Multilevel selection leads to divergent coadaptation of care-giving parents during pre-hatching parental care

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Abstract

Parental care often involves coordination between the mothers and fathers. Their coordination is partly due to flexible 'negotiation rules' that allow mothers and fathers to respond adaptively to each other in real time, but could also be due to divergent environmental conditions that cause different negotiation rules to evolve in different populations through coadaptation. We evolved experimental populations of burying beetles *Nicrophorus vespilloides* in different environments and tested for evidence that the sexes had coadapted to these divergent environments. In two populations, parents supplied pre- and post-hatching care (Full Care environment), while in two other populations, parents supplied only pre-hatching care (No Care environment). We have previously shown that parents in the No Care populations rapidly evolved superior pre-hatching care. Here we show that this is due to the coadaptation of the traits expressed by males and females while they convert carrion into an edible nest for larvae. After 15 generations of experimental evolution, we created heterotypic pairs (No Care females with Full Care males, and No Care males with Full Care females) and found they were less effective at making a carrion nest than homotypic No Care pairs—with negative consequences for brood performance. We suggest that the coadaptation of the sexes is the result of selection acting on pairs in different ways in the No Care versus Full Care environments. We discuss how social co-adaptations within cooperating pairs or teams of individuals could act as post-mating barriers to gene flow.

Keywords: intergenomic epistasis | burying beetle | reproductive isolation | experimental evolution

Introduction

Social interactions within species are universal (Frank 2007). When individuals interact, genes underlying the phenotype of one individual can influence the phenotypes of their social partners (Moore, Brodie, and Wolf 1997; Wolf, Brodie, and Moore 1999; Agrawal, Brodie, and Wade 2001), both by inducing trait expression in the social partner and by exerting selection on those traits. Theory predicts that adapting to the heritable environments created by social partners can therefore be more rapid than adaptation to abiotic environments (Drown and Wade 2014).

Adaptation to the social environment is more likely when populations are subdivided into subgroups, like animal families or partnerships (Agrawal, Brodie, and Wade 2001), because the grouping confers distinct roles on social partners and gives them the opportunity to influence each other's fitness (Hager and Johnstone 2003; Hinde, Johnstone, and Kilner 2010; Kölliker, Royle, and Smiseth 2012; Barta et al. 2014). Theoretically, this can result in the coadaptation of traits expressed in each social partner. Previous work on diverse animal families has found evidence that such favourable combinations of phenotypes do indeed exist between social partners within animal families (e.g., Zeh and Zeh 2000; Linksvayer, Fondrk, and Page Jr 2009; Hinde, Johnstone, and Kilner 2010). Evidence from bird species, for example, has identified the precise ways in which adults coordinate their provisioning activity at the nest (e.g., Savage and Hinde 2019; Griffith 2019). Some of this coordination is the result of flexible decision-making, through adherence to adaptive 'negotiation rules' which enable mothers and fathers to fine-tune their provisioning behaviours to one another (e.g., McNamara et al 1999; Savage and Hinde 2019; Smiseth 2019). However, the extent of flexibility in such negotiation rules is unclear. Are they so flexible that males and females from different populations can work together successfully to raise offspring? Or do populations evolve distinct sets of negotiation rules, under selection from divergent wider environmental conditions, which mean that hybrid pairings are unlikely to be as successful?

We addressed these questions by establishing experimental populations of burying beetles *Nicrophorus vespilloides* and allowing them to evolve in divergent environments. Burying beetles breed on dead vertebrate carcasses (Scott 1998). The parents jointly convert the dead mouse or bird into a carrion nest by removing the fur or feathers, rolling it into a ball, covering it in anti-microbial anal exudates, and burying it. Eggs are laid in the soil surrounding the sunken carrion nest, and newly hatched larvae crawl to the nest where they aggregate. To help their larvae take up residence on the carrion nest, and feed upon it, parents make an incision in the flesh prior to their arrival. Parents also guard and feed larvae after they hatch, though larvae can survive in the laboratory with no post-hatching care at all (Eggert, Reinking, and Müller 1998).

