The evolutionary demise of a social interaction: social partners differ in the rate at which interacting phenotypes are lost

Eleanor K. Bladon, Sonia Pascoal, Nancy Bird, Rahia Mashoodh, Rebecca M. Kilner

Abstract

Phenotypic plasticity enables animals to adjust their behaviour flexibly to their social environment – sometimes through the expression of adaptive traits that have not been exhibited for several generations. We investigated how long social adaptations can usefully persist when they are not routinely expressed, by using experimental evolution to document the loss of social traits associated with the supply and demand of parental care. We allowed populations of burying beetles *Nicrophorus vespilloides* to evolve in two social environments for 48 generations in the lab. In 'Full Care' populations, traits associated with the supply and demand of parental care were expressed at every generation, whereas in 'No Care' populations we prevented expression of these traits experimentally. We then revived trait expression in the No Care populations at generations 24, 43 and 48 by allowing parents to supply post-hatching care, and compared these social traits with those expressed by the Full Care populations. We found that offspring demands for care decayed in the No Care populations more rapidly than a parent's capacity to supply care. Furthermore, male care decayed before female care. We suggest that this reflects differences in the strength of selection for the expression of alternative traits in offspring, males and females, which can enhance fitness when post-hatching care is disrupted.

Impact Summary

Social interactions between animals are suggested to be increasingly vulnerable to breakdown in our changing world. Our experiments offer a rare insight into what happens next, by assessing in real time the durability of social behaviours that are no longer routinely expressed. Our results also have implications for conservation captive breeding programmes

where compensatory husbandry techniques prevent trait expression and so could inadvertently induce rapid, irreversible trait loss.

We investigated how long it took populations to lose the ability to express appropriate social behaviour when they had been prevented from doing so for many generations. We did this by evolving replicate populations of burying beetles *Nicrophorus vespilloides* in the laboratory for 48 generations. The burying beetle is a common insect that is well-known for caring for its larvae, although larvae can survive in the lab without any care at all. In two populations ("Full Care"), we allowed parents and offspring to interact during the supply of post-hatching care, as usual. In two other populations ("No Care"), parents were removed before offspring hatched and so could not interact socially with their young.

Over the course of 48 generations of experimental evolution, we periodically revived social interactions between parents and offspring in the No Care populations. We assessed the extent to which larval begging behaviours, and parental care behaviours, had decayed by comparing their expression with those in the Full Care populations. We found that larval begging behaviour eroded rapidly in No Care populations, and more rapidly than the supply of care by parents. Furthermore, paternal care decayed to a greater extent than maternal care (which was largely unchanged relative to its expression in the Full Care populations). We suggest that these differences could be due to differences in the strength of selection on each family member for alternative traits to enhance fitness.

Introduction

Phenotypic plasticity enables animals to flexibly, and rapidly, adjust their behaviour according to the environment in which they live – sometimes through the expression of adaptive traits that have not been exhibited for several generations (Davies, 2000; Lahti, 2006). The ability to revive 'ghosts of adaptations past' could prove beneficial for populations living in a changing world (Lahti, 2006; Robert and Sorci, 1999; Rothstein, 2001). For this reason, it is important to understand how long such adaptive traits can persist at the population level if

they are no longer routinely expressed. This is especially true for behavioural traits, which have been relatively under-studied in this context (Rayner et al., 2022).

The capacity for any unexpressed trait to persist is thought to be due to a combination of adaptive and neutral processes (Lahti et al., 2009, Rayner et al. 2022). Adaptive processes are involved because selection will act against the mechanisms that enable an unexpressed trait to be environmentally induced if there are associated costs with trait plasticity (DeWitt et al., 1998; Snell-Rood et al., 2018). The costs could be outweighed by any fitness benefits accrued through the occasional expression of this trait. If there are no net costs, then the trait might persist – vestigially and non-adaptively (Rayner et al., 2022). However, the greater the net fitness costs, the more rapidly we would expect the mechanism for plasticity to be lost. There are then two possibilities – either there is genetic assimilation and the trait is constitutively expressed, independent of environmental conditions (DeWitt et al., 1998; Pigliucci et al., 2006; Robinson and Dukas, 1999; Scheiner and Levis, 2021; Snell-Rood et al., 2009; Waddington, 1953), or trait expression ceases altogether and the trait is lost (Lahti, 2006; Rayner et al., 2022). The latter possibility is the focus of our work here.

Neutral processes can also explain how previously plastic traits are lost over time – especially in the longer-term. If a trait is unexpressed for many generations, then it is not exposed to selection and deleterious mutations can potentially accumulate in the underlying genes (Snell-Rood et al., 2009). Eventually, this too could cause the trait to be lost forever (Lahti et al., 2009). Nevertheless, the initial stages of trait loss have seldom been observed directly, partly because these steps involve relatively subtle nuanced change rather than wholesale disruption (Ellers et al., 2012).

In this study, we used experimental evolution to document the loss of unexpressed behavioural traits in real time. Our work focused on plastic social traits that are induced through interactions with a conspecific, namely the demand and supply of parental care. It has recently been suggested that social interactions like this are likely to be disrupted by broader environmental change, though the evolutionary implications are still largely unknown (Bailey and Moore, 2018). Theoretical work generally suggests that social interactions can enhance the pace of evolutionary change through indirect genetic effects,

not only by increasing the pace at which new socially expressed traits spread through the population but also by potentially accelerating their demise, by disrupting or preventing the expression of traits in a social partner (McGlothlin et al., 2010; Moore et al., 1997; Wolf et al., 1998). We mimicked this type of social disruption by experimentally preventing the expression of post-hatching parental care in the burying beetle *Nicrophorus vespilloides* in some populations ("No Care") for 48 generations, whilst allowing its continued expression in other populations ("Full Care"). After 24, 43 and 48 generations of experimental evolution, we revived interactions between parents and offspring in the No Care populations and compared the expression of the parental and offspring traits involved with the Full Care populations, to measure any changes in the frequency of their expression at the population level and the subsequent fitness effects.

We predicted (1) that by generation 24, and even more so by generation 43, we would see a decay in parental and/or larval traits associated with the supply and demand of parental care. We assessed this indirectly by measuring larval mass, since it increases with the supply of parental care (Bladon et al., 2020; Pascoal et al., 2018; Steiger, 2013). We isolated parental and larval contributions to larval mass independently, by cross-fostering larvae between Full Care and No Care populations. We predicted that if No Care larvae had lost the capacity to demand care, then they should be less effective than Full Care larvae at gaining mass when raised by Full Care parents. Conversely, if No Care parents had lost the capacity to supply care, then Full Care larvae should gain less mass when raised by No Care parents than when raised by Full Care parents.

Additionally, we quantified the supply and demand for parental care directly. We predicted (2) that if No Care parents had lost the ability to supply care then the duration of their care should be shorter than shown by Full Care parents (Eggert et al., 1998). We also predicted (3) that if No Care larvae had lost the ability to demand care then they should be less inclined to solicit care from parents (Smiseth and Parker, 2008; Smiseth et al., 2010).

