

1 **Multilevel and sex-specific selection on competitive traits in**
2 **North American red squirrels**

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23

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26 Abstract

27

28 Individuals often interact more closely with some members of the population (e.g. offspring,
29 siblings or group members) than they do with other individuals. This structuring of
30 interactions can lead to multilevel natural selection, where traits expressed at the group-level
31 influence fitness alongside individual-level traits. Such multilevel selection can alter
32 evolutionary trajectories, yet is rarely quantified in the wild, especially for species that do not
33 interact in clearly demarcated groups. We quantified multilevel natural selection on two traits,
34 postnatal growth rate and birth date, in a population of North American red squirrels
35 (*Tamiasciurus hudsonicus*). The strongest level of selection was typically within-acoustic
36 social neighbourhoods (within 130m of the nest), where growing faster and being born
37 earlier than nearby litters was key, while selection on growth rate was also apparent both
38 within-litters and within-study areas. Higher population densities increased the strength of
39 selection for earlier breeding, but did not influence selection on growth rates. Females
40 experienced especially strong selection on growth rate at the within-litter level, possibly
41 linked to the biased bequeathal of the maternal territory to daughters. Our results
42 demonstrate the importance of considering multilevel and sex-specific selection in wild
43 species, including those that are territorial and sexually monomorphic.

44

45 Introduction

46

47 Phenotypic selection measures the association between individuals' traits and some aspect
48 of their fitness. Measures of the strength and mode of selection provide insights into the
49 function of specific traits (Arnold 1983) and allow for predictions of how these traits might
50 evolve across subsequent generations (Robertson 1966; Price 1970; Lande 1979; Falconer
51 1981; Lande and Arnold 1983). More broadly, the thousands of estimates of selection in the
52 wild provide general lessons about the way selection often works in nature (Endler 1986;
53 Kingsolver et al. 2001; Smith and Blumstein 2008; Cox and Calsbeek 2009; Siepielski et al.
54 2009, 2013).

55 Almost all of these estimates consider selection as acting directly on an individual's
56 absolute trait value or value relative to the population mean. However, individuals often
57 interact more closely with those in their immediate environment; for instance bird nestlings
58 compete with their siblings for access to food brought by the parents (Werschkul and
59 Jackson 1979; Royle et al. 1999). When ecological conditions cause individuals to interact
60 more closely with some conspecifics than others, multilevel associations between traits and
61 fitness can arise. Under these conditions, fitness is influenced not only by the trait value of

62 the individual, but also the trait values of litters, broods or social groups (Goodnight et al.
63 1992). Such multilevel selection has been shown to be equivalent to kin-selection and
64 “neighbour-modulated selection”, where individuals influence each other’s fitness (Grafen
65 1984; Queller 1992; Bijma et al. 2007; Bijma and Wade 2008; but see: van Veelen et al.
66 2012), and may or may not correlate with selection at the level of the individual (Goodnight
67 et al. 1992). For instance, it might be beneficial for a chick to beg more loudly than its nest-
68 mates to receive more food from the parents, but louder nests may suffer higher predation
69 rates. The evolutionary consequences of multilevel selection are potentially striking; higher-
70 level selection in the same direction as individual-level selection can increase the rate of the
71 evolutionary response, but higher-level selection in the opposite direction can retard,
72 remove, or even reverse evolutionary response to selection (Bijma and Wade 2008).

73 Standard measures of selection represent how trait variation across individuals
74 relates to among-individual variation in relative fitness. These can be measured as fitness-
75 trait covariances (selection differential; Lush 1937; Falconer 1981) and partial regression
76 coefficients (selection gradient; Lande 1979; Lande and Arnold 1983). For example, a
77 selection gradient is given by:

78
79

$$80 \quad w_i = constant + \beta_{w_D,P} P_i + e_i \quad (1)$$

81
82

83 Where w_i is individual i 's relative fitness, P_i is i 's phenotype, $\beta_{w_D,P}$ is the partial regression
84 coefficient of P_i on w_i and e_i is a residual term. We use the notation from Bijma and Wade
85 (2008) for consistency with later sections. The D in $\beta_{w_D,P}$, indicates the effect is direct in that
86 it is the phenotype of individual i influencing its own relative fitness. A single regression
87 coefficient, $\beta_{w_D,P}$, is calculated across the whole population under investigation. This implies
88 that the component of an individual’s trait that is relevant to its relative fitness is its deviation
89 from the population mean.

90 In contrast, in the context of multilevel selection, an individual's trait can be modelled
91 as both a deviation from its own group mean, and the deviation of the group mean
92 phenotype from the global mean phenotype (also called "contextual analysis"; Heisler and
93 Damuth 1987; Goodnight et al. 1992; Goodnight and Stevens 1997). An alternative is the
94 “neighbour-modulated” or “social selection” approach, where individual phenotype values,
95 and the mean of their neighbours (i.e. the mean of the group excluding the focal individual)
96 are used to predict fitness (Wolf et al. 1999; McGlothlin et al. 2010). Both Queller (1992) and

97 Bijma and Wade (2008) have shown these approaches are equivalent; we use the former for
98 consistency with recent work on this topic by Bouwhuis et al. (2015).

99 Both among-individual and among-group variation may be important in determining
100 fitness. In this case, selection is modelled with two terms: i 's group mean (including i), \bar{P}_{gi} ,
101 and that individual's deviation from the group mean ΔP_i (Bijma and Wade 2008). A multilevel
102 selection analysis can, therefore, quantify both the among-group selection gradient, $(\beta_{w,\bar{P}_{gi}})$,
103 and the within-group selection gradient $(\beta_{w,\Delta P_i})$ using standard multiple regression methods
104 for estimating selection gradients (Lande & Arnold 1983):

105

106

$$107 \quad w_i = \text{constant} + \beta_{w,\bar{P}_{gi}}\bar{P}_{gi} + \beta_{w,\Delta P_i}\Delta P_i + e_i \quad (2)$$

108

109

110 This simple two-level selection model then assumes that all groups within the population
111 equally interact with one another. However, if some groups are clustered into a higher
112 hierarchical level of organization (e.g. groups that share a local neighbourhood might interact
113 more strongly) then relationships between group mean traits and group mean fitness might
114 vary among these higher levels of organization. Therefore, the basic multilevel selection
115 approach can be extended across any number of hierarchical levels of organization
116 (Goodnight et al. 1992; Bijma et al. 2007).

117 Whilst debate over multilevel selection continues (Gardner 2015; Goodnight 2015),
118 empirical data for its action is gathering. For example, Bouwhuis et al. (2015) found
119 covariance between fledging mass and survival at the between-year, within-year and within-
120 brood levels in great tits (*Parus major*), with the covariance being strongest at the broadest
121 scales. Similarly, selection has been observed at various different levels in different systems,
122 including among honey bee (*Apis mellifera*) colonies (Page and Fondrk 1995), among pairs
123 of monogamous collared flycatchers (*Ficedula albicollis*) (Björklund and Gustafsson 2013),
124 among pens of captive Japanese quail (*Coturnix japonica*) (Muir et al. 2013), among groups
125 of jewelweed plants (*Impatiens capensis*) (Stevens et al. 1995), while contrasting individual
126 and group-level selection was observed in water strider (*Aquarius remigis*) groups (Eldakar
127 et al. 2009, 2010).