In two of our experimentally evolving populations, larvae received both pre-hatching and post-hatching parental care (the 'Full Care' populations), while in the two other populations we prevented parents from supplying any post-hatching care by removing them before their larvae hatched, once the carrion nest was complete (the 'No Care' populations). During the first thirteen generations of experimental evolution, the No Care lines rapidly adapted to a life without parental care (Schrader, Jarrett, and Kilner 2015a; Schrader et al. 2017), through divergent phenotypic adaptations in both larval (Jarrett et al. 2018a; Jarrett et al. 2018b; Rebar et al. 2020) and parental traits (Jarrett et al. 2018a; Duarte et al. 2021). In particular, No Care parents rapidly evolved to lay smaller clutches on small carrion (Rebar et al. 2022), which could have resulted from males evolving to become less effective at stimulating female fecundity and/or females evolving to produce fewer eggs (Pascoal et al. 2018). In addition, No Care parents evolved to become more efficient and more effective at converting carrion into a nest for their larvae, and in this way increased their offspring's chance of surviving without post-hatching care (Duarte et al. 2021).

After fifteen generations of experimental evolution, we conducted crosses between the experimental populations to investigate the nature and extent of coadaptation in each of these two traits: clutch size and carrion nest preparation. We predicted that if: (1) clutch size resulted from local coadaptation between parents then heterotypic pairs should produce clutches that differed from those laid by either the homotypic Full Care or No Care pairs and that any differences could be explained by the population of origin of each sex. We further predicted that if: (2) changes in carrion nest preparation resulted from divergent local coadaptation, where No Care parents are better at preparing the carcass, then heterotypic pairs should be less efficient and less effective at preparing the carrion nest than homotypic No Care pairs, though more effective than homotypic Full Care pairs as heterotypic pairs have one No Care parent. However, if: (3) coadaptation involved highly flexible negotiation rules, then heterotypic pairs should be no less effective than homotypic No Care pairs at preparing the carrion nest.

Methods

Experimental evolution

The experimentally evolving populations used in this work have been described in detail elsewhere (Schrader et al. 2017). In brief, we established a large founding population of *N. vespilloides* by interbreeding wild-caught individuals from four different woodlands and dividing the offspring into four replicate experimental populations. In two populations, larvae experienced Full Care (FC) in each generation, with both parents staying in the breeding box to feed and interact with their offspring. In the remaining No Care (NC) populations, both parents were removed from the breeding box at each generation, 53 hours after they were paired, so that they never interacted with their larvae (see Schrader et al. 2017 for more details on the experimental set up). The populations were organised

into two blocks, separated by a week, to ease the workload (hence NC1, FC1 in Block 1 and NC2, FC2 in Block 2). The experimental work reported here used populations that had been exposed to 15 generations of experimental evolution.

Experimental design

The design of the experiment is shown in Figure 1. Prior to testing, the four populations went through a common garden environment of Full Care (where the parents were allowed to interact with their larvae throughout development). The aim was to standardise parental effects across all four treatments and so that any residual variation could be attributed to evolutionary change. To set up the common garden generation, the FC1 and NC1 parents were bred three weeks after eclosing as adults, and the FC2 and NC2 parents were bred at two weeks after eclosing as adults. This synchronised breeding of individuals from the two blocks ensured that the adults generated for the focal experiment were matched in age at first breeding across treatments. The populations were then crossed: homotypic crosses involved crossing the two replicate lines that had experienced the same parental manipulation (i.e., FC1 × FC2 and NC1 × NC2), while heterotypic crosses involved crossing populations that had experienced different parental manipulations (i.e., FC1 × NC1 and FC2 × NC2). The homotypic crosses served as a control for any population divergence that was not attributable to adaptation to the different parental care environments.

