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1 This article is a contribution to the Topical Collection Measuring individual reproductive success in the wild -
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3 In: Behavioral Ecology and Sociobiology
4

5 **Measuring fitness and inferring natural selection from long-term**
6 **field studies: different measures lead to nuanced conclusions**

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19 **ABSTRACT**

20 Measuring individual reproductive success in the wild is often achieved by counting the
21 number of descendants produced by individuals. In seeking to understand how reproductive
22 success can inform us about natural selection, however, we are faced with a conundrum. In terms
23 of timing, what is the most relevant measure for examining selection? We might count the number
24 of offspring born, surviving to the termination of parental care, surviving to adulthood, or only
25 those surviving to themselves reproduce. Clearly, only the latter are passing on genes and traits to
26 future generations, but this estimate may not always be available. So, are different estimates of
27 fitness consistent? Do they provide us with similar inferences of selection on phenotypic traits?
28 We examined these questions on a 29-year long-term study of individually monitored male and
29 female Columbian ground squirrels (*Urocitellus columbianus*). We used the long-term data to
30 calculate male and female fitness based on an annual measure of adult survival and the yearly
31 production of offspring counted at the stages of birth, weaning, and yearling age. We then
32 decomposed fitness into its constitutive elements including (1) adult survival to the next spring,
33 and (2) the yearly production of offspring counted at the stages previously mentioned. We then
34 compared fitness metrics to evaluate if they provided similar or contrasting information in the wild.
35 Next, we used those fitness metrics to test for selection on the date of emergence from annual
36 hibernation, a phenotypic trait previously shown to be highly variable, heritable, and associated
37 with reproduction. Finally, we partitioned selection on emergence date into additive episodes of
38 selection by looking at how selection changed from reproduction measured at birth, weaning and
39 when offspring reached yearling age. Overall, fitness metrics were well correlated, but correlations
40 weakened the further offspring were counted from birth. We generally found directional selection
41 for earlier emergence dates both in males and females. The strength of selection depended on

42 which fitness metric was used. Most of the selection gradient on emergence date was explained by
43 offspring born, and the selection differential was stronger in males than females. We evaluate how
44 the choice of fitness metrics in life-history studies may nuance our inferences about natural
45 selection.

46 **Keywords:** fitness, life history, lifetime reproductive success, mating, natural selection, survival

47 **SIGNIFICANCE STATEMENT**

48 This study explores how our inferences about natural selection acting on organismal traits vary
49 depending on our choice of fitness metrics. Focusing on the timing of emergence from hibernation
50 in Columbian ground squirrels, we show that directional selection for earlier emergence dates
51 occurs, but the strength of selection depends on whether fitness is evaluated from offspring counted
52 at birth, at weaning or later in life. These results show that the choice of timing for fitness
53 measurements may nuance inferences about natural selection in life-history studies.

54

55 **INTRODUCTION**

56 Understanding natural selection and the selective processes that shape the evolution of phenotypic
57 traits in living organisms hinges upon our ability to measure and aptly capture the adaptive
58 influence of traits with respect to specific environments (Endler 1986). Studies of natural selection
59 can be broadly separated into those that model the evolutionary process and those that attempt to
60 measure the action of natural selection in populations in nature. Models of natural selection seek
61 to reveal how the process works through mathematical expressions of change from one generation
62 to the next (Wright 1931; Crow and Kimura 1970; Orr 2009). These models often assume that
63 changes in trait frequencies are the result of natural selection, without the occurrence of other
64 processes such as migration, mutation, drift, or epigenetic modifications of gene expression.

65 In contrast, evolutionary change in real populations may actually reflect a combination of
66 these different processes. In addition, empirical studies of natural selection, in action in real
67 populations, generally measure change in trait frequencies from short-term measures of survival
68 or reproduction. This is problematic for inferences on natural selection, since fitness advantages
69 of particular trait values are often measured over a shorter time period than a complete generation

70 (Lande and Arnold 1983; Kruuk et al. 2002; Dobson et al. 2017). Such studies measure fitness as
71 success at surviving a storm or at producing juvenile offspring, and then compare the fitness metric
72 to trait values, rather than using success at producing reproducing offspring that themselves
73 reproduce in the next generation (but see Boyce and Perrins 1987).

74 Fitness is the complex metric by which one typically measures the adaptive value of
75 organismal traits in evolutionary ecology. Studies that refine the definition of fitness or seek
76 alternative measures of fitness are legion (Fisher 1930; Hamilton 1964; Grafen 1982, 2015; Endler
77 1986; Lucas et al. 1996; Queller 1996; Oli 2003; Qvarnström et al. 2006; van de Pol et al. 2006;
78 Hunt and Hodgson 2010; Dobson et al. 2012; Zhang et al. 2015; Scranton et al. 2016; Harris et al.
79 2017; Rubach et al. 2020; Levin and Grafen 2021). These studies usually propose some practical
80 means of measuring the propensity of a given gene, group of genes, trait, or group of traits, to
81 spread through populations over generations (Sæther and Engen 2015). In the present paper, we
82 use a trait-based definition of fitness: the change in frequencies of alternative forms of an
83 organismal trait over time in a population (Dobson and Viblanc 2019). This definition presents the
84 advantage that trait variation integrates sources of both genetic and non-genetic (e.g., early
85 environmental or maternal effects affecting trait expression) variation. It further has the advantage
86 of practicality since it is the phenotypic traits (e.g., individual mass, size, age) that are measured
87 in field studies, with no assumption made about their underlying genetic or non-genetic
88 architecture (e.g., the genetic variance-covariance matrix; Lande 1979; Arnold et al. 2008).

89 Regardless of the metric used, measuring fitness requires understanding how traits are
90 transmitted through the population over time. In terms of evolution, it is the fitness of a trait form
91 relative to others that counts (Ayala and Campbell 1974). For sexual organisms, such as many
92 animals and plants, transmission of traits is achieved through reproduction. Thus, most studies

93 evaluate fitness by measuring the propensity of organisms to transmit traits through the number of
94 offspring they produce. For iteroparous organisms, this includes successive reproductions, so that
95 adult survival and longevity, as well as age at first reproduction and reproductive senescence, are
96 also important components of fitness (Brommer et al. 2002; Oli et al. 2002; Bouwhuis et al. 2012).
97 However, when measuring fitness, the most important issues are what ultimately influences
98 changes in trait frequencies, and how our conclusions about trait evolution are shaped by the nature
99 of our fitness measures.

