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3 **The inbreeding strategy of a solitary primate, *Microcebus murinus***

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32 1. ABSTRACT

33 Inbreeding depression may be common in nature, reflecting either the failure of inbreeding  
34 avoidance strategies, or inbreeding tolerance when avoidance is costly. The combined  
35 assessment of inbreeding risk, avoidance and depression is therefore fundamental to evaluate  
36 the inbreeding strategy of a population, i.e., how individuals respond to the risk of inbreeding.  
37 Here, we use the demographic and genetic monitoring of 10 generations of wild grey mouse  
38 lemurs (*Microcebus murinus*), small primates from Madagascar with overlapping generations,  
39 to examine their inbreeding strategy. Grey mouse lemurs have retained ancestral mammalian  
40 traits including solitary lifestyle, polygynandry and male-biased dispersal, and may therefore  
41 offer a representative example of the inbreeding strategy of solitary mammals. The  
42 occurrence of close kin among candidate mates was frequent in young females (~37%, most  
43 often the father) and uncommon in young males (~6%) due to male-biased dispersal.  
44 However, close kin consistently represented a tiny fraction of candidate mates (<1%) across  
45 age and sex categories. Mating biases favouring partners with intermediate relatedness were  
46 detectable in yearling females and adult males, possibly partly caused by avoidance of  
47 daughter-father matings. Finally, inbreeding depression, assessed as the effect of  
48 heterozygosity on survival, was undetectable using a capture-mark-recapture study. Overall,  
49 these results indicate that sex-biased dispersal is a primary inbreeding avoidance mechanism  
50 at the population level, and mating biases represent an additional strategy that may mitigate  
51 residual inbreeding costs at the individual level. Combined, these mechanisms explain the  
52 rarity of inbreeding and the lack of detectable inbreeding depression in this large, genetically  
53 diverse population.

54

55 Keywords: sex-biased dispersal, inbreeding risk, inbreeding avoidance, mate bias,  
56 heterozygosity, survival, *Microcebus murinus*

57

## 58 2. INTRODUCTION

59 Inbreeding depression is defined as a decline of fitness in offspring of related individuals  
60 relative to offspring of unrelated individuals (Charlesworth & Charlesworth, 1987). Decreases  
61 in the fitness of inbred individuals are thought to result from a reduced reaction scope of the  
62 immune system and/or from deleterious combinations of recessive alleles in the genome due  
63 to genome-wide increased homozygosity (Charlesworth & Charlesworth, 1987, Charlesworth  
64 & Willis, 2009). Fitness-related traits found to be negatively affected by inbreeding include  
65 birth weight (Coltman et al., 1998, Coulson et al., 1998), development (Diehl & Koehn, 1985,  
66 Charpentier et al., 2006, Nielsen et al., 2012), reproductive success (Foerster et al., 2006, Zeh  
67 & Zeh, 2006), resistance to disease and environmental stress (Coltman et al., 1999, Acevedo-  
68 Whitehouse et al., 2003) and survival (Acevedo-Whitehouse *et al.*, 2003). Effects are often  
69 age-dependent, with younger individuals suffering higher levels of inbreeding depression  
70 (Stockley et al., 1993, Markert et al., 2004, Cohas et al., 2009) although inbreeding effects on  
71 adult traits have been far less studied and could also impose a substantial cost to fitness  
72 (Grueber *et al.*, 2010).

73 Inbreeding depression may commonly occur in wild populations (Saccheri et al., 1998,  
74 Keller & Waller, 2002), and its implications for extinction risk have long been debated (Lande,  
75 1988, Caro & Laurenson, 1994). It is a question of considerable significance at a time when  
76 population fragmentation and associated loss of genetic diversity may threaten the viability  
77 of many populations (Hedrick, 2000). Nevertheless, it remains difficult to evaluate the  
78 frequency and intensity of inbreeding depression across wild populations for several reasons.

79 First, it is often difficult to assess patterns of parentage in large representative samples of  
80 individuals with known life-histories in natural populations. Second, studies focusing on  
81 inbreeding effects may often target small or fragmented populations. Third, a potential  
82 publication bias towards positive results might further bias the empirical record (Chapman *et*  
83 *al.*, 2009). Multigenerational individually-based studies of vertebrates offer a unique  
84 opportunity to generate unbiased estimates of the occurrence and fitness costs of inbreeding  
85 depression across taxa (Kempnaers *et al.*, 1996, Keller, 1998, Walling *et al.*, 2011, Nielsen *et*  
86 *al.*, 2012, Szulkin *et al.*, 2013).

87 Inbreeding depression may represent a significant evolutionary pressure even where  
88 it is undetectable. Inbreeding risk may be intrinsically low in a given population due to a  
89 combination of demographic and life history factors as in large populations with non-  
90 overlapping generations, but it may also be actively contained by behavioural strategies of  
91 inbreeding avoidance, including sex-biased dispersal (Greenwood, 1980, Clutton-Brock, 1989,  
92 Pusey & Wolf, 1996) or discrimination against related mates (Tregenza & Wedell, 2000,  
93 Kempnaers, 2007). Estimating the extent of inbreeding depression is therefore insufficient  
94 to evaluate the evolutionary importance of inbreeding within and across taxa.

95 In addition, some studies indicate that animals sometimes show no inbreeding  
96 avoidance or even preferentially mate with relatives, suggesting that inbreeding is not  
97 universally detrimental and that 'inbreeding strategies' may differ across individuals,  
98 populations and species, according to the relative costs and benefits of inbreeding and of  
99 inbreeding avoidance (Bateson, 1978, Szulkin *et al.*, 2013). Avoiding mating with kin may  
100 sometimes be more costly than having inbred offspring. Moreover, kin selection theory  
101 suggests that inbreeding may be adaptive under specific circumstances, by increasing the

102 relatedness between parents and offspring (Bateson, 1978, Waser et al., 1986, Kokko & Ots,  
103 2006, Puurtinen, 2011, Szulkin et al., 2013). Overall, the current state of the field suggests that  
104 our theoretical understanding of animal inbreeding strategies remains limited and calls for  
105 further empirical efforts to examine inbreeding risk, inbreeding avoidance strategies and  
106 depression in concert.

107         This study presents a quantitative investigation of inbreeding risk, avoidance and  
108 depression in a large population of wild primates. Grey mouse lemurs (*Microcebus murinus*)  
109 are small nocturnal and solitary foragers distributed along Southern and Western Madagascar  
110 (Kappeler & Rasoloarison, 2003). They are polygynandrous with no paternal care (Eberle &  
111 Kappeler, 2004a). Individuals acquire feeding independence at around 2 months of age and  
112 can reproduce for the first time at 10 months of age and then every year thereafter. Each  
113 female is sexually receptive for one to two nights per year, and may mate with up to seven  
114 different males during those nights, while up to 14 candidate males have been observed  
115 around a receptive female (Eberle & Kappeler, 2004a). Mortality rates of grey mouse lemurs  
116 are high, especially in the first year of life (Kraus *et al.*, 2008) but some individuals survive 6 to  
117 10 years (unpublished observation), which generates a potential overlap between  
118 reproductive periods of parents and offspring and may create inbreeding risk. Natal dispersal  
119 is strongly male-biased (Schliehe-Diecks *et al.*, 2012) and secondary dispersal, where  
120 individuals disperse repeatedly, is low (Radespiel et al., 2001, Eberle & Kappeler, 2002,  
121 Kappeler & Rasoloarison, 2003, Fredsted et al., 2005) so that fathers often live close to their  
122 philopatric daughters and individual variation in relatedness levels is locally high, creating  
123 ample opportunities for inbreeding avoidance or tolerance (Radespiel et al., 2001, Fredsted et  
124 al., 2004, Fredsted et al., 2005).

125 Two previous studies have detected mate choice for dissimilar partners at immune  
126 genes of the Major Histocompatibility Complex (MHC) in females of this population  
127 (Schwensow et al., 2008, Huchard et al., 2013), but avoidance of mating with kin was only  
128 detected by one of these studies, where mated pairs were less related than random pairs. This  
129 study, however, only adopted a female perspective and did not document the extent of  
130 inbreeding risk and depression across age and sex categories (Huchard *et al.*, 2013). Here, we  
131 extend these studies and combine a 10-generation dataset of a capture-mark-recapture  
132 (CMR) study with genetic data to provide an integrative analysis of inbreeding risk and its  
133 evolutionary consequences in a natural primate population by estimating, for both the  
134 philopatric sex (females) and the dispersing sex (males) in adults and in yearlings, the extent  
135 of (1) inbreeding risk, (2) inbreeding avoidance via mating biases between actual mates and  
136 random members of the mating pool (although observing such a bias does not inform us on  
137 the choosy sex, as choice by one sex will generate a detectable mating bias in the other sex),  
138 and (3) inbreeding depression by quantifying the survival cost of inbreeding.

