Sons benefit from paternal care in African striped mice

Running head: Sons benefit from paternal care

Neville Pillay[1*] and Tasmin Lee Rymer[1,2,3]

[1] School of Animal, Plant and Environmental Science, University of the Witwatersrand, Private Bag 3, WITS 2050, Johannesburg, South Africa

[2] College of Science and Engineering, James Cook University, P. O. Box 6811, Cairns, QLD 4870, Australia; E-Mail: Tasmin.Rymer@jcu.edu.au

[3] Centre for Tropical Environmental and Sustainability Sciences, James Cook University, P. O. Box 6811, Cairns, QLD 4870, Australia

* Author to whom correspondence should be addressed; E-Mail: Neville.Pillay@wits.ac.za
Tel.: +27-11-717-6459; Fax: +27-11-717-6494

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Abstract

Mammalian paternal care is rare and is often linked to enhanced fitness under particular ecological conditions. The proximate consequences of paternal care on offspring are lacking, however. Here, we tested whether levels of paternal care predict the behavioural, cognitive and physiological development of sons in the naturally paternal African striped mouse (*Rhabdomys pumilio*). We focused on sons raised in two treatments: biparental (both parents) or uniparental (mother alone) families. We recorded levels of interactions between pups with both parents, and later assessed the behaviour, cognition and physiology of sons at three developmental stages: juvenile, sub-adult and adult (sexual maturity). Sons from biparental families showed (i) reduced anxiety as juveniles; (ii) greater exploration and social interaction at different stages; (iii) better cognition; and (iv) reduced corticosterone concentrations than sons from uniparental families. In contrast, sons from uniparental families showed greater levels of paternal care, although prolactin concentrations did not differ between treatments. Paternal care in striped mice enhances fitness of males. Here, we also show that sons benefit psychologically and physiologically through interactions with their fathers. However, sons also trade-off such benefits against their own paternal care behaviour, suggesting that fathers influence the development of their son's phenotype in complex ways.

Keywords: Cognition; Fitness; Hormones; Male parental care; *Rhabdomys*; Rodent; Trade-off

1. INTRODUCTION

Mammalian paternal care, defined as any direct or indirect nongametic investment made by the father post-fertilisation that benefits his offspring (Dewsbury, 1985; Woodroffe & Vincent, 1994), is rare, occurring in only 5-10% of species (Wright, 2006). Paternal care is an evolved behaviour, modulated by neuroendocrine processes (e.g. prolactin, Schradin & Anzenberger, 1999), which has a genetic basis (Rymer & Pillay, 2011a) and may be epigenetically regulated by parental effects acting during an individual's ontogeny (Braun & Champagne, 2014). The occurrence of paternal care in several mammalian lineages suggests some fitness-related benefits from providing (by the father) or receiving (by the offspring) care (Rymer & Pillay, 2018).

Paternal care, like maternal care, may contribute directly to offspring survival, growth and/or cognitive and behavioural development (Gubernick & Teferi, 2000; McCarty & Southwick, 1977; Outscharoff, Helmeke, & Braun, 2006; Wright, 2006). These effects are particularly discernible during the ontogeny of anxiety-like and social behaviours and cognition (Braun & Champagne, 2014). For example, male California mice (*Peromyscus californicus*) that experienced high levels of retrieval by their fathers were more aggressive in resident-intruder tests (Frazier et al., 2006), whereas young mandarin voles (*Microtus mandarinus*) that experienced paternal deprivation were more anxious in an open field test (Jia et al., 2009). Furthermore, increased paternal care during development resulted in better object recognition and spatial memory in California mice (Bredy et al., 2004), whereas paternal deprivation resulted in impaired social recognition in prairie voles (*Microtus ochrogaster*; Prounis et al., 2015).

Paternal care may also influence the physiological development of offspring, leading to changes in behaviour and cognition. For example, mandarin voles that experienced paternal deprivation showed higher corticosterone concentrations and lower play-fighting, which is

apparently a prelude to aggression in adulthood (Wang et al., 2012). Finally, by providing paternal care, males may also indirectly affect the physical, behavioural, cognitive and physiological development of offspring via alterations to female maternal care. For example, female rock cavies (*Kerodon rupestris*) increase their investment in offspring when a mate is absent, which has a concomitant effect on the growth of young: young raised by mothers only gain more weight than sons raised by both parents (Tasse, 1986).

The proximate factors underlying the expression of paternal care have been relatively well studied, but a broader understanding of the proximate outcomes in offspring benefitting from paternal care is lacking. Indeed, few studies have focused on how paternal care might be impacting aspects of offspring development (physiological, cognitive and behavioural) in the same subjects, while also considering the social environment in which offspring are raised (but see Agarwal et al., 2020). The African striped mouse (*Rhabdomys pumilio*) is a useful model for understanding the proximate consequences of paternal care because males from a population in the Succulent Karoo of South Africa are naturally paternal (Schradin & Pillay, 2004). The neuroendocrine mechanisms affecting the expression of paternal care (e.g. Schradin, 2008; Schradin & Pillay, 2004), as well as the ontogeny and function of paternal care (e.g. Rymer & Pillay, 2011b; Rymer, Schradin & Pillay, 2008), have been well studied in this species.

African striped mice from the Succulent Karoo are socially flexible and facultatively group-living, which is contingent on female responses to population density. When population density is low, females prefer to nest alone to reduce the costs associated with reproductive competition, such as increased female-female aggression (Schoepf & Schradin, 2012) and infanticide (Schradin, König, & Pillay, 2010). Females fully compensate for a lack of care provided by a mate when they raise young alone (Rymer & Pillay, 2011b). At this time, males will generally adopt a solitary roaming strategy, seeking mating opportunities and

providing no paternal care (Schradin et al., 2009), although some males will remain with a single female, providing paternal care. As population density increases, breeding sites become limited and females often become constrained to nest in small groups (Schradin & Pillay, 2004; Schradin et al., 2010). Males may then become territorial dominant breeders that show paternal care (Schradin & Pillay, 2004). Consequently, uniparental, biparental and family units occur naturally in this population.

