Flexible parental care ensures robustness of post-embryonic development

Ahva L. Potticary*, Christopher B. Cunningham, and Allen J. Moore

Department of Entomology, University of Georgia, Athens, Georgia, 30602 USA

***Corresponding Author:**

Ahva L. Potticary

120 Cedar Street

Biological Sciences West #360

Athens, GA 30602

ORCID identifier:

Ahva L. Potticary: 0000-0002-1157-5315

Christopher B. Cunningham: 0000-0003-3965-2076

Allen J. Moore: 0000-0002-1498-3322

Preprint Servers:

BioRxiv: DOI forthcoming

Classification:

Evolution

Keywords: maternal effect, transgenerational effect

This PDF file includes:
Main Text
Figures 1 - 3

Abstract

Parental care is expected to buffer offspring against poor environments, yet such environments are common and parental care is both rare and incredibly variable. Moreover, parental care is complex, composed of multiple acts that together influence offspring fitness. We tested the hypothesis that parental care can act as a buffer because it is composed of multiple component behaviors that allow for robustness of post-embryonic development in response to changing environments. We test this hypothesis in the burying beetle *Nicrophorus orbicollis*, a species with complex parental care, by determining how this species responds to a poor developmental environment due to reduced parental care. We manipulated the duration of parental care received and measured both offspring and grandoffspring traits of males and females. As expected, reduced care affected key traits that influence fitness including offspring development time and mass. However, offspring that received reduced care responded by increasing uniparental care when they became parents and, in addition, shifted the type of parenting behavior used to transfer nutrients to offspring. Parents that received reduced care spent more time directly feeding offspring, while parents that received full care invested more time maintaining the food resource. As a result, both the number and mass of grandoffspring were unaffected by the developmental experience of their parent. Our results show that flexibility in the behaviors composing parental care can overcome poor developmental environments and limit negative parental effects to a single generation, suggesting that parental care can confer robustness both within and across generations.

Significance Statement

Parental care is thought to buffer offspring against poor environments during their development, but what components of parenting shift to achieve this buffering? Here we manipulated the amount of care provided by burying beetles, an insect that directly feeds its offspring. Reduced care affected offspring development and mass. However, when these offspring reached adulthood, they modified their own care so that the effects were limited to a single generation. We suggest that these result show that parental care not only serves as a buffer within-generation but can also confer robustness against poor natal environments across generations.

Introduction

Parental care is thought to evolve to buffer developing young from harsh environments (1). Yet despite their importance for offspring development, parental care strategies show a bewildering amount of variation within and across taxa (2-7) and have arisen across a wide diversity of ecological conditions (1, 8, 9). Moreover, while parental care is often treated as a single trait, it is a category encompassing multiple behaviors, such as offspring feeding, maintenance of resources, nest defense, preparation and preservation of food, among others (10, 11). Thus, it is unclear how a composite trait like parental care buffers offspring development when the types of environmental hazards that an organism may experience are expected to be incredibly variable over space and time.

Implicit to the idea that parental care acts as a buffer is that it ensures appropriate offspring development despite variation in environmental insults. The maintenance of function despite changing environments – or robustness - is a ubiquitous feature of complex systems from artificially evolved electronic circuits to gene networks to ecological communities (12-16). Research across systems indicates that robustness in different environments is achieved though changes in the lower-level units that compose the system. For example, if a single gene stops functioning in a gene network, functionality may be maintained at the level of the network because multiple genes can produce the same product/outcome (17). As parental care is a composite trait, a robustness perspective predicts that buffering of offspring development across environments may be accomplished based on changes in different components of the system – that is, the behaviors composing parental care (18, 19). Selection for robustness itself can produce complex and unpredictable structures in lower-level units (16), as mechanisms that increase robustness against perturbation often trade-off with other types of functions (e.g., replication rate; 20). Since parental care is expected to evolve to buffer offspring, one solution for the diversity and complexity of parental care strategies is that there is selection for behavioral

robustness to protect offspring against a range of threats, rather than for a particular behavior in a specific environment. Yet, whether behavior follows similar principles of robustness as other complex systems is poorly understood.

Here we test the hypothesis that parental care can act as a buffer because it confers robustness to post-embryonic development for the subsocial burying beetle *Nicrophorus orbicollis* through alteration of the components of parental care. If parental care confers robustness, robustness theory predicts that, across environments, the form and frequency of specific parental care behaviors may change to maintain offspring developmental outcomes across a range of conditions. Burying beetles require small animal carcasses to breed, and compete with other burying beetles and species that feed on carrion for this essential resource (21). Upon securing a carcass, parents bury it partially or fully underground, depending on species (20), remove the exterior (e.g., fur), and coat it with compounds that slow decomposition through removal of bacteria and fungi and form it into a ball with an opening for larvae to occupy and within which parents predigest the carcass to form a "soup" (4, 22). Females lay eggs in the soil and upon hatching, larvae crawl to the carcass and enter the cavity. Parents begin feeding larvae immediately upon detection, and larvae of *N. orbicollis* cannot survive without at least several hours of direct regurgitation of predigested food from the parent (23). Parents enable nutrient transfer from carcasses to offspring using multiple behaviors, including burying and defending carcasses (which in part acts to ensure that the carcass is available for longer; 21, 24), destruction of bacterial and fungal consumers (25, 26), creation of larval cavities to support offspring self-feeding (23, 27, 28), and direct transfer of food to larvae through regurgitation (29). Larvae do not feed after they leave the carcass, and for this reason, adult body size is largely determined by the efficiency of resource acquisition during the larval period on carcass. Body size is a key fitness trait for *Nicrophorus*, as larger beetles are more effective competitors for carcasses (21, 30). Thus, parental care creates a direct link between a larva's developmental experience and competitive ability in later life.

