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6   TITLE: Using playback of territorial calls to investigate mechanisms of kin

7   discrimination in red squirrels

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9   SHORT TITLE: Kin discrimination in red squirrels

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27   ABSTRACT

28

29   Kin recognition can facilitate kin selection and may have played a role in the

30   evolution of sociality. Red squirrels (*Tamiasciurus hudsonicus*) defend territories using

31   vocalizations known as rattles. They use rattles to discriminate kin, though the

32   mechanism underlying this ability is unknown. Our objective was to distinguish between

33   the mechanisms of prior association, where animals learn the phenotypes of kin they

34   associate with early in life, and phenotype matching/recognition alleles, where animals

35   use a template to match phenotypes, thereby allowing them to recognize kin without an

36   association early in life. We used audio playbacks to measure the responses of squirrels to

37   rattles from familiar kin, unfamiliar kin, and non-kin. Initial analyses revealed that red

38   squirrels did not discriminate between familiar and unfamiliar kin, but also did not

39   discriminate between kin and non-kin, despite previous evidence indicating this

40   capability. Post-hoc analyses showed that a squirrel's propensity to rattle in response to

41   playback depended on an interaction between relatedness and how the playback stimuli

42   had been recorded. Red squirrels discriminated between rattles from close kin ($r = 0.5$)

43   and rattles from non-kin ($r < 0.5$) when the rattles were recorded from provoked squirrels.

44   Squirrels did not exhibit kin discrimination in response to rattles that had been recorded

45   from unprovoked squirrels. Once we accounted for how the stimuli had been recorded,

46   we found no difference in the responses to familiar and unfamiliar kin. Our study

47   suggests that kin discrimination by red squirrels may be context-dependent.

48

51

52   INTRODUCTION

53

54   Kin recognition is the ability of an individual to recognize its relatedness to other

55   individuals. This involves the expression of a recognizable signal by one individual, and

56   the perception of that signal by another (Hamilton 1964; Beecher 1982). Kin recognition

57   allows individuals to avoid inbreeding (Pusey and Wolf 1996) and to gain inclusive

58   fitness benefits (Hamilton 1964) by mediating social behaviors, such as alarm calling

59   (Sherman 1977). Evidence of kin recognition has been documented in group-living

60   animals, as well as in solitary and territorial animals (Fuller and Blaustein 1990; Sun and

61   Müller-Schwarze 1997; Hare 2004; Flores-Prado and Niemeyer 2010).

62

63   Several mechanisms have been proposed to explain how animals recognize kin,

64   including prior association, phenotype matching, and recognition alleles (reviewed by

65   Holmes and Sherman 1982, 1983, Blaustein 1983, Waldman 1987). In prior association,

66   animals learn the phenotypes of specific individuals early in life, when social interactions

67   usually involve kin (e.g., interacting with one's siblings or mother while in the natal

68   nest). In phenotype matching, animals recognize familiar or unfamiliar kin by comparing

69   them to a generalized kin template that is based on their own phenotype or on the

70   phenotypes of familiar kin encountered early in life. In recognition alleles, the animal is

71   hypothesized to express and recognize a familial trait, but unlike in phenotype matching,

72   the expression and recognition of that trait is inherited instead of learned (i.e. green-beard

73   effect, Hamilton 1964; Dawkins 1976). Distinguishing between phenotype matching and

74 recognition alleles is often impossible because both allow for the recognition of familiar

75 and unfamiliar kin.

76

77   Whereas kin recognition is the process of assessing genetic relatedness, kin

78 discrimination is the differential expression of behavior towards kin. Several studies have

79 found that kin discrimination can be context-dependent and can vary between social

80 contexts and with fluctuating environmental conditions. In a few studies on salamanders,

81 kin discrimination varied with predator density (Harris et al. 2003), food abundance and

82 larval size (Hokit et al. 1996). Another study found that female red-backed salamanders

83 (*Plethodon cinereus*) cannibalized unrelated neonates significantly more often than they

84 cannibalized their own offspring, yet they otherwise did not behave differently towards

85 the two groups of young (Gibbons et al. 2003). In eusocial insects, discrimination of

86 nestmates (i.e. kin) has been found to vary with social context (intruder introductions,

87 group interactions or dyadic interactions; Buczkowski and Silverman 2005), with

88 perceived threat to the colony (amount of nectar in the hive; Downs and Ratnieks 2000;

89 and number of intruders; Couvillon et al. 2008), and with the location of the behavioral

90 assay (either at a natural colony entrance or a test arena; Couvillon et al. 2013). These

91 studies show that multiple factors can influence kin discrimination behavior, and that the

92 absence of kin discrimination does not necessarily mean an absence of kin recognition.

93

94   North American red squirrels (*Tamiasciurus hudsonicus*) are solitary, territorial

95 animals capable of discriminating kin in certain contexts. Males and females defend

96 exclusive territories throughout the year (Smith 1968), and use vocalizations known as

97   rattles to establish and signal their presence on a territory (Smith 1978; Lair 1990);

98   physical disputes over territory boundaries are rare (Dantzer et al. 2012). Rattles have

99   individually distinctive acoustic structures (Digweed et al. 2012) and previous research

100   has shown that squirrels respond differently to the playbacks of rattles from kin and non-

101   kin, regardless of whether those rattles were from neighbors or non-neighbors (Wilson et

102   al. 2015). Nepotistic behavior in red squirrels has also been documented in several other

103   contexts. Specifically, females are known to bequeath territories to offspring (Price and

104   Boutin 1993; Berteaux and Boutin 2000; Lane et al. 2015), to nest occasionally with kin

105   during the winter (Williams et al. 2013), and, in rare circumstances, to adopt the

106   orphaned young of close kin (Gorrell et al. 2010). These examples are primarily between

107   pairs of closely related individuals that have close associations early in life (e.g., mother-

108   offspring and littermate pairs): bequeathal occurs only between mother-offspring pairs,

109   nest sharing occurs primarily between mothers and daughters (though there were a few

110   unfamiliar half-siblings nesting together), and adoption occurs only when the orphan's

111   genetic mother and adopting mother were familiar and close kin. In contrast to these

112   examples of kin discrimination, cross-fostering experiments with newborn red squirrels

113   suggest that females do not preferentially allocate parental care to genetic offspring

114   versus foster offspring (Humphries and Boutin 1996; McAdam et al. 2002). Kin

115   discrimination is therefore context-dependent in this species.

116

117   The objective of this study was to determine whether or not kin discrimination in

118   red squirrels is based on the mechanism of prior association. Previous research showed

119   that red squirrel rattles are individually distinctive (Digweed et al. 2012; Wilson et al.

120   2015) and used for kin discrimination (Wilson et al. 2015). However, the work on kin

121   discrimination did not address whether red squirrels discriminate between kin with which

122   they had prior associations early in life (familiar kin) and kin with which they had no

123   prior associations early in life (unfamiliar kin). This was because most playback stimuli

124   in the kin treatment of that study involved mother-offspring pairs, who would have

125   interacted early in life while in the natal nest (Wilson et al. 2015). Therefore, we

126   measured the responses of squirrels exposed to the playback of rattles from familiar kin,

127   unfamiliar kin, and non-kin. If red squirrels recognize kin using the mechanism of prior

128   association, we predicted that they would behave less aggressively and thus be less likely

129   to rattle in response to rattles from familiar kin than in response to rattles from unfamiliar

130   kin or non-kin. Alternatively, if red squirrels recognize kin by phenotype matching or

131   recognition alleles, then we predicted that they would be less likely to rattle in response

132   to rattles from familiar and unfamiliar kin than in response to rattles from non-kin. Upon

133   finding no evidence of kin discrimination (see results, below), we conducted a series of

134   post-hoc analyses to explore possible contextual factors that might have affected kin

135   discrimination in this study.