Male striped mice influence the behaviour of their offspring in multiple ways. Males enhance the development and growth of their offspring, most likely by providing thermoregulatory benefits (Schradin & Pillay, 2005), and fathers provide social learning opportunities for their young in response to novel food (Rymer et al., 2008). Jones, Mason & Pillay (2010) found that offspring raised by both parents showed lower levels of abnormal repetitive behaviour (e.g. stereotypic behaviour) than offspring raised by mothers alone. However, Rymer & Pillay (2011b) found that sons raised by mothers alone showed higher quality care to their own offspring than sons raised by both parents. Thus, although striped mouse fathers benefit offspring growth, paternal care might have non-genetic consequences on the paternal care behaviour of sons later. Whereas these findings collectively demonstrate the impact fathers have on the general behavioural development of their offspring, the influence of fathers on the cognitive and physiological development of their sons remains unknown.

We investigated whether and how paternal care by male African striped mice predicted the behavioural, cognitive and physiological ontogeny of their sons. We ascertained the development of sons from two different social environments (mother and father present vs. mother only) at different life stages from juveniles to adults, allowing us to explore how a present or absent father can lead to different developmental trajectories in their sons. We expected that paternal care would have a positive effect on the behavioural, cognitive and

126 physiological development of males. We made four predictions. 1. Sons raised by both
127 parents would be less anxious, both behaviourally and physiologically (i.e. lower
128 corticosterone concentrations), as suggested for mandarin voles (Jia et al., 2009; Wang et al.,
129 2012). 2. Sons raised by both parents would show greater exploratory and increased social
130 behaviour than sons raised by mothers only, as seen in California mice (Glasper, Hyer, &
131 Hunter, 2018). 3. Sons raised by both parents would show enhanced memory, because
132 growing up in a family group affects spatial cognitive abilities in female African striped mice
133 (Pillay & Rymer, 2015). 4. Since sons raised by both parents show reduced paternal care as
134 adults (Rymer & Pillay, 2011b), they would display lower concentrations of prolactin, a
135 hormone associated with paternal care in numerous species (Schradin & Anzenberger, 1999),
136 including striped mice (Schradin, 2008).
137
138 **2. METHODS**
139 We used F4–5 descendants of wild-caught parents from the Succulent Karoo (Northern Cape
140 Province, South Africa: 29.41.56 S, 18.1.60 E). They were housed at the University of the
141 Witwatersrand under partially controlled environmental conditions (14:10 light-dark cycle,
142 lights on at 05:00; 20–24 ºC; 30–60% relative humidity). We established 48 parentally
143 experienced breeding pairs housed in glass tanks (90 x 30 x 45 cm). Tanks were furnished
144 with a plastic nest box (15 x 15 x 15 cm), wood shavings for bedding, hay for nesting
145 material, and an assortment of enrichment devices (e.g. cardboard tubes, dowel sticks, tissue
146 paper). Each mouse was provided with approximately 5 g millet or sunflower seeds and
147 Epol™ mouse cubes (Epol, Pretoria West, South Africa) and approximately 5 g fresh
148 fruit/vegetables daily. Seed was sprinkled throughout the cage to stimulate foraging
149 behaviour. Water was available *ad libitum*.

The 48 pairs were randomly and equally assigned to one of two treatments: biparental, in which both parents raised their offspring to weaning at 21 days of age; and uniparental, in which the mother raised offspring alone until weaning. In the uniparental treatment, fathers were removed from the breeding tank at parturition and housed elsewhere in the breeding facility.

We video-recorded the parental care behaviour of the father and mother separately (biparental) and the mother only (uniparental) every second day from postnatal day (PND) 2 to PND 10, whereafter young started independent exploration of the tank and thus were not confined to the nest. We recorded behaviours of parents to provide a quantitative value of parental investment received by pups in each treatment. All adults were uniquely marked with non-toxic hair dye for individual identity. Observations were made for 30 min continuously during the peak of striped mouse activity periods between 10:00 to 12:00 and again between 15:00 and 16:00, generating 300 min of data in total. To ensure minimal disturbance, nest boxes were positioned in a manner to video record behaviours inside the nest from outside the tank. We recorded the duration of the following behaviours (see Schradin & Pillay, 2003) performed by mothers and fathers (biparental) and mothers only (uniparental): time spent in close proximity (< 2 cm) of pups (designated near), grooming pups (including sniffing and licking) and huddling pups; we could not differentiate between nursing and huddling pups by mothers, so we pooled these data and classified them as huddling (Rymer & Pillay, 2011b). We summed the data for each individual for the five days of sampling.

At weaning on PND 21, two sons per litter were randomly selected and housed together in plastic boxes (30 x 25 x 25 cm) with wire lids. They were maintained as described above. All sons were marked with non-toxic hair dye for identification. We used only one brother (test male) in experiments (below) but group housing of brothers initially provided social

stability for males and reduced stress following separation from the family (Mackay, Rymer, & Pillay, 2014). Test males were housed alone from PND 40, long after natural natal dispersal, which normally occurs around four weeks of age (Schoepf & Schradin, 2012).

In total, we had 24 males in the biparental treatment and 24 males in the uniparental treatment. Males were used in a series of experiments (Figure 1), covering different life stages: the juvenile (PND 25), sub-adult (PND 45–50) and adult (PND 80– > 120; sexually mature) age categories (Brooks, 1982). At each stage, age-appropriate behaviours were tested, although some behaviours were tested in more than one age category (Figure 1). All experiments were conducted between 10:00 and 12:00. Behavioural scoring was conducted by trained assistants who were blind to the experiments. After each test (or dyadic encounter), the experimental apparatus and any objects used were washed with warm soapy water and 70% alcohol and allowed to air dry to remove residual odours of the previous occupant. Any wood shavings used were replaced.