Methods

Natural History of Burying Beetles Nicrophorus vespilloides

Burying beetles show elaborate parental care that is highly variable in its duration (Jarrett et al. 2018b). Male and female beetles can detect small vertebrate carrion from a distance of several kilometres with their highly sensitive antennae (Kalinová et al., 2009). A pair converts the dead body into a nest for their young by tearing off the fur or feathers, covering the flesh with antimicrobial anal and oral exudates, rolling it into a ball and burying it underground (Cotter and Kilner, 2010). Meanwhile, the pair mates and the female lays her eggs in the soil around the nest. The larvae crawl to the nest after hatching (Müller and Eggert, 1989). Both parents tend the larvae by defending them from intruders and supplying fluids through oral trophallaxis (Eggert et al., 1998). Larvae also self-feed (Smiseth et al., 2003) and can survive (at least in the laboratory) without any post-hatching care, though they attain a lower mass at dispersal and have a poorer chance of surviving to independence (Eggert et al., 1998). Larval mass is a key correlate of fitness because larval mass predicts adult size (Jarrett et al., 2017) which, in turn, predicts fecundity in both males and females (Bladon et al., 2020; Pascoal et al., 2018).

Burying beetle husbandry in the laboratory

For all breeding experiments, each pair of sexually mature male and female beetles was bred by placing them in a plastic breeding box (17 x 12 x 6 cm) with damp soil (John Innes Compost) and a 10-15 g mouse carcass on which to breed. Larvae were counted and weighed 8 days after pairing and placed in plastic pupation boxes (10 x 10 x 2 cm), filled with damp peat. Sexually immature adults were eclosed approximately 21 days later and housed in an individual box (12 x 8 x 2 cm). Adults were fed twice a week (beef mince) until breeding, which took place 15 days post-eclosion. Adults and pupating larvae were kept on a 16L: 8D hour light cycle at 21°C.

Experimental Evolution

The *N. vespilloides* populations described in this study were part of a long-term experimental evolution project that investigated how populations of burying beetles adapt to the loss of parental care. It focused on four experimental populations: Full Care (FC; x2 replicates) and

156 No Care (NC; x2 replicates). Their establishment and husbandry have been described in detail
157 before (Duarte et al., 2021; Jarrett et al., 2018a, 2018b; Rebar et al., 2020; Schrader et al.,
158 2015a, 2015b, 2017). Briefly, these populations were established in 2014 with wild-caught
159 beetles (trapped under permit) from four woodland sites across Cambridgeshire, UK (Byron's
160 Pool, Gamlingay Woods, Waresley Woods and Overhall Grove). The NC populations were
161 routinely prevented from supplying any post-hatching care, through the removal of adults at
162 53 h post-pairing, when the carrion nest was complete but before the larvae had hatched.
163 Whereas in the FC populations adults were allowed to stay with their larvae throughout
164 development and provide care. This procedure was repeated at every generation. Each type
165 of experimental population was run in a separate block (FC1/NC1 and FC2/NC2) with breeding
166 staggered between blocks by 7 days. We used these populations to assess the level of
167 parental care populations provided towards offspring and the amount of care solicited in
168 offspring at generations 24, 43 and 48.
169
170 We have previously described the divergent adaptive evolution of these experimental
171 populations in response to the loss of care. Although the No Care populations initially showed
172 higher rates of larval mortality, they swiftly adapted (Schrader et al., 2015b). After 23
173 generations of experimental evolution, larvae had a similar chance of survival and attained a
174 similar mass at dispersal in each type of population (Rebar et al., 2020; Schrader et al., 2017).
175 Over the same time frame, No Care larvae evolved to hatch more synchronously (Jarrett et
176 al., 2017), to have disproportionately larger mandibles (Jarrett et al., 2018a), and to be more
177 cooperative with their siblings (Jarrett et al., 2018b; Rebar et al., 2020). No Care parents
178 evolved to frontload parental care, making a rounder carrion nest more quickly than Full Care
179 parents, which promoted larval survival in the absence of post-hatching care (Duarte et al.,
180 2021).
181
182 ***Experiment 1: Assessing the duration of care, and partitioning larval and parental***
183 ***contributions to brood mass***
184 We assayed the propensity for parents to supply care in the evolving populations after 24 and
185 43 generations of experimental evolution. Whilst they are providing parental care, parents
186 typically stay in close proximity to the carcass. We measured the duration of care exhibited
187 by NC and FC parents by breeding them in a box with a plastic partition that allowed parents

188 to desert the nest after parental care was completed (Figure S1; (de Gasperin et al., 2015)).
189 We therefore used time of desertion as a proxy for when parents had stopped supplying
190 parental care.
191
192 Each breeding pair was placed in the larger compartment (Figure S1), along with an 8-12 g
193 carcass. At 53 h post-pairing, we cross-fostered parents between boxes to create families that
194 either stayed with their own nest or were translocated to a foster nest of the same (control)
195 or different (cross-fostered) type of experimental population (i.e. No Care or Full Care) (see
196 Figure S2). This design enabled us to partition the contributions of parents versus offspring
197 to brood mass at dispersal. The transfers within experimental populations enabled us to
198 control for any effects due to the cross-fostering procedure itself. Following the translocation
199 of some pairs and nests, adults remained in the boxes until they entered the escape chamber
200 or the experiment ended (8 days after pairing). Thus, in all treatments, parents were able to
201 supply post-hatching care. From breeding to dispersal, we checked the escape chamber every
202 4 hours between 8am and 8pm (i.e. 64-192 h after pairing in generation 24; 56-192 h after
203 pairing in generation 43). At dispersal (8 days post-pairing), we removed the remaining
204 parents, counted the number of surviving larvae and weighed the brood.
205
206 ***Experiment 2: Assessing larval begging behaviour***
207 We quantified the frequency of larval begging behaviour using previously described methods
208 (Smiseth and Parker, 2008; Smiseth et al., 2010). At generation 48, we created Full Care pairs
209 and No Care pairs of sexually mature, unrelated, virgin beetles and put them in a standard
210 size breeding box (17 x 12 x 6 cm) with 300 ml soil and a 10-13 g mouse carcass. At 53 h after
211 pairing, we removed the male and transferred the female and her carrion nest into a new
212 standard size breeding box (also containing 300 ml soil). We checked the original breeding
213 box for freshly-hatched larvae 24 h later. Larvae were pooled by condition and distributed to
214 create four conditions: 1) Full Care female with 15 Full Care pooled larvae, 2) Full Care female
215 with 15 No Care pooled larvae, 3) No Care female with 15 Full Care pooled larvae and 4) No
216 Care female with 15 No Care pooled larvae. This design ensured that begging was not specific
217 to parent of origin. Larvae were placed directly on the focal female's carrion nest. Females
218 were included in the experiment only if their original eggs hatched successfully to prevent

219 cannibalism of larvae that appear prior to their own eggs hatching (Müller and Eggert, 1990;
220 Smiseth and Parker, 2008).
221
222 Twenty-four hours after establishing the experimental broods, when the larvae were second
223 instar and had reached peak begging activity (Smiseth et al., 2003), we removed the females
224 from their broods, and placed them in labelled containers in a -20 °C freezer for 30 min to
225 euthanise them. Meanwhile, all surviving larvae were removed from their brood ball and
226 placed on labelled pieces of damp paper towel for 25 min prior to the start of the experiment
227 to increase the solicitation of care (T Ratz. *pers. comm.*). After removal from the freezer, each
228 female was thawed for 5 min and mounted on a pin at the centre of a plastic box (11 x 17 x
229 4.5 cm) lined with damp paper towel, mimicking the stance of a parent regurgitating food
230 (Mäenpää and Smiseth, 2017).
231
232 The focal female's experimental brood was then added to the container, with individual
233 larvae placed haphazardly within it, and left for 5 min to acclimatise. We used instantaneous
234 scan sampling to detect begging activity (Mäenpää and Smiseth, 2017; Smiseth and Parker,
235 2008; Smiseth et al., 2010), recording larval activity every minute for a 10 min period. Activity
236 was classified as either 1) associating with the parent (a larva was within one-pronotum's
237 width of the female); or 2) begging (a larva was rearing up and touching the parent with its
238 legs); or 3) neither.
239
240 ***Statistical analyses***
241 All statistical tests were conducted in R version 3.6.1 (R Core Team, 2019). Data handling and
242 visualisation were carried out using base R and the 'tidyverse' suite of R packages (Wickham
243 et al., 2019). A stepwise deletion method using F tests, implemented in the base 'statistics'
244 package in R, was used to determine significance of each term and remove non-significant
245 terms sequentially (Crawley, 2007).
246
247 *Experiment 1*
248 Prediction 1: By generation 24, and even more so by generation 43, we should see a decay in
249 parental and/or larval traits associated with the supply and demand of parental care, as
250 inferred through parental and larval contributions to larval mass.

