Indirect effects of territorial neighbors on the timing of spring breeding may counteract changes in selection in North American red squirrels

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Abstract

Organisms can affect one another's phenotypes when they socially interact. Indirect genetic effects occur when an individual's phenotype is affected by genes expressed in another individual. These heritable effects can enhance or reduce adaptive potential, thereby accelerating or reversing evolutionary change. Quantifying these social effects is therefore crucial for our understanding of evolution, yet estimates of indirect genetic effects in wild animals are limited to dyadic interactions. We estimated indirect phenotypic and genetic effects, and their covariance with direct effects, in North American red squirrels (*Tamiasciurus hudsonicus*) living in an array of territories of varying spatial proximity. Additionally, we estimated variance parameters and the strength of selection at high and low population densities. Social effects of neighbors on date of spring breeding were weak at low population densities, but stronger at high population densities. Although indirect phenotypic effects were detected, the genetic component to these was not statistically significant. Nevertheless,

34 the estimated effect size was large enough to suggest that indirect genetic effects would alter

35 evolutionary change, giving less change at high densities despite stronger selection. The (uncertain)

36 potential for indirect genetic effects to alter evolution suggests that they have potentially important

37 consequences for any natural systems where organisms interact.

38 **Key words**: evolution, indirect genetic effects, KRSP, selection, social interactions, *Tamiasciurus*

39

40 ## Introduction

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42 An individual's phenotype is influenced by both its genotype and the environment it experiences.

43 When organisms live in a social world, moving, mating, competing and cooperating with conspecifics

44 (Frank 2007), the environment they experience is partly made up of the phenotypes of other

45 individuals. If the elements of the phenotype that influence other individuals have a genetic

46 component, then a portion of an individual's phenotype will be influenced by the genes of others

47 (Griffing 1967). These are known as indirect genetic effects (IGEs), of which maternal genetic effects

48 are a widely known example (Moore et al. 1997; Wolf et al. 1998; McAdam et al. 2014). With

49 maternal genetic effects, the genes of a mother influence the traits of her offspring beyond those

50 directly inherited (e.g. a mammal's genes affecting milk production may influence the growth rate of

51 her offspring; (Koch 1972; María et al. 1993). In the same way, the genes affecting hunting success of

52 an individual (so DGEs for condition) in a pack-hunting species may influence the body condition of

53 its pack mates (an IGE). Additionally, when competing for limited resources, the genes for resource

54 acquisition of one individual are expected to negatively influence the resource acquisition, and so

55 resource dependent traits, of individuals with which it competes (Wilson 2014). Therefore, IGEs can

56 be expected in almost any system where conspecifics interact with each other (McAdam et al. 2014).

57 As IGEs are heritable by definition, they contribute additional heritable variation within a

58 population alongside direct genetic effects (DGEs; i.e. an effect on an individual's genes on its own

59 phenotype; Moore et al. 1997; Bijma and Wade 2008). Similar to a genetic correlation between any

60 two traits (Lande 1979; Kirkpatrick 2009), an individual's own phenotype for some focal trait and its

61 indirect effect on that trait expressed by neighbors can be genetically correlated (a DGE-IGE

62 correlation). When IGEs are positively correlated with DGEs, which we would expect for the trait of

63 hunting success in the pack-hunter example above, IGEs can enhance that trait's response to

64 directional selection. This happens because the standard response of the focal trait to selection

65 results in a correlated evolutionary change in the social environment. This in turn causes further

66 change in focal trait mean - in the same direction - through a plastic response to the social

67 environment (Moore et al. 1997).

68   Conversely, if IGEs are negatively correlated with DGEs then the population response to

69   selection can be reduced, removed, or even reversed (Bijma and Wade 2008; Wilson 2014). Negative

70   correlations are expected for focal traits that are themselves dependent on the outcome of

71   competition for limited resources (Wilson 2014). For instance, Wade (1976) observed a decrease in

72   mean reproductive output across generations in flour beetles (*Tribolium castaneum*) that were

73   under individual selection for *increased* reproduction. This was presumably due to a negative IGE-

74   DGE correlation that caused each subsequent generation to be composed of individuals that more

75   strongly suppressed the reproduction of others through competitive interactions. Furthermore,

76   Costa e Silva *et al.* (2013) observed a strong negative DGE-IGE covariance for diameter at breast

77   height in eucalyptus trees (*Eucalyptus globulus*), indicating that trees with DGEs for fast growth had

78   IGEs that caused their neighbors to grow less. This DGE-IGE covariance nearly completely

79   counteracted the evolutionary potential set by DGEs for this trait. This means that despite tree

80   growth rates being heritable in the traditional sense (i.e. subject to DGEs), the total heritable

81   variation in the population was near zero, preventing a response to selection. Estimates of DGEs

82   alone might, therefore, provide a poor measure of the potential for a trait to respond to natural

83   selection, yet most estimates of response to selection or evolvability in the wild only consider DGEs

84   (Houle 1992). More specifically, to the extent to which resources are limited in nature, we might

85   expect DGEs to consistently overestimate the adaptive potential of resource-dependent traits

86   because of negatively covarying IGEs (Wilson 2014). As such, IGEs arising from competition

87   represent one possible explanation for the "paradox of stasis", in which natural selection on

88   heritable traits often leads to stasis rather than evolutionary change (Merilä et al. 2001), yet IGEs are

89   very rarely quantified in the wild.

90   To date, empirical studies of IGEs in animals have focussed on scenarios in which within

91   group interactions can be considered (approximately) uniform, and among-group interactions are

92   absent. This allows IGEs to be estimated from the covariance between phenotypes of group mates,

93   provided pedigree data spanning groups are available (Bijma 2010a). This approach is well suited to

94   dyadic interactions, but also to larger discrete groups (n > 2) of captive animals, where all individuals

95   within a pen are assumed to interact equally, but no interactions occur between individuals in

96   different pens. It has now been applied in a variety of taxa, such as mussels (*Mytilus*

97   *galloprovincialis*) (Brichette et al. 2001), flour beetles (*T. castaneum*) (Ellen et al. 2016), Nile tilapia

98   (*Oreochromis niloticus*) (Khaw et al. 2016), domesticated chickens (*Gallus domesticus*) (Muir 2005;

99   Brinker et al. 2015) mink (*Neovison vison*) (Alemu et al. 2014), and domestic rabbits (*Oryctolagus*

100   *cuniculus*) (Piles et al. 2017). This work has helped establish the importance of IGEs for trait

101   evolution (see: Ellen *et al.* 2014, for a review in livestock), and has led to growing interest in studying

102   IGEs in wild populations.

103   Studies of IGEs in free-living animal populations have however been largely confined to

104   dyadic interactions. Here the "group" can be simply defined as the interacting pair, be that mother

105   and offspring, a breeding pair, or antagonists in a dyadic contest. For example, Wilson *et al.* (2011)

106   demonstrated that the tendency to win fights in wild red deer (*Cervus elaphus*) is subject to both

107   DGEs and IGEs that are perfectly *negatively* correlated, resulting in a total heritable variation of zero.

108   This reconciles quantitative genetic predictions with a common sense approach that sees that the

109   tendency to win cannot evolve at the population level, as each contest must always have one winner

110   and one loser (Wilson et al. 2009, 2011). Similar consequences of IGEs have been demonstrated in

111   captive deer mice (*Peromyscus maniculatus*) (Wilson et al. 2009), and in contests between fighting

112   cattle (*Bos taurus*) (Sartori and Mantovani 2013). In addition, Brommer and Rattiste (2008) showed

113   that male common gulls (*Larus canus*) exert IGEs on the laying dates of their mate, but these effects

114   are negatively correlated with the DGE for laying date in females, which would constrain the

115   evolution of this trait.