128 These examples portray organisms interacting in relatively clearly defined groups, yet
129 animals do not always interact in such discrete units. For example populations of territorial
130 animals consist of individuals aggregated at a range of spatial scales, from individual
131 territories, to groups of neighbouring territories to entire populations (Coulson et al. 1997).
132 Selection presumably could act at each of these levels simultaneously, and possibly in

133 differing directions, but this is rarely investigated. Laiolo and Obeso (2012) found there was
134 disruptive selection at the level of the individual for song repertoire in Dupont's lark
135 (*Chersophilus duponti*), but when selection on “neighbourhoods” (small populations
136 containing 2-50 territories) was considered, selection on song repertoire was found to be
137 stabilising. This demonstrates that non-discrete units can be a basis for selection. Nunney
138 (1985) similarly demonstrated such “continuous arrays” of animals can be the basis for
139 selection for altruism as they are when structured in “trait groups”.

140 Therefore, the key question is not whether multilevel selection is possible, but its
141 form and strength across systems in the natural world (Biernaskie and Foster 2016).
142 Aggregating estimates that included scales at which there might be no genetic variance in
143 the trait might lead to an under-estimation of evolutionary change (if estimates cancel out as
144 they are in opposing directions) or an over-estimation of evolutionary change (if the levels of
145 selection are in the same direction). This may help us explain the inaccuracy of our
146 predictions of evolutionary responses to selection on heritable traits (Merilä et al. 2001).
147 Additionally, sexually antagonistic selection is quite common, and may also pose a
148 constraint on evolution (Cox and Calsbeek 2009). However, it is unknown whether this
149 antagonistic selection extends to multiple levels.

150 To study multilevel selection in an animal interacting in non-discrete groups, we
151 focused on recruitment in a wild population of North American red squirrels (*Tamiasciurus*
152 *hudsonicus*, hereafter “red squirrels”). Red squirrels defend exclusive, food-based territories
153 centred on a cache of hoarded white spruce (*Picea glauca*) cones (Smith 1968). Most of the
154 variation in lifetime reproductive success is determined by whether or not squirrels acquire a
155 territory during their first year before winter commences (McAdam and Boutin 2003b;
156 McAdam et al. 2007). Juveniles cannot oust adults from their territories, so they must find
157 vacant territories or, if resource availability is high, create new ones (Price and Boutin 1993),
158 suggesting that the population density is a key ecological agent of selection (Dantzer et al.
159 2013; Taylor et al. 2014). In most cases, juveniles leave their natal territory in search of
160 vacant territories, ranging on average around 90m, although occasionally up to 900-1000m
161 away from the natal territory (Price and Boutin 1993; Larsen and Boutin 1994; Berteaux and
162 Boutin 2000). However, in some cases the mother will “bequeath” all or part of her territory to
163 one of her offspring, typically a daughter, and search for a vacant territory herself (Price and
164 Boutin 1993; Larsen and Boutin 1994; Berteaux and Boutin 2000; Lane et al. 2015).

165 Mean litter size in red squirrels is between three and four but can range from one to
166 seven (McAdam et al. 2007). Therefore, there is potential for competition within a litter for
167 maternal resources, nearby available territories, or for access to the mother’s territory if she
168 leaves it. Furthermore, each litter is in competition with the other litters in adjacent territories
169 for vacant territories. Given the distance squirrels can range in search of vacant territories

170 (see above) there is possibly selection at greater spatial scales, for example amongst the
171 young-of-the-year for the few unoccupied territories in the area covered by several territories
172 (“neighbourhoods”), and for competition among neighbourhoods for access to vacant
173 territories within a study area (a rectangular grid of around 40 hectares, here representing a
174 sub-population). Finally, within each year the population is comprised of multiple study
175 areas, so there is possibly selection among these large spatial scales. This creates the
176 opportunity to investigate the strength of selection at different spatial scales: within-litters,
177 within-social neighbourhoods, within-study areas and within-years (amongst-study areas in
178 each year). As claiming a vacant territory is our suggested mode of competition (Taylor et al.
179 2014), we investigated selection on two traits that are relevant to this ability: birth date and
180 growth rate. Earlier born litters presumably are able to start searching for vacant territories
181 earlier than later ones (Réale et al. 2003a; Williams et al. 2014). A fast growth rate might
182 mean individuals of a given age have an advantage in terms of size when competing for a
183 vacancy (McAdam and Boutin 2003b).

184 We pursued three main questions. First, what is the strength of selection on growth
185 rate and birth date at each of these levels? Ranking each of these levels of selection also
186 allowed us to identify which was most important to red squirrels. We hypothesized that since
187 settlement distance is typically short (see above), selection will be strongest at the most local
188 scales (i.e. within-litters and within-social neighbourhoods). We also compared this multilevel
189 approach to a standard selection analysis, where we regressed recruitment on individual
190 growth rates and birth dates relative to the yearly average. Secondly, we sought to
191 determine whether, and at what scale, a putative agent of selection, the population density of
192 the study area, affected the direction and magnitude of natural selection. We hypothesized
193 that selection would be intensified by increased population density, although we did not
194 predict which scale would show the most density-dependent selection. Third, as sex-biased
195 patterns of bequeathal may influence selection strengths, we investigated whether these
196 levels of selection differed between males and females. We did not have any previous
197 expectations for which sex would experience stronger selection.

198

199 **Materials and Methods**

200

201 **Study system**

202 We collected data on a wild population of red squirrels in the southwest Yukon, Canada (61°
203 N, 138° W). We have monitored two adjacent study sites (ca. 40 hectares each), bisected by
204 the Alaska highway, continuously since 1987. For this study, we restricted our analyses
205 squirrels born from 1989-2015, as 2015 was the last cohort for which survival data were

206 available. Each year, we live-trapped new individuals (Tomahawk Live Trap, Tomahawk, WI,
207 USA) and gave them unique ear-tags, identified females with litters and ear-tagged their
208 pups, and conducted censuses (using complete enumeration) to ascertain the location and
209 survival of individuals. See McAdam et al. (2007) for further details. These study sites are
210 patches of good habitat among poorer habitat, and hence are somewhere between “islands”
211 and arbitrary areas within a continuous range. As red squirrels can live in the surrounding
212 area, we do see a very low degree of successful emigration from the study area. However,
213 estimated juvenile survival does not differ between the core and the periphery of the study
214 areas, indicating rates of dispersal outside of the study areas are not biasing mortality
215 estimates (McAdam et al. 2007).

216 Female red squirrels typically give birth to litters between March and May. Young are
217 weaned at approximately 70 days of age (Larsen and Boutin 1994), after which the pups
218 disperse in search of vacant territories or the mother may bequeath a portion or all of her
219 territory to one of her pups (Price and Boutin 1993; Larsen and Boutin 1994; Berteaux and
220 Boutin 2000).