136

137   METHODS

138

139   *Study Site and Subjects*

140   We conducted research on a population of red squirrels that has been studied

141   annually in southwestern Yukon (61°N, 138°W) since 1989 (McAdam et al. 2007). The

142   population lives in open boreal forest that is dominated by white spruce (LaMontagne and

143   Boutin 2007). All individuals in the population were marked with numbered metal ear

144   tags (Monel #1 National Tag and Band Co.) for permanent identification, and with

145   colored wires threaded through their ear tags for visual identification from afar (McAdam

146   et al. 2007). We monitored female reproductive status by live-trapping individuals in

147   Tomahawk traps baited with peanut butter, and we ear-tagged the pups when they

148   reached 25 days of age and were still in the natal nest (McAdam et al. 2007).

149

150   As part of our ongoing research program, we generated a multigenerational

151   pedigree for this population (e.g., McFarlane et al. 2015). We established maternal

152   linkages by identifying mothers and their pups while they were still within their natal

153   nests. The few cases of adoption documented in this study population mostly occurred

154   when pups had emerged from the natal nest but were not yet weaned, between 43 and 63

155   days of age (Gorrell et al. 2010). A single adoption occurred when the pup was only six

156   days old. Therefore, adoptions should not have influenced our method of establishing

157   maternal linkages. Paternal pedigree linkages (Lane et al. 2007; McFarlane et al. 2014)

158   were established since 2003 using paternity analysis involving 16 microsatellite loci

159   (Gunn et al. 2005). The paternal linkages were made with 99% confidence using

160   CERVUS 3.0 (Kalinowski et al. 2007). Any unobserved adoptions would have been

161   detected by mismatching genotypes between the pup and mother during the paternity

162   analysis.

163

164   Playback trials for this study were conducted on three sites: one was part of an

165   ongoing food supplementation experiment that started in 2004 (45 ha), and the other two

9

166   were control sites for this same large-scale experiment (40 ha each). As part of this

167   experiment, squirrels on the food-supplemented site were supplied with 1 kg of peanut

168   butter every six weeks between October and May each year. The density of squirrels in

169   2009 was low on the two control sites (1.13 and 0.76 squirrels/ha), but was higher on the

170   food supplemented site (2.45 squirrels/ha) due to higher food availability (Dantzer et al.

171   2013).

172

173   *Experimental Design*

174   The playback experiment followed a 2 X 3 factorial design in which each subject

175   was played a single territorial rattle that varied in terms of its kinship status (familiar kin,

176   unfamiliar kin, or non-kin) and neighbor status (neighbor or non-neighbor). "Kin" was

177   defined as having a pedigree relatedness coefficient (r) of at least 0.25. We used a

178   categorical kin variable because we were interested primarily in determining whether kin

179   discrimination was limited to familiar kin or whether it extended to unfamiliar kin as

180   well. Our "familiar kin" treatment referred exclusively to pairs of squirrels that shared a

181   natal nest, as this is the only time in a squirrel's life when they are interacting only with

182   kin. The familiar kin treatment included 15 mother-offspring pairs and 22 litter-mate

183   pairs (full siblings and maternal half-siblings). Male red squirrels do not provide parental

184   care and have no interactions with pups in the natal nest. Our "unfamiliar kin" treatment

185   included 12 father-offspring pairs, 14 non-litter-mate pairs (paternal half-siblings,

186   maternal half-siblings, or full siblings from different litters), and four grandparent-grand-

187   offspring pairs (Table 1). "Non-kin" were defined as having a relatedness coefficient of

188   less than 0.125. We included neighbor status in our experimental design to account for

10

189   the possibility that squirrels behave less aggressively towards their neighbors (i.e., the

190   dear-enemy effect: Fisher 1954; Temeles 1994). "Neighbors" were defined as squirrels

191   with middens located within 100 m of each other, whereas "non-neighbors" were defined

192   as squirrels whose middens were more than 200 m apart. The familiar kin treatment

193   included 24 neighbor and 13 non-neighbor trials, the unfamiliar kin treatment included 16

194   neighbor and 14 non-neighbor trials, and the non-kin treatment included 16 neighbor and

195   22 non-neighbor trials.

196

197   *Playback Stimuli*

198   Rattles used as playback stimuli were recorded from squirrels as they moved

199   freely around their territories ($N = 46$), as they emerged from a trap ($N = 17$), or as they

200   rattled in response to rattles that we broadcast from a loudspeaker ($N = 10$). All rattles

201   were recorded using a shotgun microphone (Sennheiser model ME66 with K6 power

202   supply; 40 – 20000 Hz frequency response ($\pm$ 2.5 dB); super-cardioid polar pattern)

203   connected to a Marantz Professional Solid State Recorder (model PMD 660; 44.1 kHz

204   sampling frequency; 16-bit amplitude encoding; WAVE format). The final set of

205   recordings included one recording from each of 73 different adult squirrels. The

206   recordings were from 35 males and 38 females that we recorded in 2005 (one recording),

207   2006 (nine recordings), and 2009 (63 recordings). There were 30 rattles recorded on the

208   site with the food supplementation experiment and 43 rattles recorded on the two control

209   sites.

210

211   Recordings of squirrels with living kin were assigned preferentially to the kin

212   treatments (familiar and unfamiliar kin), as there were a limited number of squirrels with

213   close relatives on our study site. The non-kin stimuli were assigned to subjects at random.

214   In trials for which recordings from 2005 and 2006 were used as stimuli, the vocalizing

215   squirrel from the recording was still alive at the time of the trial in 2009. Most recordings

216   were used only once in the playback experiment; 26 were used to test more than one

217   squirrel, though these were used in different treatments for each squirrel.

218

219   Rattles used as playback stimuli were not filtered and were not edited to

220   standardize their length. Each stimulus consisted of a single rattle that ranged between 1.5

221   and 12.3 s in duration (mean $\pm$ SD $= 4.0 \pm 2.3$ s). The mean duration of the rattle stimuli

222   ranged from 3.5 s $\pm$ 2.1 s (mean $\pm$ SD) in the non-kin non-neighbor treatment to 4.8 s $\pm$

223   3.2 s (mean $\pm$ SD) in the unfamiliar kin non-neighbor treatment, and did not differ

224   significantly among treatments (one-way ANOVA: $F_{5, 99} = 0.70$, p-value $= 0.63$). Rattles

225   were transferred to a SanDisk mp3 player (Sansa e280 model) that supported the WAVE

226   format. Our playback speaker was a custom Saul Mineroff SME-AFS field speaker, with

227   a frequency range of $10 - 22,500$ Hz. The speaker's volume setting was held constant

228   throughout the experiment. At this setting, the rattle peak amplitude averaged 68 dB $\pm$ 3.3

229   dB (mean $\pm$ SD), as measured with a digital sound level meter (RadioShack; C weighting;

230   fast response) held 1 m from the speaker. When broadcast within this amplitude range,

231   the rattles were audible to the human ear at up to 120 m away. This is comparable to the

232   only published account of rattle amplitude, which states that red squirrel rattles can be

233   heard up to 130 m away (Smith 1968). The peak amplitude ranged between an average of

234   66.7 dB ± 4.5 dB (mean ± SD) in the unfamiliar kin non-neighbor treatment and 68.8 dB

235   ± 2.8 dB (mean ± SD) in the non-kin non-neighbor treatment, and did not differ

236   significantly among treatments (one-way ANOVA: $F_{5,\,99} = 0.70$, p-value = 0.62).