139

### 140 3. METHODS

#### 141 *3.1 Study population and trapping procedures*

142 The study population is located within a 12,500 ha forestry concession of the Centre National  
143 de Formation, d'Etude et de Recherche en Environnement et Foresterie (C.N.F.F.R.E.F.) in  
144 Kirindy Forest (Kappeler & Fichtel, 2012). Since 1994, DNA samples and population parameters  
145 have been collected during monthly captures using about 160 traps at a time in an area of  
146 about 9 ha within a 60 ha grid system. Additional captures in surrounding areas were  
147 conducted once or twice a year and covered an area of about 18 ha. For trapping, Sherman

148 live traps were baited with small pieces of banana and positioned near trail intersections at  
149 dusk on three consecutive nights. Captured animals were collected at dawn and marked with  
150 subdermal transponders if captured for the first time, and otherwise simply weighed and  
151 handled according to published protocols (Eberle & Kappeler, 2002). Data on 1,298 individuals  
152 were available for analyses between 2000 and 2010.

153

### 154 *3.2 Microsatellite DNA analyses*

155 DNA was isolated from ear biopsies, using the QIAGEN QIAamp Tissue Kit for DNA Purification  
156 (Qiagen) (Eberle & Kappeler, 2004b). DNA amplification and sequencing are described in  
157 Supporting Information, Appendix S1. A total of 1073 to 1278 individuals were typed for each  
158 locus, with an average of 21.5 alleles per locus.

159

### 160 *3.3 Parentage analyses and calculation of relatedness estimates and heterozygosity*

161 Parentage analyses for determination of true parents and their spatial distribution were based  
162 on a likelihood analysis using CERVUS 3 (Kalinowski *et al.*, 2007) and COLONY v 2.0.1.9 (Jones  
163 & Wang, 2010) and are fully described in the Supporting Information, Appendix S2.  
164 Relatedness estimates were calculated with the software COANCESTRY v 1.0.0.0 (Wang, 2011)  
165 for all individuals captured between 1999 and 2010, based on the triadic individual by descent  
166 (IBD) 'TrioML' index (Wang, 2007), which uses the genotypes of a triad of individuals in  
167 estimating pairwise relatedness ( $r$ ). To estimate individual genome-wide heterozygosity, we  
168 calculated the homozygosity by loci (HL) index of Aparicio *et al.* (2006), which has been found  
169 to perform better than two other estimators of heterozygosity, internal relatedness (IR) (Amos



170 *et al.*, 2001) or uncorrected homozygosity (HO, Aparicio *et al.*, 2006). Unless otherwise stated,  
171 all analyses were run in R 3.0.2 (R Development Core Team 2013).

172

### 173 *3.4 Assignment of candidate mates*

174 A list of candidate mates was established for each individual and for each mating season in  
175 our sample (thereafter referred to as a “mating season”) following Huchard *et al.* (2013).  
176 Individuals were considered as candidate mates if they fulfilled the following three criteria: (1)  
177 they were present in captures immediately preceding or following the mating season (there  
178 are no captures during the mating season, in order to avoid potential disruption of mating  
179 patterns) to ensure that only live animals were included in the analysis; (2) the average  
180 distance between home range centres of partners is lower than the maximum distance  
181 recorded between the two parents of an offspring using long-term parentage data from this  
182 population (females: perimeter=319m; males: perimeter=336m, see Huchard *et al.* (2013));  
183 (3) they ranged within the core study area where the demographic monitoring has been  
184 regular and continuous throughout the study period. A total of 56 females and 81 males that  
185 were found to be part of a parent-offspring triad between 2000 and 2010 were included in  
186 the analyses.

187

### 188 *3.5 Estimating inbreeding risk*

189 To estimate inbreeding risk, we used parentage analyses and pedigree data to determine  
190 whether first order relatives (parent-offspring and full siblings) were present within pools of  
191 candidate mates. Inbreeding risk was quantified by its occurrence and intensity and compared  
192 between males and females and between yearlings and adults. The occurrence of inbreeding

193 risk was computed as the presence/absence of at least one first order relative in each mating  
194 season. To test for possible sex and age differences in the occurrence of inbreeding risk, we  
195 ran a binomial generalized linear mixed model (GLMM) (response variable: presence/absence  
196 of at least one first order relative during a mating season) including the fixed effects sex, age  
197 and their interaction and the crossed random effects year and individual identity, to control  
198 for the non-independence of observations from a same individual or from a same year. Then,  
199 we computed the intensity of inbreeding risk by calculating, for each mating season, the  
200 proportion of first order relatives among the candidate mates. We evaluated age and sex  
201 effects on the intensity of inbreeding through a second binomial GLMM with the same  
202 structure of fixed and random effects as the occurrence model (response variable: number of  
203 first order relatives/number of candidates in a given season).

204         Model selection was based on AIC or one of its appropriate variants (here QAICc which  
205 adjusts for small sample sizes and the presence of overdispersion, Burnham & Anderson,  
206 2002) using the dredge function from the MuMIn package (Barton, 2015) in R 3.0.2. We  
207 interpreted model selection results based on AICc differences ( $\Delta_i$ ) and normalised Akaike  
208 weights ( $w_i$ ) as described by Burnham & Anderson (2002). We further computed estimates of  
209 fixed effects for the top models.

210

### 211 *3.5 Investigating inbreeding avoidance*

212 To test whether relatedness among mates is minimized, we compared the mean observed  
213 relatedness values of the parents in our long-term dataset to a distribution of the mean  
214 relatedness values of randomly matched partners generated under the null hypothesis of  
215 random mating. We further compared the mean-corrected variance (assessed by the

216 coefficient of variation) in the relatedness of true versus randomly assigned parents for two  
217 reasons. First, if some individuals avoid inbreeding whereas others preferentially inbreed,  
218 mean observed relatedness could match null expectation but with an increased variance  
219 (Szulkin *et al.*, 2013). Second, if individuals avoid mates that are either too closely or too  
220 distantly related, thereby optimizing rather than minimizing relatedness to their mates, mean  
221 observed relatedness could match null expectation but with a decreased variance. The  
222 coefficient of variation ('CV', standard deviation divided by the mean) was used as a measure  
223 of variance to ensure that results would be statistically independent from results obtained on  
224 the mean. The correlation between mean and variance of parental relatedness was positive  
225 and high in all four samples (yearling females, adult females, yearling males and adult males)  
226 with Pearson's *r* values comprised between 0.70 and 0.80 ( $df=19998$  and  $p<10^{-15}$  in all four  
227 cases), while there was no correlation between mean relatedness and the coefficient of  
228 variation in relatedness (Pearson's *r* was comprised between -0.09 and -0.03 in all four cases).  
229 Finally, we tested whether individuals may choose partners with high heterozygosity, which  
230 may occur if these partners are more competitive than, or preferred over, less heterozygous  
231 individuals. They could be preferred if choosing a heterozygous partner brings direct benefits  
232 (such as a decreased risk of infection by sexually transmitted diseases) or indirect benefits  
233 (such as the transmission of rarer – and therefore more heterozygous - genotypes to offspring)  
234 (Fromhage *et al.*, 2009; Kempenaers, 2007).

235         The distribution of the mean and coefficient of variation of relatedness between  
236 random partners to an individual was generated by randomly matching each individual 20,000  
237 times to one mate of their pool of candidate mates for a given mating season. *P*-values were  
238 computed in two ways, due to the difficulties, and the resulting lack of consensus, regarding  
239 the calculation of two-sided *p*-values in the case of asymmetrical distributions (e.g., Gibbons

240 & Pratt, 1975, Kulinskaya, 2008), as well as to facilitate future meta-analytic approaches: first,  
241 a one-tailed p-value was computed as the proportion of cases displaying a lower (for mean  
242 and variance of relatedness) or greater (for mean heterozygosity) than the observed value.  
243 Second, an exact two-tailed p-value was computed as the proportion of cases displaying a  
244 greater value than the observed value for successful partners plus the proportion of cases  
245 displaying a lower value than the symmetrical (relative to the simulated mean) of the observed  
246 value. Results are presented using both one- and two-tailed p-values, and interpreted based  
247 on the two-tailed p-value, in order to be conservative, and consistent with other analyses  
248 presented in this study. Note that more than one individual could be chosen per mating season  
249 since mouse lemurs commonly give birth to mixed-paternity litters (Eberle & Kappeler, 2004b)  
250 and that some individuals appeared repeatedly in the dataset, which is inevitable in a system  
251 where both home ranges and generations are overlapping. We further tested whether  
252 individuals choose partners that have higher heterozygosity than randomly matched  
253 individuals following the same procedure.

