

1 Maternal and larval niche construction interact to shape
2 development, survival, and population divergence in
3 the dung beetle *Onthophagus taurus*

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15

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17

18 **ABSTRACT**

19 Through niche construction, organisms modify their environments in ways that can alter how
20 selection acts on themselves and their offspring. However, the role of niche construction in shaping
21 developmental and evolutionary trajectories, and its importance for population divergences and
22 local adaptation, remains largely unclear. In this study, we manipulated both maternal and larval
23 niche construction and measured the effects on fitness-relevant traits in two rapidly diverging
24 populations of the bull-headed dung beetle, *Onthophagus taurus*. We find that both types of niche
25 construction enhance adult size, peak larval mass, and pupal mass, which when compromised lead
26 to a synergistic decrease in survival. Furthermore, for one measure, duration of larval development,
27 we find that the two populations have diverged in their reliance on niche construction: larval niche
28 construction appears to buffer against compromised maternal niche construction only in beetles
29 from Western Australia, but not in beetles from the Eastern United States. We discuss our results
30 in the context of rapid adaptation to novel conditions and the role of niche construction therein.

31

32 **INTRODUCTION**

33 Understanding the mechanisms that facilitate persistence and local adaptation following
34 colonization of novel habitats is a fundamental objective of evolutionary ecology and conservation
35 biology. Recent work has begun to emphasize the potential significance of niche construction to
36 this process, which occurs when organisms modify their environments in ways that alter the
37 selective conditions that they or their descendants experience (Laland et al., 2016; Matthews et al.,
38 2014; Odling-Smee et al., 2003). Niche construction may result from modifications of biotic and
39 abiotic conditions, can be expressed through behavioral, physiological, and developmental
40 processes, and can facilitate the inheritance of environmental states, (e.g., though the transmission

41 of habitat conditions or symbionts). For example, when adult *Drosophila* colonize new food
42 sources on which to feed and lay their eggs, the flies also vector yeasts (Begon, 1982). The larval
43 generation then modifies the microbiotic conditions of the food source, dramatically affecting
44 yeast densities and species composition, and thereby creating a predictable microbial environment
45 conducive to larval development (Good and Tatar, 2001; Stamps et al., 2012). Furthermore, this
46 microbial environment can be shaped by the niche constructing activities of the yeast themselves:
47 by preferentially engaging in fermentation despite the presence of oxygen, yeasts produce ethanol
48 and heat, allowing them to defend sugar resources by generating an environment that is both toxic
49 and too hot for many of their interspecific competitors (Goddard, 2008; Pfeiffer and Morley, 2014).
50 Such niche construction is therefore of interest to evolutionary biologists because it offers
51 additional sources of phenotypic variation and alternate routes to adaptation. Moreover, niche
52 construction may contribute avenues for non-genetic inheritance in taxa in which modified
53 environments are passed on to subsequent generations. Lastly, evolution in response to heritable
54 environments is also thought to be faster than evolution to abiotic non-heritable environments
55 (Drown and Wade, 2014). These same properties are also integral, though not as emphasized, in
56 the overlapping frameworks of eco-evolutionary feedbacks (Hendry, 2016) and extended
57 phenotypes (Dawkins, 1982).

58 Both the ubiquity and ecological relevance of niche construction are increasingly well
59 established (Bateson and Gluckman, 2011; Laland et al., 2017, 2016; Sultan, 2015). Similarly,
60 explicit tests of niche construction and its effects on fitness-relevant traits are accumulating for
61 diverse organisms and types of niche construction, and illustrate that niche construction is
62 frequently adaptive, critical for normative development, and able to diverge among closely related
63 species (e.g., arthropods: Bailey et al., 2009; Saltz and Foley, 2011; Schwab et al., 2017;

64 flatworms: Majdi et al., 2014; Wilden et al., 2019; plant-soil systems: Schweitzer et al., 2014;
65 yeast: Goddard, 2008). Yet, whether niche construction also contributes to local adaptation among
66 populations and in the face of novel or challenging environmental conditions is poorly understood.
67 Moreover, many organisms engage in niche construction through diverse routes, yet the
68 consequences of potential interactions among multiple forms of niche construction are essentially
69 unexplored. Here, we investigate the relative contributions of, and interactions between, two forms
70 of niche construction in the development and survival of *Onthophagus taurus* (Schreber, 1759)
71 dung beetles from two rapidly diverging populations.

