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► To cite this version:

Lindelani Makuya, Neville Pillay, Siyabonga Patrick Sangweni, Carsten Schradin. Tolerant mothers: aggression does not explain solitary living in the bush Karoo rat. *Proceedings of the Royal Society B: Biological Sciences*, 2024, 291 (2032), 10.1098/rspb.2024.1534 . hal-04833503

HAL Id: hal-04833503

<https://cnrs.hal.science/hal-04833503v1>

Submitted on 12 Dec 2024

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Tolerant mothers: aggression does not explain solitary living in the bush Karoo rat

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PUBLISHED AS

Makuya, L., N. Pillay, S. P. Sangweni & C. Schradin (2024) Tolerant mothers: aggression does not explain solitary living in the bush Karoo rat. *Proceedings of the Royal Society B: Biological Sciences*, **291**, 20241534.

Abstract

Many mammal species are thought to adopt solitary living due to mothers becoming intolerant of adult offspring and the occurrence of social intolerance between adults. However, field studies on how solitary mammals interact are rare. Here we show that solitary living can occur without social intolerance. Over three years, we recorded interactions between free-living bush Karoo rats (*Otomys unisulcatus*) and conducted dyadic encounter experiments between kin and non-kin female neighbours, both in a neutral test arena and in field intruder experiments. Social interactions were rare (230/2062 observations), and aggressive in only 34% cases. In dyadic encounters, mothers interacted amicably with young offspring. Aggression between mothers and offspring was almost absent. This mother-offspring relationship remained amicable even after adult offspring had dispersed. Aggression between neighbouring adult females was low in neutral arena tests, independent of kinship and season. However, in the field, females reacted more aggressively towards non-kin than kin intruders, especially during the breeding season. Tolerance between mothers and adult

37 offspring indicates that aggression is not the mechanism leading to dispersal and solitary
38 living. We found a solitary social system characterised by social tolerance, suggesting that
39 dispersal and lack of social attraction rather than aggression can lead to solitary living.

40

41

42 **Keywords:**

43 Solitary living; social system; aggression; social tolerance; kin neighbours; natal dispersal;
44 social structure; social organisation

45

46 **Introduction**

47 To understand the diversity of social systems, many studies have focused on pair and group-
48 living species, assuming solitary living to be the ancestral state that does not require any
49 explanation for its occurrence [1-3]. Solitary, pair- and group-living refer to the social
50 organisation, describing the composition of social units [4]. Social organisation is one of four
51 components of the social system which further includes the care system, mating system and
52 social structure [4, 5]. Of all the different forms of social organisation, solitary living is the
53 most understudied [2].

54 Previous studies regarded solitary living as the ancestral default form of social organisation in
55 mammals [1]. However, recent comparative studies have shown that this is often not the case
56 [6, 7]. These studies showed that pair-living was most likely the ancestral form of social
57 organisation in artiodactyls [8], primates [9], and possibly in marsupials [10] and Eulipotyphla
58 [11], indicating that solitary living is often a derived state [12]. However, we know little about
59 the mechanisms leading to solitary living in mammals [13]. To understand the mechanisms of
60 group-living, we must also understand the alternative, which is solitary living [14].

61 It is generally assumed that solitary living in mammals is due to aggression. This opinion is
62 based on studies of the European hamster (*Cricetus cricetus*) in the 1950s [15]. In this species,
63 mothers become intolerant of their offspring when they reach puberty and adults are highly
64 intolerant of each other [15]. The assumption that social intolerance is the main reason of
65 solitary living in mammals was also based on standardized laboratory experiments on solitary
66 rodents to examine the proximate mechanisms of aggression [16-18]. However, whether this
67 is always the mechanism leading to solitary living under natural conditions is unknown. As an
68 alternative to social intolerance and aggression, a lack of social attraction combined with a
69 motivation to disperse when reaching sexual maturity could lead to solitary living. In solitary
70 mustelids, for example, individuals commonly meet in a non-aggressive context [19]. Social
71 interactions in nature have been studied in a few solitary living species such as the puma
72 (*Puma concolor*) [20] and the giant Kangaroo rat (*Dipodomys ingens*) [21]. While social
73 interactions in these solitary species were rare, these studies highlight the lack of aggressive
74 interactions when individuals met. More field studies are needed to understand the
75 mechanisms that cause solitary living.

76 Kin selection has been used to explain interactions in group-living species, but might also
77 influence solitary species, predicting close kin to be more tolerant towards each other [22].
78 Solitary species can have complex social structures in which individuals interact in a non-

79 random way [13]. They often display kinship-determined spatial patterns where kin live close
80 to each other and share part of their range and engage in non-aggressive interactions when
81 they meet [20, 21]. The kinship patterns in these solitary species are driven by philopatry, a
82 behaviour usually displayed by group living species, with individuals dispersing only short
83 distances and forming kin clusters [23]. For example, the social structure of the giant kangaroo
84 rat is formed by female kin neighbours, with shorter distances between neighbours leading
85 to increased social interactions [21]. The frequency of social interactions was positively
86 related to population density, indicating an influence of season on the social and spatial
87 structure [24]. Amicable interactions at territory boundaries are not a contradiction to the
88 theoretical assumption that aggression is a main driver of solitary living, but could simply be
89 due to the dear enemy phenomenon, where individuals are tolerant of known neighbours as
90 long as they do not cross territory boundaries [25]. To test whether solitary living can arise
91 without aggression, we need to measure aggression inside territories and at nesting sites.
92 Field experiments are needed to test the level of aggression in solitary species, and whether
93 close kin are more tolerant of each other than non-kin.