237

238   *Playback Procedure*

239   Subject squirrels were located by sight, sound, or radio telemetry, and trials were

240   commenced only if the subject was within 20 m of its midden. We used the squirrel's

241   unique color markings to confirm their identity before beginning trials. Once a subject

242   was identified, we set up the speaker approximately 10 m from the subject and concealed

243   it behind a tree, fallen log, or dense vegetation. The observer then sat on the ground

244   approximately 10 m from the subject, such that the line between the observer and subject

245   was perpendicular to the line between the subject and speaker. All trials were completed

246   by a single observer.

247

248   Trials consisted of a three-minute pre-playback observation period followed

249   immediately by the playback stimulus and a three-minute playback observation period.

250   Throughout the pre-playback and playback periods, we counted each time the subject

251   produced a rattle, looked at the speaker, and approached the speaker. We subjectively

252   scored 'looking at the speaker' when we saw head movement by the subject that ended

253   with the squirrel's head facing the speaker. We defined "approach" as 2 m of continuous

254   travel directly toward the speaker. During the playback period, we audio-recorded the

255   subject using the same recorder as described above. Ten rattles recorded from subjects

13

256   during the playback period were later used as stimuli in other playback trials on different

257   focal subjects.

258

259   We attempted to minimize confounding or obscuring factors during the playback

260   trials. For example, we did not commence a trial if the subject alarm-called as the

261   observer approached (known as a bark; Lair 1990; Digweed and Rendall 2009) or

262   interacted with another squirrel (e.g., chasing) while the observer approached. We also

263   discarded trials if the subject chased an intruding adult ($N = 2$) or juvenile ($N = 2$) squirrel

264   from their territory during the trial, if the subject moved more than 20 m away from the

265   speaker before the trial began ($N = 2$), if the observer lost sight of the subject for longer

266   than 1 min ($N = 18$ during the pre-playback period; $N = 7$ during the playback period), or

267   if the squirrel entered a nest during the pre-playback period (n = 7). Discarded trials were

268   attempted again after three days. In total, we completed 105 successful trials on 85

269   individual squirrels between 23 May and 26 July 2009. There were 63 trials conducted on

270   45 squirrels on the two control sites and 42 trials on 40 squirrels on the food-

271   supplemented site. For the 20 subjects that received two trials, each received a different

272   treatment during each trial and the trials were separated by at least three days.

273

274   *Statistical Analyses*

275   Response variables in our analyses included (1) whether or not the subject

276   produced a rattle, (2) whether or not the subject looked at the speaker, (3) whether or not

277   the subject approached the speaker, and (4) the latency for the subject to rattle. We

278   considered the first three variables to be dichotomous because it was uncommon for

14

279   squirrels to express these behaviors more than once during each observation period.

280   Latency to rattle was only measured in the playback period, and was defined as the time

281   from the start of the playback stimulus to the start of the subject's rattle (measured to the

282   nearest 10 ms using Raven Pro Sound Analysis Software version 1.3).

283

284   All statistical analyses were performed in R (version 3.2.2; R Development Core

285   Team 2009) using the packages "lme4" (Bates et al. 2015) and "survival" (Thernau and

286   Lumley 2009). Our first set of analyses tested whether subjects responded to the playback

287   stimuli by comparing response variables between the pre-playback and playback periods.

288   Separate generalized linear mixed effect models (binomial error distribution; logit link

289   function) were fitted to each of the three dichotomous response variables measured in

290   both periods. We included subject identity as a subject variable with random effects to

291   account for the repeated measures obtained during the pre-playback and playback

292   periods, as well as for the multiple trials that were conducted on each of 20 subjects.

293   Period (pre-playback or playback) was included as a categorical variable with fixed

294   effects.

295

296   Our second set of analyses tested whether subjects' responses were affected by the

297   kinship status of the playback stimuli. We used separate generalized linear mixed effect

298   models (binomial error distribution; logit link function) to test if the kinship status of the

299   playback stimulus affected (1) whether the subject rattled and (2) whether it looked at the

300   speaker. Approaching the speaker was not included as a response variable in this set of

301   analyses because it did not differ between the pre-playback and playback periods (see

15

302   results below). In each model, we included subject identity as a subject variable with

303   random effects, and the kinship status (familiar kin, unfamiliar kin, or non-kin) and

304   neighbor status (neighbor or non-neighbor) of the playback stimulus as categorical

305   variables with fixed effects. The two-way interaction between kinship status and neighbor

306   status was not significant in either model and was, therefore, removed from the final

307   model.

308

309   We used a survival analysis approach to test the effect of kinship status and

310   neighbor status on latency to rattle in the playback period. A survival analysis approach

311   was used because it is useful for analyzing time-to-event data and can deal with censored

312   values that result when the event does not occur (e.g., subject squirrels that did not rattle

313   during the three-minute playback period). We used a Cox proportional hazard model with

314   the playback period data of a reduced dataset (n = 85 trials), with kinship and neighbor

315   status as independent variables. We eliminated multiple trials from each of 20 individuals

316   by randomly selecting one trial per individual. The two-way interaction between kinship

317   status and neighbor status was not significant and was, therefore, removed from the final

318   model.

319

320   Upon finding no overall effects of kinship or neighbor status (see results below)

321   on any of the response variables, we conducted exploratory post-hoc analyses in an

322   attempt to understand the negative results and their inconsistency with previous evidence

323   of kin discrimination in red squirrels (Gorrell et al. 2010; Williams et al. 2013; Wilson et

324   al. 2015). For all exploratory analyses, we used whether or not the subject rattled as the

16

325   response variable, since this variable can be compared directly with previous studies.

326   Neighbor status was removed from the final models because there were no significant

327   interactions and the main effect of neighbor status was not significant.

328

329   There was some variation in the degree of average relatedness within the kinship

330   categories (Table 1), so our first exploratory post-hoc analysis examined the relationship

331   between the probability of rattling and known relatedness coefficients derived from the

332   pedigree. We conducted a simplified analysis that treated kinship as a continuous variable

333   and excluded familiarity. Therefore, in contrast to our earlier analyses, this exploratory

334   analysis tested for an overall effect of kin discrimination, regardless of whether kin were

335   familiar or unfamiliar.

336

337   Local density was quite variable among squirrels tested in this study, and red

338   squirrels emit rattles more frequently when surrounded by a higher density of

339   conspecifics (Dantzer et al. 2012; Shonfield et al. 2012). For the second exploratory

340   analysis we tested the effect of local density on rattle responses. Local density

341   (squirrels/ha) was calculated for each subject as the number of squirrels that owned a

342   midden within a 130 m radius (5.31 ha) of the subject's midden. We chose a 130-m radius

343   because rattles from neighboring squirrels are audible up to this distance (Smith 1968).

344

345   Recent bioacoustics research has revealed structural differences among rattles

346   recorded from (1) squirrels as they move freely around their territories, (2) rattles

347   recorded as squirrels emerge from a trap, and (3) rattles produced in response to rattles

348   that have been broadcast through a speaker (unpublished data). It is not yet clear how

349   these structural differences affect the natural inter-individual variation in rattle structure

350   (Digweed et al. 2012; Wilson et al. 2015) that is presumably used in discrimination.