We experimentally induced perturbation of the parental care environment by altering the duration of parental care that mixed foster broods received to produce larvae that experience reduced (six hours) or full care (approximately seven days). Poor developmental environments, including those arising from poor parental care, often produce cascading multigenerational effects (reviewed in 31) and thus represent an environmental perturbation. We then tracked these individuals into adulthood and determined how removal of parental care influenced developmental times, behavior, and reproductive success. Beetles that received full care were larger and developed faster during the vulnerable period on the breeding carcass than beetles that received reduced care. However, this effect did not carry through to the next generation (i.e., to the grandoffspring). Parents that received reduced care as larvae spent more time regurgitating food to their larvae than parents that had received full care. As a result, parents from full and reduced care treatments produced larvae of similar numbers and weights. Together, these results indicate that parental care behavior may not only buffer offspring development within generation but may act as a developmental buffer that mitigates the legacy of environments past. This robustness was achieved through flexible changes of parental behaviors that transfer nutrients to offspring and the ability of offspring to accept these differences.

Results

Effect of Perturbation of Care Environment on Development.

We set up 36 families, 18 in each treatment, of mixed-parentage broods with a single, unrelated female caretaker in the full and reduced care treatments. Overall larval survival was high in both treatments; from an initial 10 fostered larvae placed on carcass, 3-10 survived to dispersal from the mouse with a mean 8.3 ± 0.4 surviving larvae for full care families and 8.7 ± 0.4 for reduced care ($F_{1,34}$ = 0.430, P =

132 0.516). Three (2%) larvae died after dispersal from the full care treatment, and 3 (1.9%) died from the
133 reduced care treatment. A further 14 (9.6%) died in the pupal stage in the full care treatment, and 16
134 (10.4%) in the reduced care.

135 Larvae in the full care treatment developed faster on the carcass than larvae that received
136 reduced care ($\chi^2 = 18.86$, $P < 0.0001$, $N = 305$). Larvae that received full care completed development
137 from hatch to dispersal in 7 - 8 days (mean = 7.4) and reduced care larvae dispersed in 8 - 9 days (mean
138 = 8.3) in reduced care families; **Fig. 1*A*)**. The largest effect of reduced care was on larval weight at
139 dispersal from the carcass (**Fig. 1*B***). There was significant variation between care treatments ($F_{1,269}$ =
140 433.253, $P < 0.0001$), as well as caretakers nested within treatments ($F_{34,269} = 10.155$, $P < 0.0001$).
141 Controlling for variation amongst families, larvae that received full care weighed 500.4 ± 4.4 mg,
142 whilst larvae that received reduced care weighed 373.4 ± 4.2 mg. Larval weight at dispersal was highly
143 correlated with adult size ($r = 0.91$, $N = 248$).

144 However, even though larvae in the full care treatment developed faster on the carcass, duration
145 of total post-embryonic development (hatch to eclosion) was influenced primarily by larval weight at
146 dispersal ($F_{1,232} = 77.283$, $P < 0.0001$) and not on care treatment ($F_{1,232} = 1.311$, $P = 0.2535$; **Fig. 1*C***).
147 There was significant variation attributable to caretaker ($F_{34,261} = 9.301$, $P < 0.0001$), larval weight
148 ($F_{1,261} = 34.501$, $P < 0.0001$) and duration of parental care received ($F_{1,261} = 13.47$, $P = 0.0003$) on time
149 spent after dispersal to pupation. The same factors influenced duration of pupal development (pupae
150 to adult eclosion) with significant variation arising from larval weight ($F_{1,231} = 55.586$, $P < 0.0001$),
151 caretaker ($F_{34,231} = 3.173$, $P < 0.0001$), but not duration of parental care received ($F_{1,231} = 0.883$, $P =$
152 0.348). Thus, the duration of care received influenced the speed with which the larvae developed on
153 the carcass, but this only shifted development time.

154

155 **Robustness of Parental Care Against Perturbation.**

We next established 89 uniparental broods using the resulting offspring from the reduced and full care treatments (26 full care males, 20 full care females, 24 reduced care males, and 17 reduced care females). There is no evidence that the type of parenting received influenced the likelihood that individuals would provide parental care. Focal male caretakers were more likely to abandon before eggs hatched (reduced care males: 11/24 breeding attempts; full care males: 11/26 breeding attempts) than female caretakers (reduced care females: 2/17 breeding attempts; full care females 2/20 breeding attempts). Once larvae hatched, most focal parents stayed for the entire larval development on the carcass. When caretakers did abandon larvae, it was almost exclusively males (10/29 males, 1/33 females; $\chi^2 = 11.640$, df = 1, P = 0.0012). The one female that abandoned early had received full care; for males, four had received full care and six received reduced care.

Parental care behavior of both males and females was affected by the length of care they received as larvae. There are strong differences between uniparental females and males in this species (**Fig. 2**). Females spent more time on the carcass during the observation period than did males ($F_{1,55}$ = 17.610, P < 0.001; **Fig. 2*A***) regardless of the duration of parental care they received ($F_{1,55}$ = 1.878, P = 0.176) and there was not a significant interaction between the duration of care received and sex of the caregiver on amount of time spent on the carcass ($F_{1,55}$ = 1.332, P = 0.253). Direct care was strongly influenced by the duration of parental care received ($F_{1,55}$ = 6.761, P = 0.012); individuals that received reduced care provided more direct care themselves and this pattern was consistent for both males and females (**Fig. 2*B***); there was no interaction between the sex of the caregiver and the effect of parenting received ($F_{1,55}$ = 0.111, P = 0.741). Females provided more direct care than males under both treatments ($F_{1,55}$ = 12.699, P = 0.0008). In contrast, indirect care was not affected by the duration of parental care received ($F_{1,55}$ = 1.181, P = 0.282; **Fig. 2*C***) or sex of the caregiver ($F_{1,55}$ = 3.072, P = 0.085), and there was no statistically significant interaction between these treatments ($F_{1,55}$ = 0.705, P = 0.405).