94 Our aim was to test the general assumption that aggression and social intolerance are the
95 mechanisms leading to solitary living in our study species, the bush Karoo rat (*Otomys*
96 *unisulcatus*) from South Africa. In particular, if aggression is the main mechanism leading to
97 natal dispersal and solitary living, we predicted (1) that the behaviour of mothers changes
98 towards their offspring as the offspring become older, with mothers showing more aggression
99 towards dispersed adult offspring than non-dispersed juvenile offspring. Because our study
100 species has a kin based spatial structure with significant overlap of home ranges between
101 close kin [26], and because kinship has been largely ignored to understand social systems of
102 solitary species, we further predicted (2) that they would show higher levels of aggression
103 towards non-kin neighbours than towards kin neighbours. To test these predictions, we
104 conducted more than 2000 focal animal observations over a period of 3 years, and additional
105 tests in a neutral presentation arena in a field laboratory and field experiments where we
106 presented kin and non-kin neighbours at the nesting sites of resident females.

107

108 **Materials and methods**

109 **Study site**

110 The study was conducted in the Goegap Nature Reserve, in the Northern Cape, South Africa.
111 The study site is located in the Succulent Karoo biome [27]. The climate is semi-arid with
112 temperatures falling below 0°C in winter and exceeding 40°C in summer [28]. Mean
113 precipitation at the field site is 160mm per annum. Seasons are divided into the hot dry non-
114 breeding season (December to May) and the cold wet breeding season (June to November).

115 **Study species**

116 The 100g bush Karoo rat offers a model to study solitary living because it is diurnal, occupies
117 an open habitat, has small home ranges (0.06 ± 0.04 ha in the dry, non-breeding season and
118 0.04 ± 0.03 ha in the wet, breeding season) [26], and easily habituates to the presence of
119 observers. It inhabits the semi-arid regions of South Africa including the Succulent Karoo (less
120 than 200mm of rain per annum), one of the world's most important biodiversity hotspots
121 [29]. The bush Karoo rat builds stick-lodges inside shrubs, which offer a favorable

122 microclimate with high humidity and mild temperatures as a buffer against the harsh outside
123 environment that is characterized by unpredictable rain in cold winters, and long, hot summer
124 dry seasons [30-32]. The bush Karoo rat is a central place forager, foraging around its stick
125 lodge and taking food back to the lodge, where it can be easily observed, and where
126 experiments can be conducted. In the Succulent Karoo, around 95% of the rats are solitary,
127 although a few small groups of 2-3 closely related females occur [26]. They have a kin based
128 spatial structure, with female kin living close to each other, and home ranges of close kin
129 overlapping more with each other than of non-kin [26]. Young male bush Karoo rats behave
130 similarly to females at the beginning of the dry season and stay in an area close to their natal
131 lodge. However, they disperse in winter when food availability increases, just before the
132 beginning of the breeding season. Adult males roam over very large areas and there are no
133 resident males and therefore no male neighbours exist during the breeding season.

134 **Sampling regime**

135 *Marking and trapping*

136 Trapping was conducted at lodges that showed signs of being occupied (fresh faeces, active
137 runways, rats observed). The field site was divided into six areas, with 1-2 areas trapped
138 simultaneously by two research teams. All lodges within one area were trapped for three
139 consecutive days before moving on to the next area. Traps were set in the morning before
140 sunrise and checked after 45 and 90 minutes and then un-set during the hottest times of the
141 day. In the afternoon, traps were set at 45 minutes before sundown, checked after sundown,
142 and then un-set during the night. We used a combination of foldable Sherman traps
143 (<https://shermantraps.com/>) and locally produced heavy metal traps of the Sherman style.
144 Traps were baited with a combination of bran flakes, salt and sunflower oil and re-baited each
145 morning and afternoon. Traps were arranged around the entrances of the lodges and along
146 runways. We recorded the body weight of individuals to the nearest 0.1-gram, as well as their
147 sex, reproductive status and lodge number. We marked individuals with single, metal-band
148 ear tags that had a unique reference number (National Band and Tag Co., Newport, KY, U.S.A.)
149 [33]. To aid in visual identification during observations, individuals were marked with non-
150 toxic hair dye (Inecto Rapido, Pinetown, South Africa), in combinations (females: head and
151 chest/sides/back; males: hindquarters and chest/sides/back). Age was estimated from body
152 mass at first capture, using a species-specific growth curve [34] validated for our field data.
153 The bush Karoo rats were classified according to their age, with pups being up to two weeks
154 old and weighing less than 30 g (weaning is at 14 days; [34]), juveniles being 2-6 weeks old
155 and weighing 30 and 70 g, and adults being older than 6 weeks and weighing more than 70 g,
156 when both sexes can start reproduction [34].