2.1 Experiment 1. Emotional response in a novel environment

On PND 25, 45 and 80, we studied the emotional response of test males in open field tests. All individuals were tested once at each age. Tests were conducted under fluorescent light (361 lux), staged in glass tank arenas (45 x 30 cm and 30 cm high) with wire mesh lids. Tanks had a thin layer of coarse wood shavings.

The test male was released into the centre of the arena using a small closed plastic carrier (15 x 10 cm), which was washed between tests. Thereafter, its behaviour was video-recorded for 10 min using a video camera mounted above the arena. Using the Ethovision XT video-tracking software (Noldus, Netherlands; http://www.noldus.com), we created a 3 cm peripheral zone on the inside perimeter of the tank (Pillay, Rimbach, & Rymer, 2016) and recorded: (i) the duration of time spent by the test mouse in the centre and on periphery; and

(ii) the number of transitions between the periphery to the centre and back to the periphery. We discarded the first transition since test subjects were introduced into the centre of the arena. To assess anxiety, we analysed the time spent in the centre, as more time spent on the periphery indicates greater anxiety (Pillay et al., 2016).

2.2 Experiment 2. Social interaction in dyadic encounters and corticosterone measurements

On PND 47 and 88, we studied the social behaviour of individuals in dyadic encounters in a neutral arena. Dyads comprised of the test male and an unrelated age- and weight-matched stimulus male from our breeding colony. Males were tested with different dyad partners at each age.

Dyadic encounters were staged in glass tanks (60 x 30 x 35 cm) equipped with perforated lids. Tanks had a 1 cm deep layer of wood shavings. Each tank was divided with an opaque partition. The test and stimulus males were randomly placed on either side of the partition for 5 min to acclimate to the arena. The barrier was removed, and the behaviour of the dyad was video recorded for 15 min. The first minute of recording was discarded because of the disturbance of removing the barriers. We recorded the frequency of allogrooming of the stimulus male by the test male, duration of close body contract and aggression by the test male (e.g. box, chase and/or tumble). One of us (NP) sat in the room to terminate dyads that engaged in damaging fights, but aggression was mostly chases and occasionally boxes, and no animals were harmed in the study. After encounters, dyads were examined for any injuries, but none was recorded.

225 **2.3 Corticosterone assays**

226 On PND 88, after the dyadic encounters, we measured serum corticosterone concentrations
227 from 12 randomly selected males per treatment (n=24). We chose a subset of males because
228 the blood sampling used was invasive and potentially stressful. These males were not used
229 again in further experiments.

230 The males were anaesthetised with Isoflurane 20 min after dyadic encounters, and a
231 blood sample of 200-500 µl was collected from the saphenous vein of one leg, a procedure
232 that does not result in elevated corticosterone concentrations in laboratory mice (*Mus*
233 *musculus*, ICR strain; Abatan, Welch, & Nemzek, 2008). Males weighed > 60 g at testing,
234 and the volume of blood collected was sufficient for analysis without long-term impact
235 (Schradin et al., 2009). We timed blood collection to coincide with the peak of corticosterone
236 release after dyads, which is estimated to be 30–40 min following a stress test in Sprague–
237 Dawley rats (*Rattus norvegicus*, Cavigelli & McClintock, 2003), and to control for circadian
238 rhythm variation in hormone secretion. The time from anaesthesia to blood collection did not
239 differ statistically between the biparental (8.7 ± 0.8 min) and uniparental (8.6 ± 0.7 min)
240 males [$t(21.19) = -0.16$, $p = .874$].

241 Blood samples were left at room temperature for one hour and then centrifuged twice for
242 10 min each. The resulting serum was isolated and frozen in aliquots of 20 µl at -20 °C.
243 Corticosterone analysis was performed using a commercial radioimmunoassay (RIA) kit (MP
244 Biomedical Solon, Ohio). Intra-assay variability was 10.1%; samples were analysed in one
245 assay. All procedures were performed according to the manufacturer's instructions.

246

247 **2.4 Experiment 3. Short-term memory: recognition memory**

248 At PND 50, males were tested in novel object recognition tests, following Akkerman et al.
249 (2012). Tests were conducted in the same glass tank arenas used in open field tests. The

250 procedure comprised of three sessions: habituation, training and retention, each conducted
251 approximately 24 h apart. During habituation sessions, a test male was provided with an
252 opportunity to explore the arena for 10 min. During training sessions, two identical plastic
253 figurines (5 cm high x 3 cm wide) were placed 10 cm from either the left or right corners of
254 the arena (the side on which each figurine was placed was alternated between tests). The
255 figurines were 2 cm apart and held down with Prestik™ adhesive. We had 10 pairs of
256 figurines, which were washed and used interchangeably between tests. A male was placed
257 into the middle of the arena using a plastic carrier and its behaviour was recorded for 5 min.
258 Using the multiple-tracking module in Ethovision XT video-tracking software, we scored the
259 duration that the test male spent investigating each object (i.e. touching the object or
260 orientated towards the object up to a 1 cm away).
261 During retention sessions, test mice were again presented with two objects: one of the
262 familiar objects used during training and a novel object of the same size but different in
263 appearance. Individuals were allowed to explore the objects again for 5 min, and we scored
264 the time they spent investigating both objects.
265 The time spent investigating objects might be a function of exploratory tendencies. To
266 counter this bias, we assessed five variables: (i) Et (explore training) – time spent with both
267 identical sample objects (O1 + O2) during the training trial; (ii) Er (explore retention) – time
268 spent with the novel object and familiar object (N + O) during the retention trial; (iii) Da –
269 absolute difference between time spent with the novel object and sample object during the
270 retention trial (N - O); (iv) Nr – relative time spent with the novel object (N - O) / (N + O)
271 during the retention trial, providing a measure of discrimination of the novel object corrected
272 for exploration; and (v) Np – proportion of time spent with the novel object during the
273 retention trial N/ (N + O).
274

2.5 Experiment 4. Short-term memory: Spatial working memory in the Y maze

At PND 83, we assessed the short-term memory ability of males using a closed Y maze test. The Y maze choice apparatus was built of transparent plexiglass material and comprised of a start box (36 x 20 cm, height = 16 cm) connected by a Y maze (internal diameter 4.6 cm; 22 cm long arms) to two choice chambers (same size as the start box). The apparatus was placed in a white room with several extra-maze cues (i.e. chair, paintings on the wall).

