1   Promiscuous mating in the endangered Australian lizard *Tiliqua adelaidensis*: a potential

2   windfall for its conservation.

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4   Julie A. Schofield[1], Michael G. Gardner[1,2] Aaron L. Fenner[1], and C. Michael Bull[1]

5   [1]School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001,

6   Australia.

7   [2]Evolutionary Biology Unit, South Australian Museum, North Terrace Adelaide 5000 South

8   Australia.

9   Corresponding author: Julie.Schofield@flinders.edu.au

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13   Abstract

14   Studies have revealed an unsuspected complexity in social systems within a few lizard

15   species, including group living, long-term monogamy and individual recognition of partners

16   or offspring. Comparisons among these species and their relatives could provide valuable

17   insights, allowing us to investigate traits that are shared across social systems and identify

18   general principles relating to the evolution of sociality. The endangered pygmy bluetongue

19   lizard, *Tiliqua adelaidensis,* is a member species in the *Egernia* group, but is thought to show

20   a more solitary social structure than other members in this group. Within this study we used

21   microsatellite markers to determine the mating system of *T. adelaidensis*. Unlike many other

22   species in the *Egernia* group, we found a predominately promiscuous mating system in *T.

23   adelaidensis.* We detected multiple paternity in 75% of litters. Of the 70 males identified as

24   having fathered juveniles, only five were identified as mating with the same female in more

25   than one year and only three were identified as the father of juveniles with the same female in

26   consecutive years. The genetic evidence suggested that partners were chosen randomly with

27   respect to the level of relatedness among neighbouring lizards. However, mated lizards were

28   geographically closer to each other than expected by random chance. Multiple paternities rely

29   on the opportunity for males to encounter multiple females during the period when they are

30   receptive to mating, and this may depend on population densities. Drivers for the polygamous

31   mating system may be the single occupancy burrow and the central place territorial defence

32   of those burrows in *T. adelaidensis*. We propose a fourth mating system for the *Egernia*

33   group: polygyny within stable non-social colonies.

34

35   Key Words

36   Multiple paternity, polygamy, lizard sociality

37

Introduction:

Species within many taxonomic groups display a range of social behaviours from those that live in highly social groups, often providing some level of parental care to their young, to species where individuals are normally isolated, contacting only for reproduction, and providing little or no parental care. The mating system of a species is defined by the number and frequency of mating partners, and is often linked to the form of social organization. The mating system can also influence mating success, gene flow within and between populations, and the ability to recolonize newly available habitat and avoid inbreeding (Greenwood 1980). Many species have evolved social mechanisms and mating systems in large, continuous habitats, but now occupy isolated fragments of habitat, with consequential impacts on their dispersal and mating systems (Stow et al. 2001, Levy et al. 2010). Knowledge of the mating system and how it has changed with restricted dispersal is an important component in the sustained management of these species.

How have different mating systems and levels of sociality evolved among related species? Studies of variation among the members of a single clade can provide insights into the relevant selective factors (Oliver and Sachser 2011). Over two decades of field studies have revealed that one Australian lizard clade, the *Egernia* group of skinks, includes species with a variety of social systems (Bull 2000, Gardner et al. 2002, O'Connor and Shine 2003, Stow and Sunnucks 2004; Chapple 2003). The *Egernia* group is a monophyletic lineage that includes six primarily Australian genera *Egernia*, *Liopholis*, *Bellatorias*, *Lissolepis*, *Tiliqua*, and *Cyclodomorphus*, and one Melanesian genus *Corucia* (Gardner et al. 2008).

A large proportion of species in the *Egernia* group live in mixed sex social aggregations, often comprising related individuals, with shared refuges or home sites (Chapple 2003). Monogamy appears to be the most common mating strategy in the social, sedentary species, such as those that live in social groups on isolated rocky outcrops (e.g. *Egernia stokesii*

63 (Gardner et al 2002), *E. saxatilis* (O'Connor and Shine 2003), *E. cunninghami* (Stow et al

64 2004), and *Liopholis whitii* (Chapple and Keogh 2005), those that have large multi

65 generational groups co-occurring in extended burrow systems (e.g. *Liopholis kintorei*

66 (McAlpin et al.2011)), or those that have limited movement (e.g. *Tiliqua rugosa* (Bull 2000)).