351   Therefore, for the third exploratory analysis, we tested whether kin discrimination was

352   affected by the method by which rattles were recorded. We pooled rattles into two

353   collection method categories: 'unsolicited' included those rattles collected from squirrels

354   moving freely around their territories, and 'provoked' included those rattles collected

355   from squirrels emerging from traps and those produced in response to rattles broadcast

356   from a speaker.

357

358   The effects of local density and recording method were tested separately by fitting

359   an interaction between each of these variables and relatedness (one model with

360   categorical kinship status and one model with continuous relatedness from the pedigree)

361   in the generalized linear models that predicted whether or not a squirrel rattled in

362   response to the playback (see above). A significant interaction would indicate that red

363   squirrels discriminate kin under some circumstances (e.g., local density), but not others.

364   We similarly tested for effects of sex of the subject squirrel, and the date of the playback

365   trial on kin discrimination, but the rationale for these post-hoc analyses was weaker, so

366   we did not report these nonsignificant results. We mention them briefly here to be

367   transparent about the scope of our post-hoc analyses.

368

369   RESULTS

370   Squirrels were significantly more likely to produce a rattle during the playback

371   period (42% of squirrels) than during the pre-playback period (26% of squirrels; Figure 1;

372   Table 2). Similarly, squirrels were significantly more likely to look in the direction of the

373   speaker during the playback period (44% of squirrels) than during the pre-playback

374   period (3% of squirrels; Figure 1; Table 2). Squirrels were not more likely to approach

375   the speaker during the playback period (7% of squirrels) than during the pre-playback

376   period (2% of squirrels; Figure 1; Table 2), so this variable was not included in

377   subsequent analyses. Subject identity did not improve any of the statistical models,

378   including the models for whether the subject produced a rattle (likelihood ratio test: $X^2 <$

379   0.1, df = 1, $P > 0.9$), looked at the speaker ($X^2 = 0.9$, df = 1, $P = 0.3$), or approached the

380   speaker ($X^2 < 0.1$, df = 1, $P > 0.9$). Therefore, a subject's behavior in the playback period

381   was independent of its behavior in the pre-playback period and in other playback trials.

382

383   Kinship status (familiar kin, unfamiliar kin, non-kin) and neighbor status

384   (neighbor, non-neighbor) did not have statistically significant effects on any of the

385   response variables, including whether subjects produced a rattle, whether subjects looked

386   at the speaker, or how quickly subjects produced a rattle following the onset of the

387   stimulus (Table 3). These results indicate that red squirrels did not discriminate between

388   playbacks of kin and non-kin. Subject identity did not improve the generalized linear

389   mixed models, including the models for whether the subject produced a rattle (likelihood

390   ratio test: $X^2 < 0.1$, df = 1, $P > 0.9$) or looked at the speaker ($X^2 < 0.1$, df = 1, $P > 0.9$).

391

392   We conducted exploratory post-hoc analyses in an attempt to understand the lack

393 of kin discrimination and the inconsistency of this finding with previous evidence of kin

394 discrimination in red squirrels (Gorrell et al. 2010; Williams et al. 2013; Wilson et al.

395 2015). Our first exploratory analysis replaced kinship status with known relatedness

396 coefficients (derived from the pedigree as a continuous covariate) as the independent

397 variable in the model with rattling as a response variable to test for an overall effect of

398 kin discrimination regardless of familiarity. The results of the model with relatedness

399 coefficient from the pedigree as a covariate were very similar to the results described

400 above with kinship status. There was no effect of relatedness on any of the response

401 variables (results not shown, see footnote in Table 3).

402

403 In our second exploratory analysis we tested the effect of local density on territorial

404 responses. Local population density (i.e., the number of squirrels with middens within a

405 130-m radius of the subject's midden) varied from 0.4 to 3.2 squirrels/ha. When local

406 density was included as an independent variable, we found that local density affected the

407 difference in response between unfamiliar kin and familiar kin, but did not affect the

408 difference in response between familiar kin and non-kin (Table 4). However, this effect

409 disappeared when we ran the same model with the relatedness coefficient as a covariate

410 (Table 4).

411

412 In our third exploratory analysis we tested whether kin discrimination was affected

413 by the method by which rattles were recorded. There were 67 trials with unsolicited

414 rattles as the stimulus and 38 trials with provoked rattles. In the models with collection

415 method as an independent variable, we found no significant interaction or main effect of

416 kinship status, when kinship was included as a categorical variable (Table 5). Thus there

417 was no difference in the responses between familiar kin, unfamiliar kin, and non-kin, and

418 no mediating effect of collection method on responses to these kinship status groups.

419 However, in the model with relatedness included as a continuous variable, we found a

420 significant interaction and a significant main effect of collection method, though the main

421 effect of relatedness was not significant (Table 5). Visual inspection of these results

422 indicated that this interaction between collection method and relatedness was largely

423 driven by the differential response of squirrels to rattles of kin with a relatedness

424 coefficient of 0.5 (Figure 2). Specifically, red squirrels were more likely to rattle in

425 response to provoked rattles if they were from non-kin or less related kin (r < 0.5) than

426 from more closely related kin (r = 0.5) (Figure 2). Taking the subset of trials that used

427 provoked rattle stimuli (n = 38 trials), we found that there was a marginally non-

428 significant effect of relatedness on propensity to rattle (generalized linear mixed model: Z

429 = -1.89, P = 0.058).

430

431 DISCUSSION

432

433   We found that across all playback trials red squirrels did not discriminate between

434 familiar kin, unfamiliar kin, and non-kin. Squirrels were just as likely to rattle or look

435 towards the speaker in response to a familiar kin rattle as they were to either an

436 unfamiliar kin or non-kin rattle, and there was no overall effect of the degree of

437 relatedness on their behavioral response. Similarly, squirrels did not discriminate between

438   neighbors and non-neighbors in their behavioral response. We also found no difference in

439   the latency of red squirrels to respond to the playback for either kinship or neighbor

440   status. This lack of effect of kin status on the response of red squirrels to the playback

441   cannot be explained by the playback stimulus not being detected by the focal squirrels.

442   Subject squirrels were more likely to look in the direction of the speaker following the

443   playback and to rattle in response to the playback, though they were not more likely to

444   approach the speaker. Squirrels, therefore, detected the experimental playbacks but

445   showed no evidence of kin discrimination in how they responded. This absence of kin

446   discrimination precluded us from evaluating mechanisms of kin recognition in red

447   squirrels.

448

449   Our findings differ from two similar territorial playback experiments previously

450   done on the same population of red squirrels in Kluane (Price et al. 1990; Wilson et al.

451   2015). The difference in findings between this study and the Wilson et al. (2015) study

452   are surprising, given the similarity in the methods. Indeed, the only differences were the

453   speaker used to broadcast the stimuli (a Saul Mineroff speaker in the present study and a

454   GPX portable stereo in the previous study), the sound level of the stimuli (not measured

455   in dB in the previous study), and the sample size of playback trials (105 trials in this

456   study compared to 53 in the previous study). We found no effect of neighbor status,

457   which is consistent with the results of the Wilson et al. (2015) study, but inconsistent with

458   the Price, Boutin, and Ydenberg (1990) study, which found that squirrels were more

459   likely to rattle in response to rattles from non-neighbors compared to rattles from

460   neighbors. In the Price, Boutin, and Ydenberg (1990) study, relatedness between subject-

461   stimulus pairs was unknown, and since neighbors tend to be more closely related than

462   non-neighbors (Berteaux and Boutin 2000), it is possible that their results are due to an

463   effect of kin discrimination (as in Wilson et al. 2015) as opposed to discrimination

464   between neighbors and non-neighbors. We found no difference in responses to familiar

465   kin, unfamiliar kin, and non-kin, and no overall effect of the degree of relatedness, which

466   was unexpected given the results of the Wilson et al. (2015) study that found that

467   squirrels were more likely to rattle in response to a non-kin ($r < 0.125$) rattle than to a kin

468   ($r \geq 0.25$) rattle. Despite the lack of discrimination in the responses from red squirrels in

469   this study, the results of the Wilson et al. (2015) study, as well as other documented cases

470   of nepotism in red squirrels, provide strong support that red squirrels are capable of

471   recognizing familiar kin (Price and Boutin 1993; Berteaux and Boutin 2000; Gorrell et al.