180 Overall, the care a parent received as a larva did not result in negative effects on grandoffspring.
181 There was no statistically significant effect of caregiver sex ($F_{1,56}$ = 3.583, P = 0.064) or duration of
182 care received by parent ($F_{1,56}$ = 0.057, P = 0.812) on the length of time larvae spent on the carcass, but
183 there was a significant interaction ($F_{1,56}$ = 5.796, P = 0.019; **Fig. 3*A***). Offspring from females that had
184 received reduced care took longer to develop on the carcass than offspring from females that received
185 full care, but the opposite was true for offspring reared by males. We again included carcass mass as a
186 covariate ($F_{1,56}$ = 1.564, P = 0.216). There was no effect of caregiver sex on average larval mass ($F_{1,56}$
187 = 1.855, P = 0.179), care received by parent ($F_{1,56}$ = 1.340, P = 0.252) or their interaction ($F_{1,56}$ = 0.477,
188 P = 0.493), controlling for carcass mass as a covariate ($F_{1,56}$ = 0.047, P = 0.829; **Fig. 3*B***). Females
189 reared more offspring than did males in general ($F_{1,56}$ = 15.521, P = 0.0002), but there was no effect of
190 parental treatment on the number of offspring reared ($F_{1,56}$ = 0.095, P = 0.759; **Fig. 3*C***) and no
191 interaction between caregiver sex and the duration of parental care received ($F_{1,56}$ = 0.429, P = 0.515).
192 We included carcass mass as a covariate ($F_{1,56}$ = 1.562, P = 0.217), but given the restricted range of
193 masses used we see no effect here or in any of our analyses.
194

195 ## Discussion

196

197 We tested the hypothesis that parental care can act as a buffer because it allows for robustness of post-
198 embryonic development by altering components of care in response to perturbation. We show that the
199 architecture of parental care behaviors of burying beetles translates developmental perturbation into
200 consistent phenotypic outcomes across generations. Experimentally minimizing parental care greatly
201 impacted ecologically relevant offspring traits that are aligned with fitness, including duration of the
202 vulnerable stage on the carcass (**Fig. 1*A***; 21, 29) and body size (**Fig. 1*B***; 30). Parents that received
203 reduced parental care themselves spent more time directly transferring nutrients to offspring, as

204 opposed to parents that received full care, who invested more in indirect mechanisms to enable nutrient
205 acquisition of larvae (i.e., maintenance of a food resource). This reflects that parents differentially
206 express functionally redundant behaviors – i.e., providing self-feeding opportunities to offspring versus
207 direct provisioning – in response to perturbation. This resulted in the negative effects of a poor
208 developmental environment being constrained to a single generation. Together, these data may reflect
209 robustness of parental care to perturbation during development, even when that perturbation arises
210 from variation in the care environment itself, which is not always the case (31-33).

211 Parental care strategies show a bewildering amount of variation across taxa (2-7) and have
212 arisen across a wide diversity of ecological conditions (1, 8, 9). Our results may provide an explanation
213 for why there is so much variation in parental care (e.g., 34) given its importance to offspring
214 development (10). Theory and empirical work indicates that selection for robustness itself can produce
215 unpredictable and sometimes counterintuitive patterns in lower-level units (16), in this case the
216 behaviors composing a complex behavior, as mechanisms that increase robustness against perturbation
217 often trade-off with other types of functions (e.g., replication rate; 20). A "byzantine" complexity of
218 biological systems may be what enables their continued functionality across a wide array of
219 environmental perturbations (35, 36). Moreover, once a robust system has evolved, it may be able to
220 accommodate environmental variation and dampen changes in natural selection (12, 19). Theoretical
221 work on parental care found that stochastic environments are expected to be associated with a range of
222 care strategies (37), which indirectly supports the idea that selection for robustness may increase the
223 complexity and diversity of parental care strategies. *Nicrophorus orbicollis* live in highly heterogenous
224 and variable environments where they are generally competitively superior to other species due to
225 larger body size (21, 38, 39), and parental care is important for body size across *Nicrophorus* spp. (40).
226 This may indicate that the great variation observed both in how individual burying beetles show care
227 (34, 41) and who shows care (e.g., uniparental to biparental to communal; 4, 5, 42) may emerge from
228 selection for consistency of offspring phenotypes relative to the prevailing environmental context (38),

229 which may require variation in the form and frequency of components of parental care (**Fig. 2**).
230 Selection experiments for larger offspring across a diversity of perturbations may shed light on whether
231 selection on the robustness of offspring post-embryonic development influences the structure of
232 parental care.

233 Robustness is often misunderstood to reflect a lack of change in response to perturbation, but
234 robustness refers to consistency of function, which often requires flexibility or change at the level of
235 interacting units (13). Systems spend more time in robust states because, over time, fragile systems do
236 not last (12). Biology is hierarchical by nature. For this reason, robustness at one level of level of
237 organization, e.g., the outcome of parental care, does not imply a lack of change at other levels, e.g.,
238 the expression of component parental care behaviors. That parental care has evolved independently
239 multiple times across taxa (e.g., 7, 43) may indicate that it appears because parental care strategies
240 confer robustness, or, diverse taxa end up repeatedly evolving parental care strategies because these
241 are the most stable mechanisms for ensuring appropriate offspring development. A robustness
242 perspective further reconciles why there is such a great diversity of life history, ecological, and social
243 contexts associated with the evolution of parenting (1, 7, 37, 44). Future work could disentangle
244 whether robustness of parental care in burying beetles can also ensure functional offspring outcomes
245 under a suite of both historical and novel perturbations.

246 Organisms both construct and evolve in response to their experience of the environment (45).
247 Robust systems are expected to emerge over evolutionary time, and the architecture of such systems
248 are often enabled by modularity and functional redundancy (12, 13). We found that removing parental
249 care induced strong changes in ecologically relevant traits. Offspring that received poor parenting
250 responded by increasing uniparental care when they became parents and shifting the type of nutrient-
251 transfer behavior they showed, resulting in the amelioration of perturbation during the development of
252 parents. In conclusion, parenting may be an effective evolutionary mechanism for buffering poor past
253 and present environments, including those imposed during the parents' development. This robustness

may be enabled by dynamic changes in the units of parental care – the component behaviors themselves.