The procedure comprised of a training phase and a testing phase. At the beginning of the training phase, the entrance to one arm leading a choice chamber was randomly selected and blocked off using an opaque disc. This was designated the novel arm. A male was placed in the start box that was blocked off from the rest of the maze using an opaque partition. After a 5 min habituation period, the partition was removed, and the male was allowed to explore the maze for 10 min, excluding the novel arm. Thereafter, the male was removed from the maze and returned to its home cage. The test phase started one hour later when the barrier to the novel arm was removed. The male was returned to the start box for a 5 min habituation period, as for the training phase, and was then allowed to explore the entire maze, including the novel arm, for 10 min. The behaviour of the male was video recorded from above and no observers were present in the room during training or testing.

We recorded the total number of arm entries (defined by half a body length in an arm), the number of alternations and the percent alternations. The number of alternations was calculated from the sequence of three different arm entries (e.g. arms 3, 1, 2); repeated use of the arms (e.g. 2, 1,2) were not considered in alternation scores. The percent alternation was calculated using the formula: total number of alternations / number of arms entered * 100.

2.6 Experiment 5. Paternal care and prolactin measurements

At approximately PND 120, 12 males (i.e. those not sampled for corticosterone; above) per treatment (n=24) were paired with unrelated, age-matched females from our colony. Breeding pairs were established (as described above) and housed in glass tanks (46 x 30 x 32 cm). Pairs were provisioned with food, water and enrichment (as described above).

Paternal care (parents were uniquely marked with non-toxic hair dye to identify fathers) was recorded every second day from PND 2 to 10 for 1 h per day (as described above). We also recorded litter size.

2.7 Prolactin assays

Blood samples were collected from all males that sired and raised offspring (biparental: n=10; uniparental: n=11) when litters were 11 days old. We waited until after pups started to eat solid food (PND 10-11; Pillay, 2000) and paternal care observations were completed before sampling prolactin in males to reduce the possibility of maternal aggression directed towards males returned to the breeding cage after their removal, which would have influenced paternal care (pers. obs.). Blood was collected between 09:00 and 11:00 as described above for corticosterone analyses. However, stress can lead to increased prolactin concentrations (Ziegler, Wegner, & Snowdon, 1996). To minimise stress, males were removed from the breeding tank, taken to an adjacent room and immediately anesthetised. Blood was collected approximately 5 min later. The time from anaesthesia to blood collection did not differ statistically between the biparental (8.2 ± 0.8 min) and uniparental (9.8 ± 0.8 min) males [$t(18.95) = -1.36$, $p = .189$]. Males were then placed into holding cages and, once they were awake and fully recovered (about 30 min later), they were then housed singly in the breeding colony under the same conditions (as described above).

Collected blood was transferred into an Eppendorf tube and kept at room temperature for 60 min to clot. Samples were then centrifuged for 20 min and the resulting serum was isolated and frozen in aliquots of 20 µl at -20 °C for 1 month before analysis.

A commercial RIA kit for measuring rat prolactin was used (MP Biomedical Solon, Ohio). All procedures were performed according to the manufacturer's instructions. Samples from all males were analysed in one assay, and the intra-assay variation was 10.2%.

2.8 Statistical analyses

All analyses were performed using R studio (R version 4.00; http://www.R-project.org). All data were tested for homogeneity of variances (Levene's test) and normality (Shapiro–Wilks test). Non-normal data were transformed using the Box-Cox method (MASS package, Ripley et al., 2020). We used Welch's two-sample t-tests or analysis of variance (ANOVA) to analyse variables by treatment for most experiments. When tests were repeated at different ages (i.e. emotional responses and social interactions), linear models for repeated measures (lme4 package, Bates et al., 2020; car package, Fox et al., 2020) were used to analyse variables by treatment (fixed factor) and time (age at testing) for individuals (repeated measures). Pairwise contrasts (Tukey *post hoc* tests) were obtained for significant predictors (emmeans package, Lenth et al., 2020). The model-level significance was determined at $\alpha = 0.05$ and all tests were two-tailed. Data are presented as mean (± SE) throughout.

Ethical Note

This study complied with the current laws and regulations in South Africa, and the research adhered to the ABS/ASAB guidelines for the ethical treatment of animals in research (Bee et al., 2020). Animals were provided with environmental enrichment, and the welfare of the animals was monitored daily. The experimental procedures did not have any obvious

negative effects on the welfare of the striped mice. Dyads were carefully monitored to prevent any fights; no physical harm occurred. At the end of the study, all experimental animals were kept as part of the breeding stock of the colony. Experimental procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC 2010/55/2A, 2012/13/3, 2013/18/2A).

3. RESULTS

3.1 Parental care behaviours of mothers and fathers

Fathers in the biparental treatment spent significantly more time near their pups [$F(2, 69) = 6.56, 69, p = .002$] than their mates (biparental) and mothers alone (uniparental; Table 1). However, mothers raising their litters alone huddled [$F(2, 69) = 118.51, p < .001$] and groomed [$F(2, 69) = 70.57, p < .001$] their pups significantly more than either fathers or mothers in the biparental group (Table 1). The two groups did not differ significantly in their litter sizes [biparental = 4.9 ± 0.21; uniparental = 5.25 ± 0.26; $t(43.88) = -1.11, p = .274$].