472   2010; Williams et al. 2013), even if they do not always behave differently towards kin

473   and non-kin individuals.

474

475   Several studies have found that animals discriminate kin in some contexts, but not

476   in others (Hokit et al. 1996; Gibbons et al. 2003; Harris et al. 2003). The benefits and

477   costs of kin discrimination may fluctuate depending on environmental conditions, even

478   within the same context, so it is possible that under certain conditions red squirrels do not

479   exhibit kin discrimination in their territorial defense behavior. In an attempt to understand

480   our negative results and to reconcile the discrepancy between our results and previous

481   research, we conducted exploratory post-hoc analyses to explore possible factors that

482   might have affected kin discrimination. In the red squirrel system, changes in population

483   density and food availability (abundance of spruce cones) are important environmental

484   factors that show large fluctuations from year to year (LaMontagne and Boutin 2007;

485   Fletcher et al. 2010), and which affect survival and reproductive success in females

486   (Descamps et al. 2008). We did not have the data to be able to account for food

487   abundance (i.e., amount of food cached by each subject squirrel), but added local

488   population density to our models because of previous evidence indicating that red

489   squirrels emit rattles more frequently when surrounded by a higher density of

490   conspecifics (Dantzer et al. 2012; Shonfield et al. 2012). We found that local density

491   affected the difference in response between unfamiliar kin and familiar kin, but did not

492   affect the difference in response between familiar kin and non-kin. This result did not

493   align with our original predictions. We had expected that, if red squirrels recognize kin

494   by phenotype matching/recognition alleles, they would be less likely to rattle in response

495   to calls from familiar and unfamiliar kin than to calls from non-kin, and, alternatively,

496   would be less likely to rattle in response to calls from familiar kin than to calls from

497   unfamiliar kin or non-kin if they recognize kin by prior association. As such this model

498   suggested that, if anything, there were density-mediated differential responses between

499   familiar and unfamiliar kin rattles, but not differential responses between kin and non-

500   kin. In addition, the effect of density disappeared when we ran the models with

501   relatedness coefficients calculated from the pedigree to test for an overall effect of kin

502   discrimination regardless of familiarity, suggesting that the interaction between density

503   and kinship was most likely spurious resulting from post hoc exploratory data analysis.

504

505   We also explored whether the method by which rattle stimuli were recorded

506   affected kin discrimination. Although there was no significant interaction with kinship

507   status in our post hoc analyses, we did find a significant interaction between the degree of

508   relatedness and collection method. We are not able to determine the importance of

509   familiarity in kin discrimination by red squirrels, but the results of the model with

510   relatedness coefficients suggests that when stimuli were recorded by provoking a squirrel

511   to rattle, the subject squirrel in the trial was more likely to discriminate between kin and

512   non-kin (i.e., more likely to rattle at non-kin). This tentatively suggests that the context in

513   which the stimulus was recorded might be important and that kin discrimination in the

514   overall analysis may have been masked by the difference in responses between collection

515   methods. The interaction between collection method and kinship class was not significant

516   (Table 5), but, in this analysis, rattles from kin with relatedness coefficients equal to 0.25

517   were considered to be kin a priori (see also Wilson et al. 2015). Visual inspection of the

518   results with relatedness coefficients, however, suggests that closely-related squirrels ($r =$

519   0.5) responded differently than more distantly-related squirrels ($0 < r < 0.5$; Figure 2),

520   which might have led to heterogeneity in the responses of squirrels to rattles classified as

521   kin ($r \geq 0.25$). Future studies are needed to explicitly test the importance of the degree of

522   relatedness to kin discrimination in red squirrels. These preliminary results suggest that

523   kin discrimination by red squirrels might depend on the circumstances under which the

524   stimulus call is recorded, and suggests that if we had run the experiment using only

525   provoked rattle stimuli we might have detected an effect of kin discrimination. However,

526   we must explicitly acknowledge that this relationship was identified through exploratory

527   post hoc analyses and needs to be tested more rigorously (Simmons et al. 2011; Motulsky

528   2014). If these results are robust, however, they would suggest that a squirrel's

529   physiological state might influence the structure of its rattles, including those individually

25

530   distinctive structural features (Digweed et al. 2012; Wilson et al. 2015) that are

531   presumably used in discrimination. This raises the interesting possibility that the receiver

532   obtains information from rattles about the physiological state of the signaler, and could be

533   important in assessing the costs and benefits of discriminating kin from non-kin.

534

535   Although we found no overall evidence for kin discrimination in red squirrels, our

536   results hint at the possibility that kin discrimination in red squirrels is context-dependent.

537   We suspect that the costs and benefits of responding to territorial intrusions by kin and

538   non-kin might be mediated by both environmental and social factors. While we found no

539   evidence that kin discrimination is due to the local density of potential territory intruders,

540   we did find post-hoc evidence that kin discrimination might be mediated by the

541   conditions under which the stimulus call is recorded. Our results raise questions about the

542   information contained in the rattles and suggest that they may reflect the current state of

543   stress or aggressiveness of the squirrel. Future studies on kin recognition in red squirrels

544   or other species should explicitly test the importance of environmental or social factors

545   on kin discrimination in order to better understand the costs and benefits of preferential

546   behavior toward kin.

547

26

557

558   ACKNOWLEDGEMENTS

559

567

568   DATA ACCESSIBILITY

569   Analyses reported in this article can be reproduced using the data provided by Shonfield

570   et al. (2016).

571

572   REFERENCES

573   Bates D, Maechler M, Bolker BM, Walker S. 2015. Fitting linear mixed-effects models

574   using lme4. J. Stat. Softw. 67:1–48.

575   Beecher MD. 1982. Signature systems and kin recognition. Am. Zool. 22:477–490.

576   Berteaux D, Boutin S. 2000. Breeding dispersal in female North American red squirrels.

577   Ecology 81:1311–1326.

578   Blaustein AR. 1983. Kin recognition mechanisms: phenotype matching or recognition

579   alleles? Am. Nat. 121:749–754.

580   Buczkowski G, Silverman J. 2005. Context-dependent nestmate discrimination and the

581   effect of action thresholds on exogenous cue recognition in the Argentine ant.

582   Anim. Behav. 69:741–749.

583   Couvillon MJ, Robinson EJH, Atkinson B, Child L, Dent KR, Ratnieks FLW. 2008. En

584   garde: rapid shifts in honeybee, *Apis mellifera*, guarding behaviour are triggered by

585   onslaught of conspecific intruders. Anim. Behav. 76:1653–1658.

586   Couvillon MJ, Segers FHID, Cooper-Bowman R, Truslove G, Nascimento DL,

587   Nascimento FS, Ratnieks FLW. 2013. Context affects nestmate recognition errors

588   in honey bees and stingless bees. J. Exp. Biol. 216:3055–3061.

