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

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Abstract

Phenotypic plasticity enables animals to flexibly adjust their behaviour 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. Populations of burying beetles *Nicrophorus vespilloides* were evolved over 48 generations - in 'Full Care' populations traits associated with the supply and demand of care were expressed, 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 faster than female care. We suggest that this reflects differences in the strength of selection on offspring, males and females, for the expression of alternative traits which are more likely to enhance fitness when post-hatching care is disrupted.

Impact Summary

Social interactions between animals are suggested to be increasingly vulnerable to breakdown in this 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 over 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"), however, 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 social partner 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.

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). Selection will act against the mechanisms that enable an unexpressed trait to be environmentally induced if there are associated costs, for example with trait plasticity. The costs could be outweighed by any fitness benefits accrued through the occasional expression of this trait, but the greater the net costs are, the more rapidly we would expect the mechanism for plasticity to be lost and for trait expression to then cease altogether. In addition, if the trait is unexpressed for many generations, then it is not exposed to selection and mutations can potentially accumulate that affect the underlying genes. 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 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 (Price, 2008) but also by potentially accelerating their demise, by disrupting or preventing the expression of traits in a social partner. We mimicked this type of social disruption by experimentally preventing the expression of parental care in the burying beetle *Nicrophorus vespilloides*. After 24 and 43 generations of experimental evolution, we revived interactions between parents and offspring and assessed the expression of the parental (Experiment 1) and offspring (Experiment 2) traits involved, to measure any changes in the frequency of their expression at the population level and the subsequent fitness effects.

Methods

Natural History of Burying Beetles Nicrophorus vespilloides

Burying beetles show elaborate but variable parental care. 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 mating pair then 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). During this process of preparing the carcass nest, 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 (in the laboratory) without any post-hatching care.

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. Both adult 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 are part of a long-term experimental evolution project that investigates how populations of burying beetles adapt to the loss of parental care. It focuses on four experimental populations: Full Care (FC) (x2 replicates) and No Care (NC) (x2 replicates). Their establishment and husbandry have been described in detail before (Duarte et al., 2021; Jarrett et al., 2018a, 2018b; Rebar et al., 2020; Schrader et al., 2015a, 2015b, 2017). Briefly, these populations were established in 2014 with wild-caught

124 beetles from four woodland sites across Cambridgeshire, UK (Byron's Pool, Gamlingay
125 Woods, Waresley Woods and Overhall Grove). The NC populations were routinely prevented
126 from supplying any post-hatching care, whereas the FC populations were allowed to supply
127 post-hatching care. Briefly, FC populations were left undisturbed (as described above) and
128 parents were allowed to stay with their offspring until dispersal. However, in the NC
129 population, at 53 h post-pairing, when the carrion nest was complete but before the larvae
130 had hatched, both parents were removed to prevent post-hatching care. This procedure was
131 repeated at every generation. Each replicate pair (FC1/NC1 and FC2/NC2) was run in two
132 separate blocks, 7 days apart. We used these populations to assess the level of parental care
133 populations provided towards offspring (Experiment 1) and the amount of care
134 solicited/begging behaviour in offspring (Experiment 2).
135
136 We have previously described the divergent adaptive evolution of these experimental
137 populations in response to the loss of care. Although the No Care populations initially showed
138 higher rates of larval mortality, they swiftly adapted (Schrader et al., 2015b). After 20
139 generations of experimental evolution, larvae had a similar chance of survival and attained a
140 similar mass at dispersal in each type of population (Rebar et al., 2020; Schrader et al., 2017).
141 Over the same time frame, No Care larvae evolved to hatch more synchronously (Jarrett et
142 al., 2017), to have disproportionately larger mandibles (Jarrett et al., 2018a), and to be more
143 cooperative with their siblings (Jarrett et al., 2018b; Rebar et al., 2020). No Care parents
144 evolved to frontload parental care, making a rounder carrion nest more quickly than Full Care
145 parents, which promoted larval survival in the absence of post-hatching care (Duarte et al.,
146 2021).
147
148 ***Experiment 1: Assessing the duration of care, and partitioning larval and parental***
149 ***contributions to larval mass***
150 We assayed the propensity for parents to supply care in the evolving populations after 24 and
151 43 generations of experimental evolution. We used parental departure time to assay the
152 duration of care exhibited by NC and FC parents (De Gasperin et al., 2015). To do so, sexually
153 mature beetles were bred in a breeding box with a plastic partition that allowed parents to
154 desert the nest after parental care was completed (Figure S1). Whilst they are providing

155 parental care, parents typically stay in close proximity to the carcass. We therefore used time
156 of desertion as a proxy for when parents had stopped supplying parental care.
157
158 Each breeding pair was placed in the larger compartment (Figure S1), along with a 8-12g
159 carcass. At 53h post-pairing, we cross-fostered parents between boxes to create families that
160 either stayed with their own nest or were translocated to a foster nest of the same (control)
161 or different (cross-fostered) care condition (see Figure S2). This design enabled us to partition
162 the contributions of parents versus offspring to larval mass at dispersal. The transfers within
163 care treatments enabled us to control for any effects due to the cross-fostering procedure
164 itself. Following the translocation of some pairs and nests, adults remained in the boxes until
165 they entered the escape chamber or the experiment ended (8 days after pairing). Thus in all
166 treatments, parents were able to supply post-hatching care. From breeding to dispersal, we
167 checked the escape chamber every 4 hours between 8am and 8pm (i.e. 64-192 hours after
168 pairing in generation 24; 56-192 hours after pairing in generation 43). At dispersal (8 days
169 post-pairing), we removed the remaining parents, counted the number of surviving larvae
170 and weighed the brood to calculate average larval mass (brood mass divided by number of
171 larvae).
172
173 ***Experiment 2: Assessing larval begging behaviour***
174 We quantified the frequency of larval begging behaviour using previously described methods
175 (Smiseth and Parker, 2008; Smiseth et al., 2010). At generation 48 we created Full Care pairs
176 and No Care pairs of sexually mature, unrelated, virgin beetles and put them in a standard
177 size breeding box (17 x 12 x 6 cm) with 300 ml soil and a 10-13g mouse carcass. At 53 hours
178 after pairing, we removed the male and transferred the female and her carrion nest into a
179 new standard size breeding box (also containing 300 ml soil). We checked the original
180 breeding box for freshly-hatched larvae 24 hours later. Larvae were pooled by condition and
181 distributed to create four conditions: 1) Full Care female with 15 Full Care pooled larvae, 2)
182 Full Care female with 15 No Care pooled larvae, 3) No Care female with 15 Full Care pooled
183 larvae and 4) No Care female with 15 No Care pooled larvae. This design ensured that begging
184 was not specific to parent of origin. Larvae were placed directly on the focal female's carrion
185 nest. Females were included in the experiment only if their original eggs hatched successfully