67 Uller and Olsson (2008) predicted that females of species with low population densities

68 during the reproductive season and of species with strong pair-bonding, should have fewer

69 encounters with alternative mating partners during the female receptive phase, and thus have

70 lower levels of mutiple paternity. In several well studied social species in the *Egernia* group,

71 monogamous mate fidelity is high among years, and multiple paternity is low within years

72 (Chapple 2003, Uller and Olsson 2008).

73 The genus *Tiliqua*, embedded within the *Egernia* clade, does not appear to share the high

74 levels of social grouping of its sister taxa. Field work on *T. rugosa* has shown monogamous

75 parings during breeding seasons that can persist for over 20 yrs (Bull 2000), but no evidence

76 of more extended kin group associations (Bull and Baghurst 1998). The more mobile *T.*

77 *scincoides* appears to be primarily asocial, with males occupying individual territories and

78 mating polygamously with overlapping females (Cogger 2000, Koenig et al. 2001).

79 The pygmy bluetongue lizard (*Tiliqua adelaidensis*) is a cryptic species found in native

80 grasslands in the geographical region referred to as the mid north of the state of South

81 Australia. Individual lizards live up to 9 yrs (Milne 1999) and spend the majority of their time

82 alone, refuging in abandoned spider burrows, or basking, and at the burrow entrance from

83 where they ambush passing invertebrate prey (Hutchinson et al. 1994, Milne et al. 2003).

84 Each burrow is occupied by one individual and in both males and females their range of

85 normal activity extends no more than 5cm from their burrow entrance (Fenner and Bull

86 2011). Mating occurs in the spring months in October and November (Milne 1999, Milne et

87 al. 2003, Fenner and Bull 2009). Adult males move away from their burrows, seeking

88   females to mate with during this period (Schofield et al 2012). Video recorded matings have

89   been brief encounters between a burrow resident and another lizard moving up to the burrow,

90   apparently in search of a mate (Milne et al. 2003, Fenner and Bull 2009, Ebrahimi

91   pers.comm.). Females produce one litter of up to four live young per year and can breed in

92   consecutive years (Milne et al. 2002). Some neonates begin dispersing from the natal burrow

93   within a week after birth and by 5 weeks most of the juveniles have left the natal burrow,

94   leading to early separation of the mother and her offspring (Milne et al. 2002). Alternatively,

95   some females move to a new burrow soon after the birth, leaving the juvenile to inhabit the

96   natal burrow (Milne et al. 2002). Apart from the brief contacts during mating, and the short

97   shared occupancy of natal burrows, there are no records of social aggregations in this species.

98   Smith et al. (2009) reported restricted gene flow even between closely adjacent populations,

99   and moderate levels of genetic differentiation among sites with FST varying from 0.021 –

100   0.091. They found no evidence of population genetic bottlenecks and little evidence of

101   inbreeding due to consanguineous mating. Individual populations had observed

102   heterozygosities ranging from 0.75 to 0.82 (Smith et al 2009).

103

104   However, the secretive lifestyle of this species makes it difficult to observe whether there are

105   any social associations among neighbouring lizards, or whether the mating system is

106   polygamous as may be predicted if this is a more asocial species. We used genetic analysis to

107   identify the parents of juvenile pygmy bluetongue lizards in populations from two locations,

108   and to determine the mating system used by this species. We had two aims. The first was to

109   provide an additional comparative case within the *Egernia* clade to allow new insights into

110   the evolution of sociality within that group. The second was to provide vital information for

111   modeling population genetics and demography, and determing conservation strategies, within

112   isolated populations of this endangered species.

Methods

Field sampling

Lizards were sampled from two localities in native grassland, 11kms apart, and both within

20 km east of Burra, South Australia (33° 42'S; 138° 56'E). In the spring and summer of

2005/2006 we searched 11 – 12 ha at each locality and captured 160 lizards from locality 1

and 63 lizards from locality 2. In a second sampling period which included the two spring

and summer seasons of 2008/2009 and 2009/2010, we captured 353 individual lizards within

three 1.2 ha enclosures. One enclosure site was at locality 1 and two enclosure sites, 1 km

apart, were at locality 2. Each enclosure site had four 110 m long trap lines set in a square

(and thus enclosing an area of 1.2 ha) (Fig1). Each trap line had a 15 cm high black-plastic

drift fence and 16 bucket traps (20 litre, 38 cm deep, 28.5 cm diameter), placed immediately

under the drift fence, and spaced at 7 m intervals along its length (Figure 1). We attempted to

capture most of the resident lizards in each site first by setting the pitfall traplines and

trapping for 43,000 trap days over the entire sampling period (Schofield et al. 2012). We also

searched the inside of each enclosure each month for any occupied burrows that we could

detect, and attempted to lure individuals to the surface with mealworms following the method

of Milne et al. (1999). We sampled blood from those resident lizards that we were able to

capture.