251 We analysed generation 24 and 43 separately using a linear model with a Gaussian error
252 structure, implemented by base R regression functions. The dependent variable was brood
253 mass at dispersal (g) and the predictor variables included in the maximal model were brood
254 size, carcass mass (g), current parents' experimental population (No Care or Full Care), larval
255 experimental population (No Care or Full Care), whether the parents had been transferred
256 between carcasses and eggs or not, male leaving time, female leaving time and experimental
257 block (1 or 2) and the interaction between current parents' experimental population and
258 larval experimental population.
259
260 Prediction 2: If No Care parents had lost the ability to supply care then the duration of their
261 care should be shorter than shown by Full Care parents.
262 We first conducted an ANOVA (using the base R 'statistics package) to determine whether
263 there was a significant difference in male and female leaving time across generations. As
264 there was a significant difference, male and female parents were then analysed separately in
265 subsequent analyses. We analysed the parental leaving time data using semi-parametric Cox's
266 proportional models for interval censored data (using the icenReg R package (Anderson-
267 Bergman, 2017)). In the male maximal models we included parental experimental population
268 (No Care or Full Care), focal offspring experimental population of origin (No Care or Full Care),
269 whether the parents had been transferred or not, number of larvae, mass of mouse carcass
270 and experimental block as fixed effects. In the female maximal models, the same terms were
271 included but male leaving time was also included to determine whether females were more
272 likely to leave earlier once their partner left. Female leaving time was not included in the male
273 model because there were so few instances of females leaving before their partner (n = 9/153
274 in generation 24; 13/214 in generation 43).
275
276 *Experiment 2*
277 Prediction 3: If No Care larvae had lost the ability to demand care then they should be less
278 inclined to solicit care from parents.
279 To determine whether there were differences in begging duration between larvae originating
280 from the Full Care and No Care experimental populations, we first ran a quasi-binomial
281 generalised linear models (GLM) with begging duration as the response variable. In a second
282 model, we tested for differences in any behaviour when larvae interacted with the (dead)

283 parent by combining begging duration with time spent associating with the parent. In both
284 models, the predictor variables included in the maximal model were larval experimental
285 population (No Care or Full Care), foster female's experimental population (No Care or Full
286 Care), number of surviving larvae in the brood, experimental block and the interaction
287 between larval experimental population and foster female's experimental population.
288
289
290 **Results**

291 *Experiment 1*
292 Prediction 1: By generation 24, and even more so by generation 43, we should see a decay in
293 parental and/or larval traits associated with the supply and demand of parental care, as
294 inferred through parental and larval contributions to larval mass.
295
296 In generation 24, larval experimental population accounted for more variation in brood mass
297 at dispersal than did parental experimental population. Broods of larvae from the No Care
298 experimental populations were significantly lighter than those from the Full Care
299 experimental populations (linear regression: $F_{1,147} = 30.84$, $p = <0.001$, Figure 1A), regardless
300 of the experimental population of origin of the parents that cared for them (linear regression:
301 $F_{1,144} = 0.870$, $p = 0.353$).
302
303 By generation 43, both parental and larval experimental populations contributed to variation
304 in brood mass at dispersal. No Care broods were still lighter than Full Care broods (linear
305 regression: $F_{1,208} = 11.733$, $p = 0.001$, Figure 1B). However, by this generation the broods
306 raised by No Care parents were also lighter than those cared for by Full Care parents (linear
307 regression: $F_{1,208} = 6.138$, $p = 0.014$), regardless of the larval experimental population (Figure
308 1B). These effects were greater than, and independent of, the effects of carcass and brood
309 size (Table S1B). Together, these results support Prediction 1 by showing that parental
310 caregiving degraded over time, though more slowly than larval traits for acquiring care.

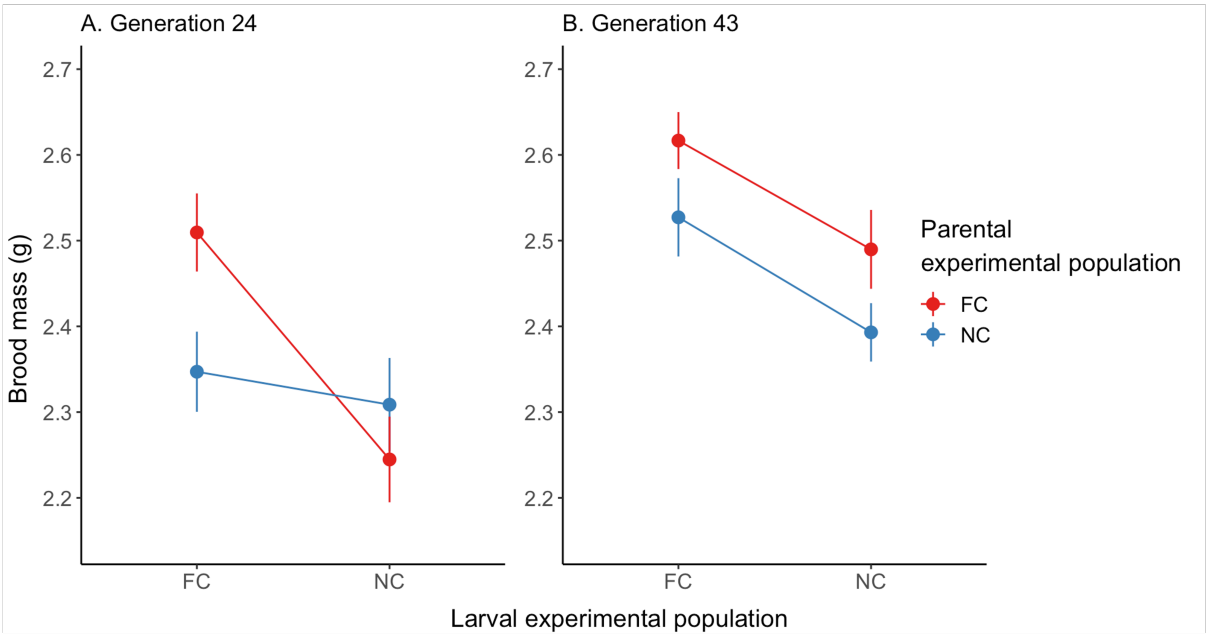


Figure 1 – The effect of parental experimental population and larval experimental population on brood mass at dispersal in A) generation 24 (n = 153) and B) generation 43 (n = 214). Points represent predicted values from the minimal model containing both parental experimental population and larval experimental population terms. In A) larval experimental population explains significant variation in brood mass at dispersal, but neither parental experimental population, nor the interaction between the two, are significant, and in B) both larval and parental experimental population explain significant variation in brood mass at dispersal.