157 *Determining dispersal*

158 The onset of dispersal was determined for the rats used during the dyadic encounter tests
159 (explained below). We determined these occurrences from the time that the rat was trapped
160 consistently over a period of more than 4 weeks at a lodge that was not its natal lodge,
161 without the mother being trapped and observed at the same lodge. Using these data, we
162 calculated the age at dispersal.

163 *Focal animal observations in the field*

164 Focal animal observations were conducted from July 2021 to October 2023 for a total of 2062
165 observations and comprising of 246 rats at lodges with identified bush Karoo rats to establish
166 1) which individuals occupied the lodges, and 2) recorded behaviours, including interactions
167 with conspecifics. The observations were done for 30 minutes in the morning after sunrise
168 and 30 minutes in the afternoon before sunset. Observations were done using focal animal
169 sampling and one zero recording for 30min. We recorded all social behaviours in 1-min
170 intervals. The social behaviours included the following groups of behaviour: (i) amicable (e.g.,
171 grooming, body contact); (ii) social investigation (i.e., sniffing); and (iii) aggression (e.g.,
172 chasing, fighting).

173 *Dyadic encounter tests*

174 Dyadic encounter experiments were used to assess whether interactions with neighbours
175 were amicable (predicted for close kin) or aggressive (predicted for non-kin). Bush Karoo rats
176 were trapped and brought inside their trap to a laboratory at the research station, located
177 100 m away, and allowed to acclimatize for 10 min before the start of the experiment. The
178 experiments were conducted in a neutral test arena that was constructed of wood chip panels
179 (80 cm x 65 cm x 94 cm) and had a partition down the middle (Figure S 1, supplementary file).
180 The testing arena was cleaned between encounters using diluted Dettol Antiseptic Liquid and
181 then air dried. All tests were done from 10 am to 12 noon, and took place from August 2021
182 until March 2023, for a total of 143 tests on 52 focal rats. Each rat was tested 2.80 ± 2.2 times
183 on average.

184 Each bush Karoo rat was introduced into the arena and allowed to settle for 5 minutes with
185 the partition down. We first tested mothers as the focal individual against their offspring
186 which were from one month to 20 months old (N = 80 tests, 3 with male offspring and 77 with
187 female offspring, using 31 focal mother rats). The offspring tested were either still living in
188 their mothers' lodge i.e., not dispersed (N = 43) or already dispersed (N = 36). We attempted
189 to test each mother with the same offspring at different ages, but because some rats were
190 not re-trapped and disappeared, some mothers were tested with different offspring at
191 different ages. Next, we tested adult female focal rats on two different days with an adult
192 female kin neighbour and an adult female non-kin neighbour respectively (N = 63 tests on 41
193 rats). Half of the rats were first tested with a kin neighbour and the other half with a non-kin
194 neighbour. We defined a neighbour as a rat that occupied a lodge not more than 25 metres
195 away from the focal rat. A total of 22 of the focal rats tested with a neighbour were used in
196 the tests with offspring. Because all stimulus animals were direct neighbours, our
197 experimental design controlled for the dear enemy phenomenon, whereby the owner of a
198 territory responds less aggressively to a familiar neighbour than towards a stranger [25].
199 Presentations lasted for 15 minutes each. The focal adult individual was heavier than the
200 stimulus female (mean weight difference $25.8g \pm 20.3g$ SD), because we wanted the focal
201 individual, which we assigned as the owner of the territory, to initiate the encounters and
202 body mass difference was expected to have a positive influence on the initiation of aggression
203 [35]. At the end of each test, bush Karoo rats were returned to their lodge. Focal animal
204 sampling was used to record the frequency of social behaviours as described for focal animal
205 observation above [36]. The behaviours were recorded for 15 minutes using a webcam. The
206 observer was present in the same room as the animals being tested but was separated from
207 the animals by a black curtain and monitored the behaviours live on a computer. Tests would
208 have been immediately terminated as soon as individuals started damaging fights (biting
209 and/or standing upright and boxing for more than 2 seconds) to avoid any injury. However,

210 this was never needed in our study. Videos of the interactions were scored using BORIS
211 (Behavioural Observation Research Interactive Software, [37]).

212 *Field intruder presentation tests*

213 Since we observed little aggression during the neutral arena tests, we conducted field intruder
214 tests directly at the lodge of the rats. A similar experiment had been done on group living
215 African ice rats (*Otomys sloggetti robertsi*) from the alpine regions of the southern African
216 Drakensberg and Maluti mountains [38]. We expected higher levels of aggression here due to
217 the focal rats defending their territory, which was not the case in the neutral arena in the
218 laboratory. Again, we tested whether focal animals are more aggressive towards non-kin than
219 kin neighbours. These tests were done 1-2 years after the dyadic encounter tests in the
220 neutral test arena, and of the individuals tested in the field, only nine had participated as the
221 focal (N = 9) / stimulus (N = 6) in the previous experiments. Stimulus individuals were trapped
222 (as described above) and then transferred into a wire mesh cage (30 x 15 cm, 12 cm height)
223 (Figure S2). This wire mesh cage allowed other bush Karoo rats to see and smell the stimulus
224 animal in the trap, but not make physical contact. The trapped individual was then presented
225 at a neighbouring rat's lodge (i.e. the focal individual). The focal individual was not caged and
226 its response to the trapped stimulus individual was recorded. The wire cage was positioned
227 within an active runway 30 cm away from the lodge. Observations started when the focal
228 animal was observed outside its lodge and lasted for 15 min thereafter. The maximum
229 duration of the presentation was 45 min; thus, when the focal animal was not seen within the
230 first 30 min, the experiment was terminated. During the 15 min of observations, we recorded
231 aggressive behaviours of the focal animal towards the caged stimulus animal, including
232 charging towards the cage and emitting aggressive chit sounds. We also recorded the latency
233 until the investigation of the stimulus individual and the first aggression as well as the total
234 time spent at the cage. Thereafter, the stimulus animal was returned to its lodge from where
235 it was trapped. Each focal was tested, once with a kin neighbour and once with a non-kin
236 neighbour on separate days. The tests were conducted in the early mornings from 6:00 to
237 9:00 from January – November 2023. We conducted a total of 54 tests for 26 focal rats with
238 each rat being tested 2.03 ± 0.87 times on average. For both the dyadic and field encounter
239 tests, a focal rat could be used as a stimulus in another experiment, and a stimulus rat could
240 be used multiple times for different focal rats. In the dyadic tests, 15 rats were used as both
241 focal and stimulus and 12 rats were used in the intruder tests.