Each captured lizard, was individually marked by toe clip and its sex, mass, snout-to-vent

length (SVL), and GPS location were recorded. Lizards were classified into neonates up to 6

months old (SVL <50mm), sub adults up to 18 months old (SVL51-80mm) or adults (SVL>

80mm), following Milne (1999). Among adults, sex was determined by the larger head size

and shorter body of males (Hutchinson et al 1994). Between late Jan and early March,

137   females produce a live litter of up to four offspring which remain in the maternal burrow with

138   their mother for periods varying from a few days to several weeks (Milne et al. 2002). We

139   recorded each case where neonates were found in the same burrow as an adult female, and,

140   where possible, we also sampled blood or toe clips from these individuals. Sub-adults within

141   enclosures could have moved there before the enclosure walls were erected, and their parents

142   may not necessarily have been within the sampling area.

143

144   DNA extraction and PCR amplification

145   A blood sample from a clipped toe was stored on FTA paper (Whatman, Maidstone), and

146   DNA was extracted following the procedure for nucleated erythrocytes (Smith and Burgoyne

147   2004). Individual genotypes for 561 lizards were determined at 15 previously described

148   polymorphic microsatellite loci: Est12 (Gardner, et al. 1999), TrL9, TrL12, TrL14, TrL15,

149   TrL16, TrL19, TrL21, TrL27, TrL28, TrL29, TrL32, TrL34,TrL35 and TrL37 (Gardner et al.

150   2008). Multiplex PCR conditions followed Gardner et al. (2008) with amplicons genotyped

151   on an ABI 3730 capillary electrophoresis DNA analyser (Applied Biosystems, Foster City,

152   CA). A fluorescently labelled size standard (GS500 (-250) LIZ) was run with the samples and

153   alleles were scored using GeneMapper software version 3.7 (Applied Biosystems) with

154   manual checking.

155

156   Hardy-Weinberg disequilibrium and linkage

157   We tested whether any individual locus had null alleles or deviated from Hardy-Weinberg

158   equilibrium (HWE), and whether there was any linkage disequilibrium (LD) between pairs of

159   loci, using GENEPOP 4.0.10 (Raymond and Rousset 1995, Rousset 2008). We obtained a

160   larger sample for these tests by combining our data from this study with genotypes for 34

161   additional lizards reported by Smith et al. (2009) from a separate but nearby locality (locality

162   6 of Smith et al. (2009) 1 km from locality 1, and 6km from locality 2). We ran the HWE and

163   LD tests separately on adults from each locality to determine if there were consistent patterns.

164   P-values were adjusted for multiple testing by the sequential Bonferroni method (Holm 1979)

165   when appropriate.

166   **Parentage analysis**

167   For each juvenile, whether it was captured in the first or second sampling period, we searched

168   for potential parents from among all of the adults sampled from the same location over both

169   sampling periods. We used adult genotypes at the 15 microsatellite loci in the program

170   CERVUS 3.03 to assign parents to genotyped juveniles. The following simulation parameters

171   were used: 100,000 cycles, 70% of the candidate parents sampled, 88% of loci typed and a

172   genotyping error rate of 1%. We accepted that we had sampled the true parents when the

173   confidence level exceeded 95%. Adults that were assigned as parents but that mismatched

174   their presumed offspring at > 2 loci were disregarded as inferred parents. Juveniles were

175   assumed to be siblings from the same litter if they were allocated the same mother and were

176   born in the same year. The simulations were also performed using an input parameter of 50%

177   of candidate parents sampled but as there was no difference we only present the results for

178   70%.

179   To assess the levels of monogamy and polygamy among adult lizards, we used CERVUS

180   3.03 and COLONY 2.0 to determine sib-groups and to predict the number of unsampled

181   parents. We assumed a polygamous mating system with no inbreeding as the populations

182   were in HWE. The marker type, allelic dropout rate and other error rates that were used can

183   be found in Online Resource 1. The probability that a parent was in the sample was tested at

184   50% and 70%. The results were the same for both, therefore probabilities were set at 70%. A

185   probability of 70% was chosen because the cryptic nature of the lizards made it unlikely we

186   had sampled of all the adults. Paternal and maternal relationships derived from the CERVUS

187   results were entered as Known Paternal and Known Maternal data sets. We then used

188   COLONY to simultaneously infer sibship and parentage using a full-pedigree likelihood

189   method (Jones and Wang 2009). Not all potential parents were sampled during our studies

190   and our estimates may not be an accurate reflection of all of the matings that had taken place.