Prediction 2: If No Care parents had lost the ability to supply care then the duration of their care should be shorter than shown by Full Care parents.

(i) Effect of parental sex

There was a significant interaction between the sex of the parent and generation of experimental evolution on the duration of care supplied (ANOVA: $F_{1,734} = 5.949$, $p = 0.015$). In support of Prediction 2, fathers left the brood significantly earlier than mothers in both generations 24 and 43 (ANOVA: $F_{2,736} = 185.38$, $p = <0.001$; Figure 2). Moreover, fathers in generation 24 left their brood earlier than fathers in generation 43 (ANOVA: $F_{1,734} = 5.949$, $p = 0.015$; Figure 2). However, contrary to Prediction 2, there was no significant difference in the duration of care supplied by mothers between the two generations.

We then sought to determine if the duration of parental care influenced average brood mass at dispersal. In generation 24 (Table S1A), the duration of both maternal (female leaving time: linear regression: $F_{1,144} = 6.393$, $p = 0.013$) and paternal care (male leaving time: linear regression: $F_{1,144} = 6.859$, $p = 0.010$) were positively correlated with average brood mass. In generation 43 (Table S2B), only the duration of maternal care contributed to average brood mass (female leaving time: linear regression: $F_{1,208} = 10.477$, $p = 0.001$, male leaving time: linear regression: $F_{1,207} = 0.004$, $p = 0.950$).

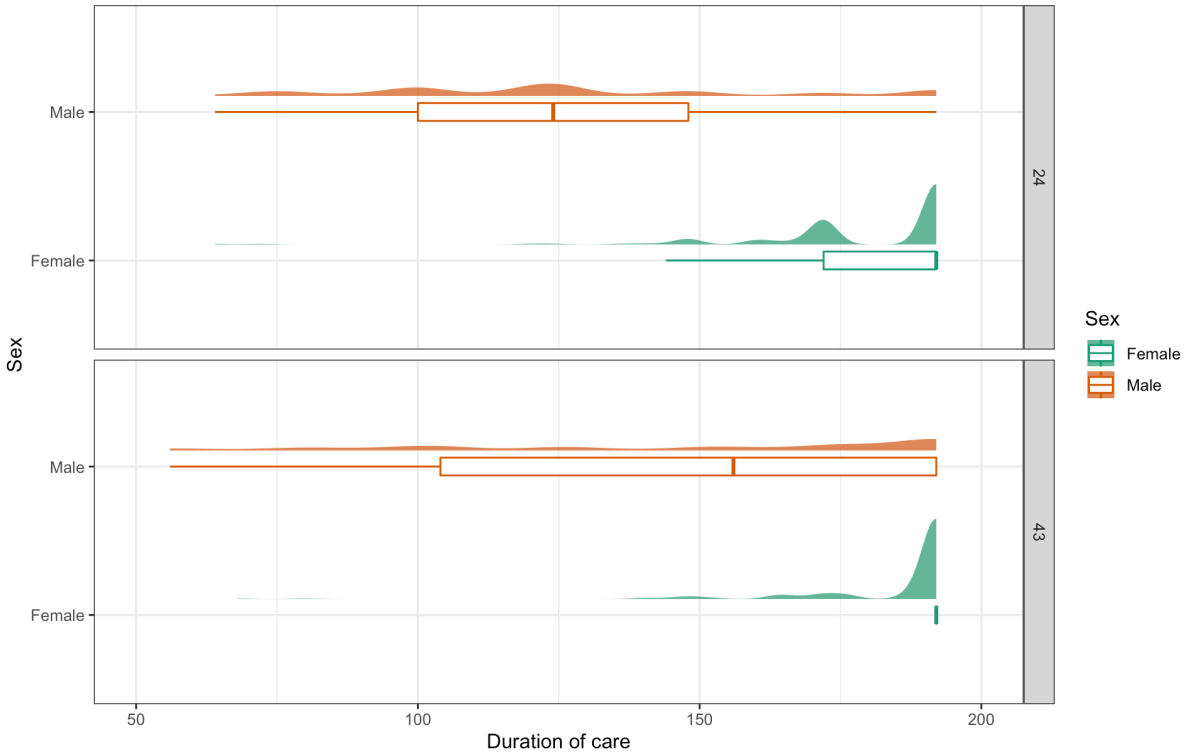


Figure 2 – Box and density plots showing the duration of maternal (female, n = 369 individuals) and paternal (male, n = 369 individuals) care in generations 24 and 43. In general, males stayed with the brood for significantly less time than females. Males in generation 24 stayed for less time than males in generation 43. Whiskers extend to the farthest data point which is no more than 1.5 times the interquartile range from the box. Density curves show the distributions of individual points.

(ii) Effects of evolutionary lines

We then used a survival analysis at each generation to determine separately how parent and larval experimental populations each independently influenced time of departure from the brood of each parent.

In generation 24, the duration of maternal care was significantly shorter when their brood was drawn from the No Care experimental populations, regardless of the female's own experimental population of origin (survival model: hazard ratio = 1.749, Wald = 2.085, p = 0.028, Figure 3). However, there was no significant effect of parental or larval experimental populations on paternal departure time (Table S2A).

By generation 43 (Table S2B), this pattern had changed. No Care males (survival model: hazard ratio = 1.758, Wald = 3.360, p = <0.001, Figure 4) provided care for significantly less time than Full Care males. Although there were no significant effects of experimental population on maternal departure time, the duration of female care was generally shorter when their partner provided less prolonged care (survival model: hazard ratio = 0.993, Wald = -2.027, p = 0.021).

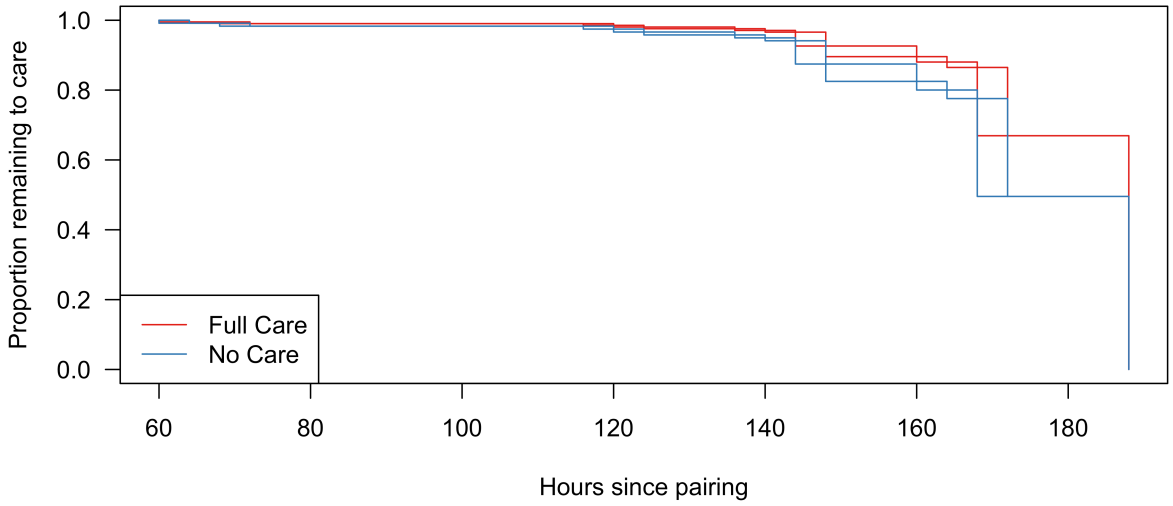


Figure 3 – Duration of female care in relation to larval experimental population at generation 24 (Full Care, n = 81; No Care, n = 72 females). The survival curves for female care were significantly affected by experimental population of their current brood (Full Care = red, No Care = blue). Two lines are displayed for each treatment because with interval censored data it is not possible to tell the exact time that the female left, only the time period within which she left. The lines depicted are predicted by the model fitted to the data: for any point between the two lines there is the same likelihood of the parent leaving.