254 We ran these simulation tests for datasets of adults and yearlings in both females and  
255 males (Table 1). We specifically compared patterns occurring in adults and yearlings to test  
256 whether the strength of mate selectivity may reflect variation in inbreeding risk across  
257 individuals belonging to different sex and age classes.

258

### 259 *3.6 Heterozygosity and survival*

260 To determine whether heterozygosity affected survival probabilities, we used a two-step  
261 approach (similar to Cohas *et al.*, 2009). We first modelled survival and recapture probabilities  
262 using capture-mark-recapture models (Lebreton *et al.*, 1992). We selected the most

263 parsimonious model out of a candidate set of models using AIC (Burnham & Anderson, 2002).  
264 In the second step, we added heterozygosity as an individual covariate to this basic model to  
265 test specific hypotheses regarding survival consequences of variation in heterozygosity using  
266 likelihood-ratio tests (LRT).

267

### 268 3.6.1 Capture-mark-recapture data

269 To model survival probabilities, we used CMR data from 1999 to 2011. We did not include  
270 data from before 1999 because too few animals from these cohorts were genotyped. As  
271 described in Kraus et al. (2008), we estimated seasonal survival using data from the main  
272 trapping season at the onset of the austral winter (April/May) and the secondary trapping  
273 session at the onset of summer (end of the dry season), before the mating season starts in  
274 October. We could not use the summer trapping season from 2004, since it was conducted  
275 too late. Hence, we created a dummy trapping season (“10 October”) and fixed its recapture  
276 probabilities at 0. The complete data set included 481 animals (294 males, 187 females) for  
277 which we have heterozygosity estimates and which were caught a total of 1031 times.

278

### 279 3.6.2 Modelling survival probabilities

280 We used the Cormack-Jolly-Seber model for open populations (CJS: Cormack, 1964, Jolly,  
281 1965, Seber, 1965) implemented in the program MARK (White & Burnham, 1999) to  
282 statistically model survival ( $\phi$ ) and recapture probabilities ( $p$ ). As for GLMMs, model selection  
283 was based on AIC or one of its appropriate variants (here QAICc which adjusts for small sample  
284 sizes and the presence of overdispersion Burnham & Anderson, 2002).

285 We first assessed the goodness-of-fit of global models using the median- $\hat{c}$  approach  
286 implemented in the program MARK. The variance inflation factor  $\hat{c}$  was estimated to be slightly  
287 above 1 ( $\hat{c}=1.09$ ), indicating a low level of extra-binomial variance. We still adjusted model  
288 selection statistics (QAIC<sub>c</sub>, QDeviance) accordingly. For the basic seasonal survival model we  
289 considered the factors sex ( $s$ ), age ( $a$ ) and time ( $t$ ). To evaluate state-determined effects of  
290 heterozygosity and to account for high mortality in the first year of life (Kraus *et al.*, 2008), age  
291 was represented by three classes: juveniles (juv, 3-9 months old, i.e., first winter), yearlings  
292 (yrl: 10-16 months old, i.e., first summer, first breeding season) and adults (ad: > 16 months  
293 old). Our candidate model set was partly based on *a priori* knowledge from an earlier study  
294 on seasonal survival of the same mouse lemur population which included the years 1995 to  
295 2005 (Kraus *et al.*, 2008). As our global model (GM), we used  $\phi^W(a*s+t) \phi^S(a*s+t) p^W(a*s+t)$   
296  $p^S(a*s+t)$  (W: winter, S: summer, \*: interactive effect, +: additive effect).

297 This analysis uses CMR data from 1999-2011 and hence only partially overlaps with the  
298 data set from the earlier study. Moreover, strong population fluctuations were observed  
299 between 2005 and 2011 (with, e.g., a mean of 23 individuals captured across capture sessions  
300 in 2005, and of 56.5 in 2008). Therefore we did not simply use the top model from that analysis  
301 for further inference, but included candidate models incorporating model terms that received  
302 some support in the confidence set of models established in that analysis (all models with a  
303 relative likelihood >0.05, Kraus *et al.*, 2008). All candidate models for winter survival included  
304 an age effect, because natal male dispersal in the Kirindy population takes place between April  
305 and September (Eberle & Kappeler, 2004b). With the CJS-model we cannot separate  
306 emigration and mortality, and hence estimates for juvenile males represent so-called  
307 “apparent survival” probabilities. We do know that female dispersal and/or secondary male  
308 dispersal are at most very rare events in this population (Eberle & Kappeler, 2004b) and thus,

309 we feel confident that estimates for these sex-age-classes closely estimate “true survival”  
310 probabilities. Hence, our candidate models for winter and summer recapture probabilities, as  
311 well as for summer survival included  $a*s+t$  (GM),  $a+s+t$ ,  $a+t$ ,  $s+t$  and  $t$ . For winter survival we  
312 used  $a*s+t$ ,  $a*s$ ,  $juv(s)ad(.)$  and  $a$ .

313 In order to limit the total number of models, we selected the most parsimonious model  
314 for each major model part (i.e., survival winter, survival summer, recapture summer,  
315 recapture winter) against the global model for the remaining model parts. We then built our  
316 basic survival and recapture model by combining the selected models for each part.

317

### 318 3.6.3 Effects of heterozygosity

319 To test for an association between heterozygosity levels and survival, we added our  
320 heterozygosity estimate (HL) as an individual covariate to the most parsimonious model for  
321 survival and recapture probabilities (the basic model). Because the basic model and those  
322 incorporating heterozygosity effects are nested, we compared these models using likelihood  
323 ratio tests (LRTs,  $\alpha=0.05$ ). Our LRTs aimed to address 3 specific hypotheses. (1) We tested for  
324 an overall effect of heterozygosity on mouse lemur survival. We excluded juvenile males from  
325 the heterozygosity effect, because for these we cannot distinguish between survival and  
326 emigration (see above). (2) Based on the idea that heterozygosity effects can be age-specific  
327 (Cohas *et al.*, 2009), we added the heterozygosity effect only for juvenile females (i.e., first  
328 winter survival). (3) To evaluate the hypothesis that heterozygosity effects are exacerbated  
329 under harsher conditions (Kempenaers, 2007), we added the heterozygosity effect only to  
330 summer survival, and, respectively, only to male summer survival, because survival was found  
331 to be substantially lower in summer and even more so in males, probably reflecting the costs

332 of intrasexual competition over reproduction (see also Kraus *et al.*, 2008). Because  
333 heterozygosity estimates were rather high, we always tested for a linear and for a quadratic  
334 effect. Linear effects were expected to show improved survival chances with increasing  
335 heterozygosity. Quadratic effects would represent an optimal heterozygosity level between  
336 inbreeding and outbreeding depression. None of the effects tested were statistically  
337 significant; therefore we did not correct  $p$  values for multiple testing.

338

## 339 4. RESULTS

### 340 4.1 Inbreeding risk

341 Inbreeding risk was moderate in males and females. First, our estimates of the number of  
342 candidate mates per female were high, ranging from 23 to 69 males (Table 1; mean=49).  
343 Similarly, the number of candidate mates per male ranged from 17 to 79 females (Table 1;  
344 mean = 51). For females, there were 21% of mating seasons (19 of 90) during which a father  
345 ( $n=13$ ), a son ( $n=4$ ) or both ( $n=2$ ) were present as potential mates. For males, there were 16%  
346 of mating seasons (19 of 116) for which first order relatives were present in the mating pool.  
347 In five cases, two first order relatives were present (four times two daughters and once the  
348 mother and a daughter). In the remaining 14 mating seasons, a mother ( $n=6$ ), a daughter ( $n=7$ )  
349 or a full-sister ( $n=1$ ) of the male was present. Within individuals, the presence of a 1<sup>st</sup> order  
350 relative in the mating pool was usually observed in one and maximum two mating seasons,  
351 with one exception: one female coexisted with her father for seven years.