191   However as we had good discrimination with our loci (Smith et al 2009) any potential bias

192   would be minimal. The mean heterozygosities of litters fathered by single or mutiple fathers

193   were compared using a paired sample t-test. Allelic richness could not be estimated with

194   confidence due to the small sample size.

195   Where we identified both parents and knew their actual locations when sampled, we

196   investigated whether individual parents showed any evidence for a preference for less related

197   individuals as mating partners. To do this we compared the relatedness of the partners to their

198   relatedness to other geographically close alternative partners. The only sample set that was

199   large enough for this analysis was in enclosure site 2 (locality 2) in the second sampling

200   period. Relatedness (r) was estimated, using the program Coancestry 1.0 (Wang 2011), with a

201   moment estimator which assumes no inbreeding (Wang 2002). We compared relatedness of

202   the two parents with the relatedness of opposite sex individuals located closest to them. For

203   each parent we considered either its relatedness to the nearest, or its mean relatedness to the

204   four nearest, non-partner individuals of the opposite sex. We then compared the relatedness

205   of partners and non-partners by paired t-test, separately for each sex.

206   **Results**

207   **Hardy-Weinberg and linkage disequilibrium**

208   Genotype frequencies deviated significantly from HWE at five of the 15 loci, but in each case

209   the deviations were only detected at single localities, one (TrL32) at locality 1, three (TrL12,

210   TrL15 and TrL37) at locality 2, and one (TrL32) at locality 6. No locus showed significant

211   deviation from HWE at more than one of the three localities (locality 1, sample size N=142;

212   locality 2, N=220; locality 6, N =34). Similarly, only two pairs of loci were significantly

213   linked in locality 1 (TrL 15/ TrL 16 and TrL 15/ TrL 21), three were linked in locality 2 (TrL

214   16/ TrL 19, TrL 19/ TrL 37, and Est12/ TrL 21) and none were linked in locality 6. Null

215   alleles were detected at five loci, but again no locus showed null alleles consistently over all

216   localities: locality1 (TrL16) locality 2 (TrL15, 21, 28 and Est12) and locality 6 (TrL 16). All

217   15 loci were used in subsequent analyses as patterns of disequilibrium and null alleles were

218   not consistent across localities and deviations may have been due to population level

219   processes (e.g. birth and death rates; different founding individuals).

220   **Parentage analysis**

221   We derived genotypes from 561 of 576 captured lizards (360 adults and 201 juveniles) (Table

222   1). Among the adults there were 189 males (52.5%) and 171 females (47.5%). Among the

223   201 juveniles (130 neonates, 71 sub adults), 140 (69 neonates, 71 sub adults) were captured

224   alone either in burrows or in pitfall traps, and 13, all neonates, were found as the only

225   juvenile in a burrow with an adult female. Juveniles found in groups of two to four

226   individuals were all from burrows and were all neonates. There were 11 groups of two, six

227   groups of three, and two groups of four individual juveniles together, with or without an

228   accompanying adult female (Table 2). A total of 39 neonate juveniles were found in burrows

229   with an adult female (Table 2). No juveniles were found in a burrow with an adult male

230   lizard.

231 Using CERVUS, 113 (56.2%) of the juveniles could be assigned to one (80 juveniles) or both

232 (33 juveniles) parents. Parentage was assigned to 51% of the neonates, and to 64% of the

233 subadults. Despite an intensive survey regime at each site, only 35% (location 1) and 69%

234 (location 2) of the parents of captured juveniles were sampled. Where only one parent was

235 identified, it was the mother in 47 cases and the father in 33 cases.

236 **Relatedness of lizards in the same burrow**

237 We were able to obtain a sample for DNA analysis from 17 of the 23 females located with

238 neonates in the same burrows. In 15 cases (88.2%) CERVUS inferred the co-located female

239 as the mother of all of the accompanying neonates. We deduced these were mothers with

240 their litters, and found a mean relatedness of 0.468 (range 0.221- 0.677) between these

241 females and their neonates.