Comparisons between the biparental (fathers and mothers combined) and uniparental (mothers alone) treatments showed that biparental groups spent significantly more time near pups than mothers alone [$t(28.68) = 6.06, p < .001$], whereas mothers raising pups alone groomed their pups significantly more than biparental groups [$t(27.74) = 6.97, p < .001$]. There was no significant difference between the groups for huddling pups [$t(38.68) = -0.72, p < .475$; Table 1].

3.2 Experiment 1. Emotional response in a novel environment

Treatment [$\chi^2_1 = 30.11, p < .001$], age [$\chi^2_2 = 467.20, p < .001$] and treatment * age [$\chi^2_2 = 8.36, p = .004$] were all significant predictors of time spent in the centre of the arena. Juvenile

males from the biparental treatment were significantly less anxious than juvenile males from the uniparental treatment, spending 1.8 times more time in the centre of the arena (Figure 2a). Time spent in the centre was similar between juvenile and sub-adult males from the biparental treatment, whereas sub-adult males increased the time spent in the centre from the juvenile to the sub-adult stage, although not significantly so (Figure 2a). Adult males from both treatments spent the greatest amount of time in the centre, and there was no significant difference between treatments for these males (Figure 2a).

Treatment [$\chi^2_1 = 15.57$, $p < .001$] and age [$\chi^2_2 = 142.66$, $p < .001$] were significant predictors of the number of transitions made by test males between the periphery and the centre of the arena. Males from the biparental treatment made more transitions at all three life stages than males from the uniparental treatment (Figure 2b), and males from both treatments showed a graded increase in the number of transitions with age (Figure 2b). The interaction between treatment and age was not a significant predictor of the number of transitions made [$\chi^2_2 = 4.79$, p = .091].

3.3 Experiment 2. Social interaction in dyadic encounters and corticosterone measurements

The frequency of allogrooming was influenced by treatment [$\chi^2_1 = 8.39$, $p = .004$] and age [$\chi^2_1 = 9.59$, $p = .002$] but not treatment * age [$\chi^2_1 = 0.35$, $p = .555$]. Males from the biparental treatment showed 1.5 times more allogrooming than males from the uniparental treatment, and allogrooming by males in both treatments decreased in adult males compared to sub-adult males (Figure 3a).

Age [$\chi^2_1 = 78.23$, $p < .001$] and treatment * age [$\chi^2_1 = 11.52$, $p < .001$] were significant predictors of the time spent sitting in close body contact, whereas treatment was not a significant predictor of close body contact [$\chi^2_1 = 1.75$, $p = .186$]. Sub-adult males from the

biparental treatment made the most body contact with dyad partners, followed by sub-adult males from the uniparental treatment (Figure 3b). Males from both treatments showed similar and reduced body contact with dyad partners when they were adults (Figure 3b).

Aggression was significantly affected by treatment [$\chi^2_1 = 23.14$, $p < .001$], age [$\chi^2_1 = 130.25$, $p < .001$] and treatment * age [$\chi^2_1 = 13.23$, $p < .001$]. Adult males from the biparental treatment were the most aggressive, whereas all other treatment and age combinations showed lower, but similar, levels of aggression (Figure 3c). Overall, males from the biparental treatment engaged in more social interactions, whether they were amicable (allogrooming, body contact) or aggressive, although behaviours changed with age.

Adult males from the biparental treatment had significantly lower corticosterone concentrations (715.96 $\pm$ 61.78 ng/ml) than males from the uniparental (973.36 $\pm$ 94.64 ng/ml) treatment [$t(18.9) = 2.28$, $p = .035$].

3.4 Experiment 3. Short-term memory: recognition memory

In the training phase, there was no significant difference in the time that males from both treatments spent exploring similar objects [Et; $t(40.8) = 0.60$, $p = .551$; Figure 4a]. In contrast, during the retention phase when a novel and familiar object was presented to the males, those from the biparental treatment showed significantly greater levels of exploration (Er) than males from the uniparental treatment [$t(42.4) = 3.70$, $p < .001$; Figure 4a]. The difference in the time spent with the novel object and familiar object (Da) was also significantly higher in the biparental treatment than the uniparental treatment [$t(38.0) = 4.41$, $p < .001$; Figure 4a]. When corrected for exploration, the time spent with the novel object (Nr) was also significantly greater in the biparental treatment than the uniparental treatment [$t(35.9) = 2.96$, $p = .005$; Figure 4b], confirming the reduced investigation (Da) of the uniparental treatment. Finally, males from the biparental treatment spent a significantly

greater proportion of time with the novel object (Np) than males from the uniparental treatment [$t(35.9) = 2.96$, $p = .005$; Figure 4b].

3.5 Experiment 4. Short-term memory: Spatial working memory in the Y maze

Alternation scores in the Y maze (i.e. the number of sequences made in the three different arms) did not differ significantly between males from the biparental and uniparental treatments [$t(42.5) = 1.19$, $p = .241$; Figure 5]. However, males from the biparental treatment made significantly more arm entries into the Y maze than males from the uniparental treatment [$t(41.5) = 3.12$, $p = .003$; Figure 5]. The percentage alternation did not differ significantly between males from the different treatments [$t(40.1) = 0.17$, $p = .865$; Figure 5].

3.6 Experiment 5. Paternal care and prolactin measurements

Males from the uniparental treatment showed significantly greater levels of grooming [$t(9.21) = 2.69$, $p = .025$] and huddling [$t(19.0) = -2.54$, $p = .020$] of their pups than males from the biparental treatment (Figure 6). There was no significant difference in the time spent near pups between the treatments [$t(18.02) = 0.33$, $p = 0.746$; Figure 6]. Litter size was not significantly different between treatments [biparental = 4.6 ± 0.34; uniparental = 4.7 ± 0.38; $t(18.9) = -0.25$, $p = 0.801$].