352 Sex and age classes differed with respect to the occurrence and intensity of inbreeding  
353 risk, as the best models included a sex by age interaction for both the occurrence and the  
354 intensity models of inbreeding risk (Tables 2 & 3, Fig 1). For females, the occurrence and



355 intensity were both higher for yearlings than for adults, with more than a third of young  
356 females having at least one close kin in their mating pool. For males, the occurrence and  
357 intensity of inbreeding risk increased with age. While occurrence reached comparable levels  
358 for adult males and females, the average intensity of inbreeding risk was approximately twice  
359 as high for adult males as for adult females. However, intensity remained low in both sexes  
360 and at all ages, since first order relatives constituted only a tiny fraction of the candidate mate  
361 sets (mean $\pm$ SD, young females: 0.93 $\pm$ 1.39%, young males: 0.18 $\pm$ 0.62%, adult females:  
362 0.39 $\pm$ 0.99%, adult males: 0.70 $\pm$ 1.44%).

363

#### 364 *4.2 Inbreeding avoidance*

365 In the present 10-generation data set, no case of breeding between first order relatives could  
366 be detected. The closest proven case of reproduction between individuals with a known  
367 common ancestor in this population was a coupling of aunt and nephew (inbreeding  
368 coefficient ( $f$ )  $\approx$  0.125). For other true parents displaying a relatively high coefficient of  
369 relatedness (TrioML > 0.20), we were not able to detect any close family relationships through  
370 the pedigree data.

371 For adults of both sexes, average relatedness (mean TrioML) of true parents tended to  
372 be lower than simulated averages (Table 4, Fig. 2). The results for yearlings showed no  
373 significant deviation from random mate choice in both females and males (Table 4, Fig. 2). A  
374 shift towards lower values of relatedness to mates was observed in the random distribution  
375 for yearling males compared to both adult males and yearling females (Fig. 2), probably as a  
376 consequence of the change in their genetic environment following natal dispersal.

377 For both yearling females and adult males, the observed coefficient of variation of  
378 relatedness estimates for true parents was significantly lower than expected under random  
379 mating (Table 4, Fig 3).

380 Finally, there was no departure from random expectations concerning the mean  
381 heterozygosity of chosen mates.

382

### 383 4.3 Heterozygosity and survival

384 The most parsimonious survival model selected from the set of candidate models was the  
385 same as in the earlier study (covering the years 1995-2005), and parameter estimates were  
386 similar, suggesting that the survival patterns found are quite representative for this population  
387 (Table 1; Kraus *et al.*, 2008). There was little model selection uncertainty in choosing the most  
388 parsimonious model for recapture probabilities and summer survival: an additive effect of sex  
389 to temporal variation was strongly supported for each of these model parts ( $p^W$ :  $w_+(s+t)=0.93$ ,  
390  $p^S$ :  $w_+(s+t)=0.87$ ,  $\phi^S$ :  $w_+(s+t)=0.75$ ). Summer survival probabilities varied between 0.38 and  
391 0.84 among years with female survival exceeding male survival (geometric means  
392  $\phi^S_{\text{females}}=0.61\pm 0.09\text{SE}$ , 95%CI=[0.40; 0.77],  $\phi^S_{\text{males}}=0.55\pm 0.10\text{SE}$ , 95%CI=[0.35; 0.73], all  
393 probabilities are given on a semi-annual time-scale). In contrast, winter survival was rather  
394 constant over the years ( $w_+(\text{no } t)=0.99$ ), and higher than summer survival, with juveniles  
395 surviving less well than adults ( $\phi^W_{\text{ad males}}=\phi^W_{\text{ad females}}=0.88\pm 0.04\text{SE}$ , 95%CI=[0.77; 0.94],  $\phi^W_{\text{juv}}$   
396  $\text{females}=0.75\pm 0.08\text{SE}$ , 95%CI=[0.56; 0.87],  $\phi^W_{\text{juv males}}=0.52\pm 0.06\text{SE}$ , 95%CI=[0.41; 0.62]). We  
397 cannot currently estimate how much of the difference between juvenile male and female  
398 survival is due to male natal dispersal. Despite important variation in heterozygosity in our  
399 sample (n=525, range: 0.00-0.60, mean $\pm$ SD=0.18 $\pm$ 0.10), we did not find any statistical

400 evidence for a linear or quadratic effect of heterozygosity on overall (excluding juvenile  
401 males), juvenile female, summer, or male summer survival (Table 6).

402

## 403 5. DISCUSSION

404 We used a ten-generation dataset to investigate the extent of inbreeding risk in a solitary and  
405 polygynandrous mammal, the grey mouse lemur. The home range of one male typically  
406 encompasses the home ranges of several females (Eberle & Kappeler, 2002, Eberle &  
407 Kappeler, 2004b, Eberle & Kappeler, 2004a), a social system that is close to the ancestral  
408 mammalian state and remains widespread in extant mammals (Lukas & Clutton-Brock, 2013).

409 Inbreeding risk was generally low and primarily resulted from reproductive overlap  
410 between generations, where the reproductive lifespan of one sex exceeded the  
411 developmental period of the other sex. Its variation across ages and sexes was directly shaped  
412 by a combination of life-history traits including age at first breeding, reproductive longevity  
413 and sex-biased natal dispersal. Across age and sex categories, the relatively frequent  
414 occurrence of close kin in the mating pool was always diluted by the large size of the mating  
415 pool, with each individual having about 20 to 70 candidate mates. Female grey mouse lemurs  
416 reach sexual maturity at around 9 months and frequently have at least one first order relative  
417 among candidate mates during their first breeding season, usually their father and  
418 occasionally a brother. Inbreeding risk decreases as a function of female age, reflecting the  
419 progressive disappearance of females' fathers. It was lowest for young males, who have just  
420 dispersed into unfamiliar areas, and subsequently increased as a function of male age and  
421 reproductive success, reflecting the presence of one or more daughters in the surrounding  
422 area.

423 Male-biased dispersal therefore appears as the primary inbreeding avoidance  
424 mechanism in grey mouse lemurs. Whereas male-biased dispersal is the ancestral condition  
425 in mammals (Lukas & Clutton-Brock, 2011) and remains considerably more frequent than  
426 female-biased dispersal across extant species (Greenwood, 1980, Pusey, 1987, Clutton-Brock,  
427 1989, Clutton-Brock & Lukas, 2012), comparative analyses indicate that female dispersal has  
428 evolved in some group-living species where females start to breed while their father is still  
429 reproductively active in their natal group (Clutton-Brock, 1989, Lukas & Clutton-Brock, 2011).  
430 This suggests that sex-biased dispersal may have primarily evolved in response to inbreeding  
431 risk, rather than under the influence of other selective pressures like kin competition or the  
432 distribution of food resources. Under this scenario, it may appear unclear why males, rather  
433 than females, disperse in grey mouse lemurs. Unlike many solitary mammals where females  
434 are intolerant of other females, grey mouse lemur females forage solitarily but rest and breed  
435 communally with female kin (Radespiel et al., 2001, Eberle & Kappeler, 2002, Eberle &  
436 Kappeler, 2006) so they may derive direct benefits from social philopatry, like many group-  
437 living mammals (Clutton-Brock & Lukas, 2012). In such conditions, females may only disperse  
438 when unrelated mates are unavailable, for example if their father holds the dominant  
439 breeding status and monopolizes most reproduction in their group by the time they reach  
440 sexual maturity, as in chimpanzees or gorillas (Clutton-Brock & Lukas, 2012). In contrast, male  
441 grey mouse lemurs cannot monopolize females and sperm competition prevails over contest  
442 competition (Eberle & Kappeler, 2004a, Eberle & Kappeler, 2004b, Huchard et al., 2012). In  
443 addition, our results indicate that young females have access to a large pool of unrelated  
444 candidate mates on top of their relative(s), explaining why the intensity of selective pressures  
445 favouring female dispersal may remain moderate.