242 In the two other cases, females were not assigned as the parent of a neonate located in the

243 same burrow, and relatedness values were low (r = -0.17 and r = -0.06). Both neonates were

244 sampled during the period of birth and neonate dispersal (26 Jan and 24 Feb). The first was a

245 single neonate and female. The second was a female with two neonates, one related and one

246 unrelated.

247 No sub adults were found sharing a burrow, either with juvenile or with adult lizards. In 18 of

248 the 19 groups of two or more neonates located together (Table 2), CERVUS assigned group

249 members to the same mother, and relatedness values among the group members suggested

250 they were siblings or half siblings (mean r = 0.412; range = 0.121 - 0.785). The other group

251 of two neonates found in the same burrow with an adult female, has been discussed above. In

252 that group the two neonates appeared to be unrelated to each other (r = -0.0004).

253 **Paternal contribution**

254   CERVUS identified from among the 201 sampled juveniles 56 sets of 2 – 4 siblings born in

255   the same year with the same mother (described as a family in this paper). For some of those

256   groups the mother was not identified from among the adults that were sampled. In 37 of those

257   sets the siblings were sampled occupying separate burrows. In 24 of these 56 families, both

258   the mother of all of the sibs, and the father of at least one sib could be identified from among

259   the adults sampled. In 18 (75%) of those 24 litters, COLONY suggested that an additional

260   male fathered one or more of the other juveniles (Table 3). For 17 litters there were at least

261   two fathers, while one litter of four sibs had at least three fathers. For the remaining 32

262   maternal families we used COLONY to deduce the possible male parent contributions to the

263   litters and inferred that at least 22 (69%) of those families had multiple fathers. There was no

264   significant difference between the mean heterozygosities of litters with single paternity (7.4)

265   and litters with multiple paternity (6.6) (Table 4)

266   We identified 43 female individuals that produced litters, and deduced that 18 of those

267   (41.9%) had produced more than one litter over the duration of this study, 11 of them in

268   consecutive years. During the second sampling period of two years, sub adult juveniles

269   sampled in the first year (2008) were assumed to have come from matings in 2007, thus we

270   had data for matings in four different consecutive years, even though sampling took place in

271   three. Most females that produced multiple litters had two litters over consecutive years,

272   while two females were detected to have produced litters in 3 and 4 consecutive years

273   respectively.

274   CERVUS identified 70 (37%) of the 189 adult males sampled in the study as fathers of

275   sampled juveniles. Seven (10%) of those males fathered juveniles with two different females

276   in the same season. This may under-represent the rate of polygyny since both males and

277   litters in the sampled populations would have been incompletely sampled. Five males were

278   identified as fathering juveniles with the same female in multiple years in the second

279   sampling period, with three of those cases (60%) being in consecutive years. Those five

280   males were also among the seven polygynous males, mating with more than one female in at

281   least one season.

Relatedness among mating partners

283   Within individuals sampled at enclosure site 2, CERVUS identified 20 juveniles for which

284   both the mother and the father could be identified. The 20 juveniles came from 17 maternal

285   litters, and were fathered by 17 males. Three of the males fathered juveniles from two of the

286   females, one female had offspring fathered by two of the males, and another female had

287   offspring fathered by three of the males. Each male-female parent combination was only

288   responsible for one juvenile, so that none of the juveniles from the 20 sampled had an

289   identical mother and father.

290   The mean relatedness between the male and female parents for each of the 20 juveniles ($r =$

291   0.063; Table 5) did not differ significantly from the mean relatedness of all male-female

292   combinations among the sampled adults in site 2 (Mann Whitney U =34560.5, z = -1.64, sig

293   0.101). There was no evidence that lizards were choosing mating partners that were less

294   related than random. Furthermore for both males and females, relatedness to their mating

295   partner was not significantly different from relatedness to the nearest other individual of the

296   opposite sex, or from the average relatedness of the four nearest other individuals of the

297   opposite sex (Table 6). That is, there was no evidence that partners were chosen non-

298   randomly from among neighbouring lizards with respect to relatedness.

299   Further evidence that relatives were not discriminated against as mating partners, came from

300   three offspring resulting from matings between partners with relatedness values of 0.500,

301   0.365 and 0.297 (Table 5). In each case other less related individuals were available as

302   partners from among neighbouring lizards (Table 5). Genotypes and lizard locations were

303 derived from samples collected when the lizard was first captured, and this may have been

304 before or after the October/ November period when mating occurs. The mean distance

305 between burrows occupied by males and females that had produced young was 27.24m (N =

306 20; SE = 6.04; range = 3 – 107m) (Table 5). The mean distance between all possible male

307 and female pairs within enclosure site 2 was 64.7m (N = 4028; SE = 0.51; range = 1 – 160).