221

222 Data collection

223 To start monitoring pups as soon as they were born, we regularly live-trapped all females
224 and examined their abdomens and nipples for signs of swelling. We estimated birth date for
225 each litter based on female stages of pregnancy during live-capture events and the size of
226 pups once we found them. For each mother we only used the first litter of the year to allow
227 better comparison among years, as second and third litters are typically only attempted in
228 “mast” years, in which white spruce (*P. glauca*) produces orders of magnitude more seed
229 (Kelly 1994; Boutin et al. 2006; Lamontagne and Boutin 2007) or after failed first litter
230 attempts (McAdam et al. 2007; Williams et al. 2014). To determine their growth rate, we
231 weighed pups twice while they were still within their natal nest, once at 1-2 days old and
232 again at about 25 days old. In this time period their growth is approximately linear (McAdam
233 and Boutin 2003a), so we calculated individual growth rate as the weight difference between
234 the two measures divided by the number of days between the measures, to give growth rate
235 in grams of mass gained per day. We excluded records where the first mass was above 50g,
236 or where the second mass was above 100g, as these were likely to be litters we found late
237 when pup growth rate is no longer linear. We also excluded records when there were fewer
238 than five days between weight measurements. Due to their conspicuous territorial behaviour
239 and our semi-annual censuses of all squirrels, we have nearly perfect knowledge of which
240 squirrels are still alive in the study areas. Each offspring born in the study areas was
241 classified as “recruited” or “did not recruit” based on whether they survived beyond 200 days

242 of age (i.e., survived their first winter). This binary variable was used as the response
243 variable in all our models.

244

245 Data analysis

246 All analyses were conducted in R ver. 3.3.2 (R Development Core Team 2016), with the
247 package “MCMCglmm” ver. 2.23 (Hadfield 2010). Figures were drawn using coefplot2
248 (Bolker 2012) and ggplot2 (Wickham 2009). To determine which levels of selection were
249 strongest, we constructed a logistic regression model, containing terms each representing a
250 different level of selection. Therefore, all terms (five for growth rate, four for birth date, see
251 below) were in the same model. The response for the model was the binary variable of
252 whether the individual recruited or not, and we used a logit link function. This meant we were
253 restricted to using absolute rather than relative fitness, but we were still able to calculate
254 selection coefficients, see below. We then calculated each of growth rate and birth date at a
255 series of levels. The first of these for growth rate was the individual’s growth rate relative to
256 the mean of its littermates. This represents within-litter selection. There is no such selection
257 for birth date as all littermates possess the same birth date. The mean of a litter of one was
258 simply the value for the single individual. The next level for growth rate was the mean growth
259 rate of its litter relative to the mean growth rate of all individuals born in nests within 130m of
260 focal nest, representing within-social neighbourhood selection. For birth date we used the
261 birth date of the litter relative to the mean birth date of all litters within its social
262 neighbourhood. The radius of the social neighbourhood was set at 130m, as this is the
263 distance within which squirrels respond to each other’s territorial calls (Smith 1968, 1978), so
264 represents the acoustic social environment an individual experiences. Furthermore, 130m is
265 similar to the distance Dantzer et al. (2012) identified (150m) in this system as being the
266 most relevant for “local” density effects. We repeated the analyses with the social
267 neighbourhood set at 60 or 200m, and found no qualitative differences in the results (see the
268 online supporting information). The next level of selection is within-study area. For this we
269 used the mean growth rate and mean birth date of an individual’s social neighbourhood
270 relative to the mean for the whole study area. We then modelled within-year selection as the
271 mean growth rate and birth date for an individual’s study area relative to the mean growth
272 rate and birth date for the entire year. We also included terms for the year’s mean growth
273 rate and birth date relative to the global mean (across all years and study areas), to control
274 for trait-fitness covariances among-years (e.g. Bouwhuis et al. 2015). Only linear terms were
275 fitted to keep models from getting overly complex and because quadratic terms have
276 previously been shown to be less important than directional selection for these traits in this
277 species (McAdam and Boutin 2003b). This method models an individual’s trait as a series of
278 deviations. For example, an individual with a growth rate of 1.6 g/day might have grown 0.2

279 g/day slower than the average pup in its litter. This average growth rate of the litter (1.8
 280 g/day) might be 0.3 g/day faster than the average of all litters within the social
 281 neighbourhood (1.5 g/day). This may be 0.15g/day slower than the study area-wide mean
 282 (1.65g/day) and 0.2g/day slower than the year-wide mean (1.85g/day). This might be
 283 0.1g/day faster than the global mean of 1.75g/day. Therefore, we modelled an individual's
 284 growth rate as the sum of a series of components ($1.6 = 1.75 + 0.1 - 0.2 - 0.15 + 0.3 - 0.2$),
 285 and estimate selection on each using separate partial regression coefficients:

286
 287

$$288 \quad \ln\left(\frac{w_i}{1-w_i}\right) = \text{constant} + \beta_{w,\Delta\bar{P}_Y} \Delta\bar{P}_{Y_m} + \beta_{w,\Delta\bar{P}_S} \Delta\bar{P}_{S_{ml}} + \beta_{w,\Delta\bar{P}_N} \Delta\bar{P}_{N_{mlk}} + \beta_{w,\Delta\bar{P}_L} \Delta\bar{P}_{L_{mlkj}} +$$

$$289 \quad \beta_{w,\Delta P} \Delta P_{mlkji} + e_i \quad (3)$$

290
 291

292 Note as this is a logistic regression we have shown the response variable as the log odds of
 293 fitness. $\Delta\bar{P}_{Y_m}$ represents the difference between the mean growth rate for the year m that i
 294 was born and the global mean growth rate. $\Delta\bar{P}_{S_{ml}}$ is the difference between the mean growth
 295 rate of i 's study area l in year m and the yearly mean. $\Delta\bar{P}_{N_{mlk}}$ is the difference between the
 296 mean growth rate of i 's social neighbourhood k in study area l in year m and the study area
 297 mean. $\Delta\bar{P}_{L_{mlkj}}$ is the difference between the mean growth rate of i 's litter j in social
 298 neighbourhood k in study area l in year m and the neighbourhood mean, and ΔP_{mlkji} is the
 299 difference between i 's growth rate and the mean of its litter j in social neighbourhood k in
 300 study area l in year m . $\beta_{w,\dots}$ terms are the partial regression coefficients for each component
 301 of growth rate on fitness. These logistic regression coefficients were converted into selection
 302 coefficients, following Janzen and Stern (1998), to allow comparison with other studies (e.g.
 303 Kingsolver *et al.* 2001). This is similar to Bouwhuis *et al.*'s (2015) analysis on brood mass
 304 and survival in great tits (*Parus major*), although for growth rate we have two additional
 305 levels (within-social neighbourhood and within- study area). The same formulation was used
 306 for birth date, except that there was no within-litter selection. We mean-centred each
 307 continuous fixed effect and transformed it by dividing by the variable's standard deviation,
 308 giving each variable a variance of 1. This allowed the effect sizes to be directly compared
 309 (Schielzeth 2010). Therefore, by directly comparing the magnitude of the coefficients for
 310 each level of growth rate and birth date, we were able to identify the levels at which selection
 311 acted most strongly.

312 Each model also included study area as a fixed effect to control for any variation in
 313 survival between the two study areas. We also entered the random effect of year, and the
 314 random effects of litter ID nested within mother ID. These accounted for variation in

315 recruitment among years, among litters and among mothers beyond the levels of growth rate
316 and birth date we are studying. As each social neighbourhood was uniquely calculated there
317 was no replication of each social neighbourhood, and so we did not include a random effect
318 for this level. The priors for the variance components followed an inverse-gamma distribution
319 ($V = 1$, $\nu = 0.002$), with the residual variance fixed at 1, because in a model with a binary
320 response the residual variance is defined by the mean. Models were run for 200,000
321 iterations, with the first 50,000 discarded and then 1/10 of the remaining iterations used for
322 parameter estimation, to reduce the influence of autocorrelation between successive
323 iterations. Trace plots of the model parameters were checked and a Gelman test for
324 stationarity was used to confirm stable convergence had been achieved ($p > 0.156$ in all
325 cases). We report the posterior distribution mode (PDM) for each parameter, and the 95%
326 credible intervals (CIs) for this estimate. Our model for the standard selection analysis
327 included individual traits relative to the yearly mean, and the yearly mean relative to the
328 overall mean, as levels of growth rate and birth date. Otherwise the model structure was the
329 same.