446           Individuals that face residual inbreeding risk despite natal male-biased dispersal may  
447 have developed additional inbreeding avoidance strategies, like secondary dispersal or  
448 avoidance of mating with kin. Secondary dispersal may occasionally occur when male grey  
449 mouse lemurs have many closely related females in their vicinity (Radespiel *et al.*, 2003),  
450 though it appears rare as no incidents have ever been recorded in this 10-year study  
451 population (unpublished observation). Mating biases appear more common, and are  
452 detectable in young females and adult males, who select mates with a narrower relatedness  
453 range than random partners. Adult females and males also show a marginally non-significant  
454 trend for mating with partners who are less related than random partners. Regarding the  
455 coefficient of variation of mate relatedness, significant results in young females and adult  
456 males may reflect the fact that these two age-sex categories face, respectively, a higher  
457 prevalence and intensity of inbreeding risk than other age-sex categories, and greater  
458 variation in the relatedness coefficients of candidate mates may confer more power to the  
459 analyses. However, it is important to realize that our analyses cannot identify the choosing  
460 sex: if one sex chooses partners with a low relatedness, or with a narrower range of  
461 relatedness, this preference will influence the results of the randomization analyses for both  
462 sexes. As a result, these mating biases may reflect the avoidance of daughter-father matings,  
463 the kin relationship that is most represented in individual mating pools, due to active  
464 discrimination by either young females or adult males. In addition, failure to detect significant  
465 mating biases for partners with low relatedness may also reflect methodological issues, and  
466 specifically the fact that mating patterns are inferred from patterns of parentage. It is possible  
467 that inbreeding depression may be more severe on early life traits (Stockley *et al.*, 1993,  
468 Markert *et al.*, 2004, Cohas *et al.*, 2009, Nielsen *et al.*, 2012) and compromise the survival of

469 inbred juveniles, resulting in their non-detectability by our sampling design, which only traps  
470 and marks recruited individuals.

471           Mate choice for partners with intermediate relatedness suggests that young females  
472 and adult males may optimize, rather than maximize genetic dissimilarity to their partners by  
473 avoiding partners that are too closely or too distantly related. Such result may reflect the  
474 spatial genetic structure of the population if young females and adult males (1) tend to avoid  
475 mating with closely related partners, and (2) have lower chances to mate with distantly related  
476 partners (compared to partners with intermediate relatedness) because mating probability  
477 and relatedness between candidate mates both decrease as spatial distance between them  
478 increases, under a scenario of isolation by distance. In the first case, variance may be more  
479 sensitive than mean to a scenario of avoidance of mating with close kin. Under such scenario,  
480 mate choice will only erase the most extreme points of the distribution of relatedness among  
481 actual partners compared to random partners, which may impact the variance of this  
482 distribution more than its mean. Consequently, future studies should integrate variance-  
483 based analyses in their design more systematically. In the second case, although assignment  
484 of candidate mates partially took into account their spatial proximity by including only  
485 individuals that were less distant than the maximal distance recorded between actual parents  
486 in our dataset, our analyses did not control for the residual effect of variable spatial distance  
487 within this range. Alternatively, mates with intermediate relatedness may balance the costs  
488 of inbreeding and the benefits of increasing the representation of genes identical by descent  
489 in future generations (Parker, 1979, Puurtinen, 2011, Szulkin et al., 2013). Theory predicts that  
490 levels of inbreeding that maximize inclusive fitness are low and compatible with a wide range  
491 of realistic inbreeding depression strengths, as well as with mate choice for intermediately  
492 related individuals (Puurtinen, 2011), as observed in a number of vertebrates (Pusey & Wolf,

493 1996, Reusch et al., 2001, Mays et al., 2008, Szulkin et al., 2013). Mating strategies observed  
494 in our population are therefore in agreement with theoretical expectations in large outbred  
495 populations. Although preferences for intermediate relatedness have not been previously  
496 reported in wild primates, they may be under-detected as studies often test for differences in  
497 mean relatedness between actual and random mates, without testing for differences in  
498 relatedness variance (Szulkin *et al.*, 2013).

499         Selecting mates based on their relatedness coefficients requires efficient kin  
500 discrimination mechanisms. While familiarity may mediate kin recognition among maternal  
501 kin, it is less clear how fathers can avoid mating with their daughters in species where both  
502 sexes mate with multiple partners and where paternity certainty is therefore low (Widdig,  
503 2007) as in grey mouse lemurs (Eberle & Kappeler, 2004b, Eberle & Kappeler, 2004a). Previous  
504 work in this population shows that individuals choose MHC-dissimilar partners (Schwensow  
505 et al., 2008, Huchard et al., 2013) and suggests that kin discrimination may rely on odour cues  
506 influenced by MHC genes in this nocturnal species characterized by an acute sense of smell  
507 (Schilling, 1979, Schilling & Perret, 1987). Additionally, a recent experiment shows that  
508 females can detect relatedness in vocalizations of unfamiliar individuals, suggesting that  
509 acoustic cues may also play an important role in kin discrimination (Kessler *et al.*, 2012).

510         Beside active mate discrimination, mate selection may also occur post-copulation via  
511 cryptic female choice. Whereas males show an impressive enlargement of testes size and  
512 roam extensively in search of mating opportunities during the breeding season (Eberle &  
513 Kappeler, 2002, Eberle et al., 2007), females actively seek multiple mates during their short  
514 period of sexual receptivity, suggesting that they benefit from such a strategy (Eberle &  
515 Kappeler, 2004a, Huchard et al., 2012). Experimental studies in both invertebrates and  
516 vertebrates, including mammals, have shown that mating with multiple males may represent

517 an efficient strategy to avoid inbreeding (Tregenza & Wedell, 2002, Simmons et al., 2006, Zeh  
518 & Zeh, 2006, Firman & Simmons, 2008), thereby offering a potential mechanism for the mating  
519 biases reported in grey mouse lemurs.

520 No mating biases, or even trends, were detected in young males. Several possibilities  
521 may explain this age effect. Young males have just left their natal area (Schliehe-Diecks *et al.*,  
522 2012) and as a result appear to have no close relatives in their mating pool. The variance of  
523 relatedness to their potential mates may consequently be too weak to detect a signal of  
524 inbreeding avoidance or they may not need to be discriminative. Moreover, young males  
525 struggle to access mates when competing with older and heavier males (Eberle & Kappeler,  
526 2004b) and the costs of inbreeding avoidance may exceed the costs of inbreeding for them.  
527 Overall, these results indicate that several inbreeding strategies co-occur within a single  
528 population and may reflect individual variation in the relative benefits and costs of inbreeding  
529 and inbreeding avoidance (Szulkin *et al.*, 2013).

530 Finally, we could not detect a positive effect of heterozygosity on mating success and  
531 on survival probability, despite a reasonable individual variance in heterozygosity and, in the  
532 case of survival analyses, irrespective of whether we considered state-dependant (age) or  
533 environmental influences (season) which are supposed to alter the magnitude of genome-  
534 wide heterozygosity effects on fitness (Balloux et al., 2004, Brouwer et al., 2007, Kempenaers,  
535 2007, Cohas et al., 2009). This lack of effect may first reflect methodological caveats, such as  
536 the use of indices of heterozygosity based on a limited number of microsatellites which may  
537 poorly reflect overall genome-wide diversity (Chapman et al., 2009, Szulkin et al., 2010, but  
538 see Forstmeier et al., 2012). However, the incomplete nature of our pedigree precluded the  
539 use of pedigree-based measures. Second, inbreeding depression may be particularly acute in  
540 early life (Stockley et al., 1993, Markert et al., 2004, Cohas et al., 2009, Nielsen et al., 2012)



541 and it is possible that inbred individuals may often die before being captured and marked, or  
542 that inbreeding depression may only be detectable on developmental traits which were not  
543 examined here. Finally, even if the genetic load responsible for inbreeding depression may be  
544 substantial in the population, our results may simply reflect the scarcity of inbred individuals  
545 in the population. The high genetic diversity of our study population indicates that it is of  
546 sufficient size and density to ensure healthy pools of largely unrelated candidate mates and  
547 fully operational inbreeding avoidance strategies. We also did not find any support for an  
548 optimal heterozygosity level balancing potential costs of inbreeding and outbreeding  
549 depression, but recent models suggest that levels of inbreeding selected under this scenario  
550 are low (Puurtinen, 2011), which may explain why they were undetectable.