100 If adaptive traits are those that spread in the population relative to other trait forms, then
101 surely offspring that die before reproducing themselves do not contribute to the fitness of a given
102 trait. Yet, for studies in nature, our ability to monitor individuals over time is often limited: many
103 animal species disperse at a young age (Greenwood 1980; Dobson 1982), and for many species
104 age at maturity may be delayed (Cole 1954; Oli and Dobson 2003), so that the contribution of
105 offspring to trait fitness through their own future reproduction is difficult to measure. Thus, as
106 second-best proxies, studies of animals often rely on metrics such as clutch size, brood size, and
107 litter size at birth or at offspring independence, to quantify the number of offspring produced that
108 potentially can contribute to the next generation. Yet, because numerous offspring die at a young
109 age, it is unclear how trait fitness is influenced by further selection or changed by environmental
110 stochasticity as time goes by (Hadfield 2008). On one hand, trait fitness may be influenced by
111 genetic correlations and selection on other traits than the one of interest. On the other hand, the
112 further fitness is measured from the production of reproductive offspring, the more environmental
113 variation may change the association between a phenotypic trait and realized fitness (the fitness of
114 alternative traits when offspring begin reproduction).

115 The purpose of our study was to assess how the evaluation of fitness changed depending
116 on the timing of measurement using a 29-year long-term data set of male and female Columbian
117 ground squirrels (*Urocitellus columbianus*). Specifically, we were interested in understanding (1)
118 how individual fitness varied depending on whether we considered the offspring born, weaned, at
119 yearling age, and at age at first reproduction; and (2) whether the association of a phenotypic trait
120 and fitness changed depending on the timing of the fitness measurement. To do this, we measured
121 individual survival *and* reproduction, and estimated the number of gene copies that a breeding
122 parent passes on to the next year, by adding the parent's survival from the current year to the next
123 (1 for surviving, 0 for not) to half the number of offspring it produced. We then compared this
124 measure to other individuals in the population to obtain an estimate of annual fitness (Qvarnström
125 et al. 2006). We also estimated fitness from adult annual survival and reproduction separately, and
126 here the number of offspring was not halved (both annual fitness and these latter measures were
127 used, for example, by Dobson et al. 2017). Offspring production may be measured at different time
128 points, such as birth, weaning, or survival to a later period. We evaluated how important the choice
129 of when to measure offspring production was with an examination of repeatability (the intraclass
130 correlation coefficient "ICC," used as a measure of consistency) of annual and reproductive fitness
131 measures when using number of offspring measured at birth, weaning, and yearling age and
132 investigated paired correlations between these.

133 We also evaluated how measuring fitness at different time periods, and using different
134 metrics, affects inferences about natural selection. For illustrative purposes, we focused on a single
135 trait, and examined the strength of selection on emergence date from hibernation, i.e., the date of
136 spring emergence above ground from hibernation. For ground squirrels, this occurs close to the
137 termination of torpor (Williams et al. 2014). We did not know the genetic variance-covariance

138 matrix for traits associated with emergence from hibernation, and thus could not differentiate
139 selection among genetically correlated traits (Lande and Arnold 1983). However, adult female
140 Columbian ground squirrels go through a single day of estrus and mating each year, about 3-5 days
141 after emergence from hibernation (Murie and Harris 1982). The genetic correlation between
142 emergence and estrus dates was 0.98 ± 0.01 SE (Lane et al. 2011), indicating selection on one of
143 these traits would undoubtedly influence selection on the other. Since our sample sizes were
144 greatest for emergence date from hibernation, we chose this trait for analyses. Columbian ground
145 squirrels are hibernating rodents, with a short and intense reproductive season lasting only a few
146 months (Murie and Harris 1982; Dobson et al. 1992). The timing of emergence from hibernation
147 is highly variable (Tamian et al. 2022), and previous studies have shown it to be both significantly
148 heritable ($h^2 = 0.22-0.34$; Lane et al. 2011), and negatively associated with annual fitness when
149 measured using offspring at emergence from their first hibernation, at about one year old (for adult
150 females; Lane et al. 2012), prerequisite conditions for responding to selection. However, nothing
151 is known about whether the strength or direction of selection gradients differs when the fitness for
152 this trait is measured at different time periods, nor whether selection gradients exist for males.
153 Thus, emergence date appeared to be a good candidate trait to investigate its association with
154 different estimates of fitness measured from different life stages.

155

156 **Materials and Methods**

157 *Study species and long-term population monitoring*

158 Columbian ground squirrels were studied from 1992 to 2020 in the Sheep River Provincial Park,
159 Alberta, Canada (50° 38' 10.73" N; 114° 39' 56.52" W; 1524 m; 2.3 ha). Individuals were fitted
160 with permanent numbered ear tags (#1-Monel metal tag; National Band and Tag Company,

161 Newport, KY) when weaned (or at first capture for immigrants). Thus, it was not possible to record
162 data blind because our study involved focal animals in the field. In each year of the study, the
163 entire population was trapped at spring emergence using $13 \times 13 \times 40 \text{ cm}^3$ live-traps (Tomahawk
164 Live Trap, Hazelhurst, WI, USA) baited with a small amount of peanut butter. Each individual
165 was then dyed with a temporary unique dorsal mark (Clairol® Hydrience black hair dye N°52
166 Black Pearl, Clairol Inc., New York, USA) for identification during field observations. We
167 followed ground squirrels daily throughout the breeding season to assess breeding phenology and
168 success. Females copulated with multiple males within 3-5 days following emergence from
169 hibernation, typically during a single day of estrus (Murie and Harris 1982; Raveh et al. 2010).
170 We determined female mating date through behavioral observations and by inspecting female
171 genitalia (presence of a copulatory plug or plug material in abdominal fur, or sperm in vaginal
172 smears; Murie and Harris 1982). Following mating events, we identified female single-entrance
173 nest burrows during gestation by visual observations of females stocking them with dry grass
174 (Murie et al. 1998), and marked them with colored and flagged metal pins (1 m in length).

175 Females in the wild gave birth to an average of three (one to seven) blind and hairless
176 offspring in a specially constructed nest burrow, after some 24-days of gestation (Dobson and
177 Murie 1987; Murie 1995). From 2000 to 2016, we caught pregnant females within 2-3 days of
178 expected parturition, about 21-22 days after mating, and brought them to an on-site field laboratory
179 (Hare and Murie 1992). Females were housed indoor in polycarbonate microvent rat cages (483 x
180 267 x 200 mm; Allentown Caging Equipment Company, Allentown, NJ), supplied with wood
181 shavings and newspaper as nesting material. Females were provided with apple, lettuce, and horse
182 feed (Equisine sweet show horse ration; Unifeed, Okotoks, Alberta, Canada) *ad libitum*. After
183 parturition, offspring were sexed and a small tissue biopsy was acquired for paternity analyses (see

184 below) by clipping a toenail bud as previously described by Hare and Murie (1992). We returned
185 mothers and their offspring to flagged nest burrows, usually within a day of birth.