242 *Statistical analysis*

243 All statistical analyses were done in R [version 4.3.1; 39]. We used an ANOVA to analyse
244 factors that influence the frequency of sniffing events, or the time spent in body contact,
245 fighting, or grooming, and included the age of offspring, and season (breeding vs non-
246 breeding) as factors for the dyadic encounter tests between mother and offspring (Table 1).

247

248 Table 1: Hypothesis and associated models tested in the dyadic and field intruder tests.

Hypothesis	Type experiment	Model	Model type
------------	--------------------	-------	---------------

The behaviour of mothers changes towards their offspring as the offspring become older.	Dyadic encounter mother offspring	-	Time in body contact = season + age of offspring.	LME
			Time fighting = season + age of offspring.	LME
			Time grooming = season + age of offspring.	LME
			Frequency of sniffing = season + age of offspring.	GLMM - Poisson
Bush Karoo rats show higher levels of aggression towards non-kin than towards kin neighbours	Dyadic encounter neighbours	-	Fight = season + relatedness + body size difference + (1 ID-focal).	LME
			Body contact = season + relatedness + body size difference + (1 ID-focal).	LME
			Groom = season + relatedness + body size difference + (1 ID-focal).	LME
			Sniff = season + relatedness + body size difference + (1 ID-focal).	GLMM - Poisson
	Field intruder test		Latency to first aggression = season * relatedness + body size difference + (1 ID-focal).	LME
			Latency to approach = season * relatedness + body size difference + (1 ID-focal).	LME
			Charging = season * relatedness + body size difference + (1 ID-focal).	GLMM - Poisson

Trills (chit) = season * relatedness + LME
body size difference + (1|ID-focal).

Time at cage = season * relatedness + LME
body size difference + (1|ID-focal).

249 * Indicates that variables were fit as fixed effects separately and as an interaction.

250 We ran linear mixed models (LME) in *lme4* to investigate factors that influenced the behaviour
251 exhibited by bush Karoo rats during dyadic encounter tests toward neighbours. For affiliative
252 behaviours, we tested the time spent sitting in body contact, and time spent grooming. For
253 aggressive behaviours we tested the duration of fights. Finally, for social investigative
254 behaviours, we fit a generalized linear mixed model (GLMM) with a Poisson distribution, and
255 we tested the number of sniffing events displayed. For all the models, the duration/frequency
256 of the behaviours were fitted in linear models as predictors, and we included season, body
257 size difference, and relatedness as fixed effects and the ID of the focal individual as random
258 effects (Table 1).

259 We used both generalized linear mixed models (GLMM) and linear mixed models (LME) to
260 analyse factors that influence the behaviour displayed by the focal rat towards a stimulus for
261 the field intruder presentation tests. We tested the latency to approach the cage and to first
262 aggression, and the number of charging events (fitted with a Poisson distribution) and the
263 number of chit sounds, and total time spent at the cage. One model was fit for each
264 behaviour. We included season, body size difference, and relatedness as fixed effects. We
265 further included the interaction between season and relatedness as fixed effects. To avoid
266 singularity, we only fitted the ID of the focal rat as a random effect (Table 1).

267

268 **Results**

269 *Mother- offspring interactions*

270 During 87 of the 2062 field observations sessions, pups were present at the mother's nest.
271 On 11 occasions, we observed amicable interactions with the mother (grooming, and body
272 contact), and no interactions occurred on 76 occasions; we never observed aggression
273 towards the offspring by the mother. Juveniles were present on 240 occasions during the field
274 observations and were observed in amicable interactions with the mother on 20 occasions,
275 and only once in an aggressive interaction.