116   While social processes in wild populations frequently involve interactions among more than

117   two individuals, it is often problematic to identify and define discrete groups where n > 2. In many

118   cases an individual will interact with multiple conspecifics, but not all at equal intensity. Some

119   interactions are frequent or strong while other interactions are brief or weak (Lusseau et al. 2003;

120   Croft et al. 2004, 2008). One possibility is that organisms interacting in larger groups have generally

121   weaker indirect effects on each of their group mates, as a consequence of their phenotype being

122   "diluted" among more group members (Muir 2005; Hadfield and Wilson 2007; Bijma 2010b).

123   However, within a continuous population (i.e. one in which distinct groups cannot be identified) it

124   seems likely the net effect of one individual on the phenotype of any other may depend on distance

125   or other factors (e.g. time associating) that mediate interaction intensity or frequency (Muir 2005;

126   Cappa and Cantet 2008). To model these situations, variation in interaction strengths can be

127   incorporated as "dilution" or "intensity of competition" factors in IGE models (Muir 2005; Cappa and

128   Cantet 2008; Bijma 2010b). Here we refer to "intensity of association" factors, as social interactions

129   are not always competitive. This approach has proven useful in forestry genetics to estimate DGEs,

130   IGEs, and their covariance, on growth traits in Eucalyptus trees (Costa e Silva et al. 2013, 2017). In

131   this context, the inverse of the distance between each pair of trees was used as the intensity of

132   association factor. The important premise here is that each focal individual has a potential indirect

133   genetic effect on the phenotype of all its social partners, but the degree to which any particular

134   partner experiences that effect is contingent on its distance from the focal individual. Incorporating

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135 intensity of association factors should be equally useful for animal focused IGE models, as it would

136 allow us to account for animals interacting with multiple different individuals, in groups of varying

137 sizes, and with different individuals at different strengths; a realistic representation of social

138 interactions in the natural world (Fisher and McAdam 2017).

139 Here we used intensity of association factors to model IGEs for the first time in a wild

140 animal. We applied this framework to a population of North American red squirrels (*Tamiasciurus*

141 *hudsonicus*, hereafter "red squirrels") that have been continuously studied since 1987. We looked at

142 a resource-dependent, but also heritable ($h^2 = 0.14$) life-history trait: parturition date (the date in

143 the spring on which a female squirrel gives birth to a litter; Réale et al. 2003; Boutin et al. 2006; Kerr

144 et al. 2007; Lane et al. 2018), which had the potential to show IGEs. Red squirrels in this population

145 live on individual exclusive territories based around a central cache of white spruce (*Picea glauca*)

146 cones called a "midden". The seeds from stored spruce cones represents their main food source

147 during reproduction in the spring (Fletcher et al. 2013a). Individuals make territorial calls ("rattles")

148 to delineate territory boundaries (Lair 1990) and deter intruders (Siracusa et al. 2017) from stealing

149 cached resources (Gerhardt 2005; Donald and Boutin 2011). Previous analyses have shown that

150 selection favors earlier parturition dates (Réale et al. 2003), while a food supplementation

151 experiment resulted in a significant advancement in the average timing of spring breeding (Kerr et al.

152 2007). Note however that females can upregulate reproduction prior to a resource pulse (Boutin et

153 al. 2006), and so typically are reproducing below capacity (Boutin et al. 2013), so may not be

154 absolutely food limited. Still, if neighbors compete for food resources, we expect superior

155 competitors to have access to more food and breed earlier. Conversely, competitively inferior

156 individuals are expected to acquire less food and so breed later.

157 Population density is a key demographic parameter with which we expect IGEs to vary.

158 Selection on birth dates is particularly strong in years of high density (Williams et al. 2014; Fisher et

159 al. 2017; although not found in Dantzer et al. 2013). Furthermore, red squirrels respond behaviorally

160 to both real and perceived increases in density (Dantzer et al. 2012), while mothers adaptively

161 increase the growth rates of their offspring under high density conditions (Dantzer et al. 2013).

162 Taken together, these findings are consistent with the expectation that, all else being equal, high

163 density means increased competition.

164 In light of the above, we had the following aims and predictions. We expected individuals to

165 have indirect effects on the parturition dates of their neighbors, and that a portion of this indirect

166 effect would be heritable, and therefore an IGE (McAdam et al. 2014; Wilson 2014). As we expect

167 there to be competition for limited resources, we expected the DGE-IGE covariance to be negative

168 (following Costa e Silva et al. 2013; see also: Piles et al. 2017). That is, superior competitors will

169   breed earlier and cause their neighbors to breed later. Due to increased competition in years of high

170   density, these indirect effects should be stronger in those years, both in the magnitude of the

171   indirect effects but also in the strength of the covariance. This covariance is particularly important,

172   since – as outlined above - it defines whether the IGEs are expected to increase or reduce the

173   expected response to directional selection. Finally, we applied the equations of Bijma and Wade

174   (2008) and Costa e Silva et al. (2013) to predict a response to selection in the presence of IGEs,

175   which since IGEs are rarely quantified for wild animal populations, is also a first. This allowed us to

176   assess how IGEs were likely to affect evolutionary responses to selection in this system.

177

178   ## Methods

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180   Data collection

181   All data were collected as part of the Kluane Red Squirrel Project in the southwest Yukon, Canada.

182   Since 1987 we have monitored two adjacent 40 ha. study areas ("Kloo" and "Sulphur"), bisected by

183   the Alaska highway. Red squirrels of both sexes defend exclusive resource-based territories of

184   around 0.3 ha (LaMontagne et al. 2013), centred around a midden, an aggregation of discarded

185   white spruce cone scales underneath which red squirrels cache intact white spruce cones. Each

186   study area is staked at 30m intervals in a grid system and we recorded the x- and y-coordinates of

187   the center of each midden (to the nearest 3m). In the spring of each year we live trapped

188   (Tomahawk Live Trap, Tomahawk, WI, USA) new individuals and gave them unique ear tags in each

189   ear. We also located females (based on vocalizations at known and new territory locations),

190   monitored them for signs of pregnancy and ear tagged their pups once they were born. Based on the

191   previously identified stages of female pregnancy and the body mass of the pups once they were

192   located, we then estimated the female's parturition date. We also conducted censuses twice yearly

193   (once in spring, once in autumn) using complete enumeration to ascertain the location of all

194   individuals holding a territory, and so estimate population density. See McAdam *et al.* (2007) for

195   further details on the study system.