Materials and Methods

Insect colony and husbandry.

We used *Nicrophorus orbicollis* from an outbred colony maintained at the University of Georgia that is regularly supplemented with wild-caught beetles from Whitehall Forest in Athens, GA, USA. All beetles were maintained in an incubator (Percival, model no. I41VLC8, Perry, IA) at 22 ± 0.1°C on a 14:10 hour light:dark cycle that simulated summer breeding conditions. We bred all individuals used here in the laboratory to ensure that parentage, age, and to standardize rearing conditions. We housed beetles individually at larval dispersal in 9-cm-diameter by 4-cm-deep circular compostable deli containers (EP-RDP5, Eco-Products) filled with ~2.5 cm of potting soil (Performance Organics All Purpose In-Ground Soil, Miracle-Gro). Larvae had no further social interactions with other burying beetles until they were randomly allocated into treatments. We defined individuals as age Day 1 on the day of eclosion. Beetles were fed *ad libitum* organic ground beef twice a week following adult eclosion.

Effect of Perturbation of Care Environment on Development.

We manipulated the duration of parental care to determine the effect of parental care on offspring traits related to fitness and their expression of care once they attained adulthood. We randomly mated focal virgins at Day 13-19 by placing an unrelated male and female in a small plastic container and observed them to ensure that mating occurred. The pair was then moved to a plastic breeding box filled with

278 approximately two cm of potting soil moistened with distilled water and containing a freshly thawed
279 mouse 20.8 ± 0.6 g SD (max = 22.2, min = 20.0; Rodent Pro, Inglefield, IN, U.S.A.). There was no
280 difference in the size of the mouse provided between treatments (20.9 g in full care treatment, 20.8 g
281 in reduced care treatment; $F_{1,34} = 0.153$, $P = 0.698$). We then returned breeding boxes to the incubator
282 and kept them on a darkened shelf to simulate underground breeding conditions. We checked breeding
283 boxes for eggs every twenty-four hours. Once eggs we observed eggs, we moved the female and brood
284 ball to a new breeding box filled with approximately two cm of moistened potting soil, while the male
285 was removed. We left eggs in the original breeding box with a small piece of ground beef. Once 90
286 hours had elapsed since egg laying, we checked the breeding box containing the eggs every hour to
287 determine when larvae had hatched and crawled to the beef. After we observed the focal females'
288 larvae hatch, we provided the focal female with an unrelated, mixed brood of 10 larvae. Burying beetles
289 identify their offspring temporally and eat any larvae that arrive too early on the carcass (46), and thus
290 we only provided females with larvae after her own larvae had hatched. For the full duration parental
291 care treatment, we allowed females to associate with larvae until at least 2 of the larvae had dispersed
292 from the breeding carcass (~7 - 8 days). For the reduced duration parental care treatment, we allowed
293 females to associate with larvae for 6 hours and then the female was removed. We checked
294 experimental treatments daily to determine larval dispersal date, subsequently, larval survival.

295 We measured several offspring traits related to fitness (47), including mass at dispersal,
296 duration of developmental periods, and adult size. We measured larval mass at dispersal to the nearest
297 0.1 mg. We also measured the duration of development on the carcass (from hatch until dispersal),
298 which when parental care and all larval growth occurs; the number of days of development from larval
299 dispersal to pupation; the number of days as pupae (from pupation to adult eclosion), and total duration
300 of development (from larval hatch until adult eclosion). When the larvae left the carcass (dispersal),
301 we placed larvae into individual containers with ~2 cm of moistened soil. We checked treatments daily
302 to determine the number of surviving larvae, length of larval period, pupation date, and adult ecolsion

303 date. We measured the pronotum length of full and reduced care offspring on Day 14 – 16 post-eclosion
304 with calipers to the nearest 0.01 mm.
305
306 **Robustness of Care Against Perturbation.**
307
308 At Day 14 – 16 following eclosion, adult beetles that received full or reduced care as offspring were
309 provided with an unrelated, virgin mate from our stock colony and a 19.6 - 21.2 g thawed mouse carcass
310 (mean = 20.6 g). These were placed into breeding container with ~2 cm of moistened soil. As variation
311 of parental care may manifest as variation of investment across the breeding cycle, breeding boxes
312 were fitted with an escape chamber to allow focal beetles to desert the breeding attempt. Breeding
313 boxes were checked daily for eggs. Beetles that escaped into the escape chamber prior to egg laying
314 were replaced in the breeding box. Beetles that escaped after egg-laying and remained in the escape
315 chamber for over 15 minutes were counted as deserters and removed. Once larvae were observed, the
316 non-focal partner was removed, and the focal male or female was left in a uniparental context to care
317 for the offspring. If the focal beetle deserted the breeding attempt prior to larval hatch, no further
318 monitoring was conducted on that breeding attempt.
319 As rates of parental provisioning may be affected by the number of larvae (48), we measured
320 parental care using both behavioral measures and larval traits associated with fitness. Behavioral
321 observations were conducted twenty-four hours after larva were first observed on the carcass for each
322 focal individual to quantify parental care. We moved breeding boxes into an observation room, and we
323 gave the focal beetle at least 30 minutes to acclimate prior to observation. All observations were
324 conducted under red lights to ensure no light effects on individual care behavior, as *N. orbicollis* are
325 nocturnal (49). In each behavioral observation, we recorded both direct and indirect parental care
326 behaviors (50) for 15 minutes. Direct care is defined as when the male or female fed larvae. Indirect
327 care is defined as activities related to carcass cleaning and maintenance, which allows for the

328 preservation of the carcass for future offspring provisioning and thus represents a type of parental care
329 without direct interactions with offspring. We also recorded time spent showing "no care", defined as
330 when the parent was on the carcass but not engaged in direct or indirect care, or the individual was off
331 the carcass. Behaviors were recorded continuously as both a duration and frequency (e.g., number of
332 times an adult fed larvae). This gave each individual three scores (direct care, indirect care, and no
333 care) which together summed to 15 minutes.