589   Dantzer B, Boutin S, Humphries MM, McAdam AG. 2012. Behavioral responses of

590   territorial red squirrels to natural and experimental variation in population density.

591   Behav. Ecol. Sociobiol. 66:865–878.

592   Dantzer B, Newman AEM, Boonstra R, Palme R, Boutin S, Humphries MM, McAdam

593   AG. 2013. Density triggers maternal hormones that increase adaptive offspring

594   growth in a wild mammal. Science. 340:1215–1217.

595   Dawkins R. 1976. The selfish gene. Oxford: Oxford University Press.

596   Descamps S, Boutin S, Berteaux D, McAdam AG, Gaillard JM. 2008. Cohort effects in

597   red squirrels: the influence of density, food abundance and temperature on future

598   survival and reproductive success. J. Anim. Ecol. 77:305–314.

599   Digweed SM, Rendall D. 2009. Predator-associated vocalizations in north american red

600   squirrels (*Tamiasciurus hudsonicus*): to whom are alarm calls addressed and how

601   do they function? Ethology 115:1190–1199.

602   Digweed SM, Rendall D, Imbeau T. 2012. Who's your neighbor? Acoustic cues to

603   individual identity in red squirrel *Tamiasciurus hudsonicus* rattle calls. Curr. Zool.

604   58:758–764.

605   Downs SG, Ratnieks F. 2000. Adaptive shifts in honey bee (*Apis mellifera* L.) guarding

606   behavior support predictions of the acceptance threshold model. Behav. Ecol.

607   11:326–333.

608   Fisher JB. 1954. Evolution and bird sociality. In: Huxley J, Hardy AC, Ford EB, editors.

609   Evolution as a process. London: Allen & Unwin. p. 71–83.

610   Fletcher QE, Boutin S, Lane JE, LaMontagne JM, McAdam AG, Krebs CJ, Humphries

611   MM. 2010. The functional response of a hoarding seed predator to mast seeding.

612   Ecology 91:2673–2683.

613   Flores-Prado L, Niemeyer HM. 2010. Kin recognition in the largely solitary bee,

614   *Manuelia postica* (Apidae: Xylocopinae). Ethology 116:466–471.

615   Fuller CA, Blaustein AR. 1990. An investigation of sibling recognition in a solitary

616   sciurid, Townsend's chipmunk, *Tamias townsendii*. Behaviour 112:36–52.

617   Gibbons ME, Ferguson AM, Lee DR, Jaeger RG. 2003. Mother-offspring discrimination

618   in the red-backed salamander may be context dependent. Herpetologica 59:322–

619   333.

620   Gorrell JC, McAdam AG, Coltman DW, Humphries MM, Boutin S. 2010. Adopting kin

621   enhances inclusive fitness in asocial red squirrels. Nat. Commun.:1–22.

622   Gunn MR, Dawson DA, Leviston A, Hartnup K, Davis CS, Strobeck C, Slate J, Coltman

623   DW. 2005. Isolation of 18 polymorphic microsatellite loci from the North

624   American red squirrel, *Tamiasciurus hudsonicus* (Sciuridae, Rodentia), and their

625   cross-utility in other species. Mol. Ecol. Notes 5:650–653.

626   Hamilton WD. 1964. The genetical evolution of social behaviour I and II. J. Theor. Biol.

627   7:1–52.

628   Hare JF. 2004. Kin discrimination by asocial Franklin's ground squirrels (*Spermophilus*

629   *franklinii*): is there a relationship between kin discrimination and ground squirrel

630   sociality? Ethol. Ecol. Evol. 16:157–169.

631   Harris RN, Vess TJ, Hammond JI, Lindermuth CJ. 2003. Context-dependent kin

632   discrimination in larval four-toed salamanders *Hemidactylium scutatum* (Caudata:

633   Plethodontidae). Herpetologica 59:164–177.

634   Hokit DG, Walls SC, Blaustein AR. 1996. Context-dependent kin discrimination in

635   larvae of the marbled salamander, *Ambystoma opacum*. Anim. Behav. 52:17–31.

636   Holmes WG, Sherman PW. 1982. The ontogeny of kin recognition in two species of

637   ground squirrels. Am. Zool. 22:491–517.

638   Holmes WG, Sherman PW. 1983. Kin recognition in animals. Am. Sci. 71:46–55.

639   Humphries MM, Boutin S. 1996. Reproductive demands and mass gains: A paradox in

640   female red squirrels (*Tamiasciurus hudsonicus*). J. Anim. Ecol. 65:332–338.

641   Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program

642   CERVUS accommodates genotyping error increases success in paternity

643   assignment. Mol. Ecol. 16:1099–1106.

644   Lair H. 1990. The calls of the red squirrel: a contextual analysis of function. Behaviour

645   115:254–281.

646   LaMontagne JM, Boutin S. 2007. Local-scale synchrony and variability in mast seed

647   production patterns of *Picea glauca*. J. Ecol. 95:991–1000.

648   Lane JE, Boutin S, Gunn MR, Slate J, Coltman DW. 2007. Genetic relatedness of mates

649   does not predict patterns of parentage in North American red squirrels. Anim.

650   Behav. 74:611–619.

651   Lane JE, McAdam AG, Charmantier A, Humphries MM, Coltman DW, Fletcher Q,

652   Gorrell JC, Boutin S. 2015. Post-weaning parental care increases fitness but is not

653   heritable in North American red squirrels. J. Evol. Biol. 28:1203–1212.

654   McAdam AG, Boutin S, Réale D, Berteaux D. 2002. Maternal effects and the potential

655   for evolution in a natural population of animals. Evolution. 56:846–851.

656   McAdam AG, Boutin S, Sykes AK, Humphries MM. 2007. Life histories of female red

657   squirrels and their contributions to population growth and lifetime fitness.

658   Ecoscience 14:362–369.

659   McFarlane SE, Gorrell JC, Coltman DW, Humphries MM, Boutin S, McAdam AG. 2014.

660   Very low levels of direct additive genetic variance in fitness and fitness

661   components in a red squirrel population. Ecol. Evol. 4:1729–1738.

662   McFarlane SE, Gorrell JC, Coltman DW, Humphries MM, Boutin S, McAdam AG. 2015.

663   The nature of nurture in a wild mammal's fitness. Proc. R. Soc. B 282:20142422.

664   Motulsky HJ. 2014. Common misconceptions about data analysis and statistics. J.

665   Pharmacol. Exp. Ther. 351:200–205.

666   Price K, Boutin S. 1993. Territorial bequathal by red squirrel mothers. Behav. Ecol.

667   4:144–155.

668   Price K, Boutin S, Ydenberg R. 1990. Intensity of territorial defense in red squirrels: an

669   experimental test of the asymmetric war of attrition. Behav. Ecol. Sociobiol.

670   27:217–222.

671   Pusey A, Wolf M. 1996. Inbreeding avoidance in animals. Trends Ecol. Evol. 11:201–

672   206.

673   R Core Team. 2016. R: A language and environment for statistical computing.

674   Sherman PW. 1977. Nepotism and the evolution of alarm calls. Science. 197:1246–1253.

675   Shonfield J, Taylor RW, Boutin S, Humphries MM, McAdam AG. 2012. Territorial

676   defence behaviour in red squirrels is influenced by local density. Behaviour

677   149:369–390.