330

331 Population density an agent of selection

332 To test whether population density acted as an agent of selection (Dantzer et al. 2013;
333 Taylor et al. 2014), we took the multilevel model built previously, and added study area
334 population density (number of live adult squirrels per hectare in that study area in that year)
335 as a fixed effect. We interacted this effect with each level of growth rate and birth date in the
336 model, to see how the influence of these competitive traits varied as density changed
337 (Bouwhuis et al. 2015). As before, we mean centred study area density and divided it by the
338 variable's overall standard deviation. Marginal R^2 s (the proportion of total variance explained
339 by the fixed effects) were calculated for each model (Nakagawa and Schielzeth 2013) to
340 determine the change in explanatory power adding our agent of selection had brought.

341

342 Sex-specific selection

343 We added sex as a fixed effect and the interaction between sex and each level of growth
344 rate and birth date to the first model for multilevel selection (without study area density) to
345 test for sex-specific selection. As sex is a two-level factor, we modelled females as the
346 default and males as a contrast, giving the regression estimate for females and the deviation
347 at each level for males. Note the values for each level of the traits are still relative to the
348 mean of all individuals in the level above, including both sexes.

349

350 Results

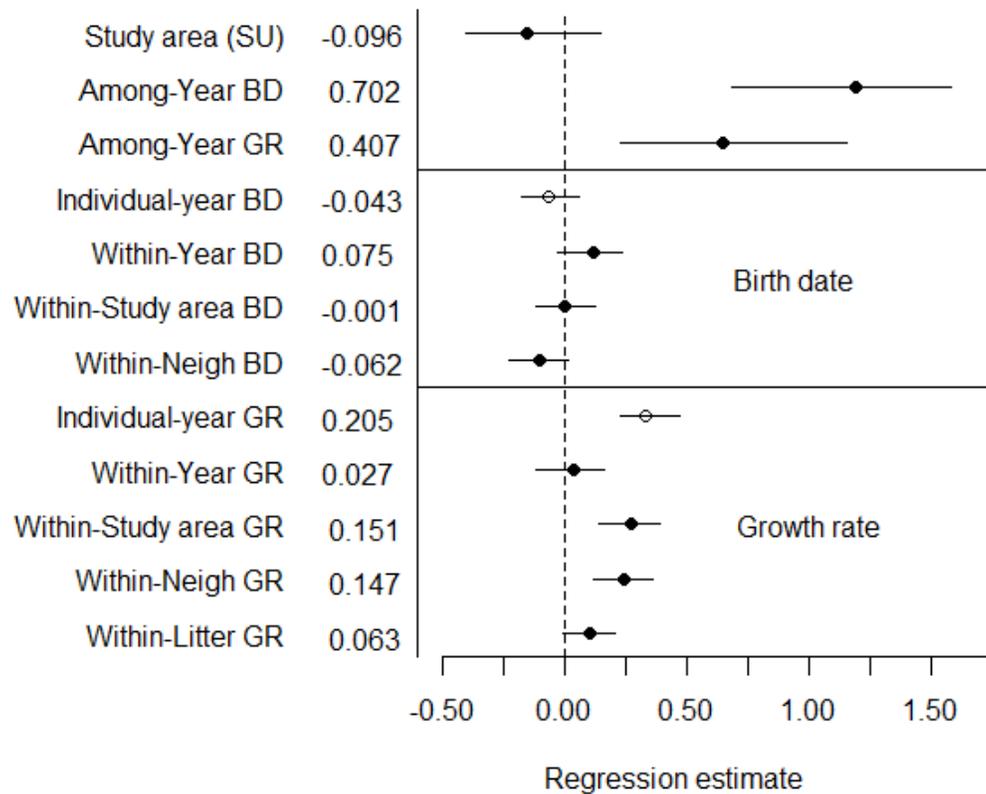
351

352 Across both study areas in all years (1989-2015) there were 2647 juveniles born that had a
353 known growth rate and birth date at each level. These came from 935 litters from 547 mother
354 squirrels. 26% of these juveniles survived to 200 days. Social neighbourhoods contained a
355 median of four litters (range: 1 – 22) and a median of 11 juveniles (range 1 – 60).

356

357 Levels of selection

358 Selection on growth rate was positive at all levels, but was strongest within-neighbourhoods
359 and became weaker at both smaller (within-litter selection) and larger hierarchical scales
360 (Fig. 1). There was also a positive among-year effect, such that years with higher growth
361 rate had higher average recruitment. None of the levels of birth date experienced consistent
362 selection, but there was a strong, positive among-year relationship; years where the mean
363 birth date was later had higher recruitment. There was considerable variation among-years in
364 recruitment (PDM = 0.749, CIs = 0.376 to 1.60), essentially no variation among-mothers in
365 recruitment (PDM = 0.02, CIs = <0.001 to 0.350), and a large amount of variation among-
366 litters (PDM = 1.26, CIs = 0.744 to 1.98). There was no difference in juvenile recruitment
367 between the two study areas (PDM = -0.164, CIs = -0.471 to 0.194). The standard selection
368 analysis indicted positive selection on growth rate (PDM = 0.330, CIs = 0.130 to 1.25) but no
369 overall selection on birth date (PDM = -0.066, CIs = -0.198 to 0.089). From Fig. 1 it is
370 apparent that these values represent an aggregation of the different levels of the multilevel
371 analysis.



372

373 Figure 1. Regression estimates and their 95% credible intervals for the influence of different
 374 levels of growth rate (GR) and birth date (BD) on the recruitment of juvenile red squirrels.
 375 Also given are the selection coefficients for each trait, obtained following Janzen and Stern
 376 (1998). Estimates from the multilevel analysis are indicated with solid points, while the
 377 estimates from the standard selection analysis (“Individual-year” terms) are indicated with
 378 open circles. Continuous variables have been transformed to the same scale, so effect sizes
 379 and selection coefficients are directly comparable. Study area is modelled as a two-level
 380 factor, with “Kloo” as the default, and so the effect here shows the difference in the “Sulphur”
 381 (SU) study area.

382

383 Agent of selection

384 Years with high population density experienced stronger within-neighbourhood selection for
 385 earlier birth dates. To a lesser degree, within-study area selection on birth date also
 386 increased with population density. Within-year selection on birth date, and all levels of
 387 selection on growth rate did not vary with changing population density (Table 1). For the
 388 majority of our traits (7/9), increasing density increased the strength of selection, as the
 389 coefficient for the interaction was of the same sign as for the main effect. However, only for
 390 within-neighbourhood selection on birth date did the interaction term not overlap with zero,
 391 although the interaction for within-study area selection on birth date only marginally
 392 overlapped zero. Adding the fixed effect of study area density, and its interaction with all

393 levels of growth rate and birth date, improved the model fit by 42% (without study area
394 density model $R^2 = 0.144$, with study area density model $R^2 = 0.204$).

395

396 Table 1. Posterior distribution mode (PDM) for the estimate of the main effect of each level
397 of growth rate and birth date, and the PDM for the interaction with each effect and study area
398 adult squirrel density (with 95% credible intervals [CIs] in parentheses). Effects for which the
399 CIs did not cross zero are highlighted in bold. When the trait main effect and the interaction
400 between density and the trait act in the same direction then increased density resulted in
401 stronger selection.