551

## 552 6. CONCLUSION

553 We simultaneously investigated the occurrence and intensity of inbreeding risk (via  
554 parentage and pedigree analyses), of inbreeding avoidance (via mating biases), and of  
555 inbreeding depression (via the survival costs of heterozygosity) in a large natural population  
556 of grey mouse lemurs. Grey mouse lemurs have retained a number of ancestral mammalian  
557 traits including a solitary lifestyle, a promiscuous mating system and male-biased dispersal  
558 (Lukas & Clutton-Brock, 2011, Lukas & Clutton-Brock, 2013) and may therefore offer a classic  
559 example of the inbreeding strategy prevailing in large populations of solitary mammals.  
560 Inbreeding risk was low, and its variation across ages and sexes suggests that male-biased  
561 dispersal is a primary inbreeding avoidance mechanism at the population level. Mating biases  
562 favouring partners with intermediate relatedness were detectable in yearling females and in  
563 adult males, the two age-classes that face the highest prevalence and intensity of inbreeding  
564 risk, respectively, suggesting that mate choice may represent a facultative secondary strategy

565 of inbreeding avoidance that mitigates residual inbreeding risk at the individual level. The  
566 effect of genome-wide heterozygosity on survival was undetectable using a ten-generation  
567 survival analysis, suggesting that inbreeding avoidance strategies were efficient in this large,  
568 open and genetically-diverse population.

569

570

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786 DATA ACCESSIBILITY

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788 Data will be made available in DRYAD upon acceptance.

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792 AUTHOR CONTRIBUTIONS

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794 EH, SSD, PMK and CK designed the study, SSD performed the labwork, SSD, EH and CK analysed  
795 the data, EH, SSD, PMK and CK drafted the manuscript.

796 Table 1. Composition of datasets for mating bias permutation tests. The number of mating seasons lists the number of individual-seasons: 42 adult females have  
 797 been present in the dataset for one to six years each, resulting in a total of 67 mating seasons (there is one mating season per year). The number of choice events  
 798 differs from the number of mating seasons because both females and males may sire offspring with multiple partners in any given season (most females only  
 799 have one litter per year but mixed-paternity litters are common (Eberle & Kappeler, 2004a, Eberle & Kappeler, 2004b)). The number of choice events therefore  
 800 corresponds to the number of offspring produced. The number of chosen individuals is the total number of opposite-sex partners that have produced offspring:  
 801 adult females have produced a total of 91 offspring, and 59 individual males have produced at least one offspring (and a maximum of 7).

Sex	Age class	No. of mating seasons [no. of individuals]	No. of choice events [no. of chosen individuals]	Mean no. of candidate mates per individual during one mating season [range]
Female	Adult	67 [42]	91 [59]	50 [23-69]
	Yearling	23 [23]	30 [25]	47 [23-69]
Male	Adult	69 [44]	109 [55]	69 [17-79]
	Yearling	47 [47]	61 [45]	50 [25-79]

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804 Table 2. Model selection statistics for age and sex effects on the occurrence and intensity of inbreeding risk. Model notation: I for the intercept, +  
 805 for an additive effect, \* for an interaction. The degrees of freedom (df), the loglikelihood (LogLik), Akaike's Information Criterion (AICc), the  
 806 difference between the AIC of the top model and the model considered ( $\Delta_i$ ) and Akaike weights are given for each model.

	df	LogLik	AICc	Delta AIC	Weight
Inbreeding risk - Prevalence					
I (Intercept)	3	-94.73	195.6	5.30	0.06
I + age	4	-94.68	197.6	7.28	0.02
I + sex	4	-94.37	196.9	6.67	0.03
I + age + sex	5	-94.35	199.0	8.72	0.01
I + age + sex + age:sex	6	-88.93	190.3	0.00	0.87
Inbreeding risk - Intensity					
I	3	-114.61	235.3	2.49	0.19
I + age	4	-114.53	237.3	4.42	0.07
I + sex	4	-114.59	237.4	4.54	0.07
I + age + sex	5	-114.52	239.3	6.50	0.03
I + age + sex + age:sex	6	-110.21	232.8	0.00	0.65

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809 Table 3. Estimates and standard error (SE) of the Generalized Linear Mixed Models (GLMMs) investigating age and sex effects on the occurrence  
 810 and intensity of inbreeding risk. Occurrence was scored as the presence/absence of at least one first order relative in the pool of candidate mates,  
 811 while intensity was scored as the proportion of first order relatives in the pool of candidate mates. Random factors included individual identity  
 812 crossed with year. The 95% confidence intervals were computed for the two top models – see Table 2.

Fixed effect	Estimate	SE
Inbreeding risk - Occurrence		
Intercept	-2.61	0.76
Age class (adult vs. yearling) <sup>1</sup>	1.83	0.87
Sex <sup>2</sup>	0.75	0.66
Age class: Sex	-3.55	1.26
Inbreeding risk - Intensity		
Intercept	6.36	0.50
Age class (adult vs. yearling) <sup>1</sup>	-0.72	0.49
Sex <sup>2</sup>	-1.13	0.59
Age class: Sex	2.34	0.83

813 <sup>1</sup>Reference category: adult

814 <sup>2</sup>Reference category: female.

815 Table 4. Summary table of the randomization tests of mating biases.

Age-sex class	Observed value	Simulated value [95%CI]	One-sided p-value	Two-sided p-value	Minimum deviation (%)*
<b>Choice for partners with low relatedness: results on mean relatedness</b>					
Adult females	0.053	0.071 [0.052-0.089]	0.029	0.071	26.39
Yearling females	0.079	0.076 [0.039-0.112]	0.605	0.840	48.16
Adult males	0.059	0.076 [0.057-0.095]	0.039	0.089	24.89
Yearling males	0.063	0.060 [0.041-0.079]	0.646	0.730	30.92
<b>Choice for partners with intermediate relatedness: results on the CV of relatedness</b>					
Adult females	1.179	1.305 [1.017-1.605]	0.202	0.395	22.21
Yearling females	0.827	1.349 [0.863-1.833]	0.004	0.033	35.95
Adult males	0.987	1.380 [1.110-1.650]	<0.001	0.004	19.57
Yearling males	1.275	1.260 [0.966-1.555]	0.576	0.926	23.51
<b>Choice for partners with high heterozygosity: results on mean heterozygosity</b>					
Adult females	0.197	0.182 [0.161-0.202]	0.070	0.137	11.39
Yearling females	0.191	0.181 [0.146-0.216]	0.292	0.585	19.59
Adult males	0.179	0.169 [0.148-0.190]	0.162	0.318	12.16
Yearling males	0.191	0.170 [0.142-0.198]	0.075	0.147	16.5

816 \*Threshold deviation between true and random pairs required for reaching statistical significance for each parameter examined given the power of our analyses.

817 We followed procedures described in Huchard et al. (2010), obtaining percentage differences by dividing the 95% CI threshold by the mean of the simulated  
 818 distribution.

Table 5. Model selection statistics for recapture probabilities in (a) winter ( $p^w$ ) and (b) summer ( $p^s$ ), and for apparent survival probabilities in (c) winter ( $\phi^w$ ) and (d) summer ( $\phi^s$ ) nested in the most general model for the remaining components ( $a*s+t$ ). Factors considered are age ( $a$ ; juv: juveniles, ad: adults), sex ( $s$ ) and year ( $t$ ). Model notation: (.) constant, \* interaction, + additive effect (parallel lines on a logit-scale). The number of estimable parameters ( $K$ ), The Quasi-likelihood adjusted deviance (QDEV), Akaike's Information Criterion (QAICc), the difference between the minimum QAICc of the top model and the model considered ( $\Delta_i$ ) and Akaike weights ( $w_i$ ) are given for each model.