196   Red squirrels collect food throughout the summer and autumn, cache it in their middens and

197   rely on it to survive over winter (Fletcher et al. 2013a). The number of cached cones is positively

198   associated with overwinter survival (juveniles: Larivée et al. 2010; juveniles and adults: LaMontagne

199   et al. 2013). Squirrels primarily forage close to their midden, with occasional forays further afield,

200   including small amounts of pilferage from other red squirrels' hoards (Donald and Boutin 2011). We

201   define the individuals a red squirrel potentially competes with as its n nearest neighbors (n was set

202   at 6 for the majority of this analysis, but see below for explorations with different numbers of

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203   neighbors). We defined neighborhoods and population densities based on our autumn census

204   (August) rather than our spring census (May), because autumn is when squirrels are potentially

205   competing for resources to hoard, and conception occurs well before May in most years. Gestation

206   varies little around 35 days (Lair 1985), hence parturition dates cannot be influenced by conditions

207   after conception. Squirrels occasionally defend a second adjacent midden, but we considered each

208   squirrel's location to be based on the location of its primary midden. We then analysed each

209   female's parturition date the following spring as influenced by her own genes (the DGE), and the

210   identities and genotypes (the IGE) of those competing individuals as identified in the Autumn census.

211   Some females gave birth in multiple years, in which case they were included each year they did so,

212   with an updated set of nearest neighbors as necessary. Females may attempt multiple litters in years

213   of high resources, or if their first litter fails (Boutin et al. 2006; McAdam et al. 2007; Williams et al.

214   2014), but we limited our analyses to each female's first litter of each year (e.g. Dantzer et al. 2013).

215   We tagged pups while they were still on their mother's territory, so maternity is known for

216   all non-immigrants. Male red squirrels provide no parental care. From 2003 onwards, paternities

217   were therefore assigned by collecting tissues samples from the ears of adults and neonatal pups. We

218   used these tissue samples to genotype all adults and pups since 2003 at 16 microsatellites (Gunn et

219   al. 2005) analysed with 99% confidence using CERVUS 3.0 (Kalinowski *et al.* 2007; see Lane *et al.*

220   2007, 2008 for further details). This method gives an estimated error rate of paternities, based on

221   mismatches between known mother–offspring pairs, of around 2% (Lane et al. 2008), which we

222   consider acceptable. Approximately 90% of yearly pups are assigned paternities with known males

223   while the remaining 10% are analysed further in Colony 2.0 (Jones and Wang 2010) to determine

224   whether they might still be full or half siblings from unknown sires using 95% confidence in

225   maximum likelihoods.

226

227   Data analysis

228   Data on the locations of squirrel territories were available from the autumns in 1991-2015, and so

229   we looked at parturition dates in the following springs (i.e. 1992-2016). All squirrels identified as

230   holding a territory in an autumn census were included in this analysis, including females that did not

231   attempt a litter in the following spring, and males. These individuals had missing values entered for

232   their parturition dates. Their inclusion was nonetheless necessary as they acted as potential

233   competitors during the autumn for those squirrels that did have a litter.

234   We used a stepwise forward model building approach as we were not confident in our

235   ability to simultaneously estimate all variance and covariance parameters in the most complex

236   model, which would limit our ability to evaluate individual parameters accurately. We initially fitted

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237   a mixed-effects linear model, with the fixed effects of study area (a two-level factor), year (as a

238   continuous linear covariate), whether or not the spruce trees "masted" (produced a super-

239   abundance of cones; Silvertown 1980; Kelly 1994) in the year of the autumn census (a two-level

240   factor), age and age^2 of the squirrel, and the random effects of year and squirrel identity, to account

241   for repeated measures on squirrels across years. If the age of the squirrel was not known, the mean

242   age of all other squirrels in that breeding season was entered. Estimating the squirrel identity

243   random effect allowed the calculation of the (conditional) repeatability of individual squirrel

244   parturition dates (Nakagawa and Schielzeth 2010). We divided raw parturition dates by the standard

245   deviation of all observations, giving a sample with a variance of 1, making the variance components

246   easier to interpret (Schielzeth 2010). We retained the same fixed effect structure for all subsequent

247   models, instead building up the random effects structure. We first added the identities of the six

248   nearest squirrels as 6 random effects (see below for our explorations of other possible neighborhood

249   sizes), assuming that all random competitor effects came from the same distribution, with a mean of

250   zero and a single variance to be estimated. This allows us to estimate an indirect phenotypic effect,

251   and the covariance between this term and the direct effect of squirrel identity. We based "nearest"

252   on location of the primary midden during the autumn census. Here the design matrix for the indirect

253   effects either contained a "1" if the individual in that column was a competitor for the individual in

254   the current row, or a "0" otherwise, indicating that its indirect effects either did or did not influence

255   the current individual respectively (Muir 2005). This used the following model, with a population

256   mean accounting for the fixed effects for i (μ_{Fi}), a direct phenotypic effect (P_{Di}) and a total indirect

257   influence arising from the sum of competitor specific indirect effects (P_{Sj}) for the 6 nearest

258   neighbors. Additionally, there are multiple measures per squirrel across years, hence we include the

259   random effect for the year t (K_t). Our model predicts a parturition date for the ith individual in a

260   given year (y_{it}) and so the residual term is specific to an individual in a year (e_{it}). Note that no

261   intensity of association factors were included in this model.

262

263
$$y_{it} = \mu_{Fi} + P_{Di} + \sum_{i \neq j}^{n}(P_{Sj}) + K_t + e_{it} \qquad\qquad 1$$

264

265   This model estimated the variance among squirrels in their parturition dates, the variance in

266   parturition dates caused by repeatable effects of neighbor identities, and the within-individual

267   covariance between direct and indirect phenotypic effects (i.e. $Cov(P_{Di}, P_{Si})$). Next we split these

268   phenotypic effects into additive genetic and permanent environment effects by the incorporation of

269   a pedigree (Kruuk 2004; Wilson et al. 2010). We estimated the DGEs and IGEs on parturition dates,

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270 their covariance, and the equivalent terms for the permanent environmental effects. This was our

271 base IGE model:

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273       $y_{it} = \mu_{Fi} + A_{Di} + PE_{Di} + \sum_{i \neq j}^{n} (A_{Sj} + E_{Sj}) + K_t + e_{it}$ 2

274

275 Where individual i's parturition date in year t, is comprised of the fixed effect mean, a direct additive

276 genetic effect (A_{Di}), a direct permanent environmental effect (PE_{Di}), both the additive genetic (A_{Sj})

277 and non-additive genetic (E_{Sj}) indirect effects of all the n neighbors (j) that i interacts with, a year

278 term (K_t), and an individual by year specific residual term (e_{it}).

279   We next associated neighbors (j) of each focal individual (i) with variable intensity of

280 association factors (f_{ij}). This allowed the indirect effect of each neighbor j actually experienced by i

281 to be mediated by their spatial proximity, with f_{ij} = 1/ (1 + distance), where distance was the

282 Euclidean distance between the center of individuals' territories, measured in units of 30m. This

283 value is bounded between 0 and 1, with low values representing individuals that were far apart and

284 high values representing individual that were close. We used the inverse of distance here, but any

285 biologically relevant measure representing intensity of social interaction could be used (Fisher and

286 McAdam 2017). To weight the strength of the indirect effects, we replaced all 1s in the indirect

287 effect design matrix (as used in Models 1 and 2 above) with these terms (Muir 2005; Cappa and

288 Cantet 2008). All individuals farther than the 6 nearest neighbors were not modelled as having an

289 indirect effect (but see below). Our model structure was as follows:

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291      $y_{it} = \mu_{Fi} + A_{Di} + PE_{Di} + \sum_{i \neq j}^{n} f_{ij} (A_{Sj} + E_{Sj}) + K_t + e_{it}$ 3

292

293   This model has not been applied to wild animals before, and we fully acknowledge that our

294 choice to consider only the 6 nearest neighbors here is somewhat arbitrary, as indeed is the scaling

295 of f_{ij}. Therefore, we also explored different numbers of neighbors, and different methods for

296 defining our f_{ij} terms. We then monitored how this influenced the estimates of the variance

297 parameters, to determine whether the model was particularly sensitive to altering these factors (see

298 also: Costa e Silva et $al.$ 2017). We present results using f_{ij} = 1/(1 + distance^2), as well as where we

299 defined the competitors as all those within 60, 130 or 200 metres, without weighting by distance, up

300 to 24 competitors, and investigations with varying numbers of neighbors (1-5, 9, 12, 15, 18 & 24), in

301 the supplementary materials (Table S1). Neither changing the number of neighbors nor rescaling

302 intensity of association terms changed the number of model parameters estimated. Therefore,

303 information criteria-based approaches for comparing model fits were not appropriate. Instead we

304   simply assessed the change in variance components, noting the size of the parameter estimates and

305   size of the standard errors. We focus on the results with the 6 closest neighbors, as this seemed the

306   median result among the variations we tried, and so the most appropriate model for our system.