276 The mean age of all offspring (N = 79) used as stimulus animals was 4.13 ± 3.72 SD months.
277 The age of non-dispersed rats in the experiments was 3.81 ± 3.25 SD months (range 1 – 14
278 months, N = 43) compared to 5.21 ± 4.01 months (range: 1 – 20 months, N = 36) of dispersed
279 rats. We calculated AIC values for models including the age of offspring vs dispersed or
280 undispersed. We found that the AIC values for models including the age of offspring as a
281 predictor were higher than for the models that included whether the offspring had dispersed
282 (Table S2, supplementary file). However, the summary tables for both these models were very
283 similar (results and conclusions did not change) and we reported the models including the
284 age of offspring in the electronic supplement. Dyadic encounters between mothers and their
285 female offspring were characterised by sniffing and body contact with little grooming and
286 nearly no aggression (Table S1, supplementary file). Social interactions were not influenced

287 by season and did not significantly change after offspring dispersed (Figure S3; Tables S3-S6:
288 supplementary file). Specifically, mothers were not more aggressive towards dispersed
289 offspring than non-dispersed (Table S4, Figure S4), nor did they decrease the level of body
290 contact (Table S6, Figure S3).

291 *Focal animal observation*

292 In 230 of the 2062 observations, conducted between July 2021 and October 2023, two or
293 more adult rats were present at the focal rats' lodge. 97 chases occurred in 79 of the 230
294 observations. Chases occurred more often during the non-breeding season (t-test; $t_{1728} = -$
295 2.99 , $p < 0.01$). The chases occurred between a male and an unrelated female (41%) or an
296 unidentified neighbour (41%), and between related females (18%).

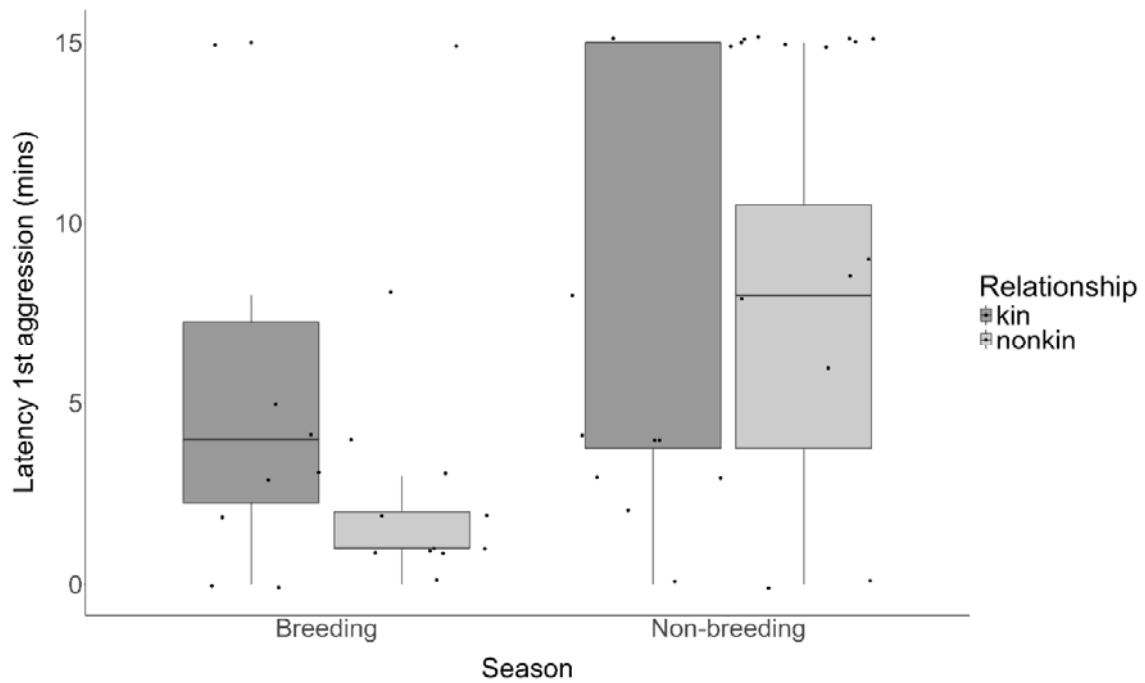
297 *Dyadic encounter tests (neutral test arena)*

298 We tested whether adult female bush Karoo rats (focal individuals) showed less aggression
299 towards kin neighbours than towards non-kin neighbours (adult females). The weight
300 difference between the individuals was not a significant predictor in any of the models. Adult
301 female bush Karoo rats showed very little aggression towards their neighbours, irrespective
302 of the season (LME; estimate = 0.016, s.e. = 0.013, CI: -0.0098/0.042, $t_{63} = 1.2$, $p = 0.22$) and
303 relatedness (LME; estimate = 0.009, s.e. = 0.015, CI: -0.02/0.04, $t_{62} = 0.6$, $p = 0.55$) (Figure S2
304 and Table S7: supplementary file). Although they tended to spend more time in body contact
305 with kin than with non-kin, this was not significant (LME; estimate = -2.36, s.e. = 1.316, CI: -
306 $5.04/0.31$, $t_{63} = -1.79$, $p = 0.08$; Figure S6 A), and there was also no significant difference
307 between seasons (LME; estimate: $0.57 + \text{s.e.} = 1.094$, CI: -1.63/2.8, $t_{61} = 0.52$, $p = 0.60$) (Table
308 S8: supplementary file). The time spent grooming neighbours was not significantly affected
309 by season (LME; estimate = 0.008, s.e. = 0.019, CI: -0.0295/0.045, $t_{63} = 0.42$, $p = 0.673$) or
310 relatedness (LME; estimate = -0.02, s.e. = 0.022, CI: -0.0645/0.025, $t_{58} = -0.93$, $p = 0.356$)
311 (Figure S6 B and Table S9: supplementary file). Bush Karoo rats spent a significant amount of
312 time investigating neighbours through sniffing in the breeding season (GLMM; estimate =
313 0.73 , s.e. = 0.27, CI: $0.22/1.29$, $t = 2.697$, $p < 0.01$) but this was not affected by relatedness
314 (estimate = 0.54, s.e. = 0.36, CI: -0.17/1.27, $p = 0.14$) (Figure S7 and Table S10: supplementary
315 file).