Rank	Model $i$	$K$	QDEV	QAIC <sub>c</sub>	$\Delta_i$	$w_i$
<i>(a) Recapture probabilities in winter <math>p^w</math> depend on:</i>						
1	$s+t$	58	1743.22	1866.45	0	0.65
2	$a+s+t$	59	1743.21	1868.70	2.25	0.21
3	$a*s+t$	60	1743.19	1870.94	4.49	0.07
4	$a+t$	58	1748.51	1871.75	5.30	0.05
5	$t$	57	1752.36	1873.34	6.89	0.02
<i>(b) Recapture probabilities in summer <math>p^s</math> depend on:</i>						
1	$s+t$	58	1745.10	1868.33	0	0.43
2	$a+s+t$	59	1743.36	1868.86	0.53	0.33
3	$a*s+t$	60	1743.19	1870.94	2.61	0.12
4	$t$	57	1751.01	1872.00	3.67	0.07
5	$a+t$	58	1749.43	1872.66	4.33	0.05
<i>(c) Survival probabilities in winter <math>\phi^w</math> depend on:</i>						
1	juv(s)ad(.)	48	1762.84	1863.76	0	0.39
2	$a+s$	48	1763.20	1864.12	0.36	0.33
3	$a$	47	1767.17	1865.89	2.13	0.14
4	$a*s$	49	1762.77	1865.92	2.16	0.13
5	$a*s+t$	60	1743.19	1870.94	7.18	0.01
<i>(d) Survival probabilities in summer <math>\phi^s</math> depend on:</i>						
1	$s+t$	58	1743.45	1866.68	0	0.50
2	$t$	57	1747.67	1868.65	1.97	0.19
3	$a+s+t$	59	1743.19	1868.68	2.00	0.19
4	$a+t$	58	1747.60	1870.84	4.16	0.06
5	$a*s+t$	60	1743.19	1870.94	4.26	0.06

Table 6. Likelihood ratio tests (adjusted for  $\hat{c} = 1.09$ ) of heterozygosity (HL) effects on survival. Models incorporating the heterozygosity effect (general models) were tested against the reduced basic model ( $\phi^W(\text{juv}(s)\text{ad}(\cdot)) \phi^S(s+t) p^W(s+t) p^S(s+t)$ ). Shown are the effects tested, and the survival model term added in the general survival model as well as the test statistic ( $\chi^2$ ), degrees of freedom (*d.f.*) and the *p* value of the LRT.

Hypothesis tested	general survival model term	$\chi^2$	<i>d.f.</i>	<i>p</i>
linear overall* effect of HL on survival	$\phi^{all}(\text{HL})$	1.05	1	0.30
quadratic overall* effect of HL on survival	$\phi^{all}(\text{HL}+\text{HL}^2)$	1.62	2	0.45
linear effect of HL on juvenile female survival	$\phi^{W, \text{juvF}}(\text{HL})$	0.51	1	0.48
quadratic effect of HL on juvenile female survival	$\phi^{W, \text{juvF}}(\text{HL}+\text{HL}^2)$	2.06	2	0.36
linear effect of HL on summer survival	$\phi^S(\text{HL})$	1.26	1	0.26
quadratic effect of HL on summer survival	$\phi^S(\text{HL}+\text{HL}^2)$	1.90	2	0.39
linear effect of HL on male summer survival	$\phi^{S, M}(\text{HL})$	1.17	1	0.28
quadratic effect of HL on male summer survival	$\phi^{S, M}(\text{HL}+\text{HL}^2)$	1.17	2	0.56

Figure 1. Sex differences in inbreeding risk. (a) Occurrence of inbreeding risk, scored as the percentage of mating seasons where a first order relative was present for yearlings and for adults. Females are depicted in black (n=90) and males in grey (n=116). (b) Intensity of inbreeding risk, scored as the average proportion of first order relatives in the mating pool of yearlings and adults. Females are depicted in black (n=90) and males in grey (n=116). Dashed error bars indicate standard deviations.



## Supporting information

### The inbreeding strategy of a solitary primate, *Microcebus murinus*

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## Appendix S1: DNA amplification and sequencing.

Twelve polymorphic microsatellites with an average number of 22 alleles ((Table S1) were used for analyses: Mm06, MmF3, Pvc 9.2, Pvc a1 (Wimmer *et al.*, 2002) as well as 33104, Mm22, Mm39, Mm40, Mm42, Mm43b, Mm51, Mm60 (Hapke *et al.*, 2003). PCR reactions had a total volume of 30µl and contained 1U Biotherm TM Taq DNA Polymerase, 3µl 10x Reaction Buffer provided by Genecraft, 4.8 mM (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>, 20.1 mM Tris-HCl (pH 8.8), 5µmM MgCl<sub>2</sub>, 0.003% Tween 20, 0.3 pmol of each primer, 0.16 mmol dNTPs, 1.3 mg/ml bovine serum albumin, 0.06 mg Triton and about 10 ng of template DNA. The PCR program used for each primer pair is described in Table S2. PCR products were processed via capillary electrophoresis (ABI 3730 XL) and subsequently analyzed in ABI GeneMapper v4.0TM.

Table S1: Overview over the microsatellite primers, their length, and the name of the PCR program used for each primer pair.

Primer name	Number of alleles	Length [bp]	PCR program
Mm51	15	98-120	Std 58
Mm42	43	123-201	Std 58
Mm43b	21	136-172	Std 58
Mm39	38	155-221	Std 58
Mm22	18	204-240	Std 58
33104	24	257-297	Std 58
Mm40	15	145-167	Std 58
Mm60	19	84-126	Std 54
Pvc a1	13	148-174	Mmu F3
Mm06	24	129-173	Mmu F3
MmF3	20	171-230	Mmu F3
Pvc 9.2	14	141-160	Pvc 9.2



Table S2: PCR programs used for the different primer pairs.

<b>Std 58</b>		<b>Std 54</b>		<b>MmuF3</b>		<b>Pvc 9.2</b>	
1 x	2 min 92°	1 x	2 min 92°	1 x	3 min 94°	1 x	3 min 94°
35 x	40 sec 92°	35 x	40 sec 92°	35 x	1 min 94°	35 x	1 min 94°
	1 min 58°		1 min 54°		1 min 48°		1 min 46°
	1 min 72°		1 min 72°		1 min 72°		1 min 72°
1 x	10 min 72°	1 x	10 min 72°	1 x	10 min 72°	1 x	10 min 72°

## **Appendix S2: Genetic determination of parentage**

We ran the parentage analysis including all sampled females and males present in the population at the time of conception of a given offspring and who were sexually mature, so that each was considered as a potential mother and father, respectively, for each offspring.

Likelihood-based paternity analysis was carried out using two different software packages: Cervus 3.0 (Kalinowski et al. 2007) and COLONY (Wang 2004). A candidate parent was considered assigned when it was identified as the most likely parent by both analyses. Cervus calculates paternity likelihood ratios and generates a statistic,  $\Delta$ , defined as the difference in the positive log likelihood ratios between the two most probable candidate parents. The statistical significance of  $\Delta$  was determined at a confidence level of 95%. The likelihood analysis in CERVUS 3 was based on simulations to estimate the resolving power of all loci and critical values necessary to assess the reliability of the parentage analysis (100,000 runs, 94 candidate parents, assumptions: sampling rate=0.95; average loci typing rate=0.85; error rate=0.05; one close relative of the true parent among the other candidate parents, combined non-exclusion probability (first parent) =  $3.7 \times 10^{-6}$ ). Sampling rate was based on behavioural observations in the study population (Eberle & Kappeler, 2004a, 2004b). In order to assess locus-specific genotyping error rates, we duplicated ca. 10% of our sample using independent amplification and sequencing (n=95 individuals). These gave a mean genotyping error rate across loci (combining allelic drop-out with other errors) of 5 %.

COLONY implements a maximum-likelihood method that assigns offspring into full-sib families nested within half-sib families and assigns candidate parents to the sib families, using the offspring and candidate parent genotypes. COLONY calculates family likelihood and searches for the best sibship configuration (with the maximum likelihood) through an iterative process, using a simulated annealing algorithm. Both software packages account for

incomplete sampling and typing errors, and COLONY further infers parental genotypes for reconstructed sibships and detects mutations and typing errors at each locus. Locus-specific error rates calculated using repeated independent amplification and typing of the same individual for a given locus (see above) were used in the analysis. Where necessary, the same specifications were used in the COLONY analysis (e.g. the proportions of mothers and fathers sampled).

Table S3. Composition of datasets for mating bias permutation tests. The number of mating seasons lists the number of individual-seasons: 42 adult females have been present in the dataset for one to six years each, resulting in a total of 67 mating seasons (there is one mating season per year). The number of choice events differs from the number of mating seasons because both females and males may sire offspring with multiple partners in any given season (most females only have one litter per year but mixed-paternity litters are common (Eberle & Kappeler, 2004a, Eberle & Kappeler, 2004b)). The number of choice events therefore corresponds to the number of offspring produced. The number of chosen individuals is the total number of opposite-sex partners that have produced offspring: adult females have produced a total of 91 offspring, and 59 individual males have produced at least one offspring (and a maximum of 7).