Concentrations of prolactin did not differ significantly between males from the biparental (20.80 ± 1.05 ng/ml) and uniparental (22.64 ± 1.61 ng/ml) treatments [$t(16.9) = 0.96$, $p = 0.353$].

4 DISCUSSION

Although paternal care is facultative in African striped mice from the Succulent Karoo, when males do care, they provide similar amounts of care to females (Rymer & Pillay, 2011b), and

447 show all the behavioral characteristics of females (e.g. grooming and huddling pups), apart
448 from lactation (Schradin & Pillay, 2003). Paternal care by striped mice enhances offspring
449 development (Schradin & Pillay, 2005) and fathers provide social learning opportunities for
450 their young (Rymer et al., 2008). Here, we provide evidence that paternal care by male
451 striped mice has important consequences for their young.

452 We investigated the influence of paternal care on the ontogeny of multiple phenotypes
453 (behavioural, cognitive and physiological) in their sons. We assessed the development of
454 males from two different social environments (mother and father present vs. mother only),
455 both of which naturally occur in striped mice from the Succulent Karoo. Our experimental
456 design allowed us to consider how early life experiences, with a present or absent father, lead
457 to different behavioural trajectories in their sons between treatments. Consistent with Rymer
458 & Pillay (2011b), in the absence of fathers, mothers showed higher levels of grooming and
459 similar levels of huddling than both mothers and fathers raising pups together, indicating that
460 females are compensating for a lack of paternal care when fathers are absent.

461 We expected that paternal care would have a positive effect on the behavioural, cognitive
462 and physiological development of males. The presence of striped mouse fathers was
463 associated with reduced anxiety-like behaviour and increased exploration in the open field
464 test, but this was age-dependent. Sons raised by both parents were less anxious and more
465 exploratory as juveniles than sons raised by both parents, which is somewhat consistent with
466 the findings of Perkeybile, Griffin & Bales (2013) for prairie voles, although juvenile prairie
467 voles that received more parental care were less anxious in the elevated plus maze, but not
468 the open field. Furthermore, we found that adult striped mouse sons were generally less
469 anxious than juveniles and sub-adults, regardless of treatment, which is contrary to CD-1
470 laboratory mice, where sub-adults were less anxious that both juveniles and adults (Macrì et
471 al., 2002). Interestingly, we found that treatment effects on anxiety dissipated with age,

472 suggesting that paternal effects only mediate anxiety during early development. Males from
473 the biparental treatment were more exploratory at all three life stages than males from the
474 uniparental treatment, although males from both treatments showed a graded increase in
475 exploratory behaviour with age. Our findings are consistent with those of California mice,
476 where paternal deprivation resulted in decreased exploratory behaviour in the elevated plus
477 maze (Glasper et al., 2018). Striped mice start to eat solid food around PND 10-11, and are
478 generally weaned at PND 16 (Pillay, 2000), when they start leaving the natal nest on foraging
479 trips. Striped mice are solitary foragers (Schradin & Pillay, 2004), and, therefore, we suggest
480 that less anxious, more exploratory juvenile striped mice benefit by spending more time
481 foraging than more anxious, less exploratory juveniles, which would enable them to increase
482 their energy reserves. That older mice were less anxious and more exploratory than younger
483 mice suggest that younger mice are more circumspect as they start exploring and learning
484 their new surroundings. Furthermore, we suggest that decreased anxiety and increased
485 exploration in striped mice raised by both parents likely provides a competitive advantage
486 into adulthood, since older male striped mice must search for both food and mates.
487 Sons raised by both parents engaged in greater social interactions in dyads than sons
488 raised by their mothers alone. Sons from the biparental group engaged in more allogrooming
489 and spent more time in body contact with a dyad partner than males from the uniparental
490 group, which is consistent with prairie voles that receive high quality care from their fathers
491 (Perkeybile et al., 2013). In contrast, adult males from the biparental group were also more
492 aggressive than males from the uniparental group. Jia et al. (2009) found that males which
493 experienced paternal deprivation were also less aggressive than males raised by both parents.
494 In general, we found that positive social behaviours declined with age in striped mice, which
495 is consistent with previous findings in striped mice in which both dominant group-living
496 males and solitary roamer males were aggressive, with higher concentrations of testosterone,

than younger philopatric helpers (Schradin et al., 2009), suggesting that male fighting ability, and thus competitive ability, increases with age.

Increased corticosterone concentrations are positively associated with increased anxiety-like behaviours in Sprague Dawley rats (Vallée et al., 1997) and African striped mice (Mackay et al., 2014), suggesting that corticosterone concentrations can reflect anxiety. Adult males from the biparental treatment had significantly lower corticosterone concentrations than males from the uniparental treatment, indicating decreased anxiety in males raised by the father and comparatively greater anxiety in males raised without their fathers. Similarly, in California mice, reduced paternal grooming was associated with elevated corticosterone concentrations (Frazier et al., 2006). Lower corticosterone is associated with aggressive behaviour in several species (e.g. Clarke & Faulkes, 1997; Leshner, 1980; Schuurman, 1980), including white-footed mice (*Peromyscus leucopus*; Oyegbile & Marler, 2006), but not California mice (Oyegbile & Marler, 2005). Corticosterone can suppress the secretion of testosterone by inhibiting transcription of genes coding for biosynthetic enzymes in testosterone (Hardy et al., 2005; Retana-Márquez et al., 2003). Likewise, testosterone can have an inhibitory effect on corticosterone (Place & Kenagy, 2000). This relationship between high testosterone and low corticosterone concentrations has been previously identified in both free-living and captive-born striped mice (Raynaud, Müller, & Schradin, 2012; Raynaud & Schradin, 2014). Male striped mice with higher testosterone concentrations have larger home ranges (Raynaud et al., 2012) and are more aggressive to their immediate neighbours that are likely to steal paternity from territorial males (Schradin, Schneider, & Lindholm, 2010). Consequently, males raised by both parents likely experience fitness benefits associated with mating and reproductive success compared to males raised by mothers only.