Burying beetle husbandry

Adult beetles were kept individually in boxes measuring 12 × 8 × 2 cm filled with compost for two weeks after eclosion until they were sexually mature. Individuals were fed ~0.3 g of minced beef twice a week. Once individuals were sexually mature, they were paired for breeding. The pair of beetles was added to a larger box measuring 17 × 12 × 6 cm half filled with fresh compost. A mouse carcass sourced from Live Foods Direct Ltd. was weighed and recorded (mean ± sd = 11.60 ± 0.87g; range = 10.00–13.18g) and placed into the box, after which the pair of beetles was added. The pair of beetles prepared the mouse carcass for the first two days, whilst the female laid in soil. At ~53 hrs, when carcass preparation and egg laying had been completed, the parents were removed in the No Care treatment. For the Full Care treatment, the parents were left in the box until the larvae dispersed, typically eight days after pairing.

Eight days after pairing, the larvae had completely eaten the carcass and were ready to complete development. We removed the larvae, counted them, and weighed the whole brood to the nearest 0.0001g. The larvae, after they had been weighed, were placed into an eclosion box, measuring 10 × 10 × 2 cm, with 25 individual cells, each 2 × 2 × 2 cm. An individual larva was placed in each cell and covered with peat that was sifted to remove large chunks of soil. Each box held one brood. Water

was sprayed over the top to prevent desiccation during subsequent development, which typically lasted 18–21 days.

Statistical analysis

All analyses were performed in R (version 3.5.1) (R Development Core Team 2018) using Bayesian models in Stan (Stan Development Team 2021) implemented in *brms* (Bürkner 2017; 2018). Additional packages used for the analysis and plotting of data in this paper include: *tidyverse* (Wickham 2017), *ggplot2* (Wickham 2009), *tidybayes* (Kay 2019), and *modelr* (Wickham 2018). Bayesian models run through *brms* provide an estimate of the slope (β) with 95% credible intervals (CI), which gives an indication as to the effect of the variable in question (i.e., if the 95% credible intervals do not overlap with 0 it indicates the variable has a significant effect). If the 95% CIs for the β estimate did not overlap with 0, we assessed the model's predictive ability by comparing the model containing the variable of interest with a simpler model that did not contain the variable (or interaction of variables) of interest. We compared these models using leave-one-out (loo) cross-validation and also calculated the relative weight of each model to evaluate which model had the highest likelihood of predicting future data (Vehtari et al. 2017). Models fit in this manner move away from the dichotomy of deciding whether a variable is significant or non-significant and assess both the strength of a variable's effect, and the predictive ability of the model.

We analysed the data taking into account the populations from which the male and female were drawn. We created a dummy variable for males and females with Full Care individuals coded as 1 and No Care as 0. The model included both the female and male dummy variables along with their interaction. All the simpler models were then considered by first removing the interaction between female and male dummy variables, then secondly removing each dummy variable in turn, and finally considering a model without either dummy variable. For all analyses, the standardised (mean = 0, sd = 1) mass of the carcass, and the standardised size of the male and the female were included as covariates. Other covariates included in specific models are outlined below. Data were split into Full Care and No Care environments, which we analysed separately for ease of interpretation. (Note, that when we refer to the 'No Care environment' and the 'Full Care environment', we mean the within-generation treatment at the end of the experiment we report (shown in Figure 1). We refer to the evolving populations as either the 'Full Care populations' or the 'No Care populations').

Testing for evidence of coadaptation between care-giving partners

We tested Predictions (1-3) by focusing on: (Prediction 1) number of eggs laid to investigate whether clutch was a product of coadaptation (Pascoal et al. 2018); and (Predictions 2 and 3) the presence of a feeding incision on the prepared carrion nest to test if pre-hatching care was a product of coadaptation or the result of flexible negotiation between parents (Walling et al. 2008). We also investigated the

fitness consequences of the feeding incision by asking how its presence or absence influenced the likelihood that a brood would succeed (i.e., produce at least one larva that survived to pupate).