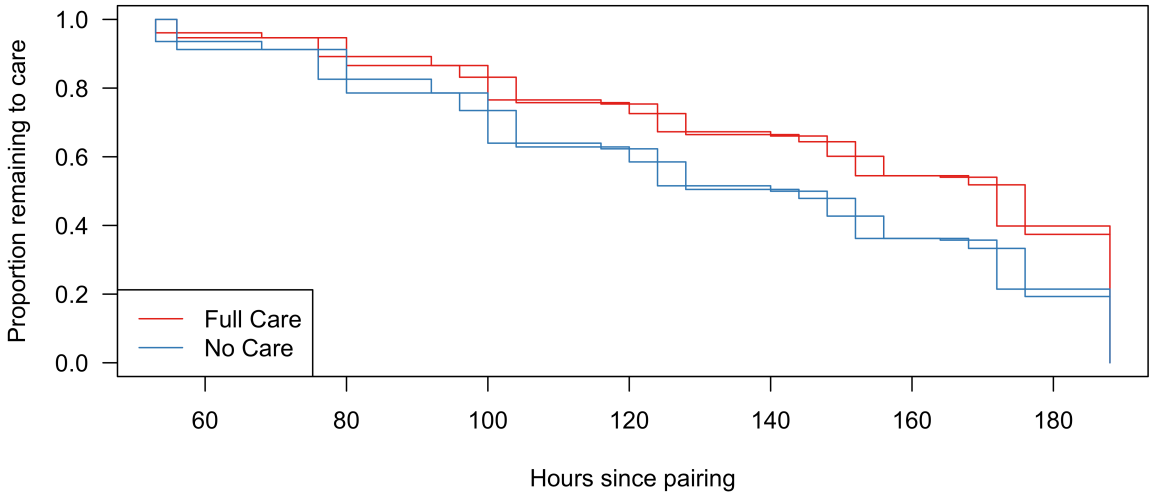
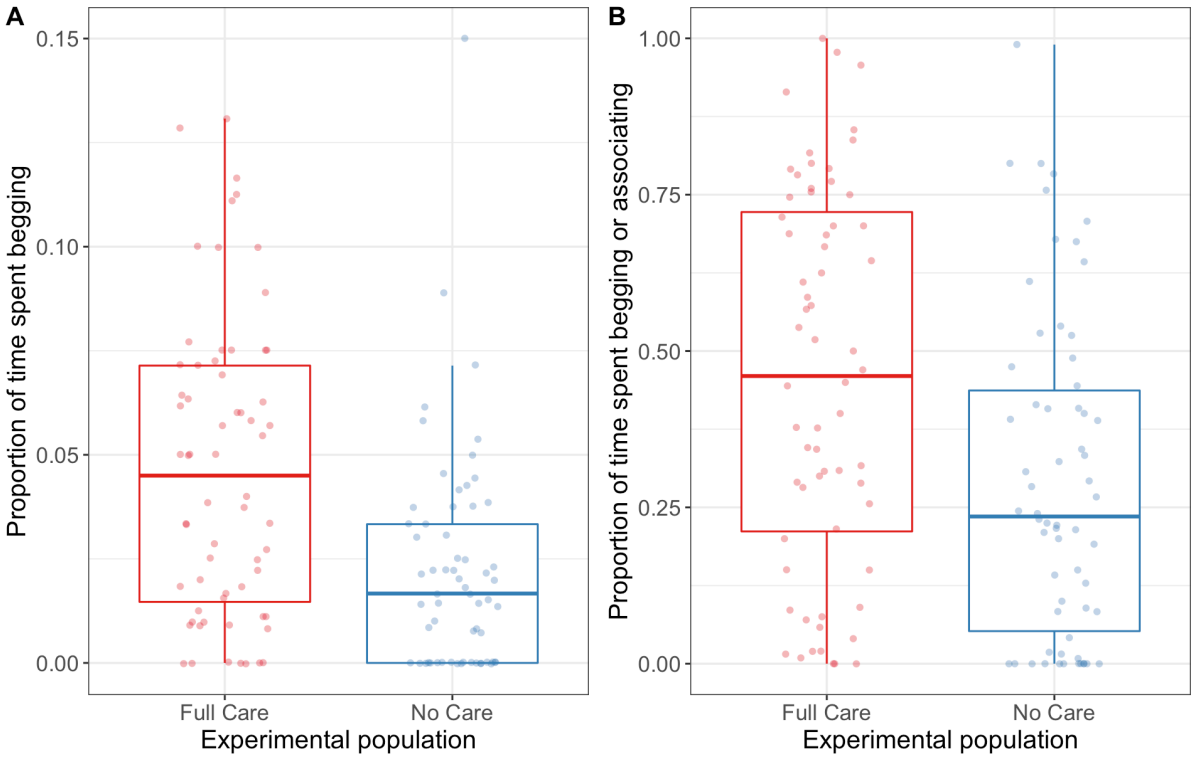


Figure 4 – Duration of male care in generation 43 (Full Care, n = 107; No Care, n = 107 males). The survival curves for male care were significantly affected by the male's experimental population (Full Care = red, No Care = blue), with No Care males caring for less time. Two lines are displayed for each treatment because with interval censored data it is not possible to tell the exact time that the male left, only the time period within which he left. The lines depicted are predicted by the model fitted to the data: for any point between the two lines there is the same likelihood of the parent leaving.

Prediction 3: If No Care larvae had lost the ability to demand care then they should be less inclined to solicit care from parents.

In support of Prediction 3, No Care larvae spent significantly less time begging towards their foster parent than Full Care larvae (linear regression: $F_{1,124} = 25.042$, $p = <0.001$) regardless of the experimental population of their foster parent (Figure 5A). The result was similar when considering total time spent interacting with the parent (linear regression: $F_{1,124} = 12.979$, $p = 0.001$, Figure 5B). No other variables significantly affected larval solicitation behaviours (Table S3).



392

393 *Figure 5 - The effect of larval experimental population on A) proportion of time spent begging and B)*
394 *the proportion of time spent either begging or associating with the parent (n = 126 broods). Whiskers*
395 *extend to the farthest data point which is no more than 1.5 times the interquartile range from the box.*
396 *Points show individual broods.*

397

398 **Discussion**

399

400 We investigated whether traits connected with offspring demand and parental supply were
401 evolutionarily lost by disrupting this social interaction for generation after generation in
402 experimentally evolving populations. We assessed the extent of loss: 1) indirectly, by
403 partitioning the contribution of the offspring and parental line of origin to average brood mass
404 at dispersal and 2) directly, by quantifying the duration of care supplied by each parent and
405 larval capacity to solicit care. We found support for all three of the predictions we tested.