Rymer & Pillay (2011b) showed that striped mouse males from a uniparental treatment displayed significantly greater levels of paternal care towards their pups than males from a biparental treatment. Again, we found that females compensate for a lack of paternal care when raising young alone, resulting in their sons displaying higher levels of paternal care than sons raised by both parents (i.e. from biparental pairs). The point to emphasise is that differences between treatments was a matter of degree, in that the biparental treatment sons did show paternal care, but to a reduced extent, and were never neglectful or aggressive to the pups. In laboratory rats, high levels of maternal grooming and licking resulted in neuroendocrine alterations in offspring associated with reduced anxiety (Liu et al., 1997). It is thus possible that the greater levels of grooming displayed by mothers in the uniparental treatment resulted in sons displaying greater levels of paternal care. Whether this was due to reduced anxiety of the sons, as suggested by Liu et al. (1997), is unclear since these males did not display reduced anxiety in other contexts (e.g. open field tests). Our findings suggest that the development and expression of paternal care in African striped mice could be regulated by epigenetic mechanisms (e.g. DNA methylation and/or histone modification and/or interactions with transposons and retrotransposons; Curley, Mashoodh & Champagne, 2011; Mashoodh & Champagne, 2019), some of which may be activated through tactile stimulation provided by the mother. Alternatively, or in addition to, the development and expression of paternal care in African striped mice could be influenced by organisational and/or activational hormonal effects (Elekonich & Robinson, 2000), which might also be epigenetically modulated. Organisational effects create permanent, irreversible changes in neural substrates (i.e. neural pathways) during pre-natal or early postnatal development, and activational effects modify neural activity in these pathways during adulthood (Elekonich & Robinson, 2000), both affecting behaviour. These hormonal effects are suggested to affect the development of parental care and other behaviours in other rodents (e.g. California mice,

546 Gubernick, Sengelaub, & Kurz, 1993), as well as striped mice (e.g. Raynaud et al., 2012;
547 Rymer & Pillay, 2013). However, the concentration of prolactin was not significantly higher
548 in males from the uniparental treatment even though they displayed more paternal care. It
549 appears that levels of prolactin in striped mice are not associated with levels of paternal care,
550 as was also suggested by Schradin & Pillay (2004). Instead, prolactin most likely maintains
551 paternal care once initiated via interactions with pups, as seen in Djungarian hamsters
552 (*Phodopus campbelli*; Ma et al., 2005), regardless of the levels of care displayed. This is
553 supported by field studies, where territorial breeding male striped mice have higher
554 concentrations of prolactin than roaming males but only in the breeding season (Schradin,
555 2008), indicating that prolactin helps maintain paternal care, but is unlikely to function in its
556 initiation. Once prolactin concentrations reach a threshold level, paternal care behaviour will
557 occur. Thus, the differences in paternal behaviour between sons in biparental and uniparental
558 treatments might be related to other factors, such as reduced anxiety (as mentioned above).
559 Biparentally raised sons had better cognitive ability. Males in the biparental treatment
560 explored the novel object for longer than males from the uniparental treatment, which is
561 consistent with the findings of Bredy et al. (2004) for California mice. The novel object
562 recognition test is commonly used to investigate aspects of learning and memory in rodents
563 (Lueptow, 2017), and our study shows a positive effect of paternal care on the short-term
564 memory of striped mice. However, we found no significant treatment effect on the spatial
565 working memory of striped mice. The Y maze is used to assess spatial memory and
566 hippocampal integrity and relies on an animal's natural instinct to explore and discover novel
567 environments (Shin et al., 2016). There was no significant difference in alternation scores in
568 the Y maze, suggesting that the spatial memory was intact, and that paternal deprivation does
569 not necessarily lead to hippocampal impairments in striped mice. The fact that males from the
570 biparental treatment made more arm entries into the maze indicates that they are more

exploratory in general, which is consistent with our findings in the open field. Food resources are patchily distributed, and often ephemeral in the Succulent Karoo (e.g. insect flushes occur after seasonal rains; Schradin, 2005). Consequently, whereas increases in spatial working memory in striped mice are unlikely to confer an advantage for locating these resources, striped mice that are more exploratory are more likely to gain access to these patchily distributed resources compared to less exploratory mice. This could be particularly beneficial for young striped mice that must invest heavily in their own growth and development when they start to eat solid food.

We showed that altering the early rearing parental environment leads to different trajectories in a suite of behavioural, cognitive and physiological phenotypes of males. Such developmental plasticity leading to different developmental trajectories and phenotypes are well known. For example, the developmental trajectory of cockroaches (*Diploptera punctata*) was phenotypically plastic, dependent on prenatal and postnatal maternal care, as well as the postnatal social environment when males were present (Holbrook & Schal, 2004). Moreover, a growing number of studies (e.g. Jonsson & Jonsson, 2014) suggest that phenotypic plasticity, as a consequence of the early life environment and an individual's experiences, shapes the development of cognition, behaviour and personality, and alters stress responsiveness (e.g. Gudsnuk & Champagne, 2011). Consequently, we suggest that striped mouse fathers have a modulating effect on offspring behavioural, cognitive and physiological development, such that offspring follow different ontogenetic trajectories based on early social interactions and the experience of paternal care.