Prediction 1: Clutch size

We analysed clutch size with a negative binomial distribution. As no environmental manipulation had occurred by the time clutch size was estimated, we did not split the data by Full Care and No Care environment, and instead included all the data from both the Full Care and No Care environments for this analysis.

Predictions 2 and 3: Feeding incision before larval hatching

We analysed the presence or absence of a feeding incision on the carcass as a dummy variable with a Bernoulli distribution where 0 = no feeding incision and 1 = feeding incision present. This was observed at 53 hours, the same time that clutch size was estimated and the time when we experimentally removed parents in the No Care environment. Therefore, there is no difference between the Full Care and No Care environments at this point of the experiment and so we used all the data from the Full Care and No Care environments for this analysis. To investigate the fitness consequences of the presence or absence of a feeding incision, we analysed whether a brood yielded at least one larvae eight days after pairing. We anticipated the relationship between the feeding incision and brood success to be dependent on the care environment, and so split the No Care and Full Care environment data and analysed each separately. A brood was considered successful if it had at least one surviving larvae at dispersal. We created a dummy variable for brood success (success vs fail) and analysed it assuming a Bernoulli distribution. The model included population of origin (i.e., whether he descended from a No Care or Full Care population) and female population of origin (i.e., whether she descended from a No Care or Full Care population) and their interaction, along with the presence or absence of a feeding incision as a two-level factor.

Results

Prediction 1: Clutch size

The model with neither male nor female population of origin had the lowest loo value and 45% of the loo weight (Table 1), indicating that neither had a strong effect on clutch size. However, when both terms were included in the model, these two terms interacted to influence clutch size. Heterotypic pairs—both No Care females paired with Full Care males, and Full Care females paired with No Care males—tended to have smaller clutches than Full Care–Full Care and No Care–No Care pairs (β = 0.16, [–0.03, 0.36], Table 1).

Predictions 2 and 3: Feeding incision before larval hatching

We found that the population of origin of each parent influenced the likelihood of a feeding incision being made before larvae hatched, and that the effect differed by sex and in relation to their partner's population of origin (interaction between sex and population of origin: $\beta = 1.06$, [–0.02, 2.15]). A feeding incision was more likely to be made before the larvae hatched if the male descended from a No Care population, and it was even more likely to be made if the female of the pair also descended from a No Care population (Fig. 2). The model with the interaction term between male and female populations of origin had a similarly low looic as the model that contained only the male population of origin (Table 1), each with ~41% of the model weight.

The presence of a feeding incision before larval hatching increased the success of the brood in the No Care environment ($\beta = 1.02$, [0.28, 1.91], Fig. 3). Indeed, in the No Care environment, the presence of a feeding incision in the carcass prior to larval hatching was the strongest predictor of brood success and could not be further explained by any other divergence between the populations of origin (Table 1).

However, in the Full Care environment, the presence of a feeding incision did not influence the success of the brood ($\beta = 0.21$, [-1.53, 2.27], Fig. 3). Brood success was potentially partly explained by the female's population of origin, but this model had no more explanatory power than the null model (Table 1).

Discussion

After only 15 generations of experimental evolution, we found evidence of divergent coadaptation between males and females in the No Care populations during their carcass preparation activities, with distinct coadapted traits functioning to promote the pair's reproductive success in a No Care environment. There was no evidence to suggest that parents employ highly flexible negotiation rules, since heterotypic pairs performed less well than the homotypic No Care pairs. We found relatively little evidence of coadaptation between parents in clutch size, however. Clutch size in these experimental populations appeared to be only weakly influenced by the male's population of origin and instead to be primarily determined by female size, carcass mass, and the female's population of origin (Duarte et al. 2021; Rebar et al. 2022).