308 Mated males and females were found closer to each other than if males and females within

309 the enclosure had mated at random with respect to geographic distance (Mann Whitney U =

310 13226.50 ,z = -5.189, P < 0.001).

311 **DISCUSSION.**

312 Compared with other lizards in the *Egernia* group our genetic results suggest a high level of

313 multiple mating within the sampled localities of pygmy bluetongue lizards. Within a season it

314 was common for females to be mated by two or more males, and males could mate with more

315 than one female in this period. Mating appeared to be indiscriminate with regard to degree of

316 genetic relatedness, and male and female mating partners could be located in burrows over

317 100 m apart. The distribution of the number of matings per male could not be estimated

318 because some litters, and thus some matings, were unsampled. Even in the sampled litters, a

319 male that had mated with the female may not have contributed to the progeny.

320

321 Chapple and Keogh (2005) proposed three distinct mating systems for the *Egernia* group (i) a

322 combination of polygyny and within season monogamy (ii) long-term genetically

323 monogamous pairings during the breeding season and (iii) long term genetic monogamy

324 within temporally stable social aggregations. Unlike other members of the group, pygmy

325 bluetongue lizards appear to be promiscuous and to display a polygamous mating system.

326

327   Several previous observations support our interpretation of the genetic analysis. Video

328   recordings of female occupied burrows (Milne et al. 2003, Fenner and Bull 2009), suggest

329   that males move across the population to seek out females in their burrows, and that

330   individual females are visited by multiple males (Ebrahimi unpubl. data 2013).

331

332   In our study, males were recorded as far as 100 m away from the females they mated with.

333   Records of mating have all been in the spring (October) (Milne et al. 2003, Fenner and Bull

334   2009). This is the time when other observations have suggested that males of this normally

335   sedentary lizard are actively moving around (and exposed to predation). It is the time of year

336   when a male lizard was found inside a brown snake stomach in 1992 when the species was

337   re-discovered (Armstrong and Reid 1992), and also the time of year when Schofield et al.

338   (2012) reported maximum capture rates of adult lizards (86% males) in pit-fall traps.

339   Combining those observations with the genetic data from the current study suggests that,

340   during a short mating period in spring, males move around the population seeking females in

341   burrows, and can mate with multiple partners. And at the same time, females in their burrows

342   accept matings from several different males. During this period the males will be exposed to

343   enhanced predation risk (Fenner and Bull 2009), and this increased predation may explain the

344   absence of some of the fathers of the juveniles from the genetic sample in this study. It would

345   also explain the disappearance of more males than females from lizard populations over a

346   spring-summer period (Fellows 2008).

347   One explanation for why females accept multiple matings may be the high risk of inbreeding.

348   Previous genetic analyses (Smith et al 2009) have shown clustering of related individuals

349   within populations, indicative of low dispersal rates. Furthermore, Fenner and Bull (2010)

350   failed to find evidence that individual pygmy bluetongue lizards discriminated among scent

351   cues from related and non-related individuals. Thus there is a high chance of a related male

352   and female coming together and of the partners not being inhibited by that relatedness in their

353   mating behaviour. Results from the current study confirm that some juveniles were produced

354   from matings between highly related males and females. In these circumstances, females that

355   mate with more than one male increase the chance that some of their offspring will be more

356   outbred.

357   One aim of our study was to contribute to understanding how mating systems evolved within

358   the *Egernia* group of Australian lizards. The promiscuous and indiscriminate mating system

359   of pygmy bluetongue lizards differs substantially from related species which have stable,

360   long-term monogamous partnerships (Bull 2000; Gardner et al. 2002), which show a highly

361   developed olfactory discrimination among related and non-related individuals (Bull et al.

362   2001) and which tend to choose single, unrelated mating partners (Bull and Cooper 1999;

363   Gardner et al. 2002).

364   One ecological factor that might drive this difference is that pygmy bluetongue lizards do not

365   dig their own refuge burrows, but instead rely on burrows dug by spiders. These are usually

366   too small for persistent sharing of burrows by more than one lizard, and the short supply of

367   burrows of optimal depth (Fellows et al. 2009) has led to a system of single occupancy

368   burrows and central place territorial defence (Fenner and Bull 2011). Specifically there is no

369   opportunity for the social aggregations commonly reported in other *Egernia* group species,

370   and for the development of within group interactions that might favour less polygamous

371   mating systems.