Numerous studies on multiple taxa have shown paternal care has a positive effect on the reproductive success of fathers (e.g. California mouse, Cantoni & Brown, 1997; spotless starling (*Sturnus unicolor*), Moreno et al., 1999); painted greenling (*Oxylebius pictus*), DeMartini, 1987); pine engraver beetles (*Ips pini*), Robertson, 1998). Similarly, several

596 studies have shown that recipients of paternal care have better survival (e.g. California mice,
597 Wright & Brown, 2002; glass frogs (*Hyalinobatrachium orientale*), Lehtinen, Green &
598 Pringle, 2014; three-spined sticklebacks (*Gasterosteus aculeatus*), McGhee & Bell, 2014). A
599 meta-analysis of 31 studies, including mammals, birds, fish, spiders and insects, found that
600 bolder, more aggressive individuals had greater reproductive success, and that more
601 exploratory, more aggressive individuals had greater survival (Smith & Blumstein, 2008).
602 However, there was also a trade-off between boldness (e.g. greater exploratory behaviour)
603 and survival, with bolder individuals having lower survival (Smith & Blumstein, 2008).
604 Although we did not find an effect of parental treatment on litter size in our study, most
605 likely because optimal conditions in captivity reduce the energetic constraints associated with
606 reproduction (Brown, 1993), our findings provide compelling evidence that the fitness of
607 sons is likely enhanced in multiple ways by paternal provisioning, since sons raised by both
608 parents were more aggressive and more exploratory, which could lead to increased
609 reproductive success. However, our results also show a trade-off between current and future
610 fitness. Whereas sons raised by both parents benefit from potential enhancements to foraging
611 and competitive ability, these same sons also demonstrate lower paternal care, which could
612 impact their sons' development and concomitant fitness, growth and survival.
613 Collectively, our findings indicate that sons gain direct behavioural, cognitive and
614 physiological benefits by being raised in a social unit with their fathers. Sons raised with their
615 fathers were less anxious, showed greater exploration, were more aggressive as adults and
616 showed enhanced short-term memory. Yet, sons raised by both parents showed reduced
617 paternal care, at least in the first litters, compared to sons raised by their mothers only.
618 Whether these phenotypic modifications are syndromic and represent a composite
619 behavioural trajectory, or are individually defined trajectories for each phenotype, requires
620 further testing. In conclusion, we demonstrated in a naturally paternal species that fathers

alter the developmental trajectory of the sons, potentially enhancing their sons' and their own fitness. Thus, although paternal care is rare in mammals, its impact can, nevertheless, be profound, as demonstrated here in striped mice. Future studies should investigate how the effects of paternal care through the phenotypes measured here enhances fitness of free-living striped mice in the harsh Succulent Karoo environment.

DATA AVAILABILITY STATEMEN

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Neville Pillay http://orcid.org/0000-0002-0778-726X

Tasmin Rymer https://orcid.org/0000-0002-9963-6345

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TABLES

TABLE 1 Mean (± SE) minutes engaged in three parental care behaviours by male and female African striped mice (*Rhabdomys pumilio*) separately (biparental treatment) and mothers alone (uniparental treatment). Rows with the same letters are not significantly different (*post hoc tests*; see text)

	Treatment		
	Biparental		**Uniparental**
Behaviour	*Father*	*Mother*	*Mother*
Near	42.26 (4.41) a	27.42 (2.33) b	38.79 (1.64) b
Groom	3.15 (5.43) a	3.46 (3.04) a	14.54 (5.36) b
Huddle	78.14 (5.87) a	112.79 (6.72) b	195.8 (3.58) c

899 **FIGURE LEGENDS**

900

901 **FIGURE 1** Timeline of events, starting from observations of parental care in biparental and
902 uniparental treatment groups (PND 2-10), to tests of the resulting male offspring, which were
903 tested in five experiments in three developmental life stages: juvenile (PND 25), sub-adult
904 (PND 45-50) and adult (PND 80 - > 120).

905

906 **FIGURE 2** Mean + SE (a) duration of time (s) spent by male African striped mice
907 (*Rhabdomys pumilio*) in the centre of the neutral arena at three different life stages; and (b)
908 number of transitions made by male striped mice between the periphery and centre at three
909 different life stages. Points show individual scores. Comparisons were made for each variable
910 by treatment. Different letters for duration or time show significant *post hoc* treatment and
911 age differences for time in the centre, and age difference only for transitions (see text).

912

913 **FIGURE 3** Mean + SE frequency of (a) allogrooming, (b) sitting in close body contact, and
914 (c) aggression displayed by male African striped mice (*Rhabdomys pumilio*) at two different
915 life stages. Points show individual scores. Comparisons were made for each behaviour by
916 treatment and age. For each behaviour, different letters show significant *post hoc* treatment
917 and/or age differences (see text).

918

919 **FIGURE 4** Mean + SE duration of time (s) spent exploring (a) two identical objects in the
920 training phase (Et), a novel and familiar object (Er), and absolute differences in duration of
921 time (s) spent exploring a novel object and sample object during the retention phase (Da), and
922 (b) proportion of time spent with novel object while exploring both objects (Nr) and in
923 relation to both objects (Np) by African striped mouse males (*Rhabdomys pumilio*) in two

924 treatments (biparental and uniparental). Points show individual scores. Comparisons were
925 made for each behaviour by treatment. For each variable, different letters show significant
926 *post hoc* treatment differences (see text).
927
928 **FIGURE 5** Mean + SE alternation scores, arm entries and percentage (%) alternation by
929 African striped mouse males (*Rhabdomys pumilio*) in two treatments (biparental and
930 uniparental). Points show individual scores. Comparisons were made for each behaviour by
931 treatment. For each variable, different letters show significant *post hoc* treatment age
932 differences (see text).
933
934 **FIGURE 6** Mean + SE time (minutes) spent engaged in three paternal behaviours (time spent
935 near pups and grooming and huddling pups) by male African striped mice (*Rhabdomys*
936 *pumilio*) from two treatments (biparental and uniparental). Points show individual scores.
937 Comparisons were made for each behaviour by treatment. For each behaviour, different
938 letters show significant *post hoc* treatment age differences (see text).