186 to prevent cannibalism of larvae that appear prior to their own eggs hatching (Müller and
187 Eggert, 1990; Smiseth and Parker, 2008).
188
189 Twenty-four hours after establishing the experimental broods, when the larvae were second
190 instar and had reached peak begging activity (Smiseth et al., 2003), we removed the females
191 from their broods, and placed them in labelled containers in a -20 °C freezer for 30 minutes
192 to euthanise them. Meanwhile, all surviving larvae were removed from their brood ball and
193 placed on labelled pieces of damp paper towel for 25 minutes prior to the start of the
194 experiment to increase the solicitation of care (T Ratz. *pers. comm.*). After removal from the
195 freezer, each female was thawed for 5 minutes and mounted on a pin at the centre of a plastic
196 box (11 x 17 x 4.5 cm) lined with damp paper towel, mimicking the stance of a parent
197 regurgitating food (Mäenpää and Smiseth, 2017).
198
199 The focal female's experimental brood was then added to the container, with individual
200 larvae placed haphazardly within it, and left for 5 minutes to acclimatise. We used
201 instantaneous scan sampling to detect begging activity (Mäenpää and Smiseth, 2017; Smiseth
202 and Parker, 2008; Smiseth et al., 2010), recording larval activity every minute for a 10 minute
203 period. Activity was classified as either 1) associating with the parent (a larva was within one-
204 pronotum's width of the female); or 2) begging (a larva was rearing up and touching the
205 parent with its legs); or 3) neither.
206
207 ***Statistical analyses***
208 All statistical tests were conducted in R version 3.6.1 (R Core Team, 2019). Data handling and
209 visualisation were carried out using base R and the 'tidyverse' suite of R packages (Wickham
210 et al., 2019). A stepwise deletion method using F tests, implemented in the base 'statistics'
211 package in R, was used to determine significance of each term and remove non-significant
212 terms, sequentially (Crawley, 2007).
213
214 *Experiment 1*
215 (1) Partitioning sources of variation in larval mass at dispersal
216 We used a linear model implemented by base R regression functions, including both
217 generations (i.e. generation 24 and 43). Predictor variables included in the maximal model

218 were generation, brood size, carcass mass (g), foster parents' evolutionary line of origin (No
219 Care or Full Care evolving population), larval evolutionary line of origin (No Care or Full Care
220 evolving population), brood identity (natal or foreign), male leaving time, female leaving time,
221 experimental block (1 or 2). We found that generation was a significant main effect and so
222 split the data by generation in subsequent analyses.
223
224 (2) Duration of parental care
225 We first conducted an ANOVA (using the base R 'statistics package) to determine whether
226 there was a significant difference in male and female leaving time across generations. Male
227 and female parents were analysed separately in subsequent analyses. We analysed the
228 parental leaving time data using semi-parametric Cox's proportional models for interval
229 censored data (using the icenReg R package (Anderson-Bergman, 2017)). In the male maximal
230 models we included parental line (No Care or Full Care), focal offspring origin line (No Care or
231 Full Care), whether the parents had been transferred or not, number of larvae, mass of mouse
232 carcass and experimental block as fixed effects. In the female maximal models, the same
233 terms were included but male leaving time was also included to determine whether females
234 were more likely to leave earlier once their partner left. Female leaving time was not included
235 in the male model because there were so few instances of females leaving before their
236 partner (n = 9/153 in generation 24; 13/214 in generation 43).
237
238 *Experiment 2*
239 Quantifying begging behaviour
240 To determine whether there were differences in begging duration between larvae originating
241 from the Full Care and No Care evolutionary lines, we first ran a quasi-binomial generalised
242 linear models (GLM) with begging duration as the response variable. In a second model, we
243 tested for differences in any behaviour when larvae interacted with the (dead) parent by
244 combining begging duration with time spent associating with the parent. In both models, the
245 predictor variables included in the maximal model were larval evolutionary line (No Care or
246 Full Care), current female's evolutionary line (No Care or Full Care), number of surviving larvae
247 in the brood, experimental block and the interaction between larval evolutionary line and
248 current female's evolutionary line.
249

Results

Experiment 1

(1) Partitioning sources of variation in larval mass at dispersal

Variation in average larval mass was explained by the evolutionary lines of origin of both the parents and larvae. Specifically, offspring were lighter when the adults caring derived from the No Care evolving populations ($F_{1,362}$ = 7.243, p = 0.008). Moreover, larvae from the No Care line were consistently lighter ($F_{1,362}$ = 15.303, p <0.001) regardless of the line from which their 'parents' originated. In addition, average larval mass was significantly affected by generation, with larvae being lighter in generation 24 compared to generation 43 ($F_{1,362}$ = 11.922, p <0.001). These effects were over and above the effects of carcass mass, brood size and female leaving time, which also significantly affected larval mass at dispersal (Table 1).

Table 1 - Results of Gaussian linear model analysing predictors of average larval mass in generation 24 and 43. Significant terms (retained in the minimal model) are shown in bold. All terms included in the maximal model are detailed and statistics given are for the last model in which the term was retained. ":" represents an interaction between terms.

Independent variable	**Estimate**	**SE**	**F**	**df**	***p***
Intercept	0.086	0.014			
Generation (24)	**-0.006**	**0.002**	**11.922**	**1**	**.001**
Carcass mass (g)	**0.006**	**0.002**	**29.031**	**1**	**<.001**
Brood size	**-0.002**	**<0.001**	**259.43**	**1**	**<.001**
Current parents' line (No Care)	**-0.005**	**0.002**	**7.243**	**1**	**.008**
Larval line (No Care)	**-0.007**	**0.002**	**15.303**	**1**	**<.001**
Transferred? (Yes)	-0.002	0.002	1.642	1	.201
Male leaving time	<0.001	<0.001	0.033	1	.856
Female leaving time	**-0.002**	**<0.001**	**6.690**	**1**	**.010**
Block (2)	-0.002	0.002	1.151	1	.284
Current parents' line:Larval line	-0.004	0.004	1.067	1	.302
Current parents' line:Transferred?	0.001	0.004	0.017	1	.897
Male leaving time:Larval line	<0.001	<0.001	0.043	1	.835
Female leaving time:Larval line	<0.001	<0.001	<0.001	1	.979
Male leaving time:Female leaving time	<0.001	<0.001	0.321	1	.571
Generation:Current parents' line	0.005	0.003	1.826	1	.177
Generation:Larval line	-0.003	0.004	0.705	1	.402

Since carcass mass was significantly positively correlated with larval mass and the larvae were on average heavier in generation 43, we ran a t-test to determine whether there was a difference between mean carcass mass between generations. The t-test showed that the carcasses were significantly heavier in generation 43 (M = 10.733, SD = 0.519) than in generation 23 (M = 10.033, SD = 0.882) (t_{369} = 7.615, p = <.001), even though carcass mass was experimentally controlled to within 3 g. This could potentially explain the generational differences in average larval mass.