316 *Intruder field presentation tests*

317 In both seasons, female bush Karoo rats were mildly aggressive towards non-kin neighbours
318 but were more tolerant of kin neighbours when presented at their lodge. There was no
319 difference in the latency to approach the cage with a kin or non-kin focal neighbour (estimate
320 = -0.98, s.e. = 1.61, CI: -4.27/2.29, $p = 0.6$; Figure S8 and Table S12), indicating resident bush
321 Karoo rat females investigated the stimulus animal regardless of kinship. However, non-kin
322 females were attacked much faster (LME; $t_{38} = 2.54$, $p < 0.05$, Figure 2 and Table S11), their
323 cages were charged more often (GLMM; estimate = 0.75, s.e. = 0.22, CI: $0.32/1.2$, $t = 3.4$, $p <$
324 0.001 ; Figure 2 and Table S13), and they produced more trill sounds (Figure S8; although this
325 was not significant because a large outlier: LME; estimate = 9.53, s.e. = 18.2, CI: -25.45/44.77,
326 $t = 1.56$, $df = 37.59$, $p = 0.61$; Table S14). Also, focal individuals spent significantly more time
327 at the cage with the non-kin than kin neighbours (LME; estimate = 4.63, s.e. = 1.37, CI:
328 $1.76/7.39$, $t_{39} = 4.06$, $p < 0.01$, Figure. 4 and Table S15). These differences occurred in both
329 the breeding and the non-breeding seasons. However, in the breeding season, female bush
330 Karoo rats showed more aggression towards intruders, regardless of kinship (LME; $t = -4.32$,

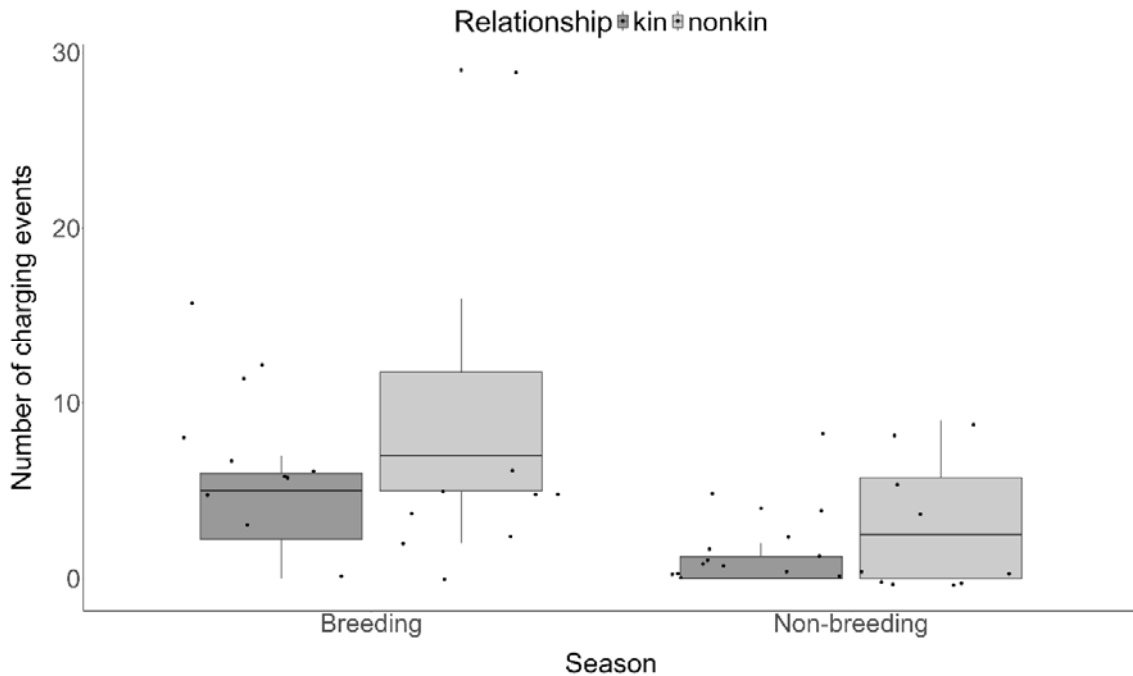
331 $p < 0.001$) by quickly attacking (LME; estimate = 4.287, s.e. = 1.82, CI: 0.61/7.95, $t_{43} = 2.349$, p
332 = 0.02), and charging at cages (LME; estimate = -1.92, s.e. = 0.44, CI: -2.899/-1.10) (Figures. 2-
333 3).



334

335 **Figure 1:** The latency to first aggression by focal females to neighbouring cage-housed female
336 bush Karoo rats during intruder tests. Boxplots show median and 1st and 3rd quartiles, the
337 whiskers represent the minimum and maximum of the outlier data, and points represent
338 individual values.