186 After a lactation period of approximately 27-28 days, offspring first emerged above ground
187 around the time of weaning (Murie and Harris 1982). We trapped females and their entire litters
188 the day the young first emerged. Mothers were determined by observation of the single lactating
189 female that associated with the natal burrow from which young emerged. Juvenile ground squirrels
190 hibernate within their colony of origin for the winter, and those juvenile males that immigrate
191 typically do so towards the end of the subsequent spring. Thus, in each year, we were able to
192 recapture all yearling males and females that survived their first hibernation.

193

194 *Paternity analyses*

195 From 2001 to 2017, paternity was estimated following the methods of Raveh et al. (2010). Briefly,
196 DNA was extracted from offspring tissue biopsies using DNeasy Tissue extraction kits (Qiagen,
197 Venlo, The Netherlands). We amplified 13 microsatellites with polymerase chain reaction (PCR),
198 using primer pairs previously developed for *U. columbianus* GS12, GS14, GS17, GS20, GS22,
199 GS25, and GS26 (Stevens et al. 1997); *Marmota marmota* BIBL18 (Goossens et al. 1998); MS41
200 and MS53; (Hanslik and Kruckenhauser 2000); and *Marmota caligata* 2g4, 2h6 (Kyle et al. 2004)
201 and 2h4 (GenBank accession no. GQ294553) amplified polymorphic microsatellite loci. We used
202 similar PCR conditions and cycling parameters as Kyle et al. (2004), but with an annealing
203 temperature of 54 °C. We tested for deviation from Hardy–Weinberg equilibrium (HWE) at each
204 locus within cohorts, and for linkage disequilibrium between pairs of loci within cohorts using
205 exact tests.

206 Paternity assignment was done using CERVUS 3.0 (Marshall et al. 1998; Kalinowski et al.
207 2007). Paternity was assigned with 95–99% trio confidence (assumed dam–sire–offspring
208 relationship). Analyses were conducted for each year separately. The input parameters for the
209 simulation step of CERVUS were 10 000 cycles, 70 candidate fathers, 90% of the population
210 sampled and 1% genotyping error. Parental assignments were accepted when the offspring had no
211 more than 2 mismatches with both parents.

212

213 *Annual survival, reproduction and fitness*

214 We calculated annual survival (*Surv*), as adult survival from the spring mating period to the time
215 of emergence in the next spring (1 for surviving, 0 for not surviving), separately for males and
216 females. Second, we quantified annual reproduction (*R*), as the number of offspring measured at
217 birth, weaning, and yearling age, separately for males and females. All immature individuals (i.e.,
218 females not observed mating or males with testes in abdominal position) were excluded from these
219 calculations. However, individuals considered mature (females observed mating and males with
220 testes in scrotal position) were all kept, even when their reproductive success was zero.

221 We calculated annual contributions to lifetime fitness, viz. annual fitness (λ_{an}), following
222 the method of Qvarnström et al. (2006), as applied to ground squirrels by Lane et al. (2011, 2012)
223 and Dobson et al. (2016, 2020):

$$224 \lambda_{an} = Surv + \frac{1}{2} R$$

225 For any given individual, *R* is halved since only half of an individual's genetic contribution is
226 passed-on to offspring.

227 The objective of our study was to determine whether fitness metrics were comparable when
228 reproduction was measured at different time points. Thus, annual fitness was calculated based on

229 R being measured as the number of offspring either produced at birth (λ_{an_b}), or weaning (λ_{an_w}),
230 or surviving up to yearling age (λ_{an_y}).

231

232 *Selection analyses*

233 We tested for directional, stabilizing or disruptive selection on emergence date using linear and
234 quadratic regression on annual fitness λ_{an} (Lande and Arnold 1983). We further ran selection
235 analyses decomposing λ_{an} into its constitutive elements including annual reproduction (fecundity
236 selection) and annual survival (viability selection). Those three fitness metrics were calculated
237 relative to the population in a given year by dividing them by the annual population mean for
238 annual fitness, reproduction and survival, respectively, and for males and females separately
239 (Lande and Arnold 1983). As selection operates among individuals, we first centered emergence
240 dates per year by subtracting from individual emergence dates the mean emergence date of the
241 population in a given year, hence translating how early or late (number of days) an individual was
242 compared to others in that same year.

243 We ran three sets of analyses where we estimated (1) directional selection gradients (β)
244 using univariate linear models, followed by (2) quadratic selection gradients (γ) from models that
245 included both a linear and quadratic term (Lande and Arnold 1983; Arnold and Wade 1984a). The
246 general form for these models is:

$$247 \quad \omega = \alpha + \beta z + \varepsilon \quad (1)$$

$$248 \quad \omega = \alpha + \beta' z + \frac{1}{2} \gamma z^2 + \varepsilon \quad (2)$$

249 where ω is the considered measure of fitness (i.e., either λ_{an} , R or $Surv$), α is the intercept, z the
250 phenotypic trait (here centered emergence date), and ε an error term. Note that γ coefficients are
251 reported as Lande and Arnold (1983)'s original formulation and do not require doubling to be

252 interpreted as stabilizing or disruptive selection gradients (Stinchcombe et al. 2008). We used
253 linear mixed models to account for individual ID as a repeated random term, and individual age as
254 a random factor because of known fitness differences that occur with age (Broussard et al. 2003;
255 Raveh et al. 2010). Statistical significance of the selection gradients for viability was estimated
256 with generalized linear-mixed models (GLMMs) with a binomial error structure (Garant et al.
257 2007; Dobson et al. 2017). Directional selection is indicated by significant linear coefficients (β),
258 the sign of the coefficients indicating the direction of selection. Stabilizing or disruptive selection
259 occurs when γ is significantly <0 or >0 , respectively (Lande and Arnold 1983; McGraw and
260 Caswell 1996). We ran the analyses separately in males and females because the variance of fitness
261 metrics was far greater in males than females (Jones et al. 2012).