We then analysed the generations separately to determine whether the relative contributions of parental and larval origins changed over the course of the 24 generations (Table 2). In generation 24, larvae accounted for more variation in larval mass at dispersal than did parents. Larvae from the No Care evolving populations were significantly lighter than those from the Full Care evolving populations ($F_{1,147}$ = 30.84, p = <.001, Figure 3A), regardless of the line of origin of the parents that cared for them ($F_{1,144}$ = 0.870, p = .353).

By generation 43, both parent and larval lines of origin accounted for variation in larval mass at dispersal. No Care larvae were still lighter than Full Care larvae ($F_{1,208}$ = 11.733, p = .001, Figure 3B). However, by this generation the larvae raised by No Care parents were also lighter than those cared for by Full Care parents ($F_{1,208}$ = 6.138, p = .014), regardless of the larval line of origin (Figure 3B). These were over and above effects of carcass and brood size (Table 2B). These data suggest that parental caregiving degraded over time, though more slowly than larval traits for acquiring care.

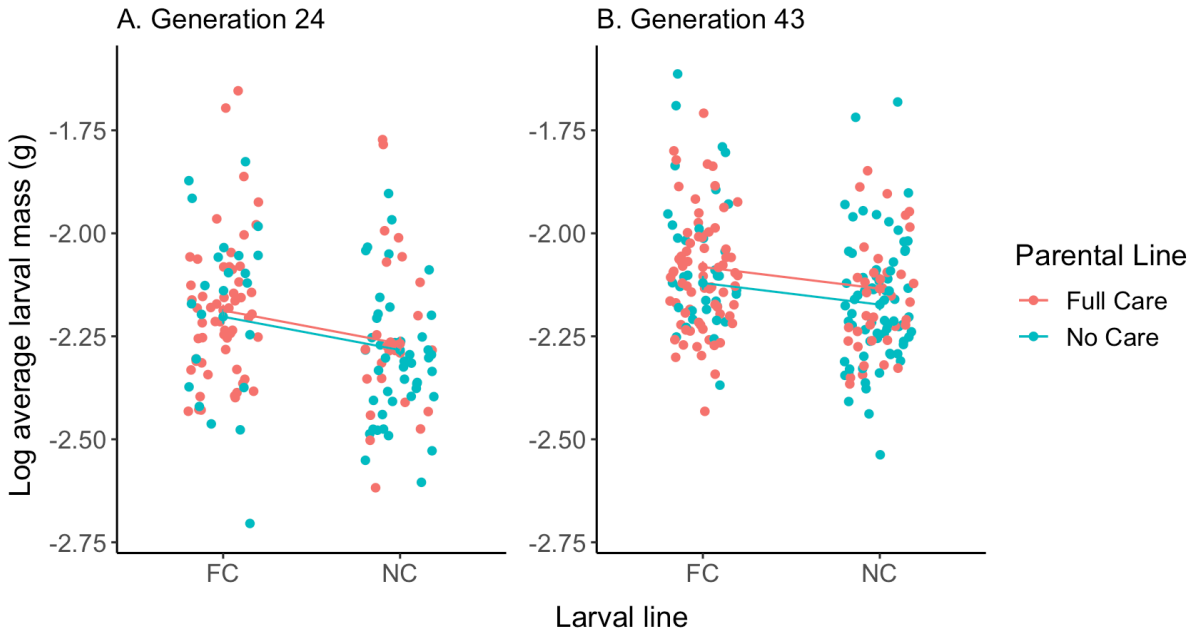

Figure 1 – The effect of parental evolutionary line and larval evolutionary line on the log of average larval mass at dispersal in A) generation 24 and B) generation 43. Points represent values predicted for individual broods from the minimal model containing both parental line and larval line terms. In A) larval line explains significant variation in larval mass at dispersal and in B) both larval line and parental line explain significant variation in larval mass at dispersal.

Table 2 – Results of a Gaussian linear model analysing predictors of log larval mass in (A) generation 24 and B) generation 43. Significant terms (retained in the 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 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	-2.495	0.109				-2.434	0.133			
Carcass mass (g)	**0.057**	**0.009**	**42.237**	**1**	**<.001**	**0.041**	**0.010**	**17.01**	**1**	**<.001**
Brood size	**-0.024**	**0.001**	**408.32**	**1**	**<.001**	**-0.017**	**0.001**	**186.19**	**1**	**<.001**
Current parents' line (No Care)	-0.014	0.015	0.870	1	.353	**-0.039**	**0.016**	**6.138**	**1**	**.014**
Larval line (No Care)	**-0.086**	**0.016**	**30.84**	**1**	**<.001**	**-0.053**	**0.015**	**11.733**	**1**	**.001**
Transferred? (Yes)	.014	.016	0.739	1	.392	-0.028	0.015	3.399	1	.067
Male leaving time	**0.001**	**<0.001**	**6.859**	**1**	**.010**	<0.001	<0.001	0.004	1	.950
Female leaving time	**0.001**	**<0.001**	**6.3934**		**.013**	**0.002**	**0.001**	**10.477**	**1**	**.001**
Block (1)	-0.014	0.016	0.790	1	.376	-0.021	0.014	2.206	1	.139
Current parents' line:Larval line	0.014	0.038	0.137	1	.712	-0.035	0.035	1.003	1	.318
Current parents' line:Transferred?	0.025	0.037	0.480	1	.490	0.021	0.036	0.379	1	.539
Male leaving time:Female leaving time	<0.001	<0.001	1.299	1	.256	<0.001	<0.001	0.686	1	.406
Male leaving time:Larval line	<0.001	<0.001	1.204	1	.274	<0.001	<0.001	0.260	1	.610
Female leaving time:Larval line	0.001	0.001	0.431	1	.513	<-0.001	0.001	0.043	1	.835
Male leaving time:Female leaving time:Larval line	<-0.001	<0.001	0.006	1	.940	<-0.001	<0.001	0.011	1	.918
Male leaving time:Current parents' line	<0.001	<0.001	0.171	1	.680	<-0.001	<0.001	0.595	1	.284
Female leaving time:Current parents' line	0.001	0.001	0.768	1	.383	-0.001	0.001	1.848	1	.176
Male leaving time:Female leaving time:Current parents' line	<-0.001	<0.001	0.179	1	.673	<0.001	<0.001	0.053	1	.819