Trait	Effect	PDM of main effect	PDM of interaction	Same direction?
Growth rate	Within-litters	0.094 (-0.029 to 0.226)	-0.114 (-0.260 to 0.066)	No
	Within-neighbourhoods	0.232 (0.094 to 0.383)	0.022 (-0.159 to 0.169)	Yes
	Within-study areas	0.239 (0.030 to 0.425)	0.007 (-0.194 to 0.223)	Yes
	Within-years	0.021 (-0.169 to 0.228)	0.103 (-0.181 to 0.384)	Yes
	Among-years	0.694 (0.156 to 1.20)	-0.287 (-0.806 to 0.294)	No
Birth date	Within-neighbourhoods	-0.174 (-0.359 to -0.029)	-0.214 (-0.476 to -0.002)	Yes
	Within-study areas	-0.131 (-0.288 to 0.095)	-0.184 (-0.407 to 0.051)	Yes
	Within-years	0.169 (-0.057 to 0.340)	0.104 (-0.205 to 0.331)	Yes
	Among-years	1.15 (0.534 to 1.71)	0.091 (-0.402 to 0.596)	Yes

402

403

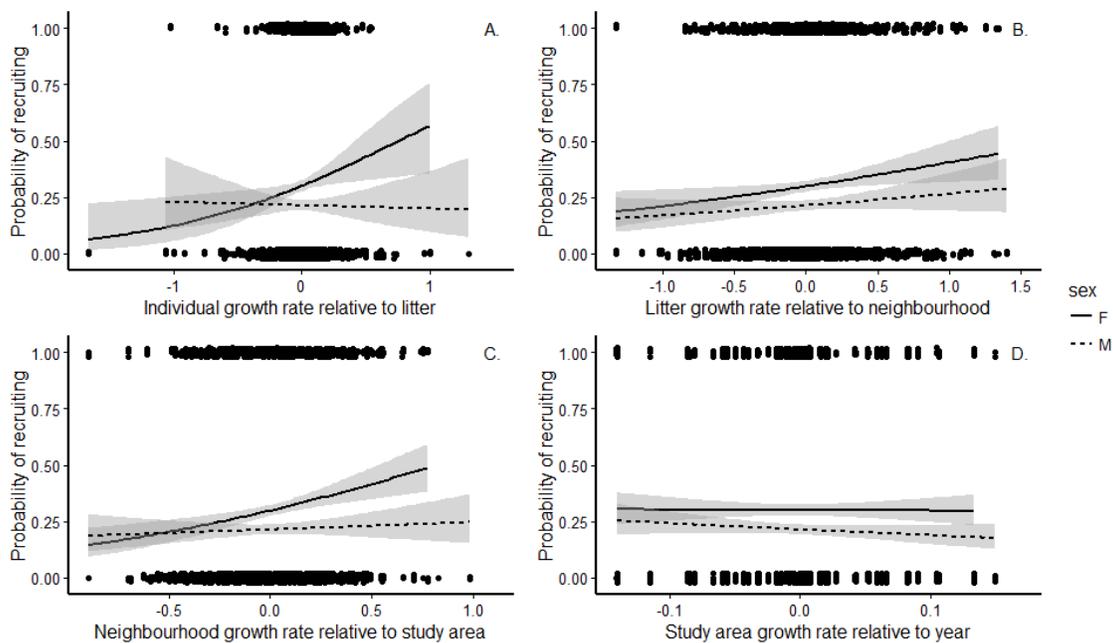
404

405 Sex-specific selection

406 Females were more likely to recruit than males (PDM = -0.747, CIs = -1.04 to -0.480; Figs.
407 2-4). Females that grew faster than their littermates were more likely to recruit, while males
408 were under very little selection for growth rate at this level (Fig. 2a; PDM = -0.403, CIs = -
409 0.740 to -0.163). Males and females were under equivalent selection for growth rate within-
410 social neighbourhoods (Fig. 2b; PDM = -0.023, CIs = -0.314 to 0.211), within-study areas
411 (Fig. 2c; PDM = -0.117, CIs = -0.415 to 0.107), and within-years (Fig. 2d; PDM = -0.032, CIs
412 = -0.356 to 0.240). The among-year relationship between mean year growth rate and

413 recruitment was positive in females, but tended to be weaker in males (Fig. 3a; PDM = -
414 0.407, CIs = -0.656 to 0.064). Males and females were under equivalent selection within-
415 social neighbourhoods for birth date (Fig. 4a; PDM = 0.053, CIs = -0.186 to 0.326). Females
416 from neighbourhoods with earlier mean birth dates tended to be more likely to recruit, but the
417 reverse was true for males (Fig. 4b; PDM = 0.311, CIs = 0.021 to 0.528). Males and females
418 were under equivalent selection for birth date within-years (Fig. 4c; PDM = 0.024, CIs = -
419 0.284 to 0.272), but females showed a marginally stronger association between growth rate
420 and recruitment among-years (Fig. 3b; PDM = -0.297, CIs = -0.657 to 0.061). Sex-specific
421 regression estimates are plotted in Fig. 5 to aid interpretation.

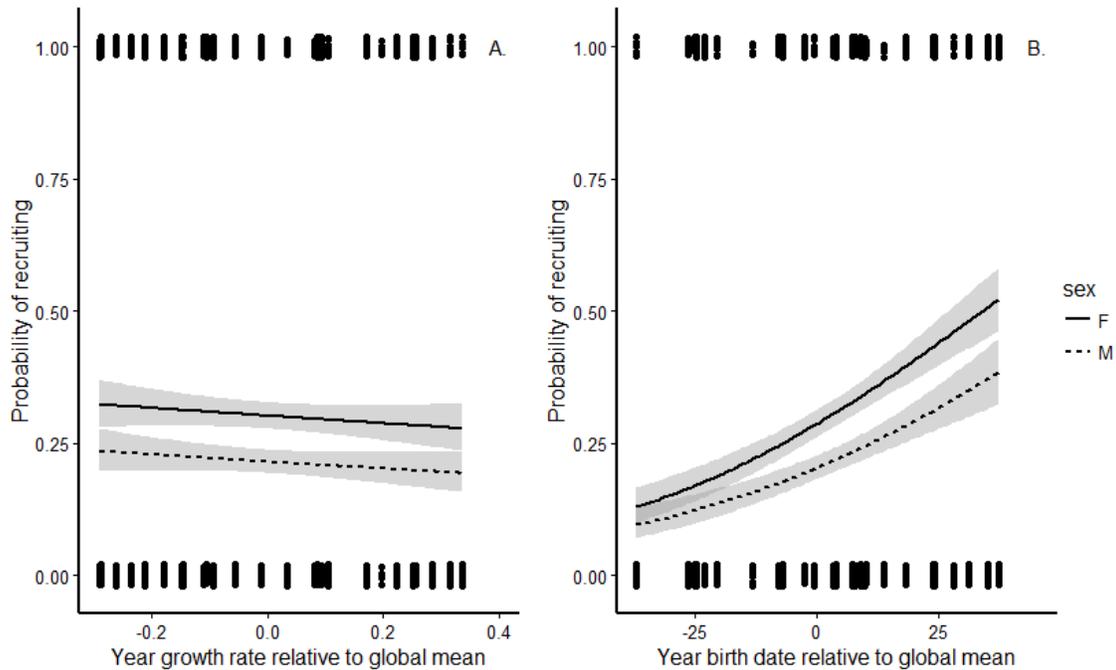
422



424

424 Figure 2. The influence of different levels of growth rate on juvenile red squirrel recruitment.
425 A: Individual growth rate relative to the litter's mean growth rate. B: Litter mean growth rate
426 relative to the social neighbourhood's mean growth rate. C: Mean social neighbourhood
427 growth rate relative to the study area's mean growth rate. D: Study area mean growth rate
428 relative to the mean growth rate for that year. Predictions from the model for females are
429 plotted as a solid line, for males as a dashed line, with the grey areas indicating the standard
430 errors around the estimates. Points have been moved a small amount at random either up or
431 down the y-axis to aid viewing, but all were either 0 or 1.