262

263 *Partitioning selection into additive episodes*

264 Following Arnold and Wade (1984a, b), we partitioned selection on emergence date into additive
265 episodes of selection by looking at how selection changed from reproduction measured at birth,
266 weaning and when offspring reached yearling age. To do so, we separated reproduction in three
267 distinct biological episodes: production of offspring at birth, offspring survival from birth to
268 weaning, and offspring survival from weaning to yearling age. To better understand how each of
269 these episodes contributed to total selection, we estimated selection differentials for each of them.
270 Selection differentials represent the change in the mean value of a phenotypic character (here
271 emergence date) produced by selection. Because reproductive success at yearling age can be
272 decomposed in the product of the number of offspring born and the number surviving in the two
273 following episodes, selection differentials corresponding to each of the episode should sum to the
274 total selection differential. Selection differentials are thus presented for each episode in absolute

275 values (shift of the emergence date in number of days), but also as a % of contribution to total
276 selection.

277

278 *Statistics*

279 All analyses were done in R v. 4.0.2 (R Core Team 2020). The 95% confidence intervals for male
280 and female survival were obtained by parametric bootstrapping (10,000 simulations, 50% of the
281 individuals resampled each time). The consistency of fitness metrics (repeatability, or intraclass
282 correlation coefficient (ICC)) was calculated at different stages using the ‘rptR’ package in R
283 (Stoffel et al. 2017), $ICC = \frac{V_G}{V_P} = \frac{V_G}{V_G + V_R}$, where V_G is the among-individual variance, V_R is the
284 within-individual (or residual) variance and V_P is the total variance in fitness. The ICC for annual
285 fecundity (number of offspring) was estimated using a Generalized Linear Model with a Poisson
286 distribution (Stoffel et al. 2017), as appropriate when working with count data (we added +1 to
287 offspring numbers to avoid zero values). Correlation between fitness metrics measured at different
288 time points are Spearman rank correlation tests, as not all distributions were Gaussian. Because
289 information at birth and paternity analyses were not available in all years, sample sizes vary and
290 are indicated as n the number of reproductive events, N_1 the number of individuals and N_2 the
291 number of years in the results.

292

293 **RESULTS**

294 **Male and female annual survival**

295 Sexually mature (scrotal) male survival from one breeding season to the next was on average 65%
296 ($CI_{95} = [59\% - 71\%]$, $n = 209$ reproductive events, $N_1 = 78$, $N_2 = 21$). In contrast, sexually mature

297 females (i.e., those that estrus cycled and mated) had survival rates of 73% (CI₉₅ = [70% – 76%],
298 n = 732 reproductive events, N₁ = 223, N₂ = 28), on average.

299

300 **Male and female annual fecundity**

301 Adult male annual reproductive success varied from 0 to 29 offspring born (mean ± SD =
302 6.2 ± 5.7, n = 154 reproductive events, N₁ = 61 fathers, N₂ = 16 years), 0 to 26 offspring weaned
303 (4.2 ± 4.8, n = 190, N₁ = 73, N₂ = 19), and 0 to 16 offspring surviving to yearling age (1.7 ± 2.6, n
304 = 190, N₁ = 73, N₂ = 19). Adult female annual reproductive success was lower, and varied from 0
305 to 7 offspring born (3.0 ± 1.2, n = 454 reproductive events, N₁ = 167 mothers, N₂ = 21 years), 0 to
306 7 offspring weaned (2.1 ± 1.5, n = 759, N₁ = 228, N₂ = 29), and 0 to 5 offspring surviving to
307 yearling age (0.9 ± 1.1, n = 732, N₁ = 223, N₂ = 28). Overall, offspring survival from birth to
308 weaning was 75% (CI₉₅ = [72% – 78%], N = 684) for male offspring and 77% (CI₉₅ = [72% –
309 78%], N = 655) for female offspring; and from weaning to yearling age 43% (CI₉₅ = [40% – 47%],
310 N = 775) for male offspring and 42% (CI₉₅ = [39% – 46%], N = 742) for female offspring. Annual
311 fecundity was strongly consistent in males (ICC = 0.71, CI₉₅ = [0.60 – 0.78]), but less so in females
312 (ICC = 0.08, CI₉₅ = [0.03 – 0.13]).

313

314 **Male and female annual fitness**

315 When calculated using all of the offspring born (λ_{anb}), weaned (λ_{anw}) or surviving to yearling
316 age (λ_{any}), annual fitness was strongly consistent in males (ICC = 0.67, CI₉₅ = [0.60 – 0.73]), but
317 less so in females (ICC = 0.49, CI₉₅ = [0.45 – 0.54]). The correlation between annual fitness metrics
318 generally weakened as the period between life stages increased, from 0.95 between birth and

319 weaning to 0.73 between birth and yearling age in males (Fig. 1A) and from 0.74 to 0.65 in females
320 (Fig. 1B).

321

322 **Selection on emergence date**

323 *Annual fitness (λ_{an}) selection*

324 In males, we found directional selection for earlier relative emergence date when annual
325 fitness was calculated based on offspring born ($\beta = -0.024$; Fig. 2A), and on offspring surviving to
326 weaning ($\beta = -0.018$; Fig. 2C), but not when calculated based on offspring that survived to yearling
327 age ($\beta = -0.007$; Fig. 2E) (Table 1). The strength of selection appeared to decrease from $|\beta\lambda_{an_b}|$
328 $> |\beta\lambda_{an_w}| > |\beta\lambda_{an_y}|$. In females, we also found directional selection for earlier relative emergence
329 date when annual fitness was calculated based on offspring born ($\beta = -0.007$; Fig. 2B), and on
330 offspring surviving to yearling age ($\beta = -0.013$; Fig. 2F), but not when calculated based on
331 offspring that survived to weaning ($\beta = -0.004$; Fig. 2D). Note, that the directional selection
332 coefficient for relative emergence date almost doubled for females between annual fitness
333 calculated based on offspring born compared to when fitness was based on offspring surviving to
334 yearling age ($-0.013/-0.007 = 1.86$), but decreased in males ($-0.007/-0.024 = 0.29$).

335 Both in adult males and adult females, we found no evidence of substantial disruptive or
336 stabilizing selection on relative emergence date, regardless of whether annual fitness was measured
337 based on offspring born, or on offspring surviving to weaning, or yearling age (Table 1).

338

339 *Viability selection*

340 In males, we found directional viability selection for later emergence date ($\beta = +0.022$), but no
341 evidence of non-linear viability selection on emergence date (Table 1). In females, there was no

342 evidence of significant directional viability selection, but suggestion of weak stabilizing viability
343 selection on emergence date that approached significance (Table 1).