406

407 Our indirect measurements showed that in both generations, No Care larvae (on carrion nests
408 prepared by No Care parents) consistently attained a lighter mass at dispersal than Full Care
409 larvae (on carrion nests prepared by Full Care parents), regardless of whether they were
410 raised by parents from the Full Care or No Care experimental populations. We can think of
411 three possible explanations for these findings, which are not mutually exclusive.

412 First, offspring from the No Care experimental populations may have developed
413 within smaller eggs, meaning that larvae were smaller from hatching. We do not know
414 whether the eggs produced by the No Care populations were smaller after 24 and 43
415 generations of experimental evolution, but we have previously shown that development in a
416 No Care environment initially yields smaller adults, and that smaller females lay smaller eggs
417 (Jarrett et al., 2017).

418 A second explanation is that the carrion nests produced by No Care parents might
419 have provided an inferior environment for offspring development compared with the carrion
420 nests made by Full Care parents. We reject this explanation because we have demonstrated
421 the opposite to be true (Duarte et al., 2021).

422 The third explanation is that No Care larvae were less effective than Full Care larvae
423 at acquiring resources from parents: the demand for care in the No Care experimental
424 populations was lost because it was not expressed in these populations for so many
425 generations. This interpretation is supported by the direct behavioural measurements, which
426 showed that by generation 48, No Care larvae were less likely to solicit care from parents than
427 Full Care larvae. It also accounts for our findings in generation 24 that all females shortened
428 their duration of care when given No Care larvae to look after. The brood's failure to solicit
429 care effectively may have contributed to this earlier departure time. On balance, we conclude

430 that the larval capacity to solicit care started to disappear after generations of not being
431 expressed and that this loss might have been evident after just 24 generations (although it is
432 possible that egg size also contributed to a lower average brood mass at dispersal in the No
433 Care larvae, at this point).
434
435 Turning to the parents, here we found no evidence for a loss of post-hatching parental
436 behaviour at generation 24, whether we used indirect (brood mass) or direct measurements
437 (departure time). No Care and Full Care parents contributed to a similar extent to average
438 brood mass at dispersal, which was positively correlated with the duration of care supplied
439 by each parent. By generation 43, however, this pattern had changed. Here there was
440 evidence of decline in the No Care male's supply of post-hatching care, which was now
441 significantly shorter than the care provided by the Full Care males. Furthermore, the duration
442 of male care no longer predicted brood mass at dispersal. In contrast there was no difference
443 between No Care and Full Care females in the time they spent caring for larvae in generation
444 43, and the duration of maternal care was still positively associated with average brood mass
445 at this point. Nevertheless, shorter periods of male care also curtailed the duration of care
446 supplied by females.
447
448 Overall, our experiments show that social behaviours start to disappear when the opportunity
449 to express them is eliminated for multiple generations. Larval demand for care started to
450 disappear first, followed by the provision of post-hatching care by males. Although supply of
451 maternal care decreased in response to weaker demands from larvae, and reduced paternal
452 effort, we found no evidence that No Care mothers had lost any intrinsic capacity to care for
453 larvae - despite being prevented from providing post-hatching care for 43 generations.
454
455 Why did we observe contrasting rates of behavioural loss between larvae, males and females?
456 The key to answering this question lies in understanding the mechanisms underpinning these
457 behavioural traits (Heinen-Kay and Zuk, 2019). We can immediately rule out any wider
458 environmental differences, since the experimental populations were kept under identical
459 conditions in the same lab. One possibility is that the genes underpinning each of these traits
460 are simply vulnerable to mutation at different rates or to different extents (Snell-Rood et al.,
461 2009). We have no evidence yet to test whether or not this proposition is true. A different

462 possibility, which is perhaps more likely given the relatively short timescale of this
463 experiment, is that the demand for care and the supply of post-hatching care are each traded
464 against the expression of alternative behavioural traits that offer a different route to gaining
465 fitness (Heinen-Kay and Zuk, 2019). For example, the expression of larval solicitation
466 behaviours could trade-off directly with the expression of self-feeding (Smiseth et al., 2003).
467 In natural populations, there might be standing genetic variation in the threshold at which
468 environmental cues tip the balance between the expression of each larval trait. Selection
469 from the No Care environment could have favoured larvae that were more inclined to express
470 self-feeding at the expense of expressing solicitation behaviours. The recalibration of this
471 trade-off at the population level to favour larval self-feeding in a No Care environment could
472 explain the corresponding loss of larval traits for demanding care.
473
474 We can make a similar population-level argument for changes in the expression of male post-
475 hatching care over time. This might be due to a re-balancing of the trade-off with the supply
476 of pre-hatching care and, specifically, the effort devoted to making the carrion nest (de
477 Gasperin et al., 2015). The existence of a trade-off like this is plausible since nest maintenance
478 activity after hatching is known to be negatively genetically correlated with the direct supply
479 of larval care in males (Walling et al., 2008). Furthermore, we have shown elsewhere that by
480 generation 20 the adults from the No Care experimental populations had evolved to put more
481 effort into making the carrion nest than those in the Full Care populations (Duarte et al., 2021)
482 and that this change in nest-making proficiency was due more to males than to females
483 (Jarrett et al., 2022). Additionally, males naturally leave 2 to 5 days earlier than females during
484 the post-hatching period – perhaps because they trade off the potential benefits to their
485 current brood of staying with the potential to sire more offspring away from the carcass
486 (Boncoraglio and Kilner, 2012). This trade-off is stronger for males than females due to the
487 division of labour between the sexes. Male duties of care focus on nest preparation and
488 defence and are completed sooner than female duties of care which focus more on post-
489 hatching care (Walling et al 2008).
490
491 Whether an equivalent trade-off exists for females is less clear. The female trait contributing
492 most to fitness before hatching is most likely to be egg size and it is theoretically possible that
493 there is a negative genetic correlation between egg size and the supply of post-hatching care.

494 However, even if such a correlation exists, it is likely to be concealed by a condition-
495 dependent positive phenotypic correlation between egg size and post-hatching care. Females
496 that receive less post-hatching care are smaller and lay correspondingly smaller eggs (Jarrett
497 et al., 2017).
498
499 If trade-offs between competing behavioural routes to fitness explain how some traits are
500 lost, then presumably it is the relative strength of selection to recalibrate these trade-offs
501 that explains why rebalancing happened most rapidly in larvae, and then in males, but not in
502 females. Larval mortality in the No Care populations was very high in the first few generations
503 of experimental evolution (Schrader et al., 2017). Any larvae that did not self-feed in the No
504 Care environment would rapidly have been selected against. Selection for recalibrating the
505 male's trade-off arguably is somewhat weaker, since their nest-building behaviour less
506 directly affects larval survival. The strength of selection on the putative egg-care trade-off in
507 females could be negligible, especially if its expression is masked by female condition.
508
509 The general principle emerging from this study is that rapid trait loss is associated with the
510 expression of alternative traits which can enhance fitness more effectively when populations
511 enter a changed environment. This is consistent with previous work analysing trait loss in
512 sexual partners (Heinen-Kay and Zuk, 2019) and with the evolution of trait loss in mutualistic
513 partnerships, where the actions of the partner species compensate for trait loss in the focal
514 species (Ellers et al., 2012). This general principle explains the loss of traits associated with
515 parental care in domesticated bird and mammal species, because breeders often intervene
516 to nurture offspring themselves or to cross-foster them to other strains that provide better
517 offspring care. As a consequence, wild Norway rats have been shown to be more efficient in
518 pup retrieval than domestic mothers (Price and Belanger, 1977), and some strains of
519 domesticated canary (Vriends, 1992) and zebra finch (Blackwell, 1988) are now incapable of
520 raising offspring to independence.
521
522 For traits like this, a return to the ancestral environment, even after relatively few
523 generations, is not sufficient to restore ancestral levels of trait expression. There are
524 important implications here for conservation captive breeding programmes, which could
525 inadvertently induce rapid trait loss through any artificial compensatory husbandry