In common with many other species (Henshaw et al. 2019), it is well established that burying beetles divide the duties of parental care between the sexes. Males carry out more of the pre-hatching duties of care, taking the lead in preparing the carrion nest (Walling et al. 2008; De Gasperin et al. 2016), while females are more involved with post-hatching care (Walling et al. 2008). Both parents benefit from this coordinated behaviour (Eggert, Reinking, and Müller 1998; Jarrett et al. 2018a; Rebar et al. 2020; Duarte et al. 2021), suggesting that task specialisation is related to cooperation rather than due

to conflict (Cooper and West 2018). Indeed, previous work on burying beetles suggests that task specialisation causes the sexes to be dependent upon each other, in the sense that they each perform most effectively when paired (Pilakouta et al. 2018). Theoretical work suggests that task specialisation could even enhance the capacity for cooperation between the sexes during biparental care (Cooper and West 2018). The burying beetle's natural history, particularly the even sharing of the benefits that result from task specialisation and the fact that effective carrion preparation is critical to larval survival (Cooper and West 2018), nicely fits the modelling assumptions behind the theory and makes this suggestion all the more plausible.

Our experiments suggest that within this broadscale division of labour, the activities of each sex can evolve rapidly, to be adaptively fine-tuned both to the social behaviour of their partner and to wider environmental conditions. Turning to the social environment first, we have previously shown that parents in the No Care lines evolved rapidly to prepare the carrion nest more rapidly, by rolling the flesh into a more perfect sphere, and by inserting a feeding incision before their larvae hatched (Jarrett et al. 2018a; Duarte et al. 2021). Here we have found that males are the drivers behind the parental adaptation to prepare the carrion more efficiently in the No Care lines. The feeding incision was consistently inserted sooner when No Care males prepared the carrion nest, irrespective of the line of origin of the female. When the female was drawn from the No Care lines too, the feeding incision was more likely to be inserted before larval hatching, indicating that the female somehow facilitated the No Care male's actions. A feeding incision was less likely to be inserted when No Care females were paired with Full Care males. The sexes therefore appear to be coadapted in their carcass-preparation behaviour, in an apparent instance of intergenomic epistasis (Wolf, Brodie, and Wade 2000; Wolf 2000).

Parents not only coadapted to each other's behaviour but also evolved in response to selection on the pair, from the wider experimental environment we generated in the No Care populations. By removing parents 53h after pairing, we imposed selection on the pair to complete the preparation of the carrion nest more rapidly. By depriving larvae of post-hatching care, pairs were also placed under selection to make a better nest for their larvae (Duarte et al. 2021). The cooperative interactions between parents during preparation of the carrion nest thus moved individual beetles slightly further along the social continuum towards a higher level of individuality, where adaptations are a function of the two individual parents, by inducing multilevel selection (Cooper and West 2018). In short, we infer from our results that there has been selection in the No Care populations on the joint efforts of the breeding pair to prepare the carrion nest effectively together, and that this has favoured a distinct combination of socially interacting genes in the two sexes (*cf* Linksvayer, Fondrk, and Page Jr 2009).

Previous theoretical analyses have suggested that divergent coadapted social traits within the family could function as a post-mating mechanism for reproductive isolation—because they cause hybrids to perform less well (e.g., Zeh and Zeh 2000; Gavrilets 2000; Martin & Hosken 2003; Brandvain & Haig 2005). Evidence from burying beetles is consistent with this view. *Nicrophorus vespilloides* has recently been split into two species, with *N. hebes* now recognised as a distinct bog-breeding specialist that lives mostly in Canada (Sikes et al. 2016). Speciation is sufficiently recent that *N. hebes* can still hybridise with Alaskan populations of *N. vespilloides* to produce viable offspring. Nevertheless, hybrids perform less well, partly because hybrid larvae are less viable (at least in a No Care environment). Experimental work indicates that hybrid pairings produce fewer eggs and have lighter broods than pure-bred populations (Sikes et al. 2016). The reduced performance of hybrids could also be due to coadaptation between care-giving adults, although this possibility remains to be tested explicitly.