72 *Onthophagus* dung beetles are promising organisms to investigate the developmental,
73 ecological, and evolutionary consequences of niche construction because individuals modify their
74 environment during key phases of their life cycle and in ways that could impact both their own
75 fitness and that of their descendants. For example, adult female *Onthophagus* dig tunnels
76 underneath dung pats, and provision them with accumulations of dung (i.e., brood balls) that
77 comprise all the food available for larvae to complete development. Furthermore, within each
78 brood ball, mothers provide their offspring with a maternal fecal pellet called a *pedestal* onto which
79 they lay a single egg (Estes et al., 2013; Parker et al., 2019). Upon hatching, the larva consumes
80 the pedestal, thereby obtaining maternal gut microbiota crucial for normal development (Schwab
81 et al., 2016, 2017). Lastly, the depth at which mothers bury brood balls affects thermal conditions
82 experienced by larvae: deeper brood balls ensure a more constant thermal environment, which
83 enhances larval growth (Snell-Rood et al., 2016). Mothers therefore construct important features
84 of the developmental niche of their larval offspring.

85 Importantly, larvae engage in significant environment-modifying behaviors of their own.
86 For example, throughout development larvae not only feed upon their brood ball, but also defecate

87 within it, working their feces into the brood ball's remainder, then eat the resulting mix anew.
88 Furthermore, recent work has documented diverse developmental and fitness consequences of
89 larval niche construction across *Onthophagus* species , and proposed that larval modifications to
90 the brood ball, in particular those involving larval fecal matter, help establish an external rumen
91 (Schwab et al., 2017), as has been documented in other species (Costa, 2006; Swift et al., 1979;
92 Thompson et al., 2013). Specifically, once established, this external rumen may pre-digest dung
93 through the help of larval microbiota prior to ingestion, or re-ingestion, by larvae. In support of
94 this hypothesis, the microbiota derived from artificial brood balls modified by larvae could digest
95 a wider range of potential carbon sources and did so more substantially than microbiota derived
96 from unmodified artificial brood balls (Schwab et al., 2017). Evolutionary changes in these
97 environment-modifying behaviors of larvae and their mothers could provide alternative routes to
98 adaptation.

99 Lastly, many *Onthophagus* species have been introduced deliberately or accidentally to
100 novel habitats, offering opportunities to investigate the role of niche construction in the
101 colonization of and adaptation to novel ecological conditions. Here, we focus on *O. taurus*, a
102 species native to the Palearctic (Ziani et al., 2015). In the 1970s, this species was deliberately
103 introduced from the Mediterranean region to Western Australia (WA), and introduced accidentally
104 to the Eastern U.S.A. (EUS; Hoebeke and Beucke, 1997; Tyndale-Biscoe, 1996). Since their
105 establishment in both exotic locations, *O. taurus* populations have diverged heritably in diverse
106 traits (e.g., genital morphology: Macagno et al., 2011, fecundity: Macagno et al., 2015; hormone
107 physiology: Moczek and Nijhout, 2002; allometry: Moczek et al., 2002), including traits relevant
108 to niche construction: WA females dig shallower breeding tunnels than EUS females, thereby
109 exposing their offspring to higher and less stable temperatures (Macagno et al., 2016).

110 Interestingly, larvae from WA but not EUS use developmental plasticity to adaptively respond to
111 stressfully high temperatures (Macagno et al., 2018).

112 In this study, we took advantage of the presence and ease of manipulation of both larval
113 and maternal niche construction alongside the existence of rapidly diverging exotic *O. taurus*
114 populations to determine whether (i) larval and maternal niche construction interact, and (ii) EUS
115 and WA populations have diverged in terms of their reliance on either one or both types of niche
116 construction. Using a full factorial design, both types (maternal and larval) of niche construction
117 were manipulated simultaneously. We manipulated maternal niche construction by exposing
118 larvae to constant or variable temperatures, thereby simulating deep or shallow brood ball burial
119 by mothers (as used previously in Snell-Rood et al., 2016). We manipulated larval niche
120 construction by either allowing or preventing larvae from accruing the benefits of their brood ball
121 modifications (as used previously in Schwab et al., 2017). We predicted that the two populations
122 would differ in the degree to which offspring fitness is influenced by larval and maternal niche
123 construction. Specifically, because maternal niche construction (i.e., deep brood ball burial) is
124 more prevalent among EUS beetles, we predicted that EUS offspring would suffer more (i.e., grow
125 less and slower) from the experimental reduction of maternal niche construction than their WA
126 counterparts. Second, because larvae from WA may have experienced a longer history of exposure
127 to highly fluctuating thermal conditions, this population may have experienced selection for
128 genotypes better able to tolerate reduced investment in maternal niche construction, potentially by
129 increasing its reliance on larval niche construction. We therefore predicted that WA offspring may
130 be able to better cope with fluctuating temperatures than their EUS counterparts, except when
131 larval niche construction is experimentally reduced. Our results provide partial support for these
132 predictions.