344

345 *Fecundity selection*

346 In males, we found directional selection for earlier relative emergence date when fecundity was
347 calculated from the number of offspring born ($\beta = -0.032$, Fig. 3A), weaned ($\beta = -0.033$, Fig. 3C),
348 and surviving to yearling age ($\beta = -0.033$, Fig. 3E) (Table 1). Selection gradients were of similar
349 magnitude regardless of the period considered. We found no evidence of disruptive or stabilizing
350 selection regardless of whether fecundity was calculated from the number of offspring born,
351 weaned, or surviving to yearling age (Table 1).

352 In females, there was mild directional selection for earlier relative emergence date when
353 fecundity was calculated from the number of offspring born ($\beta = -0.007$, Fig. 2B), but not when
354 calculated from offspring weaned ($\beta = +0.002$, Fig. 3C), or surviving to yearling age ($\beta = -0.012$)
355 (Table 1). However, we found weak stabilizing selection for emergence date based on offspring
356 surviving to yearling age that approached significance ($\gamma = -0.005$; Table 1, Fig. 3E).

357

358 *Partitioning selection into additive episodes*

359 In both males and females, selection on the number of offspring born provided a major selective
360 advantage favoring earlier emergence date (Table 2). For males, selection on the number of
361 offspring born accounted for 93% of the total selection differential and shifted the mean emergence
362 date by 2.5 days, while selection acting between birth and weaning or between weaning and
363 yearling age were minor, accounting for only 5 and 2% of the total selection differentials. For
364 females, selection differentials were much lower than in males (0.4 days vs. 2.6 days shift due to

365 total selection). While selection on the number of offspring born, as well as from weaning to
366 yearling, also shifted emergence dates earlier (by 0.6 and 0.3 days respectively), selection from
367 birth to weaning counteracted this by shifting emergence dates later by 0.5 days.

368

369 **DISCUSSION**

370 Our results in the Columbian ground squirrels show that, overall, measures of fitness are
371 relatively well correlated when fitness is measured from offspring counted at birth, at weaning,
372 and at yearling age. In general, annual measures appeared quite strongly inter-correlated at
373 different stages. Not surprisingly, the correlation between fitness measures changed, generally
374 waning over the progression of life stages of the offspring. Overall, the strongest correlations were
375 for fitness measured from birth and weaning offspring numbers, and associations became weaker,
376 as time went by (from birth to yearling surviving offspring). These results might have been
377 expected, since offspring mortality was low between birth and weaning, and much higher between
378 weaning and yearling age.

379 Columbian ground squirrel offspring are raised over a short lactation period of 27 days
380 (Murie and Harris 1982), during which they are secluded in specialized burrow systems and
381 protected by their mothers (Murie and Harris 1988). As expected, mortality is relatively low during
382 this period. After weaning, they only have a few weeks of foraging to grow and gain sufficient
383 body mass to survive their first hibernation (Dobson 1992; Dobson et al. 1992). Offspring with
384 insufficient body condition are unlikely to survive to the next spring (Murie and Boag 1984;
385 Dobson et al. 1986). Thus, not surprisingly, overwinter mortality of juveniles is high (Dobson and
386 Murie 1987; Zammuto 1987; Neuhaus and Pelletier 2001), and the correlation between fitness
387 metrics wanes rapidly as the period between life stages of the offspring increases. Nonetheless, it

388 is noteworthy that for annual fitness measures these correlations remain relatively high (> 0.60),
389 so that measuring fitness from the number of offspring weaned in a given year already appears as
390 a reasonable first approximation for estimating fitness. For instance, the correlation between
391 annual fitness measured at weaning and when offspring were yearlings was 0.81 for adult males
392 and 0.75 for adult females.

393 Sources of variation concerning the changes in correlation between measures between birth
394 and yearling age of offspring remain to be determined. Such variations could include stochastic
395 events that occur on an annual scale and accumulate over time, or other sources of biological
396 variation known to affect early offspring survival such as changes in the social environments (e.g.,
397 kin numbers; Viblanc et al. 2010; Barra et al. 2021). It is possible that selection on the offspring
398 before they begin to reproduce at a later time could operate in a way that could weaken or
399 strengthen associations of fitness estimates (whether or not an evolutionary response to selection
400 occurs). Nonetheless, it is noteworthy that, overall, the correlations between fitness measured from
401 offspring counted at birth, weaning, or yearling age were all high (> 0.65) for annual fitness
402 measures, suggesting that measuring fitness from offspring surviving to the first year is a good
403 proxy to measuring actual fitness from offspring susceptible themselves to passing on traits (as for
404 adult females in Lane et al. 2012; Dobson et al. 2016).

405 How did measuring fitness from an annual perspective at different time points affect our
406 inferences on natural selection? To answer this question, we focused on the entire data set,
407 calculating fitness from both male and female offspring, and regressed individual annual fitness
408 on date of emergence from hibernation, a phenotypic trait known to be heritable (Lane et al. 2011),
409 responsive to environmental fluctuation (Dobson 1988), and negatively associated with fitness
410 (Lane et al. 2012). There appeared to be little or no stabilizing or disruptive selection (no quadratic

411 effects), but only directional selection, acting on emergence date. In general, individuals emerging
412 earlier from hibernation had higher estimated fitness, and this was especially true for males.

413 In males, selection coefficients decreased when based on measurements from offspring
414 counted at birth, weaning to yearling age. This likely occurred because of relatively strong
415 mortality during the juvenile period, and supports the hypothesis that environmental stochasticity
416 may dilute the association between a phenotypic trait and fitness due to passage of time since the
417 environmental event (*viz.*, emergence from hibernation) increases. This is important, since
418 although the directional selection coefficients measured at different time points are consistent in
419 sign (*i.e.*, negative), the conclusions made would differ substantially. For instance, in males, based
420 on fitness calculated from the number of offspring counted at weaning, significant linear (β)
421 coefficients would give rise to a conclusion of directional selection (Lande and Arnold 1983)
422 acting on emergence date, whereas non-significant coefficients based on fitness calculated from
423 the number of offspring counted at yearling age would not (if relying on significance thresholds,
424 but see below). Similarly, in females and based on statistical significance, one might conclude on
425 directional selection on emergence when using offspring counted at birth, but not when using
426 offspring counted at weaning or at a yearling age, despite the selection gradients being strongest
427 when using number of offspring that reach a yearling age (Table 1).