334 We measured offspring traits related to fitness reared by males or females that had received full
335 or reduced care as larvae themselves. We did not manipulate the number of offspring but instead
336 allowed parents to cull the number without interference. Of the remaining 61 uniparental broods, we
337 measured the number of offspring reared, the mass of the offspring at dispersal, and the amount of time
338 larvae spent on the carcass from hatching to dispersal. Measurements of development times and larval
339 mass were the same as used in the experimental manipulation of the duration of parental care (see
340 previous section).

341

342 **Statistical Analyses.**

343

344 We performed all statistical analyses using JMP Pro (v. 16.0.0, http://jmp.com) and produced figures
345 in SigmaPlot (v. 14.5, http://www.sigmaplot.co.uk). Results are means ± SE unless otherwise noted.
346 Effects on continuous offspring traits were analyzed with nested ANOVA, with family nested within
347 treatment. We analyzed categorical data with Pearson's Chi-square goodness-of-fit test. We analyzed
348 parental care behaviors and larval traits of grand offspring with two-way ANOVA with caretaker sex
349 and parenting experienced as factors.

350

351 **Acknowledgements and Funding Sources**

We would also like to thank Kathryn Kollars for assistance constructing escape chambers and for developing the beetle graphics used in this study. We would also like to thank Patricia Moore and Hans Otto for discussions and/or feedback on the manuscript, and Emily Shelby for assistance with figures. ALP and AJM conceived of study and developed methods. ALP, AJM, and CBC collected life history data. ALP conducted behavioural observations. AJM performed statistical analyses. ALP and AJM created figures. ALP, AJM, and CBC all contributed to writing and editing the manuscript. ALP was funded by a USDA cooperative agreement to AJM.

References

1. E. O. Wilson, *Sociobiology: the New Synthesis* (Harvard University Press, Cambridge, 1975).
2. A. L. Potticary, R. A. Duckworth, A neuroendocrine perspective on the origin and evolution of cooperative breeding. *Ornithology* **138** (2021).
3. H. J. Brockmann, "Cooperative breeding in wasps and vertebrates: the role of ecological constraints." in The Evolution of Social Behavior in Insects and Arachnids, J. C. Choe, B. J. Crespi, Eds. (Cambridge University Press, Cambridge, 1997), pp. 347-371.
4. M. P. Scott, The ecology and behavior of burying beetles. *Ann. Rev. Entomol.* **43**, 595-618 (1998).
5. K. M. Benowitz, E. C. McKinney, A. J. Moore, Difference in parenting in two species of burying beetle, Nicrophorus orbicollis and Nicrophorus vespilloides. *J. Ethol.* **34**, 315-319 (2016).
6. A. Cockburn, Prevalence of different modes of parental care in birds. *Proc. Biol. Sci.* **273**, 1375-1383 (2006).
7. J. E. Mank, D. E. L. Promislow, J. C. Avise, Phylogenetic perspectives in the evolution of parental care in ray-finned fishes *Evolution* **59**, 1570-1578 (2005).
8. W. Jetz, D. R. Rubenstein, Environmental uncertainty and the global biogeography of cooperative breeding in birds. *Curr. Biol.* **21**, 72-78 (2011).
9. S. T. Emlen, The evolution of helping. 1. An ecological constraints model *Am. Nat.* **119**, 29-39 (1982).
10. N. J. Royle, S. H. Alonzo, A. J. Moore, Co-evolution, conflict and complexity: what have we learned about the evolution of parental care behaviours? *Curr. Op. Behav. Sci.* **12**, 30-36 (2016).
11. T. Székely, V. Remeš, R. P. Freckleton, A. Liker, Why care? Inferring the evolution of complex social behaviour. *J. Evol. Biol.* **26**, 1381-1391 (2013).
12. A. Wagner, Robustness, evolvability, and neutrality. *FEBS Lett.* **579**, 1772-1778 (2005).
13. H. Kitano, Biological robustness. *Nature Rev. Gen.* **5**, 826-837 (2004).
14. A. R. Ives, S. R. Carpenter, Stability and diversity of ecosystems. *Science* **317**, 58-62 (2007).