134 **MATERIALS AND METHODS**

135 *Beetle provenance and husbandry*

136 Adult *Onthophagus taurus* were collected from two exotic ranges: Eastern United States (EUS)
137 individuals were collected near Chapel Hill, North Carolina, U.S.A (35°54'47.3"N, 79°3'20.9"W),
138 whereas individuals from Western Australia (WA) were collected near Busselton (33°38'52"S,
139 115°20'45"E) and Serpentine (32°21'54"S, 115°58'51.6"E). These beetles, and their offspring,
140 were used to establish laboratory colonies, maintained at 25°C in a sand/soil mixture at a 16 h:8 h
141 light:dark cycle, and fed homogenized organic cow manure twice a week (as described in Moczek,
142 2006). In nature WA populations are generally active from December to February, while EUS
143 populations are active from approximately late May to August (Moczek, 2003). To execute our
144 experiment at the same time for both populations we therefore used wild-caught EUS but lab-
145 reared WA (F₁ and F₂) starter populations to rear larvae for our experiment, allowing us to
146 experiment on both populations simultaneously. We opted for this approach rather than the
147 alternative option of rearing the two populations separately in order to avoid introducing the
148 confounding effects of timing and seasonal variation that are beyond our control. To rear larvae
149 for experimentation, adult beetles were haphazardly selected from their respective colonies and
150 placed into separate plastic breeding containers (26 cm tall × 20 cm diameter) filled ~75% with a
151 packed moist sand and soil mixture, provided with ~0.5 L cow manure, and allowed to breed and
152 produce brood balls for five days (as described in Moczek and Nagy, 2005). A total of 85 breeding
153 containers (30 WA, 55 EUS) were set up between May and December 2017, generating 895 larvae
154 used in this experiment. Sampling intentions and sample sizes for each metric can be found in the
155 supplementary materials (Tables S1–S2).

156 To standardize larval age, we only used brood balls that contained an egg upon inspection.
157 Approximately nine hundred brood balls in total were used for the experiment, which were
158 inspected daily until each egg was observed to have hatched. Freshly hatched larvae were left in
159 their native brood ball for 24–48 hrs before transfer to an artificial brood ball, as explained below.
160 This allowed larvae to consume the pedestal made of maternal excrement that serves as the main
161 conduit for maternal to larval microbiome transmission (Estes et al., 2013).

162

163 *Rearing of experimental animals*

164 Larvae for all treatments were transferred into 12-well tissue culture plates containing standardized
165 artificial brood balls (ABBs) following Shafiei et al. (2001). To standardize the quality of ABBs,
166 the dung used to generate them was collected from a single organic farm (Marble Hill Farm,
167 Bloomington, Indiana, U.S.A., 39°3'8 N, 86°36'12" W), homogenized on-site, frozen, thawed
168 and homogenized again in two sets, thereby ensuring that dung used in the experiment was of
169 equivalent quality both within and among treatment groups.

170

171 *Manipulation of niche construction*

172 To assess the respective roles of maternal and larval niche construction in *Onthophagus*
173 development, we experimentally simulated different levels of maternal investment by imposing
174 alternate temperature regimes on developing larvae, while simultaneously manipulating the ability
175 for larvae to modify their own brood ball environment. To assess possible interactions between
176 both forms of niche construction on larval performance, we executed this experiment in a fully
177 factorial manner. Lastly, to assess the potential contributions of each form of niche construction
178 and their interactions to population divergence, we replicated this effort across both EUS and WA

179 populations. Experimental manipulations of maternal and larval niche construction followed
180 previously established protocols (Schwab et al., 2016, 2017; Snell-Rood et al., 2016) as detailed
181 next.