372 There are at least two conservation implications of our results for this endangered lizard.

373 First, the indiscriminate partner choice and close spatial proximity of relatives in existing

374 populations, suggest that individual lizards will not actively avoid mating with highly related

375 partners. This means there may be a greater risk of inbreeding as populations decline, and as

376 the genotypic range of potential partners is reduced. Continued monitoring of genetic

377 diversity in populations, particularly those with low population density, will be important.

378 Our result contrasts with the earlier studies on another Egernia group member, *E.*

379 *cunninghamii.* Stow and Sunnucks (2004) reported a reduction in mating between relatives in

380 highly fragmented areas where potential partners were limited. Second, and conversely, that a

381 promiscuous mating system may prove advantageous during any translocations or

382 reintroductions. This is because it could ensure the rapid mixing of genotypes among founder

383 individuals at unoccupied sites, or the rapid integration of new genetic material into existing

384 populations.

385

386 The success of translocations could be measured by a high reproductive output with the

387 maintenance of genetic diversity over time (Griffith et. al 1989, Gregory et al 2012). In

388 polygnous mating systems females are the limiting factor. The introduction of more females

389 than males could reduce the male search time for a mate and thus reduce predation risk to

390 males especially in species that mate indiscriminately. However when considering

391 reproductive potential in monogamous or pair bonding species equal numbers of each sex

392 would result in maximal reproduction (Sigg et al. 2005). To ensure breeding compatibility in

393 these species the translocation of previously mated individuals would be ideal. In species

394 with kin recognition and mating avoidance or long term genetic monogamy within temporally

395 stable social aggregation a selection of less related individuals would benefit reproduction

396 and genetic diversity in translocations (Gregory et. al 2012). The success of captive breeding

397 and translocation efforts for any species may hinge upon understanding both the baseline

398 genetic diversity of source and translocated populations and the mating systems they display

399 (Haig 1998; Sigg et al. 2005; Grueber and Jamieson 2008 Gregory et. al 2012).

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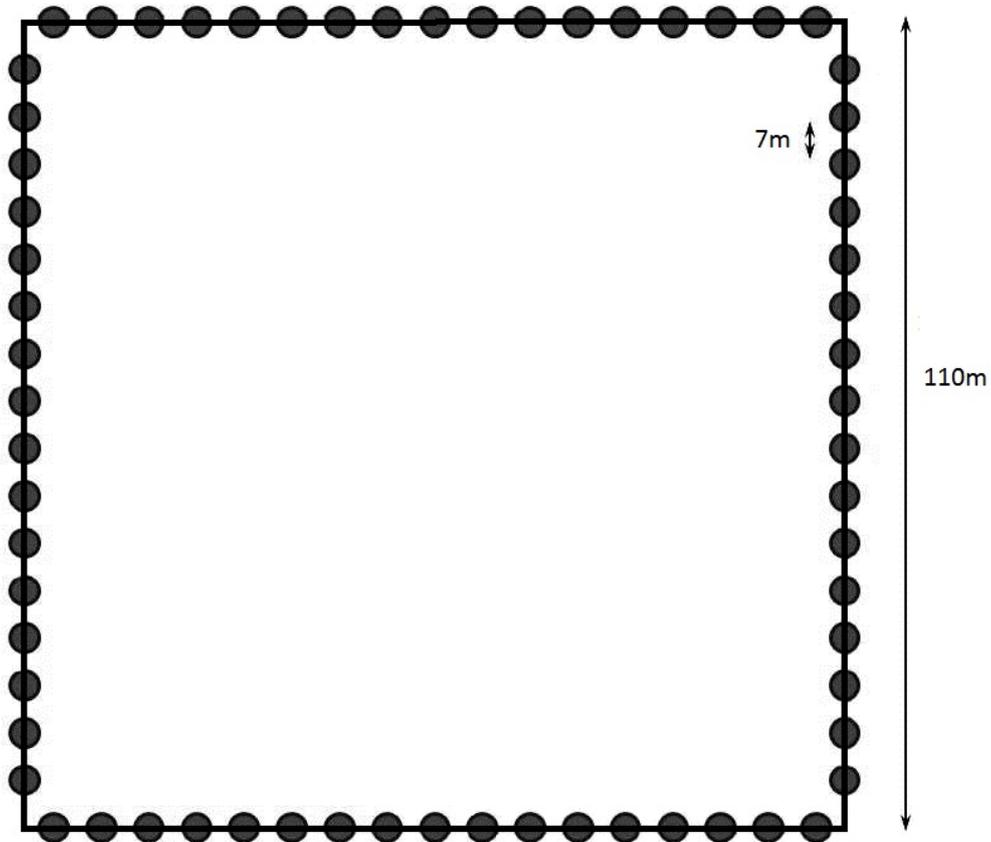
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522 Figure 1. Sampling grid used at the 3 study sites, where black circles represent pitfall traps
523 and the lines denote plastic drift fence.