Duration of parental care

(i) Effect of parental sex

There was a significant interaction between the sex of the parent and generation on parental departure time from the brood ($F_{1,734} = 5.949$, $p = .015$). Fathers left the brood significantly earlier than mothers in both generations ($F_{2,736} = 185.38$, $p = <.001$; Figure 4). Moreover, fathers in generation 24 left their brood earlier than fathers in generation 43 ($F_{1,734} = 5.949$, $p = .015$; Figure 4). There was no difference in the brood departure times of mothers between the two generations.

We then sought to determine if time with the brood (duration of parental care) influenced average larval mass at dispersal. In generation 24 (Table 2A), the duration of both maternal (female leaving time: $F_{1,144} = 6.393$, $p = .013$) and paternal care (male leaving time: $F_{1,144} = 6.859$, $p = .010$) were positively correlated with average larval mass. In generation 43 (Table 2B), only the duration of maternal care contributed to average larval mass (female leaving time: $F_{1,208} = 10.477$, $p = .001$, male leaving time: $F_{1,207} = 0.004$, $p = .950$).

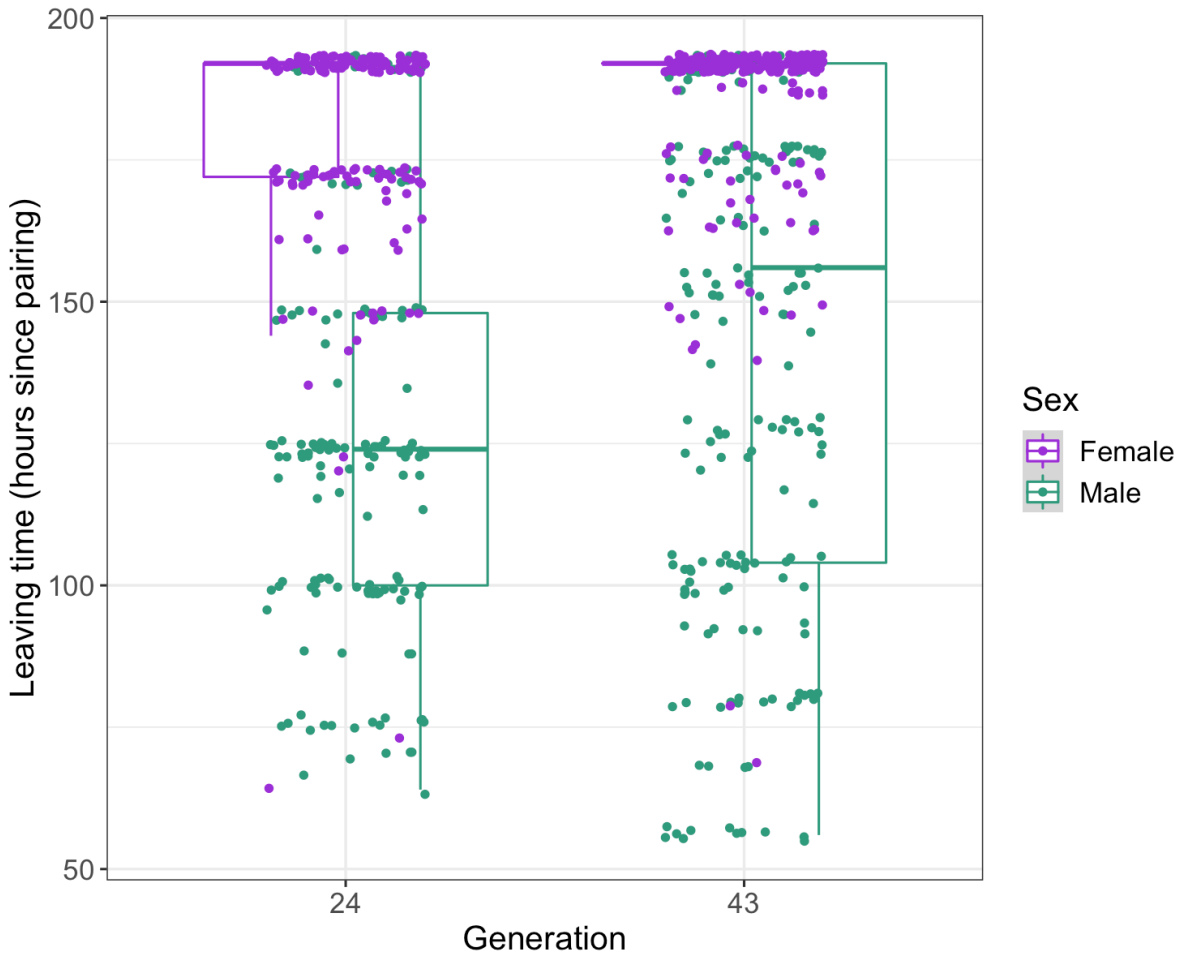

Figure 2 – Boxplot showing the duration of maternal (female) and paternal (male) care in generations 24 and 43. In general, males left significantly earlier than females. Males in generation 24 left earlier than males in generation 43. Whiskers extend to the farthest data point which is no more than 1.5 times the interquartile range from the box. Points show individual adults.

(ii) Effects of evolutionary lines

We then used a survival analysis at each generation to determine separately how parent and larval evolutionary origins 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 evolutionary lines, regardless of the female's own line of origin (hazard ratio = 1.749, Wald = 2.085, p = .028, Figure 6A). However,there was no significant effect of parental or larval evolutionary line of origin on paternal departure time (Table 3A).

By generation 43 (Table 3B), this pattern had changed. No Care males (hazard ratio = 1.758, Wald = 3.360, p = <.001, Figure 5A) provided significantly shorter periods of care than Full Care males. Though there were no significant effects of maternal evolutionary line on maternal departure time, but the duration of female care was generally shorter when their partner provided less prolonged care (hazard ratio = 0.993, Wald = -2.027, p = .021, Figure 6B).