307   Using the inverse of distance^2 squared led to a large increase in the standard errors of the DGE

308   estimate, which only occurred in this model, hence we considered simply the inverse of distance as

309   more appropriate.

310   Having decided on our scaling of f_{ij} and to focus on 6 neighbors, a final step was to add a

311   control for (non-socially determined) environmental heterogeneity in resource abundance. We

312   predicted a negative covariance between neighbors due to competition for resources (especially

313   during high-density conditions), however, this could be masked by (positive) spatial autocorrelation

314   in resource availability within a study-area arising from non-social factors. This in turn could

315   generate a net signal of positive phenotypic covariance among-neighbors (Stopher et al. 2012; Regan

316   et al. 2016). Accounting for any such effect with a spatial autocorrelation term should limit the

317   potential for non-social effects to bias our estimates of the influence individuals have on each other

318   through competition (Costa e Silva and Kerr 2013; Costa e Silva et al. 2013). However, the multiyear

319   data set adds considerable complexity (since the spatial distribution of territory quality is not

320   consistent year-to-year; LaMontagne et al. 2013) and we were unable to obtain convergence from

321   our data with a model in which a separate spatial autocorrelation term for each year. As a simpler

322   alternative, we assigned each red squirrel within each year to one 150 m x 150 m square within a

323   grid of non-overlapping squares that encompassed the study area (hereafter referred to as

324   "squares"). Each square was given a unique label comprising its location and the year, and so by

325   fitting this as a random effect we could account for any similarity among red squirrels within each

326   150 m x 150 m area in each year. We repeated this analysis with squares of 75 m x 75 m or 300m x

327   300 m. These results were qualitatively similar to the analysis with the intermediate size squares,

328   and so are presented in the supplementary materials (Table S1).

329   We tested the significance of the DGE-IGE covariance using a likelihood ratio-test (LRT)

330   between a model with the covariance freely estimated and one with it fixed to zero, and tested the

331   significance of the IGEs using a LRT between the model with the IGEs (and a zero covariance) and a

332   model without IGEs. We then tested the significance of the direct-indirect phenotypic covariance,

333   and the indirect phenotypic variance, in the same way, in models without IGEs. We assumed the LRT

334   statistic was distributed as a 50:50 mixture of χ^2_1 and χ^2_0 when testing single variance components

335   (following Self and Liang 1987) but as χ^2_1 when testing covariances. Note that, even where terms

336   were non-significant, they were retained as our best estimate of the corresponding parameter for

337   our estimates of predicted responses to selection (as described below). We report DGE-IGE

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338   correlations, although if the variance of either the DGEs or the IGEs was very small (<0.0001), then

339   we assumed it was essentially zero, and so then we report the correlation as "undefined". Although

340   they were not directly relevant to the biological hypotheses being tested, the statistical significance

341   of the fixed effects was tested using conditional Wald tests (see: Gilmour et al. 2015). This approach

342   to testing the significance of fixed effects in mixed linear models performs well in situations with

343   limited sample sizes (Kenward and Roger 1997).

344

345   Influence of population density on indirect effects

346   We consider population density during the resource caching period to be key to resource

347   acquisition. Consequently, for any given year of parturition the relevant measure of density was

348   obtained from the census in the autumn of the year *prior* to parturition. As the study area has grown

349   marginally since the start of the project, we restricted counts to individuals holding a territory within

350   a defined area 38ha that has been constant throughout the entire study period. Across both study

351   areas in all years the median population density in the autumn was 1.69 squirrels ha^{-1} (Fig. 1). We,

352   therefore, labelled each study area within each year with a density higher than this as "high density"

353   (1994, 1998-2000, 2006 and 2015 for both study areas, 1991-1993, 1995-1997, 2001 and 2002 for

354   Sulphur only and 2011-2014 for Kloo only), and so the remainder as "low density" There were,

355   therefore, 24 instances of high density conditions, and 26 instances of low density conditions. There

356   are several instances of study areas having exactly the median density, hence why there are more

357   low- than high-density conditions.

358   Building on the model with the 150 m x 150 m squares, we fitted an interaction between

359   population density (high or low) and each random effect. This gave us separate density-specific

360   estimates of each of the variances (DGEs, IGES, and non-genetic versions) and covariances, the

361   among-year variances and the among-square variances for low- and high-density study areas. To

362   obtain stable model convergence, we were required to fix the direct permanent environment effect

363   in low-density years to 0.1 x 10^{-4}, but since this term was estimated to be very small in all other

364   models, this is likely not problematic. We also refitted a simpler formulation of the model in which

365   indirect effects were not partitioned into genetic and non-genetic terms, to estimate and compare

366   the magnitude of phenotypic indirect effects between low- and high-density conditions. There was a

367   single residual variance in each model. We also included density as two-level factor in the fixed

368   effects, and an interaction between this term and each of the other fixed effects, to allow them to

369   vary between low- high-density conditions. We tested for significance of indirect effects in both high

370   and low-density conditions in the same way as for the full model. When testing the significance of

371   terms for low-density, we maintained the full structure (i.e. IGEs and their covariance with DGEs) for

372   high-density conditions, and vice versa.

373

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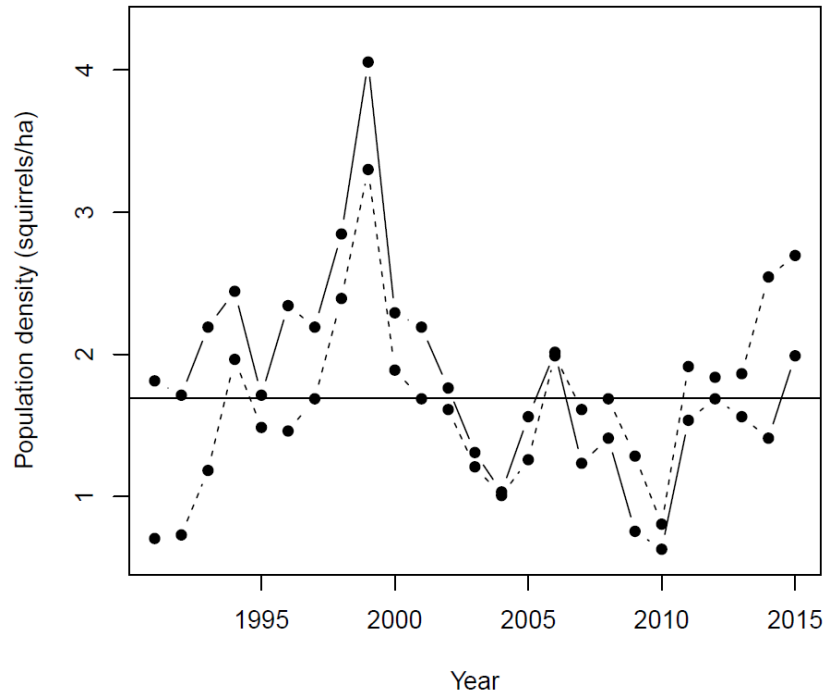
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376

377   Figure 1. Estimated population densities across both study areas in our study ("Kloo" is solid

378   line, "Sulphur" is dashed line). Points above the line (the median density: 1.69 squirrels ha.[-1])

379   were counted as "high density", points below the line as "low density".