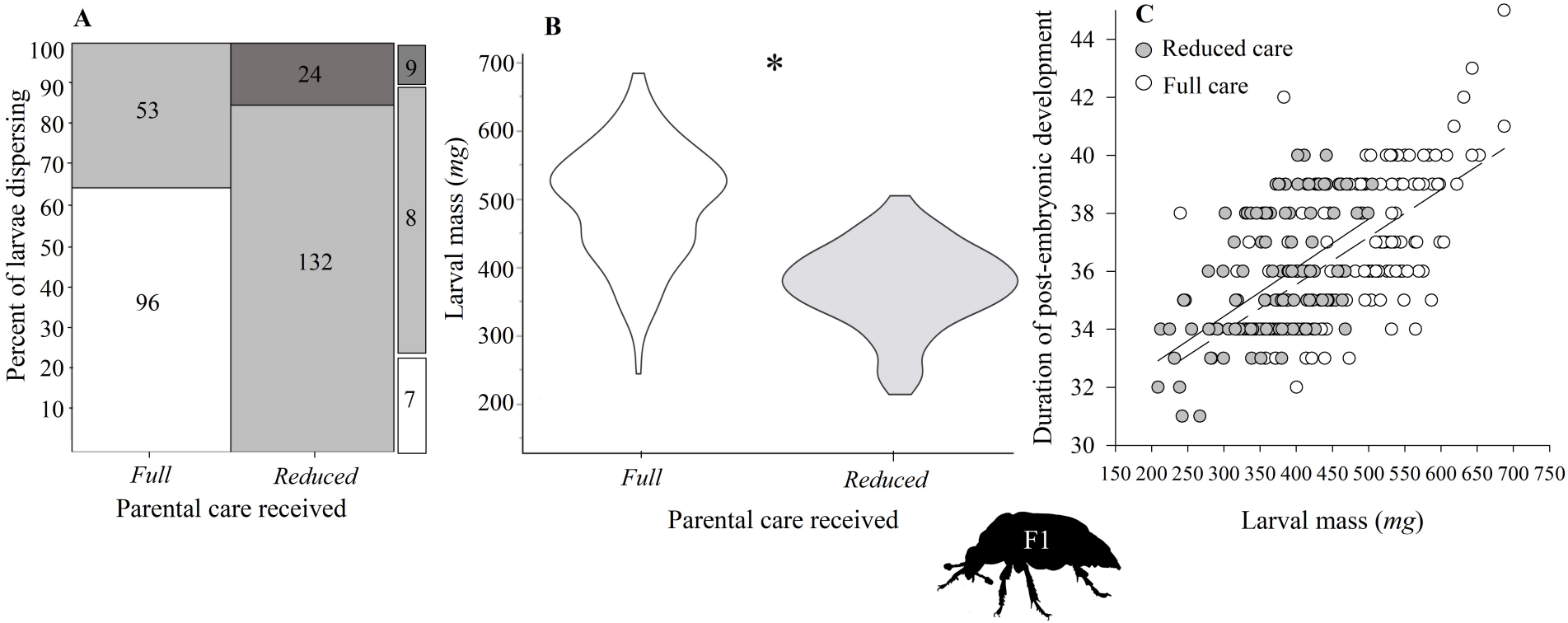
15. K. H. ten Tusscher, P. Hogeweg, Evolution of networks for body plan patterning; interplay of modularity, robustness and evolvability. *PLoS Comp. Biol.* **7**, e1002208 (2011).
16. A. Thompson, P. Layzell, R. S. Zebulum, Explorations in design space: unconventional electronics design through artificial evolution. *IEEE Trans. Evol. Comput.* **3**, 167-196 (1999).
17. P. Holme, Metabolic robustness and network modularity: a model study. *PloS One* **6**, e16605 (2011).
18. K. Chenard, R. Duckworth, "The special case of behavioral plasticity?" in *Phenotypic Plasticity & Evolution: Causes, Consequences, and Controversies,* D. W. Pfennig, Ed. (CRC Press, Boca Raton, 2021), 10.1201/9780429343001-16, pp. 301-325.
19. A. L. Potticary, R. A. Duckworth, Multiple environmental stressors induce an adaptive maternal effect. *Am. Nat.* **196**, 487-500 (2020).
20. C. O. Wilke, J. L. Wang, C. Ofria, R. E. Lenski, C. Adami, Evolution of digital organisms at high mutation rates leads to survival of the flattest. *Nature* **412**, 331-333 (2001).
21. I. C. Robertson, Nest intrusions, infanticide, and parental care in the burying beetle, *Nicrophorus orbicollis* (Coleoptera: Silphidae). *J. Zool.* **231**, 583-593 (1993).
22. S. P. Shukla *et al.*, Microbiome-assisted carrion preservation aids larval development in a burying beetle. *Proc. Natl. Acad. Sci.* **115**, 11274-11279 (2018).
23. A. Capodeanu-Nägler *et al.*, Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles. *Front. Zool.* **15**, 33 (2018).
24. S. T. Trumbo, D. S. Sikes, Resource concealment and the evolution of parental care in burying beetles. *J. Zool.* **315**, 175-182 (2021).
25. W. Haberer, S. Steiger, J. K. Müller, Dynamic changes in volatile emissions of breeding burying beetles. *Physiol. Entomol.* **39**, 153-164 (2014).
26. A. N. Arce, P. R. Johnston, P. T. Smiseth, D. E. Rozen, Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *J. Evol. Biol.* **25**, 930-937 (2012).
27. A. Duarte, D. Rebar, A. C. Hallett, B. J. M. Jarrett, R. M. Kilner, Evolutionary change in the construction of the nursery environment when parents are prevented from caring for their young directly. *Proc. Natl. Acad. Sci.* **118**, e2102450118 (2021).
28. A.-K. Eggert, J. K. Müller, "Biparental care and social evolution in burying beetles: lessons from the larder" in *The Evolution of Social Behaviour in Insects and Arachnids,* J. C. Choe, B. J. Crespi, Eds. (Cambridge University Press, Cambridge, 1997), pp. 216.
29. A.-K. Eggert, M. Reinking, J. K. Müller, Parental care improves offspring survival and growth in burying beetles. *Anim. Behav.* **55**, 97-107 (1998).
30. M. Otronen, The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). *Ann. Zool. Fenn.* **25**, 191-201 (1988).
31. F. A. Champagne, Epigenetic mechanisms and the transgenerational effects of maternal care. *Front. Neuroendocrinol.* **29**, 386-397 (2008).
32. M. J. Meaney, Maternal care, gene expression, and the transmission of Individual differences in stress reactivity across generations. *Ann. Rev. Neurosci.* **24**, 1161-1192 (2001).
33. R. M. Kilner *et al.*, Parental effects alter the adaptive value of an adult behavioural trait. *eLife* **4** (2015).
34. I. Meierhofer, H. H. Schwarz, J. K. Müller, Seasonal variation in parental care, offspring development, and reproductive success in the burying beetle, *Nicrophorus vespillo*. *Ecol. Entomol.* **24**, 73-79 (1999).
35. A. Wagner, *Robustness and Evolvability in Living Systems* (Princeton University Press, Princeton, 2013), vol. 24.
36. J. M. Carlson, J. Doyle, Complexity and robustness. *Proc. Natl. Acad. Sci.* **99 Suppl 1**, 2538-2545 (2002).