Table 3 – 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 line (No Care)	0.073	1.076	0.170	0.430	0.680	**0.564**	**1.758**	**0.168**	**3.360**	**<0.001**
	Brood evolutionary line (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 line (No Care)	0.548	1.730	0.284	1.934	0.426	0.082	1.086	0.335	0.246	0.794
	Brood evolutionary line (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 leaving time	-0.007	0.993	0.004	-1.671	0.057	**-0.007**	**0.993**	**0.004**	**-2.027**	**0.021**

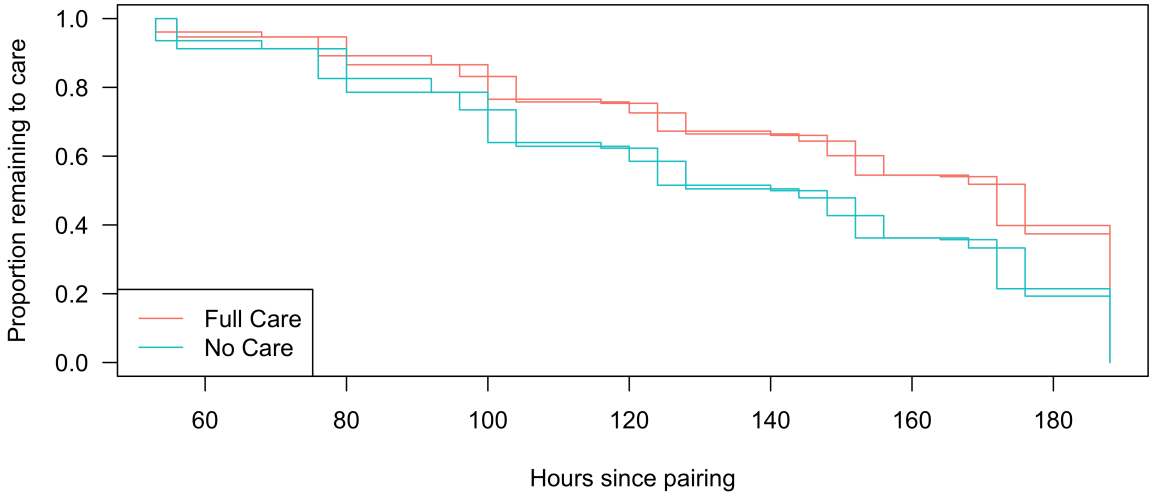

Figure 5 – Duration of male care in generation 43. The survival curves for male care were significantly affected by the male's evolutionary line, with No Care males caring for less time. Two lines are displayed for each treatment because with interval censored data it is not possible to tell the exact 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.

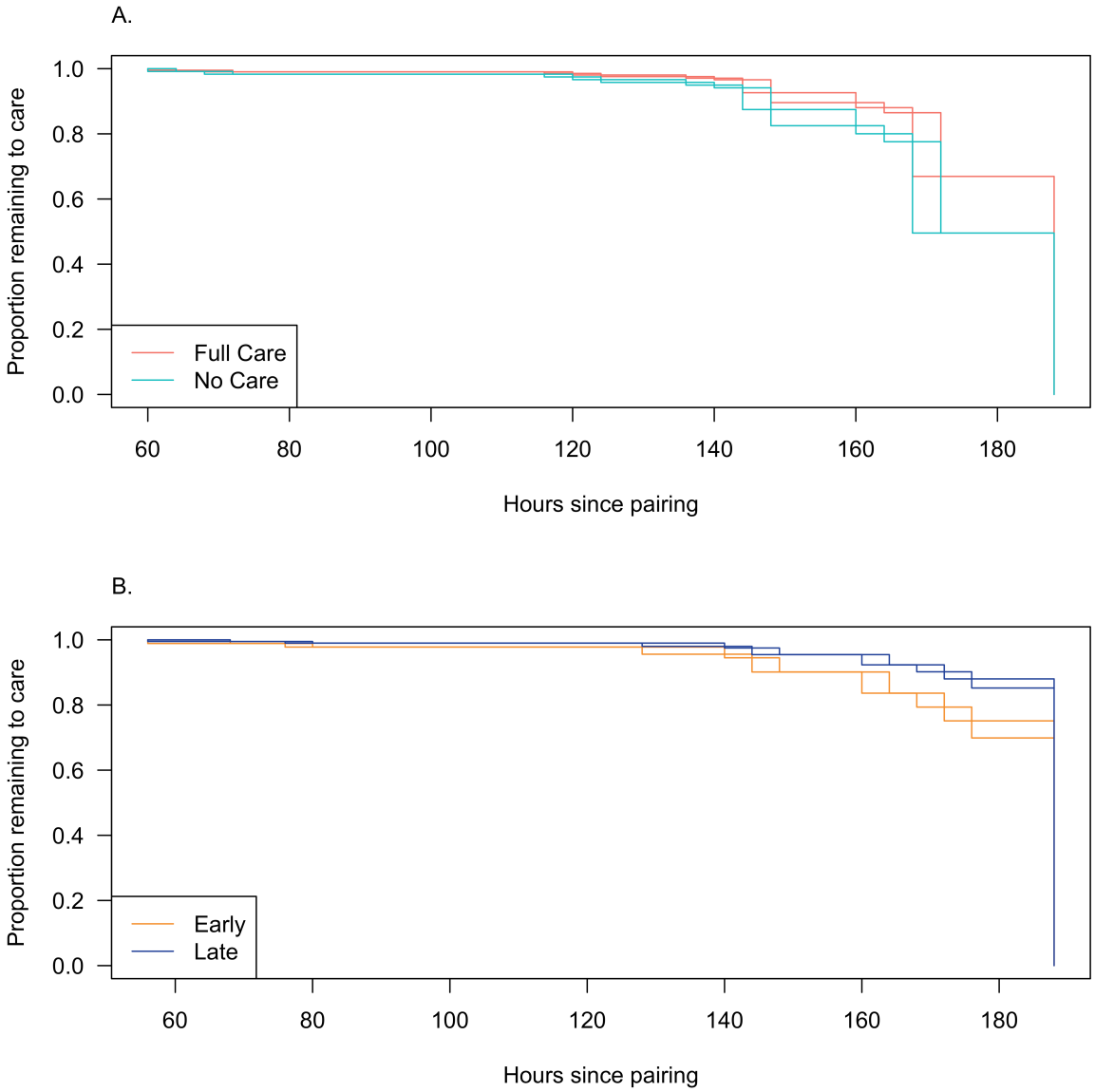

Figure 6 – Duration of female care in relation to A) larval line of origin at generation 24 and B) departure time of their male partner at generation 43. The survival curves for female care were significantly affected by A) the evolutionary line of their current brood (in generation 24 only), and B) the duration of their male partner's care (in generation 43 only). Note that the duration of male care was analysed as a continuous factor, but is depicted in the figures as the 2nd quantile (Early) and 4th quantile (Late) care only for ease of viewing the data. 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 it 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.