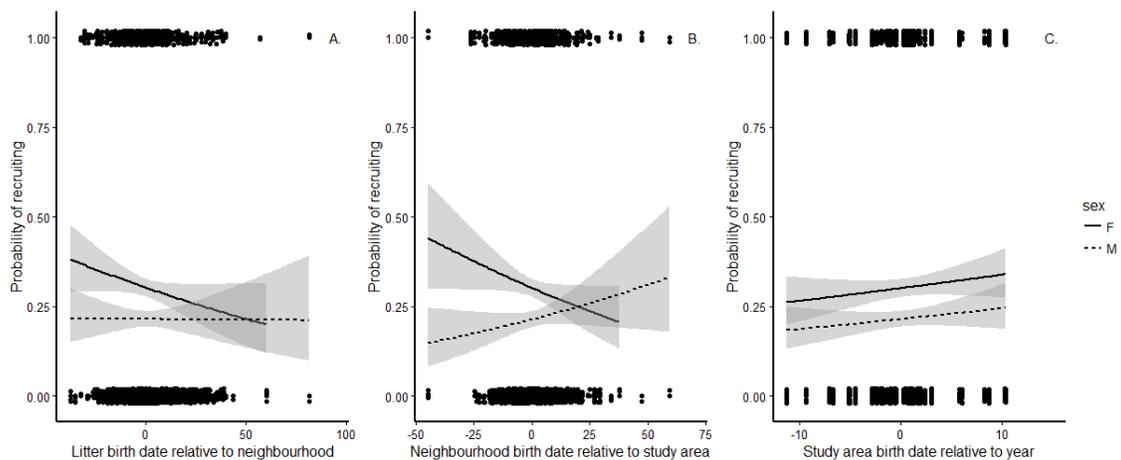
432



433

434 Figure 3. Among-year effects of A: growth rate, and B: birth date, on juvenile red squirrel
435 survival. Predictions from the model for females are plotted as a solid line, for males as a
436 dashed line, with the grey areas indicating the standard errors around the estimates. Points
437 have been moved a small amount at random either up or down the y-axis to aid viewing, but
438 all were either 0 or 1.

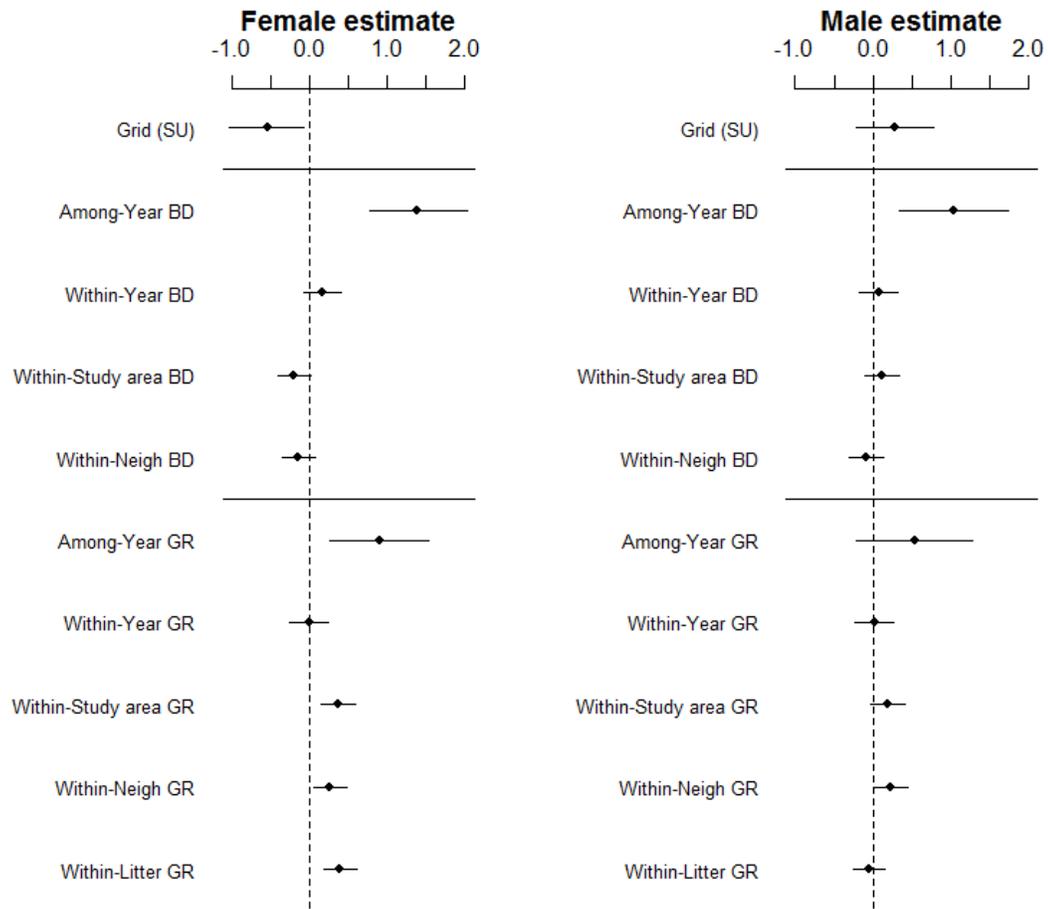
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440

441 Figure 4. The influence of different levels of birth date on juvenile red squirrel recruitment. A:
442 Litter birth date relative to the social neighbourhood's mean birth date. B: Mean social
443 neighbourhood birth date relative to the study area's mean birth date. C: Study area mean
444 birth date relative to the mean birth date for that year. Predictions from the model for females
445 are plotted as a solid line, for males as a dashed line, with the grey areas indicating the
446 standard errors around the estimates. Points have been moved a small amount
447 either up or down the y-axis to aid viewing, but all were either 0 or 1.

448



449

450 Figure 5. Regression estimates and their 95% credible intervals for the influence of different
451 levels of growth rate (GR) and birth date (BD) on the recruitment of female (left plot) and
452 male (right plot) juvenile red squirrels. Note these were modelled in one model using a sex
453 interaction term, but are plotted here as separate estimates for clarity. Variables have been
454 transformed to the same scale, so effect sizes are directly comparable. Study area is
455 modelled as a two-level factor, with “Kloo” as the default, and so the effect here shows the
456 difference in the “Sulphur” (SU) study area.