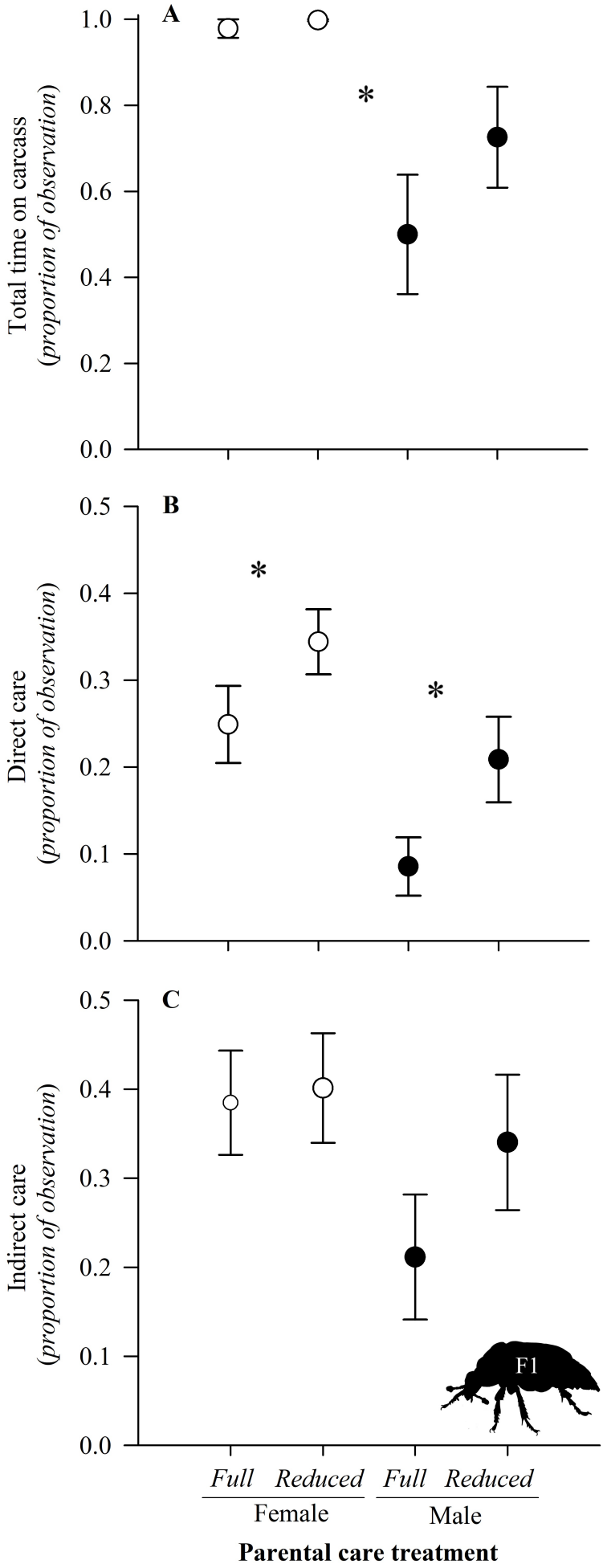
37. M. B. Bonsall, H. Klug, The evolution of parental care in stochastic environments. *J. Evol. Biol.* **24**, 645-655 (2011).
38. J. C. Creighton, Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behav. Ecol.* **16**, 1031-1036 (2005).
39. S. D. Schrempf, K. W. Burke, J. D. Wettlaufer, P. R. Martin, Behavioral dominance interactions between *Nicrophorus orbicollis* and *N. tomentosus* burying beetles (Coleoptera: Silphidae). *PeerJ* **9**, e10797 (2021).
40. B. J. M. Jarrett, M. Schrader, D. Rebar, T. M. Houslay, R. M. Kilner, Cooperative interactions within the family enhance the capacity for evolutionary change in body size. *Nature Ecol. Evol.* **1** (2017).
41. M. P. Scott, Facultative adjustment of the duration of parental care by burying beetles. *J. Insect Behav.* **11**, 597-603 (1998).
42. S. Trumbo, Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (*Nicrophorus*). *Ecol. Entomol.* **17**, 289-298 (1992).
43. N. Royle, P. Smiseth, M. Kölliker, *The Evolution of Parental Care* (Oxford University Press, Oxford, 2012), 10.1093/acprof:oso/9780199692576.003.0001.
44. J. W. Y. Wong, J. Meunier, M. Kollicker, The evolution of parental care in insects: the roles of ecology, life history and the social environment. *Ecol. Entomol.* **38**, 123-137 (2013).
45. R. C. Lewontin, "Gene, organism, and environment" in *Evolution from Molecules to Men,* D. S. Bendall, Ed. (Cambridge University Press, Cambridge, 1983).
46. J. A. Oldekop, P. T. Smiseth, H. D. Piggins, A. J. Moore, Adaptive switch from infanticide to parental care: How do beetles time their behaviour? *J. Evol. Biol.* **20**, 1998-2004 (2007).
47. J. E. Lock, P. T. Smiseth, A. J. Moore, Selection, inheritance, and the evolution of parent-offspring interactions. *Am. Nat.* **164**, 13-24 (2004).
48. C. M. Rauter, A. J. Moore, Time constraints and trade-offs among parental care behaviours: effects of brood size, sex and loss of mate. *Anim. Behav.* **68**, 695-702 (2004).
49. D. S. Wilson, W. Knollenberg, J. Fudge, Species packing and temperature dependent competition among burying beetles (Silphidae, *Nicrophorus*). *Ecol. Entomol.* **9**, 205-216 (1984).
50. C. A. Walling, C. E. Stamper, P. T. Smiseth, A. J. Moore, The quantitative genetics of sex differences in parenting. *Proc. Natl. Acad. Sci.*, 18430-18435 (2008).