Social coadaptations can cause post-mating reproductive isolation in other species too (e.g., Zeh and Zeh 2000; Gavrilets 2000; Martin & Hosken 2003; Brandvain & Haig 2005). A common factor is a high level of coordination between interacting individuals. This is true whether coordination results from cooperation or conflict. It is seen in viviparous species, for example, through the highly specific structures that have evolved to coordinate the supply of resources from mother to offspring in viviparous fish (Schrader & Travis 2008; Furness et al. 2019) and placental mammals (Roy 2022). We have shown here that the highly coordinated activities jointly undertaken by male and female burying beetles in converting carrion into an edible nest can also become coadapted, and cause hybrid pairings to perform less well. In future work, it would be interesting to test whether the coadaptations involved in other types of coordinated cooperative social activities, such as the construction of nests or burrows or the joint defence of a key resource, could also potentially function as a post-mating barrier to gene flow between populations.

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Figures

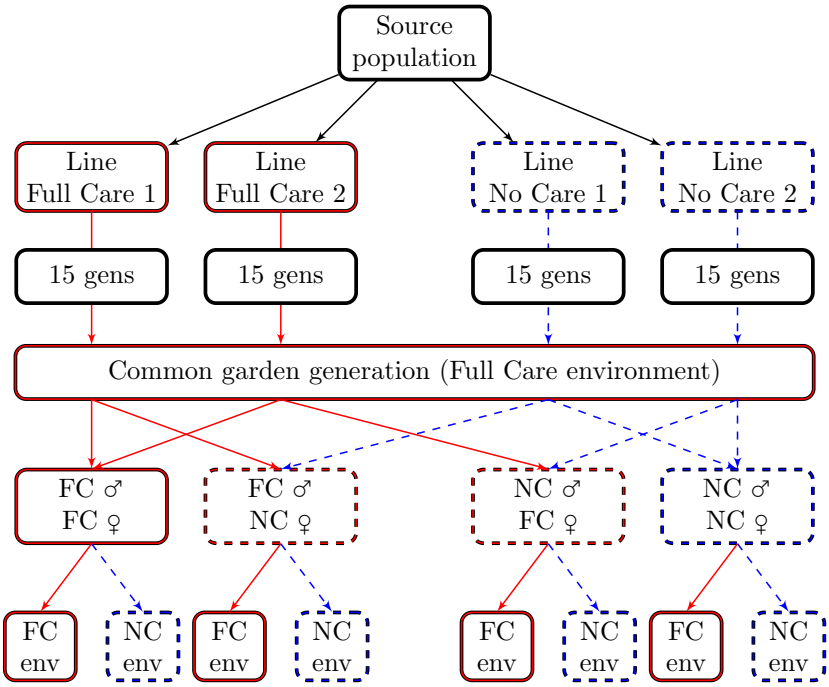

Figure 1 | The experimental design. Two manipulations were used to generate two replicate Full Care and two replicate No Care evolving populations. After 15 generations of breeding in these contrasting environments, individuals from each population were passed through a common garden regime, in which all broods received Full Care to minimise variation between lines due to parental effects. The populations were then crossed: the two control groups involved crossing the populations that had evolved under similar social environments during development (F1 with F2, and N1 with N2), and while the two experimental groups involved crossing lines that had experienced different social environments during development (F1 with N1, and F2 with N2). The broods from each type of cross were exposed to both Full Care and No Care current environments during larval development.