457

458 Discussion

459

460 Multilevel-selection

461 Natural selection on red squirrel growth rates and birth dates was most prominent for both
462 traits within-social neighbourhoods. Being born earlier than neighbouring litters, and/or
463 growing faster than them increased the chances of juveniles recruiting. This level of
464 selection is above the level of the individual squirrel yet is much more local than selection
465 acting across the entire population. Pups who grew faster than their littermates, and from

466 social neighbourhoods that grew faster than others in the study area, were also more likely
467 to recruit. Consistent selection on birth date was only apparent when we added our putative
468 agent of selection, study area density, to the model, indicating that an earlier birth date is
469 primarily beneficial for recruitment when there are many other competing individuals.
470 Therefore, there are interactions among-litters, within a social neighbourhood that are
471 important for whether a juvenile red squirrel recruits or not, and these interactions increase
472 in importance when population density is higher. Consistent selection on birth date was also
473 not apparent from our standard selection analysis, as this value represents an aggregation
474 of the within- and among-study area effects, which were in opposite directions. In contrast,
475 the standard selection analysis did reveal consistent selection favouring faster growth. Our
476 multilevel selection approach revealed that this overall selection was primarily driven by
477 selection acting at the more local scales.

478 That the within-neighbourhood scale was the most important (although for females
479 within-litter selection on growth rate was stronger, see below) suggests differences among-
480 litters within the social neighbourhood has the largest influence on recruitment in juvenile red
481 squirrels. An evolutionary response to group selection such as this requires non-zero
482 relatedness among-group members ($r > 0$), or alternatively for there to be IGEs among
483 individuals (Bijma and Wade 2008). Litters have a non-zero r (mean of between 0.25 and 0.5
484 depending on the number of fathers, notwithstanding any inbreeding) and as such selection
485 among litters can be expected to result in an evolutionary response. Indeed, previous
486 research has indicated that the majority of evolutionary potential in our system appears to be
487 through selection on litter-level characteristics and indirect maternal effects on these
488 characteristics, as this is where the genetic variance in fitness is (McFarlane et al. 2015) and
489 where selection is strongest (this study, see also: McAdam et al. 2002; McAdam and Boutin
490 2004). We also note that the response to selection will be influenced by these maternal
491 effects and their correlations with other components of maternal fitness (Thomson et al.
492 2017), which we have not estimated here. Future studies and predictions on the evolutionary
493 potential of this population should take this in account, as models of evolutionary change
494 incorporating such indirect effects can lead to counter-intuitive results (Mousseau and Fox
495 1998; Wolf et al. 1998; Bijma and Wade 2008).

496 Within social-neighbourhood selection being more important than within-study area
497 selection suggests that our definition of a social neighbourhood as all individuals within
498 130m reflects the level at which red squirrels compete for space and resources to recruit.
499 Further, this is congruent with the work of Dantzer et al. (2012), who demonstrated that
500 density within 150m was the most relevant measure in this system. Red squirrels can hear
501 territorial vocalisations by others from up to 130m (Smith 1968), and mothers use these
502 vocalisations to assess local density and increase the growth rate of their pups through

503 stress-mediated maternal effects (Dantzer et al. 2013). The within-neighbourhood scale did
504 not correspond to a discrete and mutually exclusive ‘group’, but instead represented the
505 unique interactions between each individual and its surrounding neighbours. We add to the
506 results of Laiolo and Obeso (2012) to show that this form of selection can occur based on
507 individually unique social environments, rather than discrete units such as a unique pair or
508 colony (see also: Nunney 1985). For all territorial animals, and those that live in
509 hierarchically structured populations, groups of competing or cooperating animals exist at
510 different scales (Hill et al. 2008). These can be relatively clearly defined, such as a
511 population containing distinct clans formed by discrete family units as found in sperm whales
512 (*Physeter macrocephalus*; Cantor et al. 2015), or defined based on spatial scale as we have
513 done in the current study. Therefore, multilevel selection may be widespread in situations
514 where it has yet to be considered. Genetic relatedness within a social neighbourhood or
515 IGEs among neighbours is required for among-neighbourhood selection to produce a
516 response (Bijma and Wade 2008). Juvenile red squirrels typically do not disperse far from
517 the natal nest (mean around 90m; Price and Boutin 1993; Larsen and Boutin 1994; Berteaux
518 and Boutin 2000), which could lead to clusters of related individuals. Explicit calculation of
519 this parameter will allow us to predict the response to this level of selection.

520

521 Study area density as an agent of selection on birth date

522 Our putative agent of selection, the density of the study area, was important in determining
523 the strength of selection on birth date at the within-social neighbourhood level, and to a
524 lesser extent the within-study area level, although not for growth rate at any level. Being born
525 earlier than neighbouring litters increased survival, which was especially important when the
526 study area was at a high density, but was less important when density was low. This
527 strengthens the idea that an early birth date is selectively advantageous because it allows
528 juveniles to locate vacant territories within their social neighbourhood.

529 While previous studies have shown that local density is often negatively related to
530 fitness components (e.g. Coulson et al. 1997; Wilkin et al. 2006), we have identified a trait
531 whose effects on fitness are mediated by population density (MacColl 2011; see also:
532 Dantzer et al. 2013; Bouwhuis et al. 2015). Although our initial analysis suggested no
533 consistent selection on birth date, adding population density to the model revealed both that
534 early-born litters were more likely to recruit, and that this effect was stronger at higher
535 densities. This is likely because there is among-year variation in the strength of selection,
536 related to changes in population size (McAdam and Boutin 2003b), so by accounting for this
537 we were able to detect the effect. Birth date is moderately heritable ($h^2 = 0.16$; Réale et al.
538 2003) and so as predicted by the breeder’s equation should be advancing (Lande 1979).

539 However, despite initial results suggesting a genetic change occurred over a 10-year period
540 (Réale et al. 2003b) additional data and a re-analysis indicated no change in birth date
541 (Lane et al. *In rev*), which seems to be caused by selection acting on environmental
542 deviations rather than the genetic basis to birth date.

543

544 Selection on growth rate

545 In our analysis, population density was not an agent of selection on growth rate. Dantzer et
546 al. (2013) previously found that a female's reproductive success was increased if her litter
547 was fast growing when local density was high, but not when it was low, in contrast to our
548 results. They used relative fitness rather than raw survival as their response variable, which
549 shows higher variance when recruitment is lower, which occurs in high-density years. This
550 may have enabled them to detect stronger selection on growth rate in high density years
551 where we did not. In addition, Dantzer et al. (2013) also included litter size in their selection
552 analysis, whereas we included only growth rate and birth date. The degree of competition for
553 vacant territories depends on both the number of vacancies as well as the number of
554 potential competitors (Taylor et al. 2014). While population density represents the inverse of
555 territory vacancy rates, the number of juveniles competing for each vacant territory might
556 also depend on the availability of food resources affecting the rate of offspring production.
557 This mechanism remains to be tested.

558 Goodnight et al. (1992) stated that if both individual and group-level selection
559 coefficients are the same, the selection is "hard". The absolute value of the individual's trait
560 is selected upon, unrelated to the social environment, with the agent likely to be some
561 environmental factor (Goodnight et al. 1992). Considering the selection coefficients were all
562 the same direction for growth rate, and that population density did not greatly influence the
563 strength of selection, selection on growth rate may act in this way. Possibly, faster growing
564 pups are generally of higher "quality", and so more likely to survive over winter. This too is a
565 mechanism that remains to be tested. Note that the overlapping CIs for the selection
566 coefficients is not necessarily good evidence that selection at different scales is equivalent,
567 as selection strengths fluctuate across years (McAdam and Boutin 2003b).