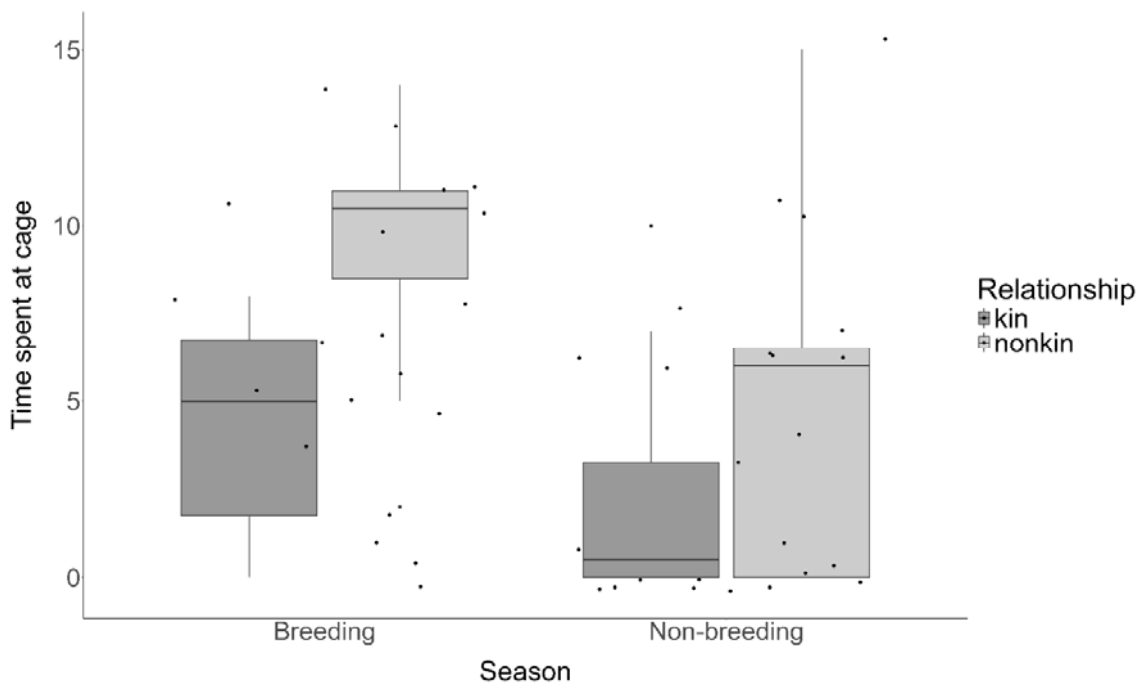
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340

341 **Figure 2:** The number of charging events by focal females to neighbouring cage-housed bush
 342 Karoo rats during intruder tests. Boxplots show median and 1st and 3rd quartiles, the
 343 whiskers represent the minimum and maximum of the outlier data, and points represent
 344 individual values.

345



346

347 **Figure 3:** The time spent at the cage by focal females to neighbouring cage-housed bush Karoo
 348 rats during intruder tests. Boxplots show median and 1st and 3rd quartiles, the whiskers

349 represent the minimum and maximum of the outlier data, and points represent individual
350 values.

351

352 **Discussion**

353 It is typically assumed that individuals of solitary species are intolerant of each other [40].
354 Here we tested whether female bush Karoo rats live solitarily because of high intra-specific
355 aggression. However, we observed no aggression between mothers and their juvenile or even
356 adult offspring. The mother-offspring relationship did not change when the offspring became
357 older and had dispersed from their mother. In dyadic encounter tests, mother and offspring
358 were found to be in regular body contact which did not decline as offspring became older.
359 During field observations, mothers interacted rarely with pups and juveniles, but when they
360 did so, it was mainly amicable. Thus, both experimental and field observation data indicate
361 that aggression rarely occurs between mothers and offspring, aggression does not increase
362 as offspring age and have become solitary. Thus, aggression is unlikely to be the mechanism
363 leading to solitary living. This makes the alternative a likely explanation, i.e. the motivation to
364 disperse when reaching sexual maturity together with an absence of social attraction leads to
365 dispersing individuals settling alone inside an unoccupied lodge.

366 It is typically assumed that aggression is the main mechanism leading to offspring dispersal
367 and solitary living [41]. The association between aggression and dispersal in which
368 subordinate individuals (juveniles) are driven out by dominant individuals (adults) is well
369 known [41, 42]. However, there are equally many studies showing that aggression and
370 dispersal are not always associated with one another [41]. In our study, mothers did not react
371 more aggressively towards their dispersed offspring than towards their offspring still living
372 with them. Female bush Karoo rats reach sexual maturity at six weeks of age [34] and are thus
373 expected to leave their mother's lodge (i.e. disperse) earliest at this age. Accordingly, females
374 dispersed at 2 months of age but with a large age variation that needs investigation in the
375 future. For example, food availability, population density, and start vs end of breeding season
376 are factors expected to influence dispersal. Our study showed the importance of dispersal in
377 becoming solitary while there was no indication that aggression by the mother drives natal
378 dispersal.