428 Decomposing annual fitness into its annual survival and reproductive components
429 generally revealed consistent patterns. Interestingly, we found mild positive directional viability
430 selection on emergence date for males, but no viability selection for females: males that emerged
431 later had better annual survival. Positive selection on viability in males might occur because males
432 emerge from hibernation early to establish mating territories (Manno and Dobson 2008), but early
433 emergence has a survival cost (Turbill et al. 2011; Constant et al. 2020). For females, a weaker

434 pattern of selective advantage was revealed for earlier emergence from hibernation, but the pattern
435 was only significant when the combined index of annual fitness was used. In general, for both
436 sexes, fecundity selection on emergence date revealed overall similar patterns as annual fitness,
437 except perhaps for females where mild stabilizing selection was found using offspring that survive
438 to yearling age as the fecundity fitness estimate.

439 When fecundity selection was separated into distinct episodes of selection (Arnold and
440 Wade 1984a, b), we found that selection at birth accounted for the vast majority of the selection
441 differentials on emergence date both in males and females. For males dates of emergence from
442 hibernation, selection based on numbers of young at birth was the strongest (by nearly 2.5 days,
443 Table 2), and might have reflected sexual selection (primarily number of mates) and maternal
444 effects during gestation. Selection during lactation, based on numbers of offspring at weaning,
445 however, was weak. This is consistent with the males contributing virtually nothing to parental
446 care in this species, and reproductive success being determined foremost by the number of mated
447 females early on in the season (CS et al., unpublished data). Finally, ecological influences on male
448 date of emergence from hibernation may have been best reflected by numbers of offspring that
449 survived their first hibernation, but this influence was trivial. For females, selection on emergence
450 from hibernation was relatively weak and only approached significance. Partitioning this pattern
451 into episodes of selection did not appear to produce important insights, other than a slight fitness
452 advantage of emerging earlier from hibernation (by less than half a day).

453 Taken together, our results over a 29-year long-term study of Columbian ground squirrels,
454 showed that annual fitness measures, whereas generally closely related, may lead to nuanced
455 conclusions on the strength (but not direction) of selection acting on heritable phenotypic traits.
456 We documented an overall dilution of fitness associations, and in most cases a waning association

457 between fitness and emergence date, as time passed likely due to added stochastic processes
458 affecting offspring survival. Importantly, focusing on the significance of associations between
459 phenotypic traits and fitness led to contrasting conclusions depending on when fitness is measured.
460 The values of the selection coefficients, however, were fairly consistent using different fitness
461 estimates. Taking a step back from a traditional hypothesis-testing approach and focusing on the
462 magnitude of effect sizes, as more and more frequently is recognized in evolutionary ecology
463 (Yoccoz 1991; de Valpine 2014), likely provides more meaningful information on the strength and
464 patterns of selection acting on phenotypic traits in living organisms.

465

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479

480 **DATA AVAILABILITY**

481 All data generated or analyzed during this study are included in this published article and its
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483

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493

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495

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502

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679

680 **FIGURE CAPTIONS**

681 **Fig. 1** Pairwise correlation plots for (A) male and (B) female annual fitness metrics calculated
682 from offspring counted at birth (λ_{an_b}), weaning (λ_{an_w}), or surviving to yearling age (λ_{an_y}). The
683 distribution of data is given on the diagonal. Significant Spearman correlation coefficients are
684 given for *** $P < 0.001$

685

686 **Fig. 2** Selection on emergence date from regression of annual fitness(λ_{an}) on mean year-centered
687 emergence dates in males (left panels) and females (right panels). Fitness was calculated for
688 fecundity based on offspring counted at birth (λ_{an_b}) (A-B), weaning (λ_{an_w}) (C-D), or surviving
689 to yearling age (λ_{an_y}) (E-F). Significant regressions are indicated by black lines, and gray ribbon
690 representing 95% confidence intervals

691

692 **Fig. 3** Fecundity selection on emergence date from regression of relative annual reproductive
693 success on year-centered emergence dates in males (left panels) and females (right panels).
694 Fecundity was calculated from offspring counted at birth (A-D), weaning (C-F), or surviving to
695 yearling age (E-H). Significant regressions are indicated by black lines, and gray ribbons
696 representing 95% confidence intervals

697

699 **Table 1** Linear (β) and non-linear (γ) selection gradients for viability selection (adult survival), fecundity
700 selection (adult reproduction, number of offspring produced) and annual fitness selection (λ_{an} , see methods) on
701 emergence date in Columbian ground squirrels. Coefficients estimated from LMMs are provided with their
702 standard errors. Significant coefficients at $P < 0.05$ are indicated by an asterisk. Significance of coefficients were
703 obtained with gaussian error structure for fecundity and annual fitness, and binomial error structure for viability
704

		MALES			FEMALES		
VIABILITY		Coefficient \pm SE	z	P	Coefficient \pm SE	z	P
<i>Linear</i>	β (ED)	0.022 \pm 0.009	2.650	0.008*	-0.009 \pm 0.004	-1.507	0.132
	β (ED)	0.022 \pm 0.012	1.584	0.113	-0.016 \pm 0.006	-1.857	0.063
	<i>Non-linear</i>	γ (ED ²)	-0.000 \pm 0.002	-0.410	0.682	-0.001 \pm 0.001	-1.909
FECUNDITY		Coefficient \pm SE	t	P	Coefficient \pm SE	t	P
Birth							
<i>Linear</i>	β	-0.032 \pm 0.009	-3.675	<0.001*	-0.007 \pm 0.003	-1.932	0.054
	β	-0.027 \pm 0.010	-2.593	0.010*	-0.008 \pm 0.004	-2.243	0.025*
<i>Non-linear</i>	γ	0.001 \pm 0.001	1.020	0.309	-0.001 \pm 0.001	-1.806	0.072
Weaning							
<i>Linear</i>	β	-0.033 \pm 0.010	-3.410	<0.001*	0.002 \pm 0.005	0.344	0.731
	β	-0.027 \pm 0.011	-2.368	0.019*	0.001 \pm 0.005	0.114	0.909
<i>Non-linear</i>	γ	0.001 \pm 0.001	0.962	0.337	-0.001 \pm 0.001	-1.017	0.310
Yearling							
<i>Linear</i>	β	-0.033 \pm 0.015	-2.180	0.031*	-0.012 \pm 0.010	-1.119	0.264
	β	-0.024 \pm 0.018	-1.337	0.183	-0.017 \pm 0.011	-1.568	0.117
<i>Non-linear</i>	γ	0.002 \pm 0.002	0.912	0.363	-0.005 \pm 0.002	-2.101	0.036*
ANNUAL FITNESS		Coefficient \pm SE	t	P	Coefficient \pm SE	t	P
Birth							
<i>Linear</i>	β	-0.024 \pm 0.007	-3.211	0.002*	-0.007 \pm 0.003	-2.624	0.009*
	β	-0.017 \pm 0.009	-2.022	0.045*	-0.008 \pm 0.003	-2.819	0.005*
<i>Non-linear</i>	γ	0.002 \pm 0.001	1.362	0.175	-0.001 \pm 0.001	-1.602	0.110
Weaning							
<i>Linear</i>	β	-0.018 \pm 0.007	-2.405	0.017*	-0.004 \pm 0.003	-1.116	0.265
	β	-0.012 \pm 0.009	-1.341	0.181	-0.005 \pm 0.003	-1.376	0.169
<i>Non-linear</i>	γ	0.001 \pm 0.001	1.166	0.245	-0.001 \pm 0.001	-1.318	0.188
Yearling							
<i>Linear</i>	β	-0.007 \pm 0.008	-0.858	0.392	-0.013 \pm 0.004	-2.873	0.004*