380

381   Calculating variance parameters and the predicted response to selection

382   To assess the contribution of indirect effects to phenotypic and genetic variances, we estimated the

383   variance in individuals' phenotypic effects on the population mean parturition date ($\hat{\sigma}^2_{PE}$,

384   incorporating both consistent direct and indirect phenotypic effects; for models without genetic

385   terms), and variance in individuals' heritable influence on the population mean parturition date ($\hat{\sigma}^2_H$;

386   for models with genetic terms, commonly referred to as the "total heritable variance"). Following

387   Bijma (2011) and Costa e Silva *et al.* (2013) these are:

388

389                     $$\hat{\sigma}^2_{PE} = V_{PD} + 2n\overline{f_{ij}}Cov(P_D, P_I) + (n\overline{f_{ij}})^2 V_{PI}$$ 4

390

391 $$\hat{\sigma}_H^2 = V_{AD} + 2n\overline{f_{iJ}}\, Cov(A_D, A_I) + (n\overline{f_{iJ}})^2 V_{AI} \qquad\qquad 5$$

392

393 Where n is the number of neighbors (excluding the focal individual, so 6), $\overline{f_{iJ}}$ is the mean intensity of

394 association factor, V_{PD} and V_{AD} are the direct phenotypic and additive genetic variances

395 respectively, $Cov(P_D, P_I)$ and $Cov(A_D, A_I)$ are the phenotypic and genetic direct-indirect

396 covariances respectively, and V_{PI} and V_{AI} are the indirect phenotypic and additive genetic variances

397 respectively. The $\overline{f_{iJ}}$ was calculated as 0.330 across the entire data set, 0.298 at low densities and

398 0.352 at high densities, which means a squirrel's 6 nearest neighbors were on average 60.9m, 70.7m

399 and 55.2m from it across the whole dataset, at low densities, or at high densities respectively. Note

400 that $\hat{\sigma}_H^2$, unlike traditional heritability, can exceed 1; see Bijma (2011) for the mathematical

401 demonstration of this, and Ellen *et al.* (2014) for empirical examples in livestock.

402   In order to calculate the predicted response to selection in the presence of IGEs among

403 related individuals, we combined equation 15 of Bijma and Wade (2008), with the relative strength

404 of multilevel selection (g) set to zero, and equation S5_2 of Costa e Silva et al. (2013), setting total

405 phenotypic variance as 1:

406
407 $$\Delta \bar{P} = \beta_{W_D P}\{r\hat{\sigma}_H^2 + (1-r)[V_{A_D} + n\overline{f_{iJ}}Cov(A_D, A_I)]\} \qquad\qquad 6$$

408

409 Where the change in the mean phenotype ($\Delta \bar{P}$) is predicted by the selection gradient of the

410 phenotype on relative direct fitness ($\beta_{W_D P}$), multiplied by a term encompassing the genetic variance

411 parameters. We estimated $\beta_{W_D P}$ across all females in the whole dataset by regressing relative

412 fitness (number of pups born to each individual in a given year that recruited to the population as

413 adults, divided by the population average for that year) on standardised parturition dates (mean

414 centred and divided by the standard deviation across the whole dataset). We then repeated this

415 with an interaction with high/low density, to give different estimates for $\beta_{W_D P}$ in each condition.

416 Densities (and competitive neighborhoods) in the autumn of a given year were associated with

417 parturition dates and fitness in the following spring (year +1). We then multiplied the predicted

418 change by the standard deviation of parturition date, to give the result as "days per generation".

419 Relatedness (r) is the mean coefficient of relatedness among the 7 squirrels in each neighborhood

420 (the focal squirrel and its 6 nearest neighbors, note as n was already defined as the number of

421 neighbors excluding the focal individual, $n-1$ in eq. 15 of Bijma and Wade (2008) is altered to n). We

422 used the pedigree to calculate r as 0.094 across the entire data set, 0.087 at low densities and 0.098

423 at high densities. We compare this to a case where IGEs were equal to zero, and so the response to

424 selection was equal to $\beta_{W_D P}$ V_{AD}.

425

Results

426

427

428   In total, 1862 unique red squirrels were recorded a total of 4362 times in autumn censuses as

429   holding territories, and so were included in the analysis. There were 555 unique females that had at

430   least one litter, with a mean of 2.1 (range = 1-8, standard deviation = 1.3) recorded parturition dates

431   each. There were 364 females that had no recorded parturition dates, and 943 males. Variance

432   components from the stepwise-forwards model building process are presented in Table 1.

433   Individuals showed a limited degree of consistency in their parturition dates, with the direct

434   variance among-individuals in parturition date in model 1 of Table 1 accounting for 5.2% of the

435   observed phenotypic variance. We then added indirect phenotypic effects of neighbors (model 2),

436   which were significant (LRT, χ^2_1 = 8.235, p = 0.002), but the covariance between the direct and

437   indirect phenotypic effects was not (cor = -0.143, LRT, χ^2_1 = 0.695, p = 0.405), indicating that

438   individuals that give birth earlier do not influence their neighbors in any particular direction.

439   Individuals' consistent differences in their own phenotypes and consistent effects on neighbors ($\hat{\sigma}^2_{PE}$)

440   was calculated as 8.1% of the observed phenotypic variation in model 2.

441   Parturition date showed direct heritability, with V_{AD} in model 5 accounting for 4.8% of the

442   observed phenotypic variance (note this differs from previous estimates of h^2 for this trait in this

443   system, but see Wilson 2008 for how h^2 and V_A/V_P can differ). The estimate for the IGEs was not

444   different from zero (LRT, $\chi^2_{0.5}$ = 0.003, p = 0.480), as was the DGE-IGE covariance (cor = undefined,

445   LRT, $\chi^2_{0.5}$ = 0.119, p = 0.729). We calculated the total heritable variance of parturition date, $\hat{\sigma}^2_H$, as

446   6.8%, a modest increase over V_{AD}. The very small DGE-IGE covariance indicates that genotypes for

447   early parturition dates did not affect their neighbors in any consistent direction. Parturition dates

448   differed among squares and greatly among years, with the square term accounting for 4.0% of the

449   total variance, while variance among-years accounted for 32.0%.

450   Estimates of the fixed effects are presented in Table 2. In short, red squirrels on the

451   "Sulphur" study area had marginally later parturition dates than those on "Kloo", but there was no

452   linear temporal trend across years. Parturition dates were significantly earlier following mast years

453   by approximately 40 days. Older squirrels had earlier parturition dates, although the positive

454   quadratic effect indicates a nonlinear effect of age in which squirrels began to breed later at older

455   ages.