678   Simmons JP, Nelson LD, Simonsohn U. 2011. False-positive psychology: undisclosed

679   flexibility in data collection and analysis allows presenting anything as significant.

680   Psychol. Sci. 22:1359–1366.

681   Smith CC. 1968. The adaptive nature of social organization in the genus of tree squirrels

682   Tamiasciurus. Ecol. Monogr. 38:31–64.

683   Smith CC. 1978. Structure and function of the vocalizations of tree squirrels

684   (Tamiasciurus). J. Mammal. 59:793–808.

685   Sun L, Müller-Schwarze D. 1997. Sibling recognition in the beaver: a field test for

686   phenotype matching. Anim. Behav. 54:493–502.

687   Temeles EJ. 1994. The role of neighbours in territorial systems: when are they "dear

688   enemies"? Anim. Behav. 47:339–350.

689   Thernau T, Lumley T. 2009. survival: Survival analysis, including penalised likelihood.

690   Waldman B. 1987. Mechanisms of kin recognition. J. Theor. Biol. 128:159–185.

691   Williams CT, Gorrell JC, Lane JE, McAdam AG, Humphries MM, Boutin S. 2013.

692   Communal nesting in an "asocial" mammal: social thermoregulation among

693   spatially dispersed kin. Behav. Ecol. Sociobiol. 67:757–763.

694   Wilson DR, Goble AR, Boutin S, Humphries MM, Coltman DW, Gorrell JC, Shonfield J,

695   McAdam AG. 2015. Red squirrels use territorial vocalizations for kin

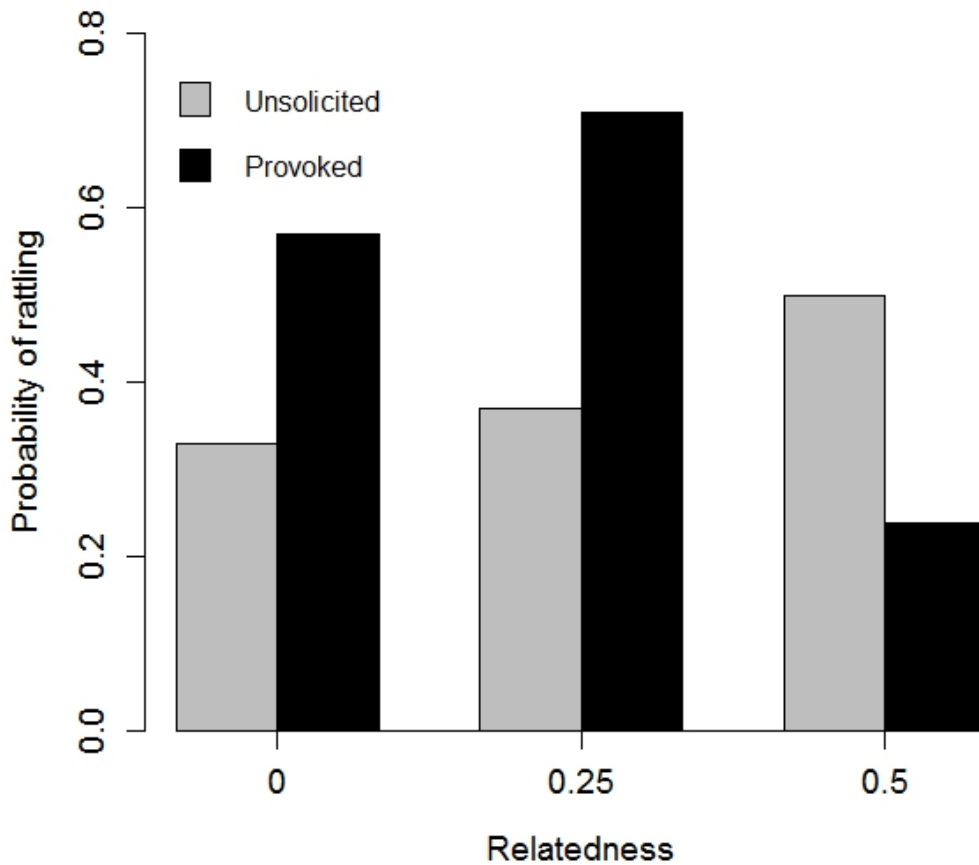696   discrimination. Anim. Behav. 107:79–85.

697
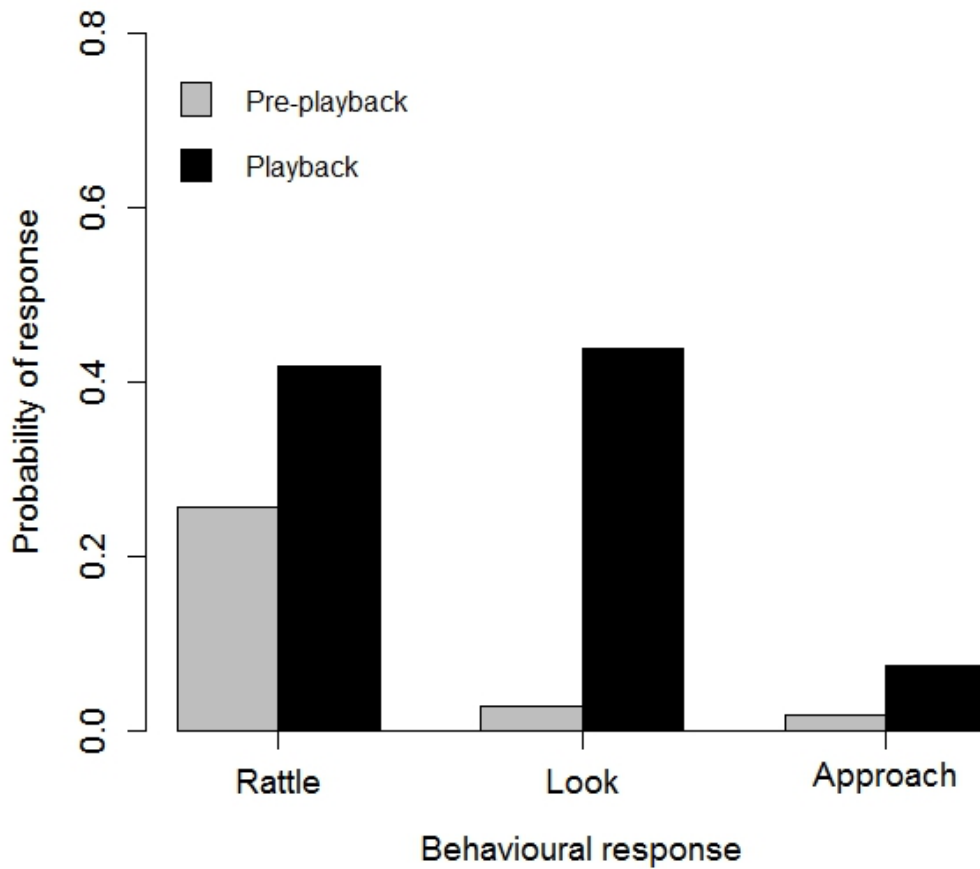
698

699 **Figure 1** – Probability of behavioral responses (producing a territorial 'rattle' call,

700 looking at the speaker, and approaching the speaker) of the subject before and during the

701 playback period (N = 105 trials).

702

703

Figure 2 – Probability of a rattle response from the subject squirrel during the playback

period by relatedness coefficient calculated from the pedigree and the collection method

of obtaining the rattle stimulus. Unsolicited rattles were recorded from squirrels moving

freely around their territories (N = 67 trials), and provoked rattles (N = 38 trials) were

recorded from squirrels as they emerged from a live-trap or from squirrels responding to

a rattle playback.