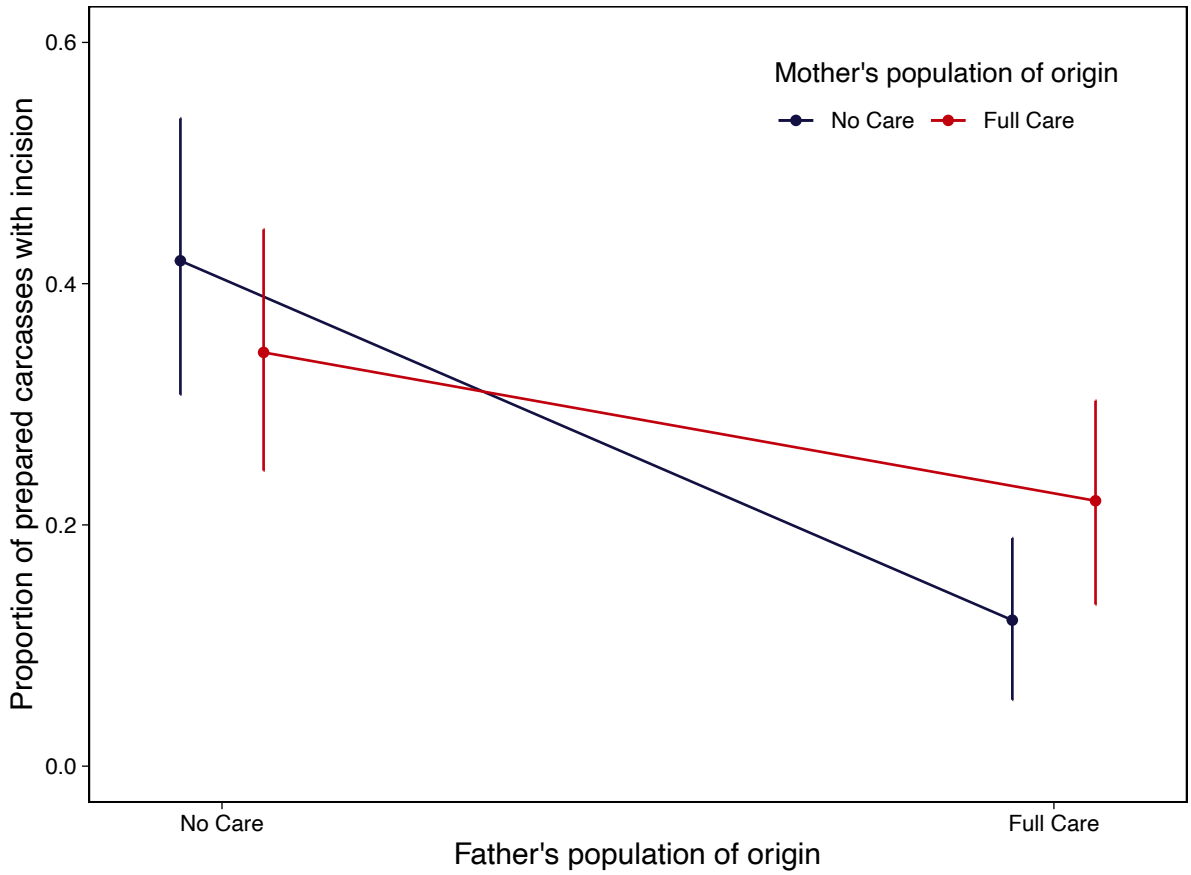

Figure 2 | The predicted proportion (and 95% credible intervals) of prepared carrion nests found with an incision hole at 53 hours after pairing (from the model containing the background of the male and female and their interaction). Males drawn from the No Care lines were more likely to have made an incision, particularly when partnered with a female from the No Care lines.