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526 Table 1. Capture summary of the individuals from which successful genotypes were derived
527 over the two sampling periods

Sampling period	Juveniles	Males	Females	Total
1 (2005 - 2006)	27	83	91	201
2 (2008 – 2010)	174	106	80	360

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529 Table 2. The number of groups containing juvenile lizards, and the total numbers of juvenile
530 lizards in each group size category that were detected with or without a female present in the
531 same burrow.

Number of juveniles per group	1	2	3	4
Number of groups with female present	13	5	4	1
Number of groups with no female present	140	6	2	1
Total number of groups	153	11	6	2
Total juvenile lizards	153	22	18	8

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534 Table 3. Number of litters with multiple fathers as identified by CERVUS and inferred
535 multiple father litters by COLONY in brackets

	Full sibs groups	Range of litter size	half sib groups	Range of litter size	Total number of families
Sampled families	6	2-3	18	2-4	24
Inferred families	10	2	22	2-4	32

536

537 Table.4 Levels of heterozygosity found in litters with multiple and single paternities

	Number of individuals	Number of litters	Observed Heterozygosity
Single paternity	11	5	7.38
Multiple paternity	9	4	6.57

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540 Table 5. Comparison of distance to partner and relatedness between the mated pairs and the 4
541 nearest individuals of the opposite sex.

Female partner	Male partner	Relatedness	Relatedness of female to nearest male non	Average relatedness of female 4 nearest	Relatedness of male to nearest female	Average relatedness of male to 4 nearest	Distance between mated pairs

		partner	males		females		
2325	2707	-0.0451	-0.160	-0.006	-0.115	-0.133	13.345
2351	2403	-0.0748	-0.036	-0.051	0.002	0.140	5.099
2352	2713	0.3614	0.059	0.116	-0.106	-0.086	30.806
2400	2706	0.1707	0.288	0.069	-0.087	0.053	64.899
2401	2431	-0.1018	-0.092	0.016	0.026	0.052	11.705
2410	2616	-0.1817	-0.026	-0.042	0.059	-0.078	10.630
2413	2355	0.2933	-0.085	-0.006	0.288	0.086	11.705
2413	2335	-0.0081	-0.085	0.178	-0.028	-0.044	107.331
2413	2632	0.4858	-0.074	-0.005	0.451	0.069	21.213
2453	2709	-0.1315	0.573	0.175	-0.077	-0.086	19.2094
2453	2707	-0.1128	0.573	0.175	-0.007	-0.118	22.3607
2500	2761	0.2117	-0.106	-0.038	-0.132	0.027	82.0549
2524	2626	0.171	-0.178	-0.005	0.064	0.040	8.5440
2533	2340	-0.127	0.025	-0.042	0.140	0.106	22.361
2540	2761	0.1311	0.093	0.005	0.037	0.104	17.117
2559	2431	-0.111	-0.205	-0.118	0.026	0.007	10.198
2619	2639	0.0259	-0.047	-0.072	0.138	0.018	31.6228
2651	2330	0.1096	0.003	0.057	-0.034	0.053	31.064
2683	2627	0.1291	-0.103	-0.075	-0.062	0.060	3
2744	2694	0.0597	-0.141	-0.065	0.197	0.259	20.615
Mean		0.06258	0.014	0.013	0.039	0.027	27.244

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Table 6. Paired t-test comparing the relatedness among breeding individuals and the 4 nearest
of the opposite sex.

Relatedness of mate pairs to:	df	t	sig (2-tailed)
Mated male and average of 4 nearest females	19	0.95	0.353
Mated female and average of 4 nearest males	19	1.17	0.257
Mated male and nearest females	19	0.522	0.608
Nearest males	19	0.746	0.465

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