710

711 TABLES

712

713 **Table 1** – Kin relationships within each kinship status category and the number of trials

714 completed. 'Familiar' kin denotes individuals with early life associations (i.e., shared a

715 natal nest).

Kinship	Kin relationship (subject-stimulus)	No. of trials	Relatedness coefficient
Familiar kin	Mother-offspring	6	0.5
	Offspring-mother	9	0.5
	Full siblings (littermates)	10	0.5
	Maternal half-siblings (littermates)	12	0.25
Unfamiliar kin	Father-offspring	3	0.5
	Offspring-father	9	0.5
	Full siblings (non-littermates)	3	0.5
	Maternal half-siblings (non-littermates)	7	0.25
	Paternal half-siblings (non-littermates)	4	0.25
	Grandparent-grand offspring	2	0.25
	Grand offspring-grandparent	2	0.25
Non-kin	None	38	<0.125
Total		105	

716

717   **Table 2** – Responses of subject squirrels to the playback rattle. Responses include

718   whether subjects produced a rattle, looked at the speaker, and approached the speaker.

Response variable	Effect	Estimate ± SE	Z	p
Rattle[1]	Intercept	-1.06 ± 0.22	-4.75	**< 0.0001**
	Period (playback)	0.73 ± 0.30	-2.46	**0.014**
Looking at speaker[2]	Intercept	-3.85 ± 0.74	-5.18	**< 0.0001**
	Period (playback)	3.54 ± 0.72	4.89	**< 0.0001**
Approach the speaker[3]	Intercept	-3.94 ± 0.71	-5.52	**< 0.0001**
	Period (playback)	1.44 ± 0.80	1.80	0.072

719   Responses were modeled using three separate linear mixed models (binary response, logit link) with subject

720   identity as a random effect. Estimates are on a log-odds scale and the effects of the factor in the design are

721   reported as the effect of the level in parentheses (*e.g.,* playback) relative to the reference category (pre-

722   playback). Significant p-values are in bold (significance level $\alpha = 0.05$).

723   [1]Random effect of squirrel identity: variance = 0

724   [2]Random effect of squirrel identity: variance = 0.72

725   [3]Random effect of squirrel identity: variance = 0

726

727 **Table 3** – Effects of kinship and neighbor status on the behavioral response from the

728 subject squirrel.

Response variable	Effect	Estimate ± SE	Z	p
Rattling[1,2]	Intercept	-0.24 ± 0.36	-0.68	0.498
	Kinship (non-kin)	-0.03 ± 0.48	-0.06	0.952
	Kinship (unfamiliar kin)	-0.12 ± 0.50	-0.25	0.804
	Neighbor status (non-neighbor)	-0.08 ± 0.40	-0.20	0.845
Looking at speaker[1]	Intercept	-0.46 ± 0.37	-1.24	0.216
	Kinship (non-kin)	0.12 ± 0.48	0.26	0.797
	Kinship (unfamiliar kin)	0.23 ± 0.50	0.45	0.653
	Neighbor status (non-neighbor)	0.22 ± 0.40	0.54	0.589
Latency to rattle[3]	Kinship (non-kin)	-0.06 ± 0.41	-0.15	0.879
	Kinship (unfamiliar kin)	-0.20 ± 0.46	-0.43	0.667
	Neighbor status (non-neighbor)	0.17 ± 0.36	0.47	0.637

729 [1]Responses were modeled using a generalized linear mixed model (binary response, logit link), with subject

730 identity as a random effect. The random effect for both models (rattling and looking at the speaker) had

731 among-individual variance of zero. Estimates are on a log-odds scale and the effects of each factor in the

732 design are reported as the effect of the level in parentheses (*e.g.,* non-kin) relative to the reference category.

733 In this case the reference category is 'familiar kin' for kinship, and 'neighbor' for neighbor status.

734 [2]The model with rattling as a response variable was also run with relatedness coefficient (calculated from

735 the pedigree) in place of kinship status as the kin variable as part of our post-hoc exploratory analyses, but

736 the results with respect to statistical significance did not differ from those above and are not shown.

737 [3]Latency to rattle (range: 4.7-173.9 s, average ± SE: 66.5 ± 8.1s) was modeled using a Cox proportional

738 hazard model with a reduced dataset (n=85 trials).

739

740   **Table 4** – Effect of local population density and either kinship status or relatedness

741   (coefficient calculated from the pedigree) on the probability of a rattle response from the

742   subject squirrel following the playback.

Model	Effect	Estimate ± SE	Z	p
Model with kinship status	Intercept	-1.45 ± 0.89	-1.63	0.103
	Kinship (non-kin)	1.25 ± 1.23	1.01	0.311
	Kinship (unfamiliar kin)	2.51 ± 1.29	1.95	**0.051**
	Local density	0.70 ± 0.49	1.45	0.148
	Kinship (non-kin) x Local density	-0.77 ± 0.68	-1.14	0.256
	Kinship (unfamiliar kin) x Local density	-1.88 ± 0.85	-2.20	**0.028**
Model with relatedness coefficient	Intercept	0.46 ± 0.80	0.58	0.565
	Relatedness	-2.86 ± 2.25	-1.27	0.204
	Local density	-0.44 ± 0.45	-0.98	0.329
	Relatedness x Local density	1.66 ± 1.31	1.27	0.206

743   Probability of a rattle response was modeled using a generalized linear mixed model (binary response, logit

744   link), with subject identity as a random effect. The random effect for both models had an among-individual

745   variance of zero. Estimates are on a log-odds scale and the effects of each factor in the design are reported

746   as the effect of the level in parentheses (*e.g.,* non-kin) relative to the reference category. In this case the

747   reference category for kinship is 'familiar kin'. Significant p-values are in bold (significance level α =

748   0.05).

749

750 **Table 5** – Effect of rattle stimulus collection method (unsolicited or provoked) and either

751 kinship status or relatedness (coefficient calculated from the pedigree) on the probability

752 of a rattle response from the subject squirrel following the playback.

Model	Effect	Estimate ± SE	Z	p
Model with kinship status	Intercept	-0.34 ± 0.41	-0.81	0.416
	Kinship (non-kin)	-0.36 ± 0.60	-0.60	0.552
	Kinship (unfamiliar kin)	0.23 ± 0.62	0.37	0.709
	Method (provoked)	0.18 ± 0.69	0.26	0.793
	Kinship (non-kin) x Method (provoked)	0.80 ± 0.98	0.82	0.415
	Kinship (unfamiliar kin) x Method (provoked)	-1.06 ± 1.07	-0.99	0.324
Model with relatedness coefficient	Intercept	-0.75 ± 0.40	-1.87	0.062
	Relatedness	1.40 ± 1.20	1.17	0.242
	Method (provoked)	1.31 ± 0.66	1.98	**0.048**
	Relatedness x Method (provoked)	-4.32 ± 1.95	-2.21	**0.027**

753 Probability of a rattle response was modeled using a generalized linear mixed model (binary response, logit

754 link), with subject identity as a random effect. The random effect for both models had an among-individual

755 variance of zero. Estimates are on a log-odds scale and the effects of each factor in the design are reported

756 as the effect of the level in parentheses (*e.g.,* non-kin) relative to the reference category. In this case the

757 reference category is 'familiar kin' for kinship and 'unsolicited' for collection method. Significant p-values

758 are in bold (significance level $\alpha = 0.05$).

759