Sex	Age class	No. of mating seasons [no. of individuals]	No. of choice events [no. of chosen individuals]	Mean no. of candidate mates per individual during one mating season [range]
Female	Adult	67 [42]	91 [59]	50 [23-69]
	Yearling	23 [23]	30 [25]	47 [23-69]
Male	Adult	69 [44]	109 [55]	69 [17-79]
	Yearling	47 [47]	61 [45]	50 [25-79]

Figure S1. Mean relatedness between true parents compared to the distribution of the simulated mean relatedness between randomly matched parents. Observed values are indicated by the black dotted lines. The scale of axes varies across datasets.

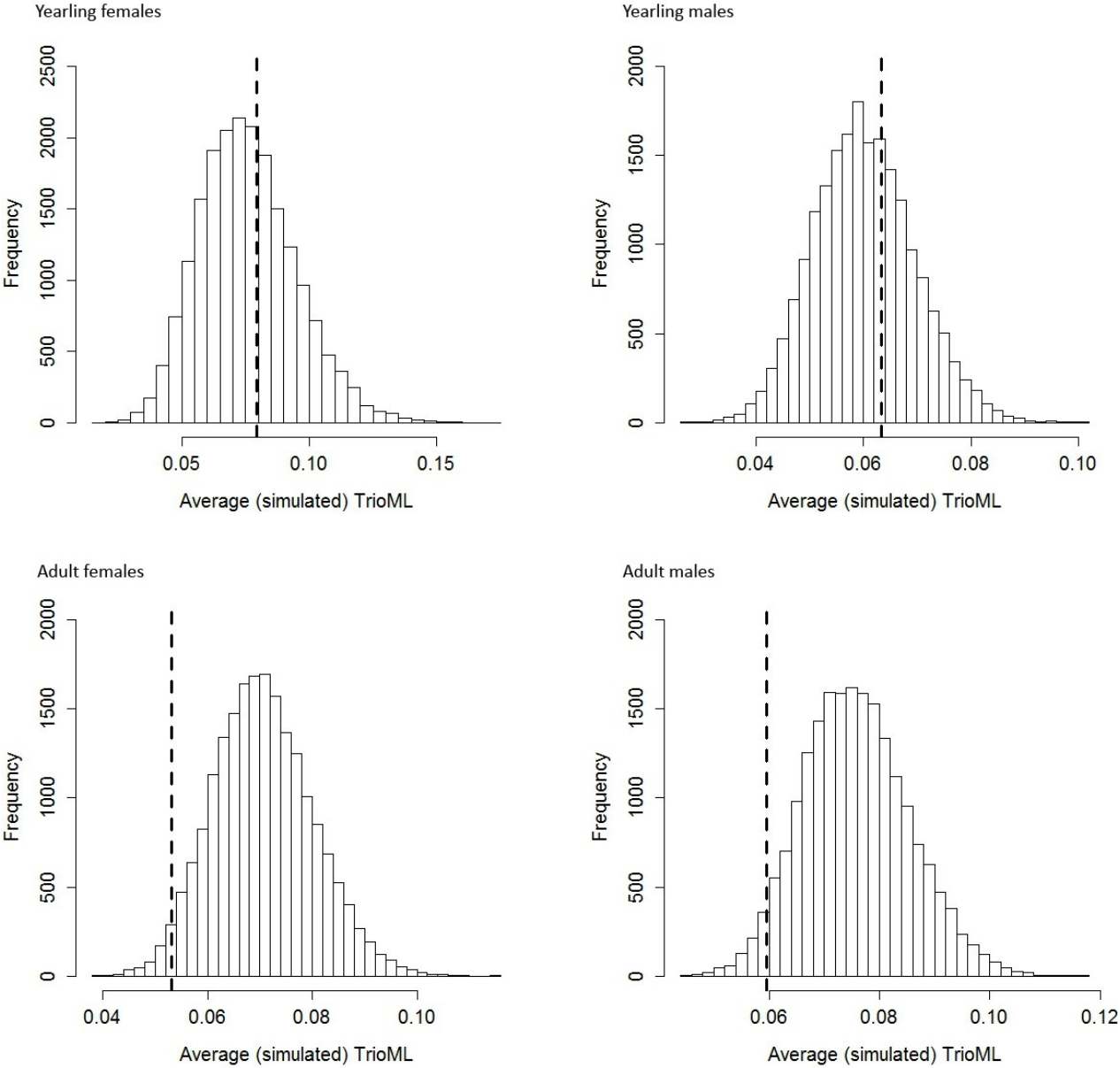
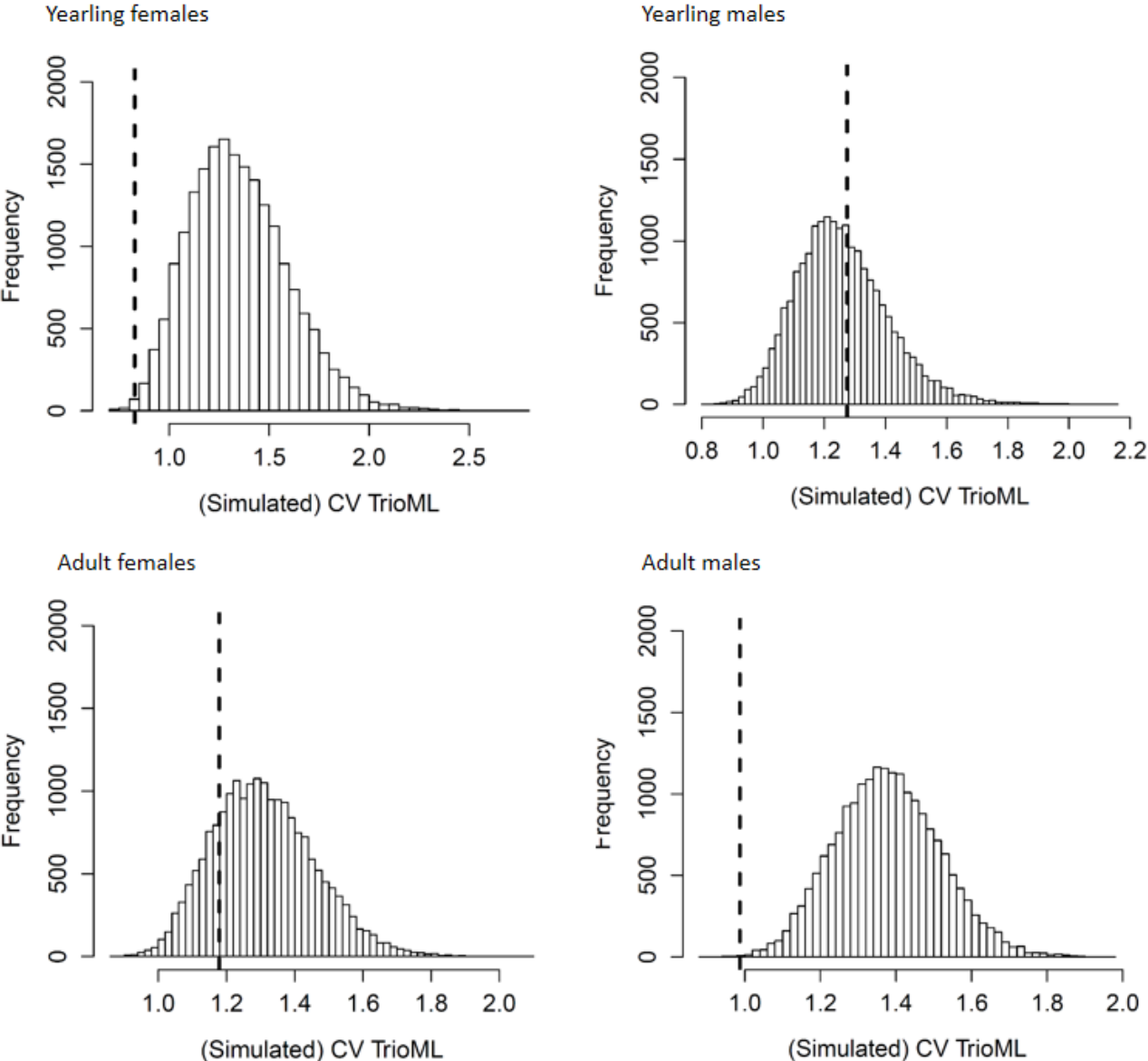


Figure S2. Coefficient of variation in relatedness between true parents compared to the distribution of the simulated coefficient of variation in relatedness between randomly matched parents. Observed values are indicated by the black dotted lines. The scale of axes varies across datasets.



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