456

457   High vs low density comparison

14

458   Results from models 6 and 7, where variance components were separately estimated in low- and

459   high-density conditions are presented in Table 1 and summarised in Fig. 2. Testing for the presence

460   of indirect phenotypic effects (model 6) indicated that in high density conditions, there were

461   significant phenotypic indirect effects (LRT, $\chi^2_{0.5}$= 7.005, p = 0.004), although the negative covariance

462   was not different from zero (cor = undefined, LRT, $\chi^2_{0.5}$ = 0.305, p = 0.581). In low density conditions

463   however, both the variance in indirect phenotypic effects (LRT, $\chi^2_{0.5}$ = 0.049, p = 0.412) and the

464   direct-indirect phenotypic covariance (cor = undefined, LRT, $\chi^2_{0.5}$ = 0.839, p = 0.360) were not

465   different from zero. From model 6, $\hat{\sigma}^2_{PE}$ was calculated to be 18.4% in low-density years, and 17.5%

466   in high-density years.

467   These indirect effects were split into genetic and non-genetic components for model 7. In

468   high density conditions, the IGEs were considerably stronger than across the whole dataset, and

469   more than one standard error from zero, although still not significantly different from zero (LRT, $\chi^2_{0.5}$

470   = 0.607, p = 0.218). The covariance between DGEs and IGEs was negative but not different from zero

471   (cor = -0.401, LRT, $\chi^2_{0.5}$ = 0.688, p = 0.407). Unsurprisingly, given that we detected no phenotypic

472   indirect effects in low-density conditions, the IGEs (LRT, $\chi^2_{0.5}$ = 0.000, p = 0.500) and the DGE-IGE

473   covariance in these conditions were also not different from zero (cor = undefined, LRT, χ^2_{1} = 0.566, p

474   = 0.452). Although we reiterate that neither covariance was statistically significant, based on our

475   parameter estimates in low-density conditions $\hat{\sigma}^2_{H}$ was 14.3%, which was higher than V$_{AD}$, as this

476   calculation includes the positive DGE-IGE covariance estimate (despite the lack of variance in IGEs

477   rendering the correlation undefined). In high-density conditions $\hat{\sigma}^2_{H}$ was 14.2%, which is similar to

478   the estimate at low densities, as the additional genetic variance from IGEs is counteracted by the

479   negative DGE-IGE covariance (see eq. 5). We stress that, as the estimates for the IGEs and their

480   covariances with the DGEs were not significantly different from zero, the estimates of $\hat{\sigma}^2_{H}$ should be

481   interpreted with caution.

482   The square term revealed that there was some variation attributable to spatial location in

483   both conditions, accounting for 4.2% in low-density, and 3.1% in high-density conditions, of the

484   variance in model 7. Finally, there was also substantial among-year variance in both conditions,

485   accounting for 32.2% and 38.4% for the observed variance in low and high-density conditions

486   respectively. Estimates for fixed effects from model 7 are presented in the supplemental materials

487   (Table S2).

488 **Table 1**. Variance component estimates (with their approximate standard errors in brackets) for each element of the variance-covariance structure from
489 our models. Terms that were bound to values very close to zero will not have a standard error estimated, and so have "-" instead. Models without a given
490 term have "NA" entered in that cell. Terms highlighted in bold were >2 times greater than their standard errors, while terms underlined were between 1
491 and 2 times greater than their standard errors. Variance in direct genetic effects are indicated by V_{AD}, in indirect genetic effects by V_{AI}, and their covariance
492 by $Cov(A_D,A_I)$. Equivalent notation with "P" instead of "A" refers to variance in purely phenotypic effects, or if the model includes genetic effects,
493 permanent environment effects. V_S is the among-square variance (with squares of size 150mx150m), V_Y is the among-year variance, and V_R is the residual
494 variance.

Model		V_{AD}	V_{AI}	$Cov(A_D,A_I)$	V_{PD}	V_{PI}	$Cov(P_D,P_I)$	V_Y	V_S	V_R
Stepwise model building process										
1. Direct phenotype only		NA	NA	NA	**0.052** (0.012)	NA	NA	**0.320** (0.099)	NA	0.273 (0.015)
2. Direct and indirect phenotypes		NA	NA	NA	**0.026** (0.012)	**0.017** (0.003)	-0.003 (0.005)	**0.315** (0.098)	NA	0.205 (0.015)
3. Direct and indirect, genetic and non-genetic effects		**0.039** (0.012)	< 0.001 (0.002)	> -0.001 (0.006)	< 0.001 (-)	**0.015** (0.005)	0.002 (0.020)	**0.317** (0.099)	NA	0.201 (0.015)
4. As above, with inverse distance weighting		**0.047** (0.012)	0.005 (0.026)	-0.003 (0.016)	<0.001 (-)	**0.089** (0.037)	0.006 (0.021)	**0.318** (0.099)	NA	0.217 (0.015)
5. As above, with square term		**0.048** (0.012)	<0.001 (-)	0.005 (0.015)	<0.001 (-)	**0.063** (0.023)	-0.018 (0.020)	**0.320** (0.099)	**0.040** (0.014)	0.192 (0.016)
High vs. low density comparison										
6. Phenotypic indirect effects split by density	Low density	**0.050** (0.020)	NA	NA	<0.001 (-)	0.034 (0.044)	0.021 (0.022)	**0.322** (0.126)	<u>0.038</u> (<u>0.024</u>)	
	High density	**0.053** (0.018)	NA	NA	<0.001 (-)	**0.067** (0.029)	-0.011 (0.019)	**0.379** (0.150)	<u>0.032</u> (<u>0.018</u>)	0.198 (0.018)
7. IGE model split by density	Low density	**0.047** (0.020)	<0.001 (-)	0.027 (0.030)	<0.001 (-)	0.021 (0.045)	-0.003 (0.036)	**0.322** (0.126)	<u>0.042</u> (0.024)	
	High density	**0.053** (0.018)	<u>0.038</u> (0.037)	-0.018 (0.023)	<0.001 (-)	0.033 (0.046)	-0.001 (0.026)	**0. 384** (0.151)	<u>0.031</u> (0.018)	0.189 (0.017)

Predicted response to selection

Across all years, selection favoured squirrels with earlier part dates (linear selection gradient β = -0.249), and while selection varied breeding earlier was favoured whether squirrels were breeding under high (β = -0.286) or low (β = -0.239) densities. The standard deviation of parturition date was 23.32. From eq. 6, this gives a predicted response when accounting for IGEs across the entire data set of -0.342 days generation^{-1}. Despite stronger selection at high densities, we calculated a slower predicted evolutionary response, due to the negative DGE-IGE covariance. At low densities, updating all parameters except n, the predicted response was -0.553 days generation^{-1}, while at high densities it was -0.183 days generation^{-1}. Predictions solely based on additive genetic variance multiplied by the selection gradient would have been -0.279, -0.262, and -0.353 days generation^{-1}, for the whole data set, low densities and high densities respectively, therefore matching selection strengths.

Table 2. Estimates and relevant statistics for fixed effects from final model with all years. Study area was a two-level factor, with Kloo as the reference level, hence the shown estimate is for the deviation of the "Sulphur" study area. Following a mast was a two-level factor, with not following a mast as the default, hence the estimate is for the deviation in parturition dates following a mast year. The denominator degrees of freedom are indicated by df_d, while the numerator degrees of freedom were 1 in all cases.