526 techniques that are introduced to promote breeding success in captivity. Interventions like
527 this could prevent the successful reintroduction of species in the wild by causing the loss of
528 key traits needed for survival and reproduction (Araki et al., 2007; Bowkett, 2009; Williams
529 and Hoffman, 2009).
530
531 Finally, this study also suggests that although the loss of the interacting phenotype in one
532 individual (e.g. larva, or male) is sufficient to prevent expression of the corresponding
533 phenotype in its social partner (e.g. mother, or female partner), it is not sufficient to cause
534 that trait to decay in the short-term. Nevertheless, unexpressed social traits are likely to be
535 lost in the longer-run, either because of costs to plasticity or because mutations can easily
536 accumulate in traits unseen by natural selection (Snell-Rood et al., 2009, 2018). A complex
537 interplay of neurotransmitter receptor gene expression regulates care in *Nicrophorus*
538 *vespilloides* (Cunningham et al. 2021) and in future work, it would be interesting to investigate
539 the vulnerability of these genes to mutation.
540
541 In general, our study suggests the likelihood of reviving 'ghosts of adaptation past' will depend
542 on how long it has been since those adaptive traits were last expressed, and how likely it is
543 that they have been superseded by alternative existing traits that more effectively promote
544 fitness in the new environment. The flexibility of many behavioural traits, and the multiple
545 behavioural routes that are consequently available for achieving similar fitness goals, could
546 mean that some behavioural traits are rapidly substituted and lost following a change in the
547 wider environment.
548
549
550 **Author contributions**
551
552 RMK and EKB designed the experiments; EKB, SP and NB collected the data. EKB and RM
553 analysed the data. EKB, RM and RMK were involved in writing and editing the manuscript.
554
555
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557

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733 **Supplementary material**

734

735 *Table S1 – Results of a Gaussian linear model analysing predictors of brood mass in (A) generation 24 and B) generation 43. Significant terms (retained in the*
736 *minimal model) are shown in bold. All terms included in the maximal model are given and statistics are given for the last model in which the term was*
737 *retained.* ":" represents an interaction between terms.

Independent variable	**Estimate**	**SE**	***F***	**df**	***p***	**Estimate**	**SE**	***F***	**df**	***p***
	A) Generation 24					**B) Generation 43**				
Intercept	-0.945	0.268				-0.695	0.307			
Carcass mass (g)	**0.134**	**0.022**	**36.811**	**1**	**<0.001**	**0.102**	**0.023**	**19.661**	**1**	**<0.001**
Brood size	**0.067**	**0.003**	**540.18**	**1**	**<0.001**	**-0.080**	**0.003**	**748.16**	**1**	**<0.001**
Current parents' experimental population (No Care)	-0.049	0.037	1.767	1	0.186	**-0.093**	**0.036**	**6.775**	**1**	**0.010**
Larval experimental population (No Care)	**-0.168**	**0.037**	**20.274**	**1**	**<0.001**	**-0.131**	**0.035**	**13.744**	**1**	**<0.001**
Transferred? (Yes)	0.026	0.038	0.463	1	0.497	**-0.074**	**0.036**	**4.358**	**1**	**0.038**
Male duration of care	**0.001**	**0.001**	**4.921**	**1**	**0.028**	<0.001	<0.001	0.173	1	0.678
Female duration of care	**0.002**	**0.001**	**6.005**	**1**	**0.016**	**0.003**	**0.001**	**8.565**	**1**	**0.004**
Block (2)	**-0.114**	**0.038**	**8.885**	**1**	**0.003**	**-0.070**	**0.033**	**4.488**	**1**	**0.035**
Current parents' line:Larval experimental population	0.059	0.088	0.448	1	0.505	-0.066	0.081	0.659	1	0.418

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Table S2 – Results of semi-parametric Cox's proportional models of parental leaving times in A) generation 24 and B) generation 43. Terms retained in the minimal model are shown in bold. All terms included in the maximal model are given and statistics given are for the last model in which the term was retained ":" represents an interaction between terms.

Parent	**Independent Variable**	**Parameter Estimate**	**Hazard Ratio**	**SE**	**z**	***p***	**Parameter Estimate**	**Hazard Ratio**	**SE**	**z**	***p***
		A) Generation 24					***B) Generation 43***				
Male	Male evolutionary population (No Care)	0.073	1.076	0.170	0.430	0.680	**0.564**	**1.758**	**0.168**	**3.360**	**<0.001**
	Brood evolutionary population (No Care)	0.022	1.022	0.218	0.099	0.908	-0.322	0.725	0.177	-1.823	0.062
	Carcass mass (g)	0.011	1.011	0.013	0.081	0.927	0.106	1.112	0.105	1.009	0.317
	Transferred? (Yes)	0.151	1.163	0.169	0.893	0.402	-0.116	0.891	0.167	-0.691	0.505
	Brood size	0.003	1.003	0.013	0.224	0.833	-0.007	0.993	0.017	-0.380	0.659
	Block (1)	0.155	1.168	0.197	0.791	0.382	**0.365**	**1.441**	**0.169**	**2.160**	**0.026**
Female	Female evolutionary population (No Care)	0.548	1.730	0.284	1.934	0.426	0.082	1.086	0.335	0.246	0.794
	Brood evolutionary population (No Care)	**0.559**	**1.749**	**0.268**	**2.085**	**0.028**	-0.132	0.876	0.266	-0.497	0.639
	Carcass mass (g)	-0.071	0.932	0.151	-0.469	0.673	-0.170	0.843	0.188	-0.904	0.390
	Transferred? (Yes)	0.020	1.020	0.389	0.052	0.946	0.027	1.027	0.350	0.077	0.929
	Brood size	0.038	1.038	0.022	1.675	0.061	0.024	1.024	0.024	0.986	0.318
	Block (1)	0.254	1.289	0.230	0.849	0.355	0.096	1.100	0.295	0.324	0.736
	Male duration of care	-0.007	0.993	0.004	-1.671	0.057	**-0.007**	**0.993**	**0.004**	**-2.027**	**0.021**

747 *Table S3 – Quasi-binomial GLMs fitted to explain variation in the proportion of time spent begging and the proportion of time spent either begging or*
748 *associating with the parent. 'Begging' was defined as occurring when a larva reached up to touch the parents with its forelegs and 'associating' was defined*
749 *as a larva being within a pronotum's width of the parent's body. All terms included in the maximal model are shown, as well as their contribution to the final*
750 *model. Terms retained in the minimal model are shown in bold. ":" represents an interaction between terms.*