568 Although our standard selection analysis indicated strong selection on growth rate,
569 some of this selection occurred at the within-study area level. Response to this selection
570 requires genetic variance within-years (among-study areas), which we do not believe is
571 likely. Therefore, this portion of the selection gradient will not contribute to any evolutionary
572 response. This may be a common phenomenon, where standard selection analyses assume
573 that all the selection measured is aligned with the available genetic variation. Our results
574 suggest that might not be the case, which may contribute to the lack of evolutionary

575 response observed in populations where directional selection has been estimated on a
576 heritable trait (Merilä et al. 2001). A thorough multilevel quantitative genetic analysis would
577 be required, however, to completely determine how the scale of selection and the scale of
578 genetic variation together affect rates of evolution of growth rates and birth dates.

579

580 Sex-specific selection at the level of the litter for growth rate

581 Combining multilevel and sex-specific selection revealed contrasting relationships within-
582 litters for selection on growth rate. Females were under strong, positive selection within the
583 litter, while males were under no selection at this level. Furthermore, females typically were
584 more likely to recruit than males, a relatively common pattern in birds and mammals
585 (Clutton-Brock et al. 1985), and one that has been detected previously in this system
586 (LaMontagne et al. 2013). We suspect that selection was strong within-litters for females as
587 red squirrel mothers sometimes (19% of mothers; Lane et al. 2015) bequeath their territory,
588 or part of it, to one of their offspring (Price and Boutin 1993; Larsen and Boutin 1994), and
589 this offspring is most commonly a daughter (Berteaux and Boutin 2000). If squirrels do
590 disperse from the natal territory, the distance of settlement is not typically very large (see
591 above), and does not differ between the sexes (Cooper et al. *In rev*). Therefore, growing
592 more quickly than its littermates to obtain a larger size is perhaps important for a female
593 squirrel to out-compete its littermates for either the natal territory, or one of the (likely few)
594 available territories near to the nest. As bequeathal is biased towards females, fast growing
595 males may have no better chance of acquiring the natal territory than slower growing males,
596 as the territory tends to go to a female regardless. This may explain the lack of selection for
597 growth rate in males within-litters. Berteaux and Boutin (2000) found that individuals having
598 a territory bequeathed to them were not heavier than those that did not, however this was a
599 population-level analysis, with a smaller sample size than ours, and so may have failed to
600 identify this level of within-litter competition. Alternatively, fast-growing females may have
601 been smaller at birth, but grew more quickly than their siblings. This, however, would oppose
602 the general pattern that individuals that experience catch-up growth suffer reduced longevity
603 (Lee et al. 2012). Young and Badyaev (2004) noted that sex-biased allocation of parental
604 resources is more common when parents are limited in their ability to acquire or store
605 resources. While red squirrels do not appear limited in their ability to store resources, in most
606 years they will be strongly limited in their ability to acquire resources. In most years this is
607 unlikely to be true. Sex-biased allocation of resources depends on changes in the cost
608 differential of sons and daughters across different environments (Young and Badyaev 2004).
609 Such a cost differential change is not obvious in red squirrels at present, but could be
610 explicitly tested.

611 We note that the absolute growth rate of individuals did not differ between the sexes
612 (1.73 and 1.75 g/d for females and males respectively; t-test, $t = -0.821$, $df = 2392$, $p = 0.41$),
613 suggesting this selection has not resulted in the evolution of sex-biased growth rates.
614 Sexually antagonistic selection is quite common (Cox and Calsbeek 2009), for instance,
615 some *Anolis* lizard species show sexual eco-morph divergence so that the sexes occupy
616 different ecological niches (Butler et al. 2000, 2007), while body size in female yellow pine
617 chipmunk (*Tamias amoenus*) was typically positively related to fitness, but was selectively
618 neutral in males (Schulte-Hostedde et al. 2002). Sexually antagonistic selection is not
619 necessarily absent in sexually monomorphic species such as the red squirrels, as a sex-
620 specific response may not be possible (Cox and Calsbeek 2009). Although viability selection
621 typically shows the least degree of sexual antagonism (Cox and Calsbeek 2009), we still
622 found evidence for sexually antagonistic selection on recruitment. Similar results have been
623 found in *Drosophila melanogaster*, where when selection on females is prevented,
624 populations evolved towards a slower rate of growth that is favoured in males (Prasad et al.
625 2007). Cox and Calsbeek (2009) noted that many studies either focus on only one sex, or
626 pool the sexes, despite the fact that sexually antagonistic selection can strongly constrain
627 evolution. Therefore, we can only agree with their assertion that more studies should look for
628 sex-specific patterns of selection. Intriguingly, this sexually antagonistic selection was not
629 apparent at any other level we considered or in previous individual-based selection analyses
630 for these traits (e.g. McAdam and Boutin 2003b). Therefore, considering both sex-specific
631 selection and multilevel selection simultaneously may be necessary in future selection
632 analyses.

633

634 Selection on birth date is opposite at local scales vs. among-years
635 Offspring from litters born earlier than others in their social neighbourhood had an increased
636 chance of recruitment, yet the among-year effect was in the opposite direction: years that
637 have on average later birth dates had higher mean recruitment. This led to the standard
638 selection analysis suggesting very limited selection on birth dates. This among-year effect is
639 driven by annual variation in resource abundance. In most years, litters tend to be born later
640 (Boutin et al. 2006). The recruitment in these years is then increased as there are far more
641 resources available, allowing juveniles to create territories where there were none previously
642 and cache food there, increasing survival over winter (McAdam and Boutin 2003b). We also
643 note that selection on growth rates is stronger in the year *after* one of high cone abundance
644 (i.e. after a mast year), likely due to high densities, but that episodes of strong selection are
645 rare (McAdam and Boutin 2003b). Therefore, consistent within-year selection may not
646 always be apparent if among-year variation is not accounted for. Among-year relationships
647 between environmental conditions and reproductive dates alongside selection within each

648 year for these dates to shift earlier have been found in collared flycatchers (*F. albicollis*) and
649 red deer (*Cervus elaphus*), where females alter reproductive dates based on local
650 temperature or previous autumn rainfall respectively (Brommer et al. 2005; Nussey et al.
651 2005). Therefore, the masking of within-year selective forces by among-year variance in
652 environmental conditions may be common, and so controlling for it necessary when
653 investigating selection (see also van de Pol and Wright 2009 for analogous within- and
654 among-individual effects).

655

656 Conclusions

657 We have detected multilevel selection on recruitment in a natural population of red squirrels.
658 Selection was typically strongest when considering all individuals within the acoustic social
659 neighbourhood, although females also experienced strong within-litter selection on growth
660 rate. We also found evidence that population density acted as an agent of selection on birth
661 date during juvenile recruitment, but we found no evidence of density-dependent selection
662 through growth rate. If, as our results suggest, interactions are strongest at the within-
663 neighbourhood level, then evolutionary dynamics will strongly depend on traits and genetic
664 parameters at this level, alongside the individual level (Goodnight et al. 1992; Bijma and
665 Wade 2008). Our results highlight 1) the range of scales at which natural selection might act
666 in a solitary organism, 2) how identifying the agent of selection helps us understand a
667 system, 3) that sex-specific selection can occur only at particular levels of organisation, and
668 4) coefficients of selection being in the same or opposite direction across levels may lead to
669 the over- or under-estimation of selection. A better understanding of how natural selection
670 acts across a range of scales will enhance our ability to understand and predict trait
671 evolution in natural populations.

672

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681

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