Experiment 2

No Care larvae begged towards their foster parent for significantly less time than Full Care larvae ($F_{1,124}$ = 25.042, p = <.001) regardless of the evolutionary line of their foster parent (Figure 7A). The result was similar when considering both total time spent interacting with the parent as for begging alone ($F_{1,124}$ = 12.979, p = .001, Figure 7B). No other variables significantly affected larval solicitation behaviours (Table 5).

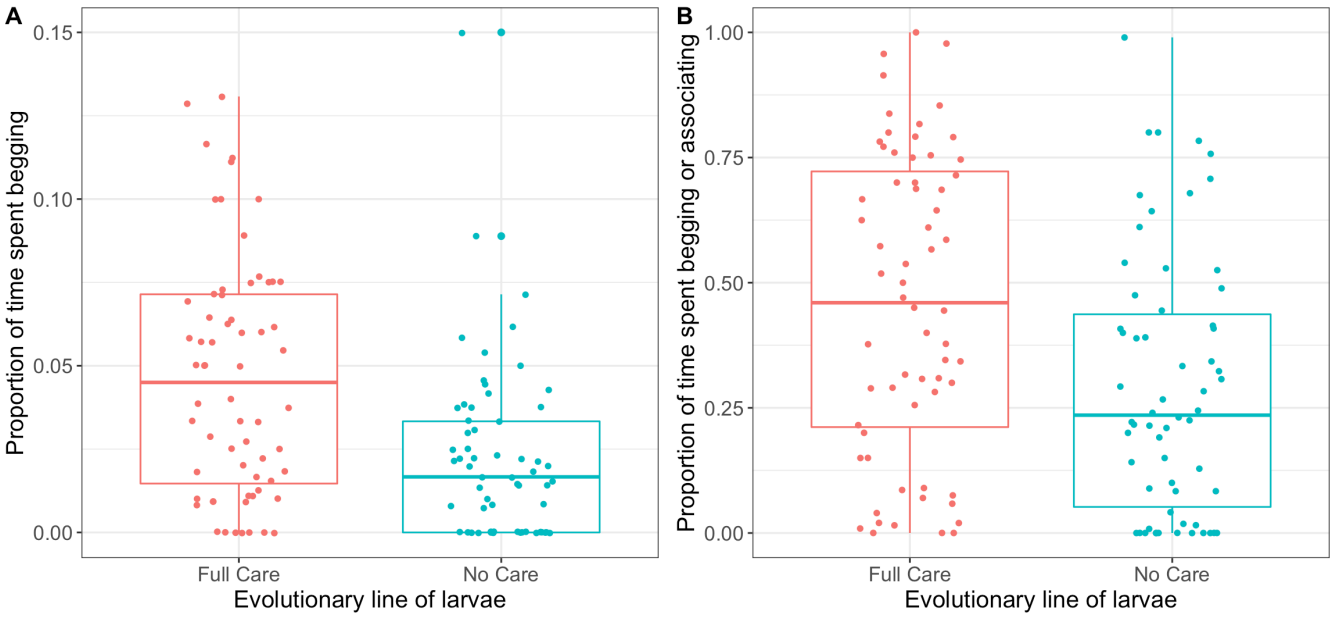

Figure 7 - The effect of the evolutionary line of the experimental larvae on A) proportion of time spent begging and B) the proportion of time spent either begging or associating with the parent (n=126 broods). Whiskers extend to the farthest data point which is no more than 1.5 times the interquartile range from the box. Points show individual broods.

Table 5 – Quasi-binomial GLMs fitted to explain variation in the proportion of time spent begging and the proportion of time spent either begging or 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 as a larvae 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 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	**Larval Line (No Care)**	**-0.850**	**0.176**	**25.042**	**1**	**<0.001**
	Parents' Line (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
	Larval Line:Parent Line	-0.191	0.353	0.294	1	0.589
Interacting with the parent (begging and associating combined)	**Larval Line (No Care)**	**-0.788**	**0.221**	**12.979**	**1**	**0.001**
	Parents' Line (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
	Larvae Line:Parent Line	0.210	0.449	0.219	1	0.641

Discussion

We investigated the rate at which traits connected with offspring demand and parental supply were evolutionarily lost by disrupting this social interaction for generation after generation in experimentally evolving populations. We assessed the extent of loss: 1) indirectly, by partitioning the contribution of the offspring and parental line of origin to

average larval mass at dispersal and 2) directly, by quantifying larval capacity to solicit care and the duration of care supplied by each parent.

Our indirect measurements showed firstly that larvae were lighter in generation 24 than in generation 43. Although carcass mass was controlled to within 3 g, even within this range we found that carcass mass significantly affected larval mass, and in generation 24 the mean mass of the mice used was slightly lower. Therefore, it is likely that this generational difference in mass is simply attributable to the difference in carcass mass. Crucially we found that in both generations, No Care larvae (on carrion nests prepared by No Care parents) consistently attained a lighter mass at dispersal than Full Care larvae (on carrion nests prepared by Full Care parents), regardless of whether they were raised by parents from the Full Care or No Care lines during the study. We can think of three possible explanations for these findings, which are not mutually exclusive. First, offspring from the No Care lines may have developed within smaller eggs, meaning that larvae were smaller from hatching. We do not know whether the eggs produced by the No Care populations were smaller after 24 and 43 generations of experimental evolution, but we have previously shown that development in a No Care environment initially yields smaller adults, and that smaller females lay smaller eggs (Jarrett et al., 2017). A second explanation is that the carrion nests produced by No Care parents might have provided an inferior environment for offspring development compared with the carrion nests made by Full Care parents. We reject this explanation because we have demonstrated the opposite to be true (Duarte et al., 2021). The third explanation is that No Care larvae were less effective than Full Care larvae at acquiring resources from parents: the demand for care in the No Care lines was lost because it was not expressed in these populations for so many generations. This interpretation is supported by the direct behavioural measurements, which showed that by generation 48, No Care larvae were less likely to solicit care from parents than Full Care larvae. It also accounts for our findings in generation 24 that all females shortened their duration of care when given No Care larvae to look after: the brood's failure to solicit care effectively may have contributed to this earlier departure time. On balance, we conclude that the larval capacity to solicit care started to disappear after generations of not being expressed and that this loss might have been evident after just 24 generations (although it is possible that egg size also contributed to a lower average larval mass at dispersal in the No Care larvae, at this point).

