonds in more species with a variety of social systems, which will

435 then enable us to come to a better understanding of the physiological mechanisms of social
436 bonding, and whether different mechanisms exist.

437

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