Term	Estimate	Standard error	df_d	F-statistic	P-value
Intercept	-6.684	32.355	23.3	0.06	0.810
Study area (SU)	0.176	0.054	5.72	10.68	0.002
Year	0.006	0.016	23.1	0.16	0.693
Following a mast (yes)	-1.71	0.288	22	35.57	< 0.001
Age	-0.002	< 0.001	1091.5	145.85	< 0.001
Age^2	< 0.001	< 0.001	1164.0	95.59	< 0.001

Discussion

Indirect effects are present and change with population density

Red squirrels live in territories surrounded by conspecifics, with whom they engage in social interactions through vocalizations, competition for resources, and mating interactions. Our analyses show that these interactions can lead to indirect effects on female squirrel reproductive traits. These are detected here as a repeatable influence of competitor identity on the parturition date of focal individuals. Our results also suggest that these indirect effects are more important determinants of focal phenotypes when density is high. Specifically, at high densities, there is significant variation in

the extent to which squirrels influence each other's parturition dates, but this is not the case at low densities.
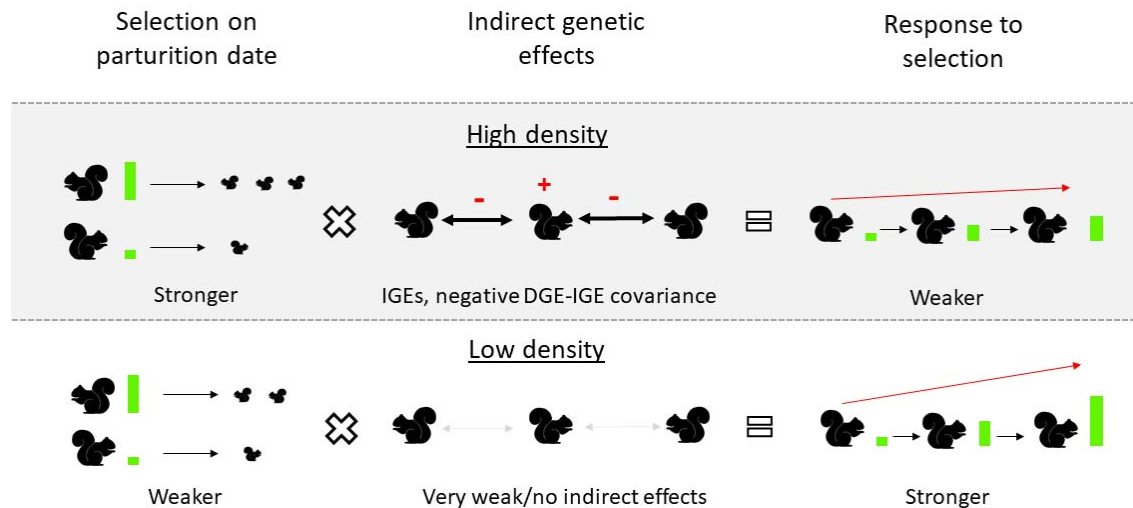

Figure 2. A summary of our results for the analysis of indirect genetic effects at high and low densities. At high density selection was stronger (β = -0.286), and there were direct genetic effects (variance = 0.053). There were significant phenotypic indirect effects, and non-zero but statistically non-significant indirect genetic effects (variance = 0.038). The DGE-IGE covariance was estimated to be negative (estimate = -0.018). These parameters gave a slow response to selection (response = -0.183 days.generation^{-1}). At low density selection was weaker (β = -0.239), while there were direct genetic effects of a similar magnitude to at high densities (variance = 0.047), but indirect phenotypic and genetic effects were absent (variance = < 0.0001). The DGE-IGE covariance was estimated still (estimate = 0.027). These parameters gave a moderate response to selection (response = -0.553 days.generation^{-1}, giving earlier parturition dates). Larger green bars represent earlier parturition dates. Red symbols represent the DGE-IGE covariance, showing that individuals with genes to be early (red plus) cause their neighbors to give birth later (red minus).

The social effects on parturition date we documented indicate that in some contexts an individual's phenotype is partly under the control of those it socially interacts with, even in a solitary and territorial species. Work on Eucalyptus trees (Costa e Silva et al. 2013) implicated competition for limited resources as the source of indirect effects, and our results are consistent with this idea. Earlier studies have shown that red squirrel females may be food limited to some degree, aside from

in years following a mast event. For example, earlier parturition dates and lower levels of oxidative stress were found when food was supplemented (Kerr et al. 2007; Fletcher et al. 2013b; Williams et al. 2014), and individuals are more likely to survive over winter with a larger food cache (Larivée et al. 2010; LaMontagne et al. 2013), suggesting that not all individuals have enough stored food. However, female squirrels appear to reproduce below capacity in non-mast years, and upregulate their reproduction *before* pulsed resources are available (Boutin et al. 2006, 2013), and so they are likely not completely food-limited. The additional insight from the current study is that, for focal individuals, competitive effects on phenotype depend not simply on high density, but also on the identities – and so phenotypes - of their nearest neighbors.

Our analysis did not explore the specific mechanism (or trait(s)) that mediate indirect phenotypic effects from competition. However, based on prior knowledge of the system we speculate that red squirrels might influence each other's parturition dates through vocal cues rather than, for instance, physical interactions. Red squirrels give territorial calls ("rattles"), to which neighbors behaviorally respond to (Shonfield 2010) and which function to maintain their territory from conspecifics (Smith 1978; Lair 1990; Siracusa et al. 2017). Additionally, red squirrels rattle more when they have a higher local population density (Dantzer et al., 2012; Shonfield et al. 2012), while red squirrel mothers increase the growth rate of their pups when playback of territorial vocalizations leads to the perception of higher local population density (Dantzer et al. 2013). This is through upregulation of maternal glucocorticoids, part of the stress axis (Dantzer et al. 2013). Other life history traits, such as parturition date, may be influenced by rattles at high densities, allowing individuals to influence each other's parturition dates. Therefore, acoustic interactions among-neighbors, which enable neighbors to influence each other's reproduction, may be a source of indirect effects, particularly in high-density conditions.

Indirect effects with a limited heritable basis

While our analyses provide statistical support for indirect effects of competitors on a focal individual's parturition date, we were unable to conclusively demonstrate that these indirect effects were underpinned by genetic variation. Estimated effect sizes were larger at high densities, in line with our predictions and the phenotypic effects. If we incorporated the estimates of variance attributable to IGEs, and the DGE-IGE covariances, into our predicted response to selection, we expected a faster response to selection in low-density conditions (-0.553 days generation^{-1}) when selection was weakest, while a slower response to selection in high-density conditions (-0.183 days generation^{-1}) when selection was strongest, compared to across the whole data set (-0.342 days generation^{-1}). Therefore, a negative DGE-IGE covariance counteracted stronger selection at high

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densities to give a slower evolutionary response. However, while the point estimates of predicted change indicate IGEs are potentially strong enough to make a meaningful difference to evolutionary dynamics, we acknowledge they are estimated with high uncertainty.