Turning to the parents, here we found no evidence for a loss of post-hatching parental behaviour at generation 24, whether we used indirect (larval mass) or direct measurements (departure time). No Care and Full Care parents contributed to a similar extent to average larval mass at dispersal, which was positively correlated with the duration of care supplied by each parent. By generation 43, however, this pattern had changed. Here there was evidence of decline in the No Care male's supply of post-hatching care, which was now significantly shorter than the care provided by the Full Care males. Furthermore, the duration of male care no longer predicted larval mass at dispersal. Shorter periods of male care also curtailed the duration of care supplied by females. We could detect no equivalent evidence of decay in the supply of care provided by females. There was no difference between No Care and Full Care females in the time they spent caring for larvae in generation 43, and the duration of maternal care was still positively associated with average larval mass at this point.

Overall, our experiments show that social behaviours start to disappear when the opportunity to express them is eliminated for multiple generations. Larval demand for care started to disappear first, followed by the provision of post-hatching care by males. Although supply of maternal care decreased in response to weaker demands from larvae, and reduced paternal effort, we found no evidence that No Care mothers had lost any intrinsic capacity to care for larvae - despite being prevented from providing post-hatching care for 43 generations.

Why did we observe contrasting rates of behavioural loss between larvae, males and females? The key to answering this question lies in understanding the mechanisms underpinning these behavioural traits (Heinen-Kay and Zuk, 2019). We can immediately rule out any wider environmental differences, since the evolving populations were kept under identical conditions in the same lab. One possibility is that the genes underpinning each of these traits are simply differently vulnerable to mutation. We have no evidence yet to test whether or not this proposition is true. A different possibility, which is perhaps more likely given the relatively short timescale of this experiment, is that the demand for care and the supply of post-hatching care are each traded against the expression of alternative behavioural traits that offer a different route to gaining fitness (Heinen-Kay and Zuk, 2019). For example, the expression of larval solicitation behaviours could trade-off directly with the expression of self-

feeding (Smiseth et al., 2003). In natural populations, there might be standing genetic variation in the threshold at which environmental cues tip the balance between the expression of each larval trait. Selection from the No Care environment could have favoured larvae that were more inclined to express self-feeding at the expense of expressing solicitation behaviours. The recalibration of this trade-off at the population level to favour larval self-feeding in a No Care environment could explain the corresponding loss of larval traits for demanding care.

We can make a similar population-level argument for changes in the expression of male post-hatching care over time. This might be due to a re-balancing of the trade-off with the supply of pre-hatching care and, specifically, the effort devoted to making the carrion nest (De Gasperin et al., 2015). The existence of a trade-off like this is plausible since nest maintenance activity after hatching is known to be negatively genetically correlated with the direct supply of larval care in males (Walling et al., 2008). Furthermore, we have shown elsewhere that by generation 20 the adults from the No Care populations had evolved to put more effort into making the carrion nest than adults in the Full Care populations (Duarte et al., 2021) and that this change in nest-making proficiency was due more to males than to females (Jarrett et al. *in prep*). Whether an equivalent trade-off exists for females is less clear. The female trait contributing most to fitness before hatching is most likely to be egg size and it is theoretically possible that there is a negative genetic correlation between egg size and the supply of post-hatching care. However, even if such a correlation exists, it is likely to be concealed by a condition-dependent positive phenotypic correlation between egg size and post-hatching care. Females that receive less post-hatching care are smaller and lay correspondingly smaller eggs (Jarrett et al., 2017).

If trade-offs between competing behavioural routes to fitness explain how some traits are lost, then presumably it is the relative strength of selection to recalibrate these trade-offs that explains why rebalancing happened most rapidly in larvae, and then in males, but not in females. Larval mortality in the No Care populations was very high in the first few generations of experimental evolution (Schrader et al., 2017). Any larvae that did not self-feed in the No Care environment would rapidly have been selected against. Selection for recalibrating the male's trade-off arguably is somewhat weaker, since their nest-building behaviour less

directly affects larval survival. The strength of selection on the putative egg-care trade-off in females could be negligible, especially if its expression is masked by female condition.

The general principle emerging from this study is that rapid trait loss is associated with the expression of alternative traits which can enhance fitness more effectively when populations enter a changed environment. This is consistent with previous work analysing trait loss in sexual partners (Heinen-Kay and Zuk, 2019) and with the evolution of trait loss in mutualistic partnerships, where the actions of the partner species compensate for trait loss in the focal species (Ellers et al., 2012). This general principle explains the loss of traits associated with parental care in domesticated bird and mammal species, because breeders often intervene to nurture offspring themselves or to cross-foster them to other strains that provide better offspring care. As a consequence, wild Norway rats have been shown to be more efficient in pup retrieval than domestic mothers (Walling et al., 2008), and some strains of domesticated canary (Vriends, 1992) and zebra finch (Blackwell, 1988) are now incapable of raising offspring to independence.

For traits like this, a return to the ancestral environment after relatively few generations is not sufficient to restore ancestral levels of trait expression. There are important implications here for conservation captive breeding programmes, which could inadvertently induce rapid trait loss through any artificial compensatory husbandry techniques that are introduced to promote breeding success in captivity. Interventions like this could prevent the successful reintroduction of species in the wild by causing the loss of key traits needed for survival and reproduction (Araki et al., 2007; Bowkett, 2009; Williams and Hoffman, 2009).

Finally, this study also suggests that although the loss of the interacting phenotype in one individual (e.g. larva, or male) is sufficient to prevent expression of the corresponding phenotype in its social partner (e.g. mother, or female partner), it is not sufficient to cause that trait to decay in the short-term. Nevertheless, unexpressed social traits are likely to be lost in the longer-run, because mutations can easily accumulate in traits unseen by natural selection. In general, our study suggests the likelihood of reviving ‘ghosts of adaptation past’ will depend on how long it has been since those adaptive traits were last expressed, and how likely it is that they have been superseded by traits that more effectively promote fitness in

the new environment. The flexibility of many behavioural traits, and the multiple behavioural routes that are consequently available for achieving similar fitness goals, could mean that some behavioural traits are rapidly substituted and lost following a change in the wider environment.

Author contributions

RMK, EKB and SP designed the experiments; EKB, SP and NB collected the data. EKB and RM analysed the data. EKB, RM and RMK were involved in writing and editing the manuscript.