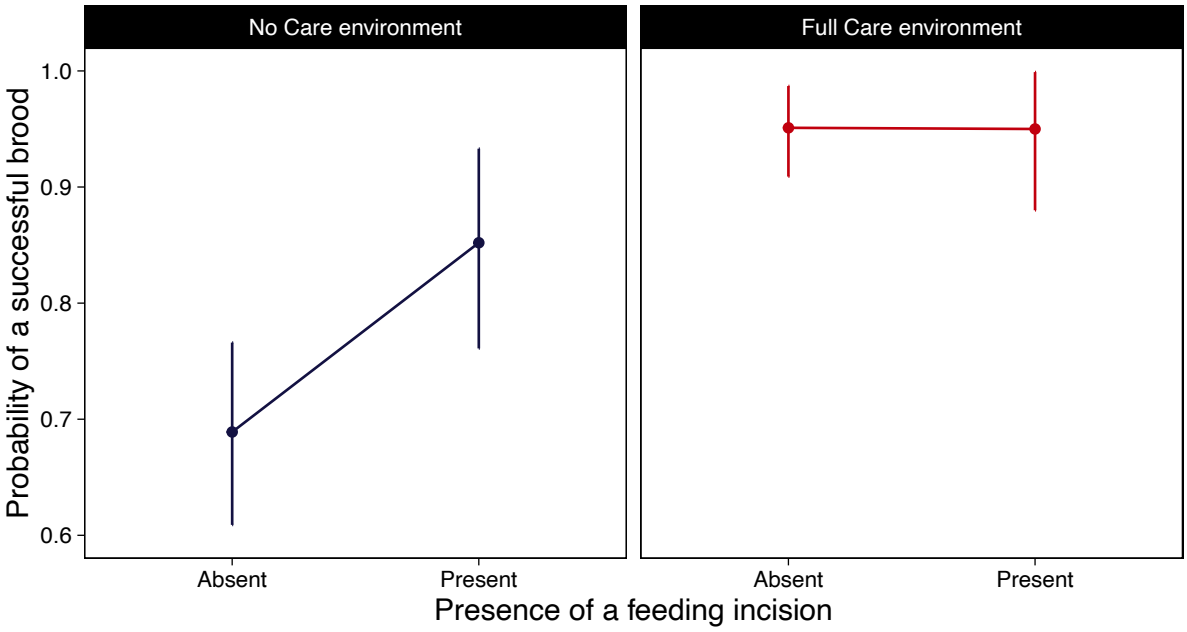

Figure 3 | The predicted proportion (and 95% credible intervals) of successful broods when the feeding incision in the carcass made by the parents prior to larval hatching was either present or absent. In the No Care environment, the presence of a feeding incision greatly improved the likelihood of a successful brood. In the Full Care environment, a feeding incision did not change the already high likelihood of a brood being successful.

Tables

Table 1 | Summary of the model comparisons for each response variable: clutch size, feeding hole or brood success. A Δloo of 0 indicates it has the lowest looic value, with the difference and standard errors of the difference listed for each model. The looic weight is also listed for each model and sums to 1 for each set of models.

Model	**Clutch size**		**Feeding hole**		**Brood success**		**Brood success**		**Brood success**	
					No Care with hole		*No Care without hole*		*Full Care*	
	Δloo	loo weight	Δloo	loo weight	Δloo	loo weight	Δloo	loo weight	Δloo	loo weight
Female × Male	3.03 ± 3.11	0.10	0.11 ± 4.21	0.41	4.67 ± 2.77	0.05	2.21 ± 3.01	0.05	1.72 ± 1.79	0.14
Female + Male	3.37 ± 0.47	0.08	1.84 ± 0.80	0.17	4.21 ± 1.28	0.06	3.54 ± 1.13	0.08	2.67 ± 1.85	0.09
Female	1.90 ± 0.45	0.18	17.71 ± 8.65	0.00	1.67 ± 1.25	0.21	1.89 ± 0.96	0.19	0	0.33
Male	1.85 ± 0.21	0.18	0	0.42	2.07 ± 0.37	0.18	1.85 ± 0.86	0.19	1.45 ± 4.50	0.16
Null	0	0.46	15.56 ± 8.46	0.00	0	0.50	0	0.49	0.24 ± 3.68	0.29