379 Aggressive interactions are often considered as the main underlying reason for solitary living
380 and the reduction of aggression as a first step towards the evolution of sociality [43]. Dyadic
381 encounters between adult neighbouring bush Karoo rats in a neutral arena were
382 characterised by few interactions, independent of kinship, with sniffing being the
383 predominant form of social investigation. Nearly no aggression occurred, not even between
384 unrelated females. However, close kin were more likely to spend time in body contact with
385 each other than non-kin. Thus, while female bush Karoo rats differentiated between kin and
386 non-kin neighbours, in both cases aggression was limited. But there was also no social
387 attraction between adults. Within the same field site, and in similar experiments to ours,
388 group-living striped mice were highly aggressive towards non-kin and interacted amicably
389 with kin [44]. We had expected much more aggression in the solitary bush Karoo rat than in
390 the sociable striped mice, and our ethical clearance protocol included that as soon as
391 damaging fighting started, experiments would be terminated. We expected this to be the case
392 regularly when non-kin met, but we never had to stop the encounter experiments. Therefore,

393 absence of social attraction could be sufficient to lead to solitary living in bush Karoo rats,
394 without the need for social intolerance and aggression.

395 Interactions between individuals are not random and instead reflect relatedness or familiarity
396 [45]. In field intruder tests, where we expected to find more aggression towards conspecifics,
397 due to the defence of resources [46], aggression was much more common during the
398 breeding than the non-breeding season, and females were much more aggressive towards
399 non-kin than kin. This indicates that female bush Karoo rats can differentiate between kin and
400 non-kin, even when their kin had been living in a different lodge (solitarily) for several months.
401 Kin recognition thus occurs post-dispersal and indicates that mothers remember adult,
402 dispersed offspring. This tells us that remembering kin (the mechanism for kin recognition)
403 persists.

404 Female territoriality functions to defend resources and offspring [47, 48]. This theoretical
405 consideration can explain why we observed nearly no aggression during dyadic encounter
406 tests in a neutral test arena because no resources could be defended but we did observe some
407 aggression during intruder tests when individuals defended their lodge. Does this indicate
408 defence of resources such as food and shelter, or defence of offspring? More aggression was
409 observed during the breeding season, which is also when more lodges are available due to
410 low population density at the start of the season, and when food is highly abundant. Thus,
411 our data support the female hypothesis of Wolff and Peterson [46] that territorial aggression
412 is linked more to the defence of offspring (maternal aggression) than the defence of food
413 resources. Reproductive competition can be high between female mammals [49], often
414 leading to female infanticide [50], and is considered as one of the main reasons for solitary
415 living [51]. Several other mammal species show maternal aggression, with females being
416 aggressive at their nests in the breeding season while showing amicable behaviour at
417 communal foraging grounds [reviewed by 46]. For example, female Arctic squirrels
418 (*Urocitellus parryii*) and grey squirrels (*Sciurus carolinensis*) defend their territories near their
419 nests but show considerable overlap in foraging areas. This behaviour is not limited to
420 mammals but has been shown also in female social lizards (e.g., White's skinks, *Egernia whitii*)
421 where female aggression increased during pregnancy and after birth [47]. Aggression in
422 female bush Karoo rats thus rather functions to protect their offspring than to establish a
423 solitary social organisation.

424

425 **Conclusion**

426 While solitary species have been considered to be generally asocial and aggressive, we have
427 evidence that the solitary bush Karoo rat shows low levels of aggression. Instead, its social
428 system is characterised by social tolerance, which suggests that lack of social attraction after
429 dispersal and not aggression leads to dispersal and solitary living. Female bush Karoo rats
430 were not aggressive to their offspring even after they had dispersed from the maternal lodge
431 and lived solitarily for months. In the solitary bush Karoo rat, maternal aggression is not the
432 mechanism driving offspring to solitary living. Tolerance of conspecifics is a condition for
433 sociality. Therefore, solitary species that can tolerate their conspecifics may provide a model
434 to understand the evolution of sociality in mammals.

435

436 **Ethical note**

437 We adhered to the ASAB/ABS Guidelines for the Use of Animals in Research [52]. Bush Karoo
438 rats were captured and handled using protocols approved by the Animal Research and Ethics
439 Screening Committee of the University of the Witwatersrand (AESC clearance number: 2018-
440 03-15B). We further received additional ethical clearance for both the dyadic encounter tests
441 and the field intruder presentation tests (AESC clearance numbers 2021/05/02/B and 22-12-
442 024B respectively).

443 **Data availability**

444 The data are available as supplementary material.

445

446 **Author contributions**

447 L.M., C.S., and N.P. developed and designed the study. L.M., C.S. and S.P.S. collected the data.
448 L.M. analysed the data, all authors discussed the results. L.M. and C.S. wrote the first draft of
449 the manuscript. All co-authors contributed to the final draft of the manuscript.

450 **Competing interests**

451 The authors declare that they have no competing interests.

452 **Funding**

453 L.M. was supported by the joint CNRS-WITS PhD program and the National Research
454 Foundation (NRF) through N.P.

455 **Acknowledgements**

456 This study was made possible by the administrative and technical support of the Succulent
457 Karoo Research Station (registered South African NPO 122-134). This study is part of the long-
458 term Studies in Ecology and Evolution (SEE-Life) program of the CNRS.

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