<i>Non-linear</i>	β	-0.000±0.010	-0.048	0.962	-0.014±0.004	-3.188	0.001*
	γ	0.002±0.001	1.161	0.247	-0.002±0.001	-1.719	0.086

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707 **Table 2** Episodes of fecundity selection (Arnold and Wade 1984a, b). The trait under selection was date of
 708 emergence from hibernation, an important phenological trait that is strongly associated with successful
 709 reproduction and fitness (Lane et al. 2012). Selection differentials are given in days
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	MALES		FEMALES	
	<i>Selection differential</i> S_k	<i>Contribution to total selection</i>	<i>Selection differential</i> S_k	<i>Contribution to total selection</i>
SELECTION AT BIRTH				
<i># Offspring born</i>	-2.435	93%	-0.593	144%
SELECTION FROM BIRTH TO WEANING				
<i>% Surviving offspring birth to weaning</i>	-0.142	5%	0.492	-120%
SELECTION FROM WEANING TO YEARLING AGE				
<i>% Surviving offspring weaning to yearling age</i>	-0.043	2%	-0.310	75%
TOTAL SELECTION	-2.619	100%	-0.411	100%

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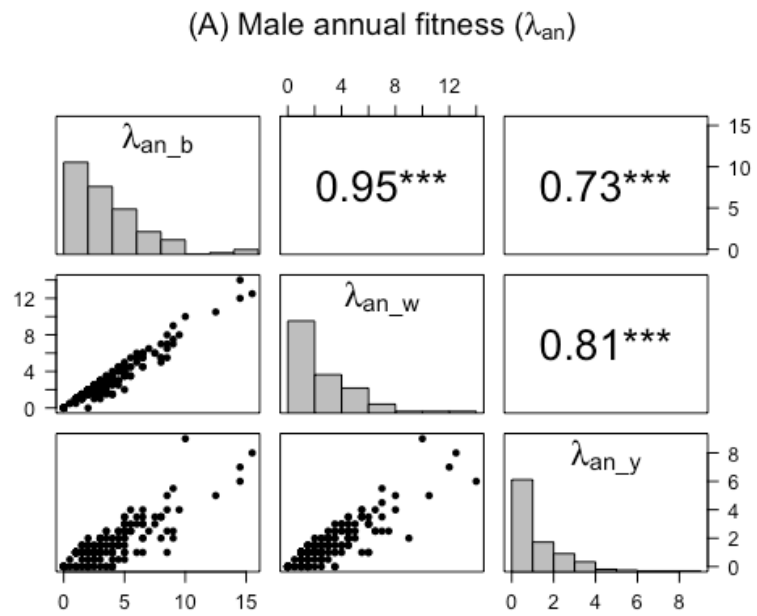
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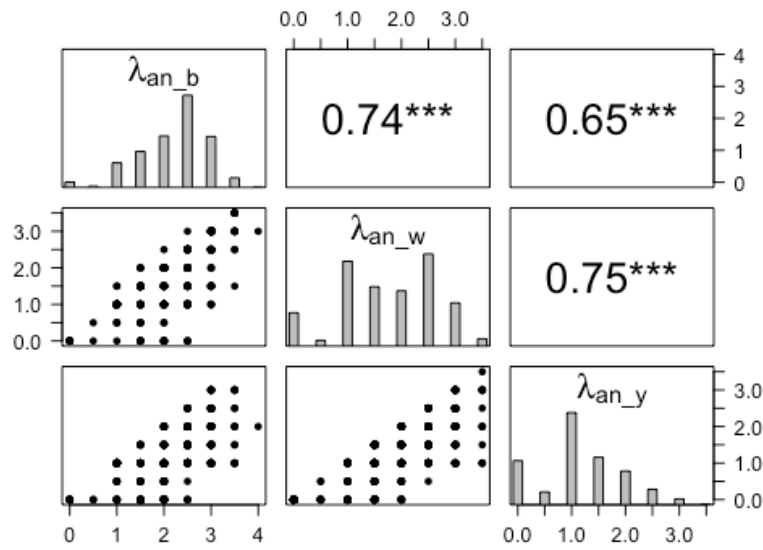
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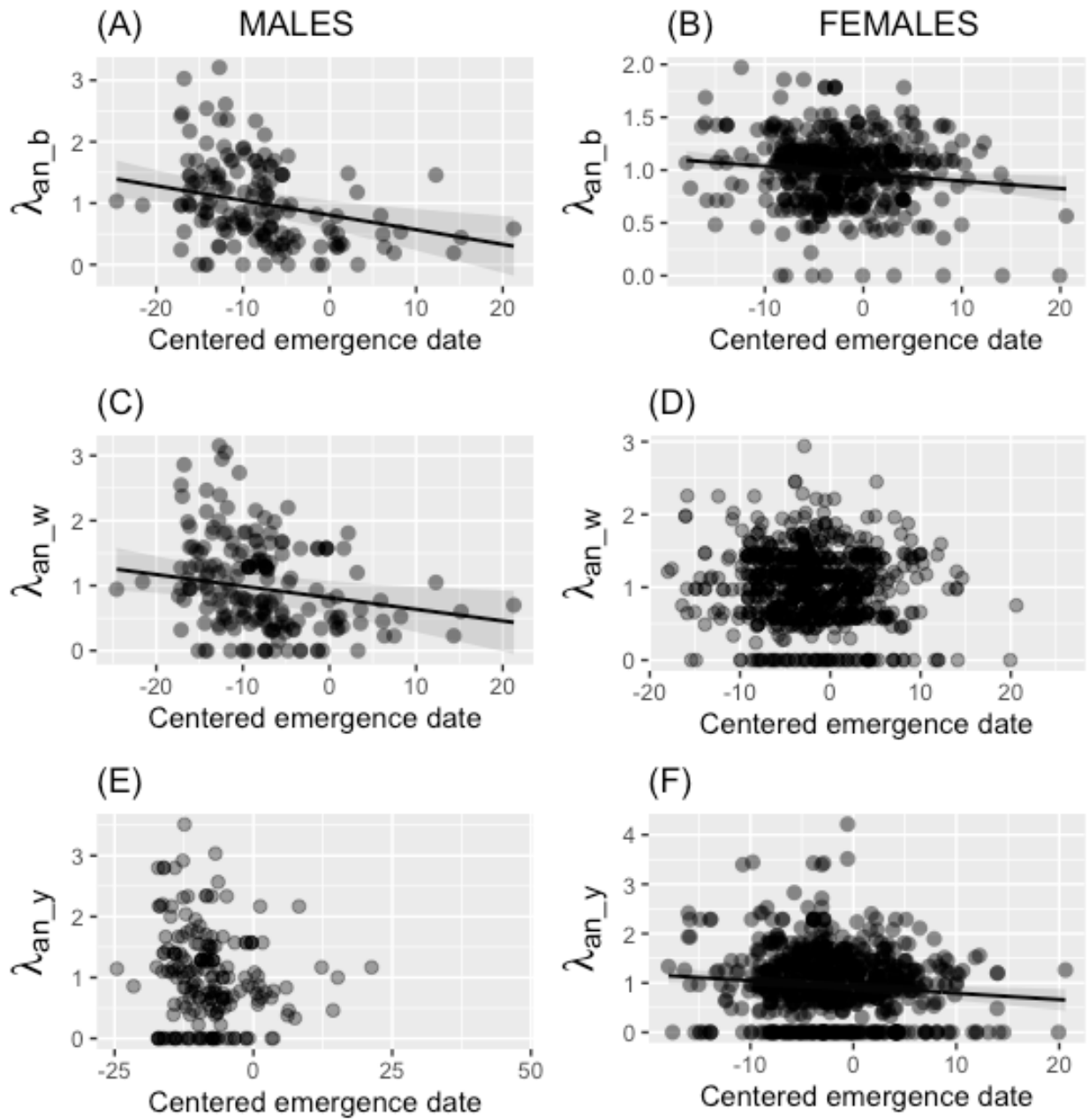
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(B) Female annual fitness (λ_{an})