Dependent variable	**Independent variable**	**Estimate**	**SE**	***F***	**d.f.**	***p***
Begging	**Brood experimental population (No Care)**	**-0.850**	**0.176**	**25.042**	**1**	**<0.001**
	Foster Female's experimental population (No Care)	-0.109	0.163	0.450	1	0.503
	Brood size	-0.041	0.037	1.243	1	0.267
	Block (1)	0.036	0.169	0.046	1	0.830
	Brood Line:Foster Female's experimental population	-0.191	0.353	0.294	1	0.589
Interacting with the parent (begging and associating combined)	**Brood experimental population (No Care)**	**-0.788**	**0.221**	**12.979**	**1**	**0.001**
	Foster Female's experimental population (No Care)	0.170	0.222	0.585	1	0.446
	Brood size	0.047	0.053	0.795	1	0.375
	Block (1)	0.319	0.226	2.009	1	0.159

	Brood Line:Foster Female's experimental population	0.210	0.449	0.219	1	0.641

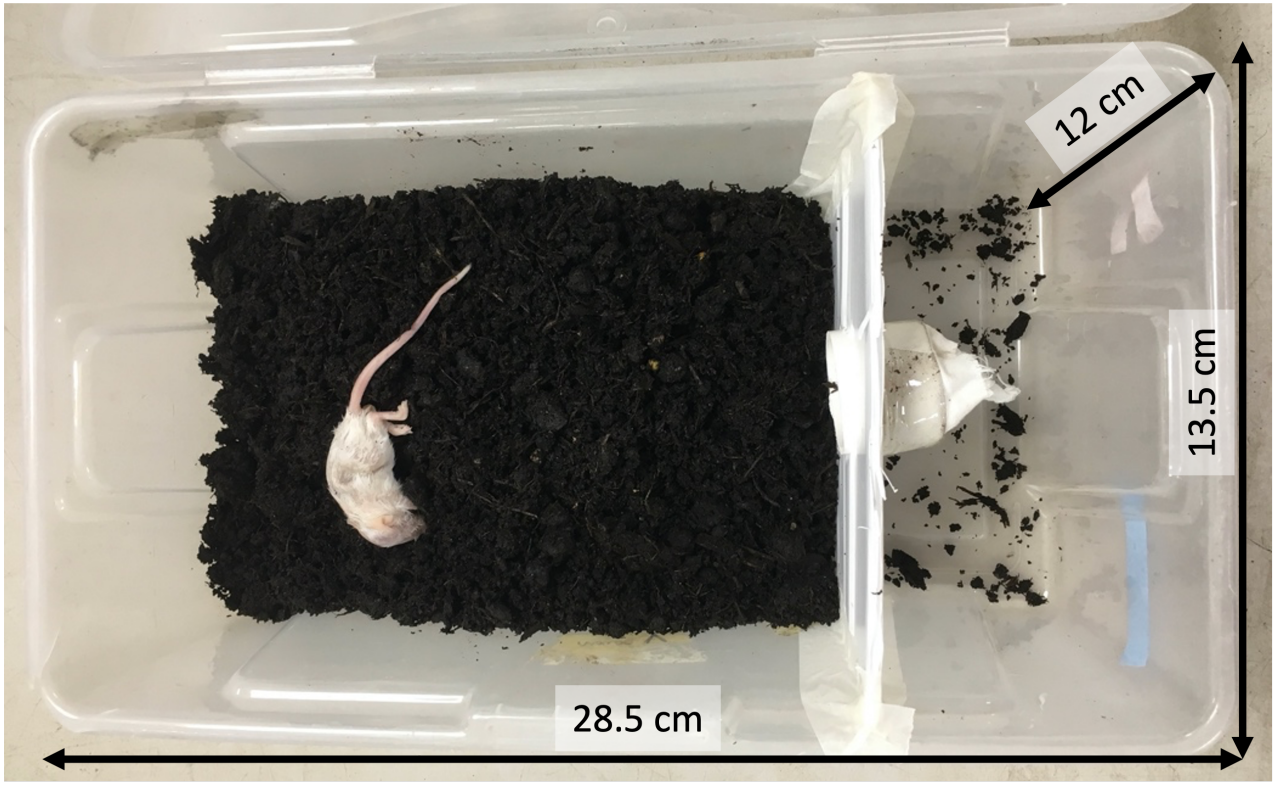

Figure S1: The breeding boxes used in Experiment 1 with breeding compartment and "escape chamber".

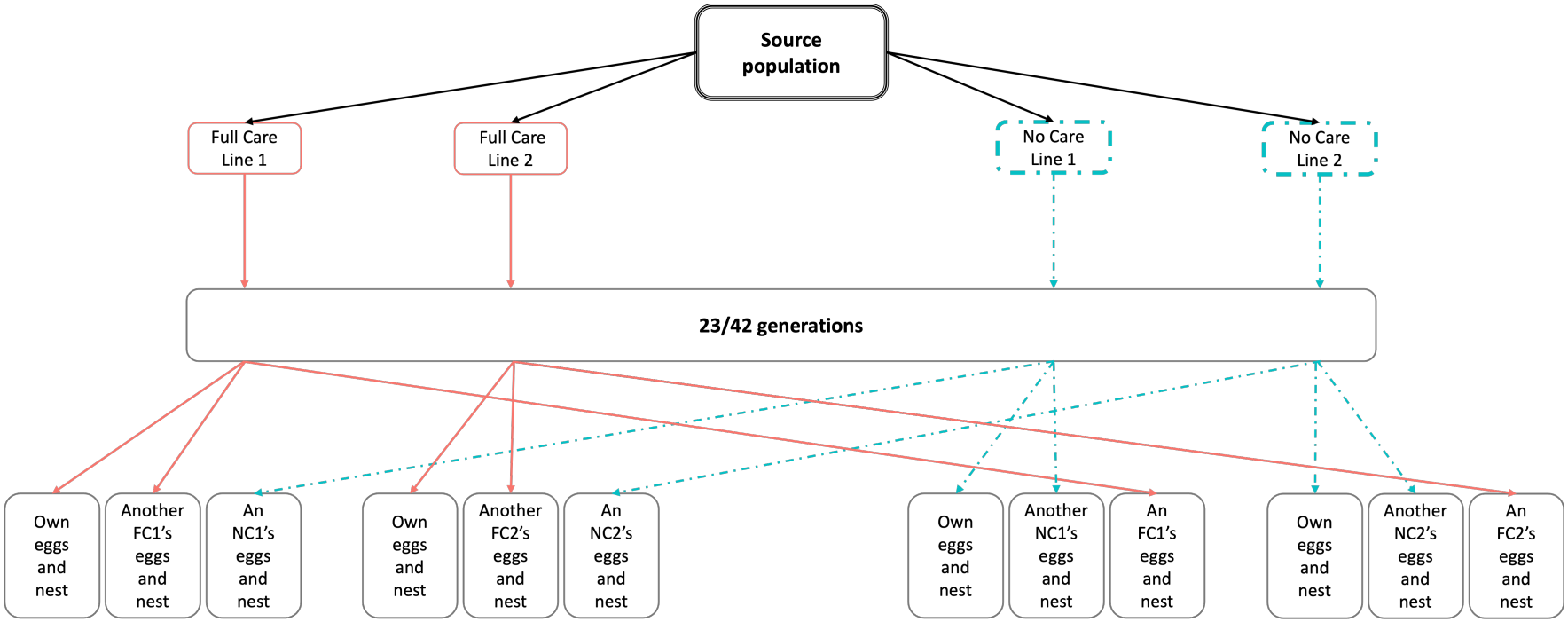

Figure S2: The design of the experimental evolving populations and Experiment 1