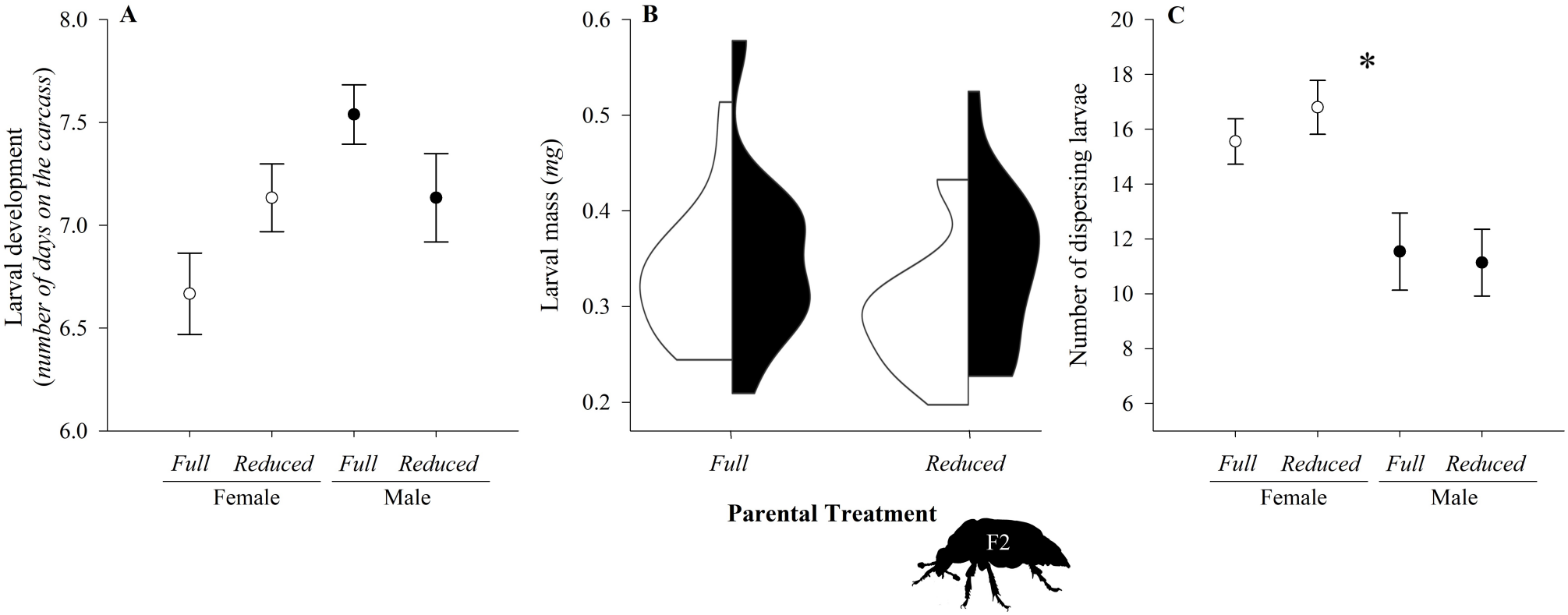
Figure legends

Figure 1. Care influences larval developmental time and size. ***(A)*** Mosaic plot indicating the percent of larvae dispersing from the carcass after 7, 8, or 9 days (white, light grey, or dark grey, respectively). Larvae that received full care developed faster on the carcass than larvae receiving reduced care. Sample sizes noted within mosaic plot. ***(B)*** Violin plot of distribution of final larval weight between individuals that received the full or reduced care treatment (Full care: N = 149, Reduced care: N = 156). Asterisks indicate statistically significant results of contrasts (with $P < 0.05$). ***(C)*** Duration of

477 larval period (in days) from larval hatch until ecolsion, or emergence of adult beetles, was statistically
478 dependent on larval weight at dispersal and not on care treatment (N = 233). Grey circles and solid line
479 refer to larvae that received reduced care, while open circles and the dashed line refer to individuals
480 that received full care.
481
482 **Figure 2. Parental behavior of females and males affected by the care they received as larvae**. ***(A)***
483 Females spent more time on the carcass during the observation period than did males regardless of how
484 much care they received. In addition, ***(B)*** both females and males that experienced reduced care
485 increased the amount of direct care given to their offspring but ***(C)*** not indirect care. Error bars are
486 mean ± SEM. N = 15 observations for all treatments except Full care males, where N = 14 observations.
487 Asterisks indicate statistically significant results of contrasts (with $P < 0.05$).
488
489 **Figure 3. Sex of parent influences offspring outcomes but not developmental perturbation.** (***A***)
490 There was no statistically significant effect of caregiver sex or duration of care received by parent on
491 the length of time larvae spent on the carcass, but there was a significant interaction. ***(B)*** There was no
492 statistically significant effect of caregiver sex, the care they received as a larva, or their interaction, on
493 larval mass. ***(C)*** Females reared more offspring than did males, but there was no effect of parental
494 treatment on the number of offspring reared. Namely, the care a parent received as a larva did not result
495 in negative effects on grandoffspring. Full care males: N = 13 breeding attempts, Full care females: N
496 = 18, and N = 15 for both Reduced care males and females. Female data are indicated with open circles
497 or violin plots, while male data are closed circles or violin plots. Error bars are ± SEM. Asterisks
498 indicate statistically significant results of contrasts (with $P < 0.05$).
499


A
Percent of larvae dispersing
100
90
80
70
60
50
40
30
20
10
53
96
24
132
9
8
7
Full
Reduced
Parental care received
B
Larval mass (mg)
700
600
500
400
300
200
*
Full
Reduced
Parental care received
C
Reduced care
Full care
Duration of post-embryonic development
44
42
40
38
36
34
32
30
150 200 250 300 350 400 450 500 550 600 650 700 750
Larval mass (mg)
F1


A
Total time on carcass
(proportion of observation)
1.0
0.8
0.6
0.4
0.2
0.0
*
B
Direct care
(proportion of observation)
0.5
0.4
0.3
0.2
0.1
0.0
*
*
C
Indirect care
(proportion of observation)
0.5
0.4
0.3
0.2
0.1
0.0
F1
Full
Reduced
Full
Reduced
Female
Male
Parental care treatment


A
Larval development
(number of days on the carcass)
8.0
7.5
7.0
6.5
6.0
Full
Reduced
Full
Reduced
Female
Male
B
Larval mass (mg)
0.6
0.5
0.4
0.3
0.2
Full
Reduced
C
Number of dispersing larvae
20
18
16
14
12
10
8
6
*
Full
Reduced
Full
Reduced
Female
Male
Parental Treatment
F2