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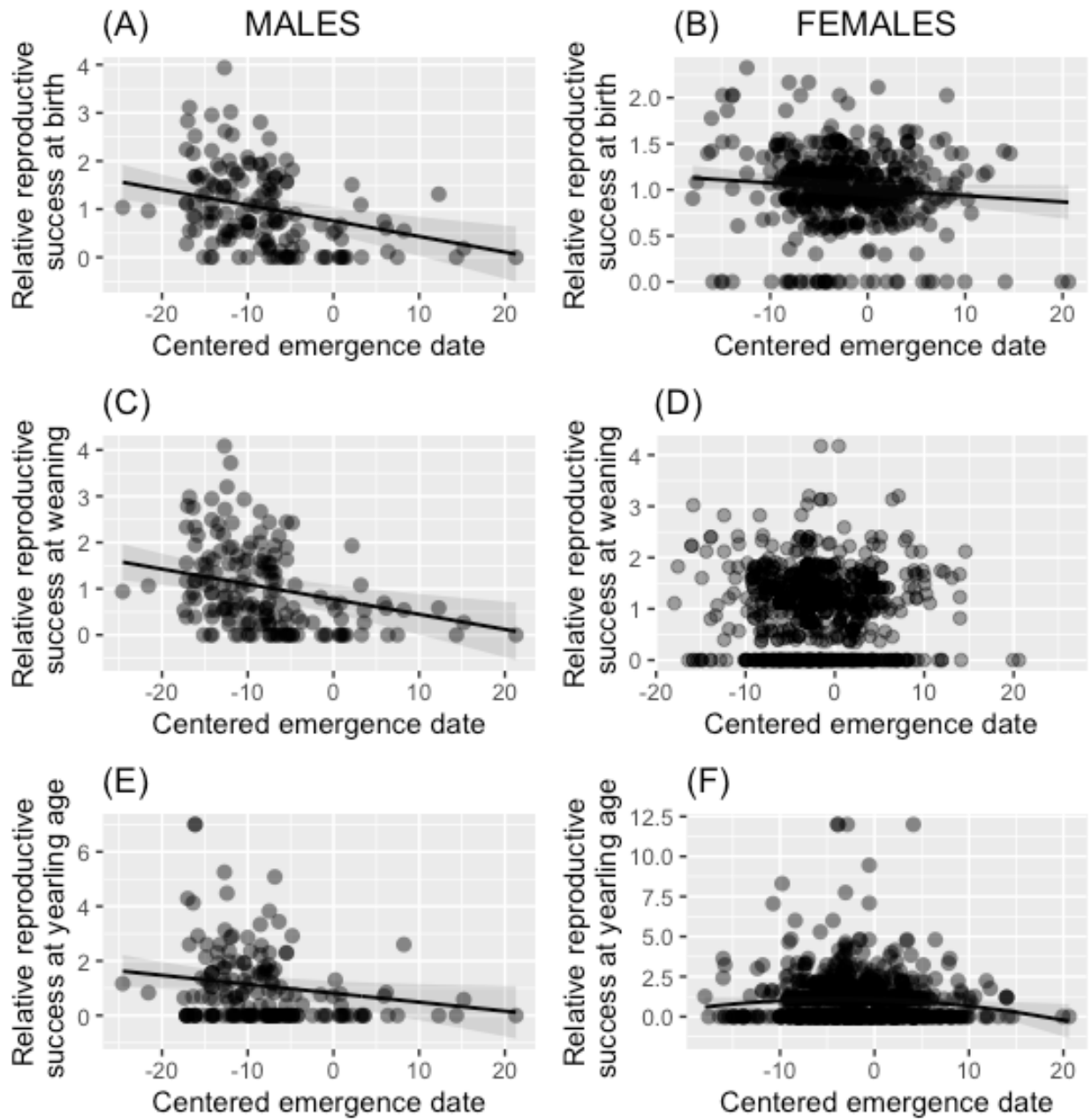
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734 **Fig. 2**

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739 **Fig. 3**

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