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Supplementary material

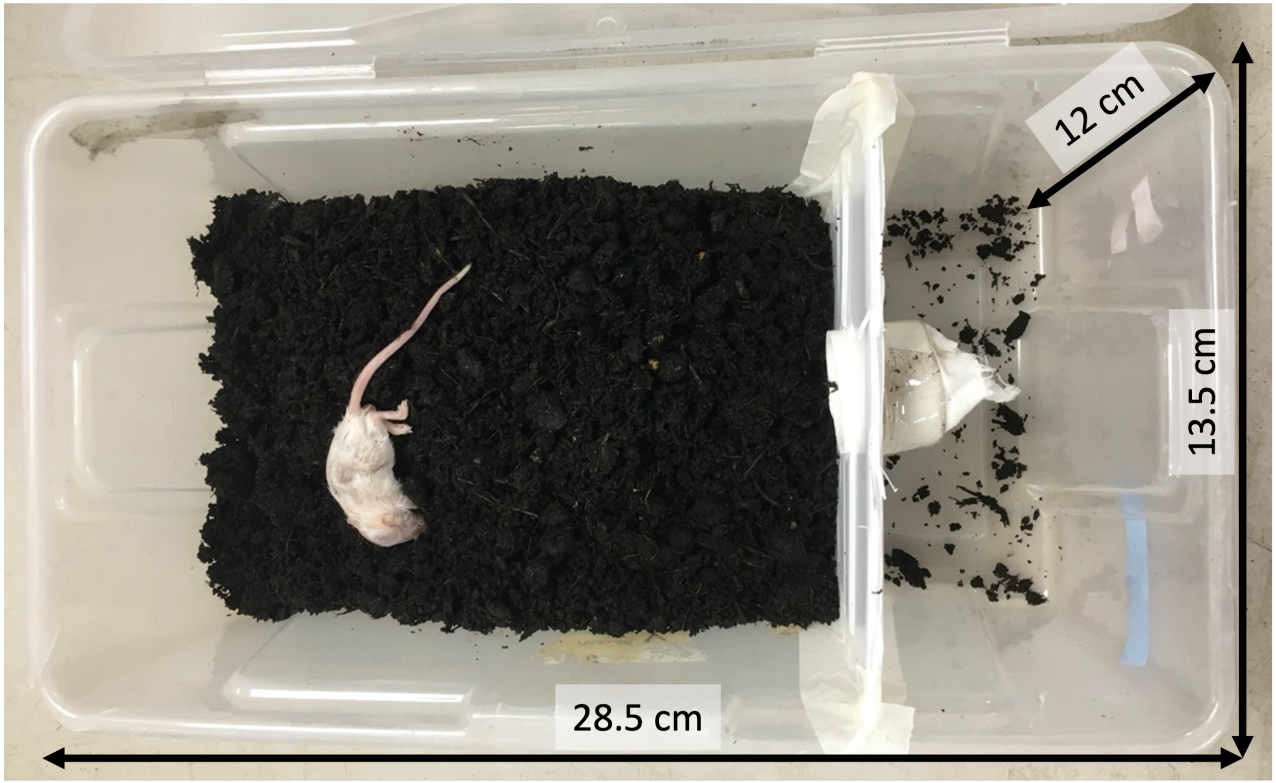

Figure S1: The breeding boxes used in Experiment 1 with breeding compartment and "escape chamber".

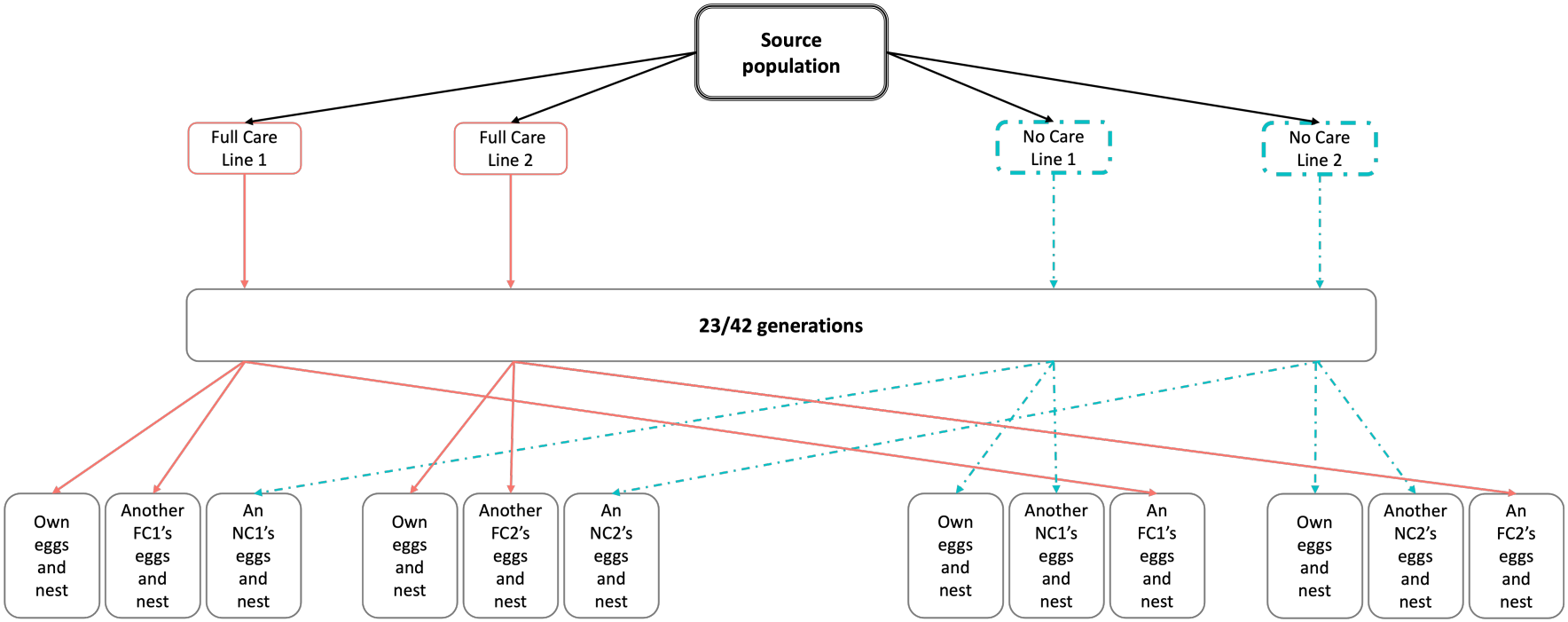

Figure S2: The design of the experimental evolution lines and Experiment 1