Previous work on livestock has shown that IGEs negatively correlated with DGEs can reduce or even reverse the expected response to selection (Costa e Silva et al. 2013; Muir et al. 2013; Ellen et al. 2014), as we have found. The evolutionary stasis of heritable traits under directional selection is a well-known observation in need of an explanation in the study of trait evolution in wild populations (Merilä et al. 2001; Kokko et al. 2017). IGEs that consistently counteract selection responses (compared to a DGE-only scenario) would reduce evolutionary change, as we have shown under high-densities, and so could contribute to a lack of evolutionary change. Whether this is a general explanation for evolutionary stasis remains to be explored (Wilson 2014).

The contribution of the variance of the IGEs, and the DGE-IGE covariance to the expected response to selection is not certain, as these estimates were not statistically significant. If IGEs are not different from zero, then the expected response to selection may not differ from that predicted by the breeder's equation (Bijma and Wade 2008). We note that the non-significance of our IGE variance estimates may have been driven by a high degree of uncertainty (large standard errors), rather than the magnitude of the effect, as in high density years the V_{AI} was quite close in absolute size to V_{AD}, and their contribution to total heritable variance and the predicted response to selection was large enough to change the expected response in different conditions. As such, the value of incorporating these estimates into predictive models is possibly large, but uncertain.

Predictions about the speed of evolution based on V_{AD} or σ_H^2 can be misleading, because, as we have demonstrated, a negative DGE-IGE covariance detracts from the response to selection (see eq. 6), even if a large variance in IGEs gives a larger σ_H^2 (eq. 5). This is similar to how negative genetic correlations between traits under equivalent selection can limit their evolution (Lande 1979; Kirkpatrick and Lande 1989). Additionally, there are several reasons why not all of σ_H^2 may be utilized in the response to selection. As can be seen from eq. 6, the response to selection in the presence of IGEs is not simply σ_H^2 multiplied by selection. Neighborhoods or groups made up of unrelated individuals, small group sizes, and a near-zero or negative covariance between DGEs and IGEs will also cause the σ_H^2 to be somewhat discounted when calculating the response. However, given their prevalence among livestock (Ellen et al. 2014), IGEs may well influence the response to selection more broadly, hence they need to be considered more often when attempting to predict the microevolution of populations, and to explain phenomena such as evolutionary stasis (Merilä et al. 2001; Ellen et al. 2014; McAdam et al. 2014). This is true even in populations of wild animals that do

not live in clearly defined groups, but in an irregular network of territories (see also Nunney (1985) for related work on the evolution of altruism in "continuous arrays" of animals).

Altering competition indices and neighborhood size

Varying the intensity of association factors (i.e. how strongly we weighted neighbors at different distances) and the size of the neighborhood did alter the balance between the estimated direct and indirect effects, as well as estimated relative contribution of genetic and environmental influences (see Table S1). Weighting the closest individuals more strongly, by only including the 1-3 nearest neighbors, or using the inverse of distance or distance^2, or by only including individuals within 60 m, gave similar results. In all these variants the variance arising from DGEs increased marginally compared to the model where all neighbors were weighted equally. This effect was more pronounced when using the inverse of distance^2 to define the intensity of association factors. We note that the standard errors of estimates for direct additive genetic variance (V_{AD}) in the model using the inverse of distance^2 were greatly increased, causing the estimate to be within two standard errors of zero (i.e. nominally non-significant). This was the only model explored where this occurred. Weighting further individuals as strongly as close individuals, either by not including any intensity of association factors for the 6 closest individuals, or by including all individuals within 200 m and weighting them equally, gave very low estimates for the IGEs. This could suggest that individuals at greater distances do not consistently influence their neighbors.

Increasing the number of neighbors considered in the analysis beyond six led to larger estimates for the variance arising from the non-genetic indirect effects (V_{PI}). A larger estimate for the V_{PI} was also present in the model before the square term was added. This suggests the apparent non-genetic influence of neighbors at large spatial scales, as indicated by V_{PI}, may be driven by shared environmental factors at the larger scale causing sets of neighbors to be consistently different from other sets, rather than by social interactions of the focal individual causing their neighbors to be consistently different. Note also that increasing the number of interacting individuals is expected to increase the difficulty in estimating indirect effects (Muir 2005). Decreasing the number of neighbors tended to increase the variance attributed to the DGE, while IGEs showed a non-linear trend, peaking in magnitude with 4 neighbors and then falling back down towards zero. At these neighborhood sizes, V_{PI} was typically estimated near zero, but grew in size once 5 or more neighbors were considered. A neighborhood of size 6 was therefore the smallest neighborhood where any indirect effects were more than two standard errors from zero, although these was no evidence these had a genetic component, at least across the whole dataset. Overall,

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these results do not indicate that inferences from our model with the six closest neighbors, weighted by the inverse of distance, are inappropriate for the system.

The approach we used, based on the work of Muir (2005) and Cappa and Cantet (2008) can be applied to organisms in a range of social structures. Due to the relatively recent increase in usage of techniques such as social network analysis (Krause et al. 2007, 2014; Croft et al. 2008), estimates of pairwise associations within populations of animals have been made in many systems. These values can be used as the intensity of association factors, as we used the inverse of distance, to scale indirect effects (Fisher and McAdam 2017). To estimate IGEs, this must be twinned with information on the phenotypes and relatedness of the individuals in the population. We had a large dataset with good information on phenotypes and relatedness of individuals, yet high uncertainty around moderately large estimates of IGEs did not distinguish them from zero. The requirement to phenotype, genotype and assess the social relationships of many individuals within a population may well limit the range of study systems this approach can be used in. However, with decreases in the cost of tracking technologies and in the cost of assessing the genetic relatedness of animals (Bérénos et al. 2014), more study systems will begin to be able to apply this and similar models.

Conclusions

Previous to this study, IGEs had only ever been estimated for wild animals in the context of pairwise (dyadic) social interactions. We extended this to estimate IGEs on a life-history trait with links to fitness in a population of wild animals that do not interact in discretely defined groups. We also incorporated varying strengths of closeness of association between individuals to more accurately represent the heterogeneous and complex nature of social interactions in the natural world. We found that indirect effects were present, were stronger at high densities, and may (but equally may not) have a heritable component. Predicting selection responses from a model that incorporated IGEs indicates that they can both slow down (at high population density) and speed up (at low density) the expected response to selection for earlier parturition dates. This is despite selection actually being stronger at high densities. However, these patterns are based on point estimates for genetic parameters that are characterised by high uncertainty and, as noted, we cannot exclude the possibility that the indirect effects have a non-genetic basis. Nonetheless, significant indirect phenotypic effects were detected and appear to increase in importance at high density. This is consistent with competition for limited food resources being the source of neighbor influences on focal life-history traits. Exactly how this competition is mediated remains to be determined. The estimation of indirect effects, and IGEs specifically, should be extended to more systems where densities and resource availabilities vary (either naturally or artificially) to determine whether the

patterns we have observed are general. While we did not conclusively demonstrate IGEs are present, we think wider estimation of effect sizes is useful even if power is limiting to make strong inferences in any single case. The method we have used is flexible enough to be applied to alternative systems, hence we look forward to the accumulation of more estimates of IGEs in the wild to detect general patterns.

Acknowledgements

Author contributions

AGM, AJW, and DNF conceived of the research question. SB initiated the long-term study and all authors contributed to field logistics, data collection and the writing of the manuscript. DNF drafted the manuscript and conducted the data analysis, with guidance from AJW and AGM. All authors approved of the final manuscript for submission.

Funding statement

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