182 High maternal investment in burial depth reduces thermal fluctuations and thus
183 developmental stress experienced by larvae (Snell-Rood et al., 2016), an interaction that can be
184 studied in the laboratory by rearing larvae at controlled constant or fluctuating temperatures
185 (Schwab et al., 2016, 2017; Snell-Rood et al., 2016). We replicated this relationship between
186 maternal niche construction and larval development by rearing larvae at one of two temperature
187 regimes. First, to simulate high levels of maternal investment, we reared larvae at constant 25°C
188 with a 12 h:12 h light:dark cycle in a Precision® Low Temperature Incubator model 815.
189 Alternatively, to simulate low maternal investment in burial depth, a subset of larvae was reared
190 in a fluctuating thermal regime of 12 h at 31°C in the light and 12 h at 19°C in the dark, as
191 described in Snell-Rood et al. (2016), in a Precision® Dual Programmed Illuminator Incubator
192 model 818. Temperature inside incubators was monitored using thermometers and Thermochron
193 iButton temperature loggers. The incubator required approximately 1 h 25 m to cool from 31°C to
194 19°C, and 1 h 40 m to warm from 19°C to 31°C. This range in temperatures was based on
195 temperatures measured in the field underneath dung pads using the same Thermochron iButton
196 loggers (Maxim Integrated, San Jose, CA) buried at 0 cm, 15 cm, or 30 cm depth, reflecting the
197 extremes of variation in beetle burial depth in Indiana (as determined by Snell-Rood et al., 2016).
198 Despite the major temperature fluctuations imposed on the second treatment group, the mean
199 temperature experienced by both groups was 25°C.

200 Manipulating *larval* niche construction involved experimentally manipulating the amount
201 of time larvae had available to modify their own brood ball. Larval niche construction significantly

202 enhances larval growth and survival, and may do so by altering the microbial community that
203 establishes in the brood ball environment. This interaction can be studied in the laboratory by
204 altering the residence time of larvae in their individual brood balls. Following a previously
205 established protocol (Schwab et al., 2017) we experimentally *reduced* larval niche construction
206 (NC[-]) by relocating larvae into a new artificial brood ball (ABB) every 48 h, whereas larvae
207 subject to normal levels of niche construction (NC[+]) were allowed to modify the same ABB
208 throughout their entire development. To control for the potential effects of handling, larvae
209 belonging to this second group were removed from their brood ball every 48 h for approximately
210 3 s, approximating the time it takes to transfer NC[-] larvae to a new ABB, but were instead
211 returned to the same, original ABB.

212

213 *Developmental measures*

214 To assess the developmental consequences of compromising larval and maternal niche
215 construction, we collected data on a number of metrics throughout ontogeny. To evaluate if and to
216 what extent larval and maternal niche construction affect the speed of development, the lengths of
217 time (in days) to reach both the third (final) instar and pupal stages were recorded. To assess the
218 effects of both forms of niche construction on mass gain, we weighed larvae at their approximate
219 peak mass (i.e., 10 days into the third instar; Moczek and Nijhout, 2002) and on day 3 of the pupal
220 stage. All individuals were monitored for survival at 48 h intervals until adulthood, and all weights
221 were collected using a scientific balance with 0.0001 g readability (Mettler Toledo AL54, Mettler-
222 Toledo Inc., Columbus, OH). Lastly, we used thorax width as a proxy for adult body size in order
223 to evaluate the effect of both forms of niche construction on overall growth. Adults were preserved
224 in 70% ethanol after their cuticle had hardened for a minimum of 10 days, and were measured

225 using a Leica MZ6 dissecting microscope (Leica, Heerbrugg, Switzerland), a Scion digital camera
226 (Scion Corp., Frederick, MD, USA), and ImageJ v1.44p software as described in Moczek (2006).

227

228 *Statistical analyses*

229 Data analyses were performed in R (R Core Team, 2017) using RStudio (RStudio Team, 2016)
230 and packages *lme4* (Bates et al., 2015), *lmerTest* (Kuznetsova et al., 2017), *dplyr* (Wickham et al.,
231 2017), and *alr4* (Weisberg, 2014). To quantify the effect of the treatments on the different
232 variables, we used linear and generalised linear mixed-effects models. Prior to analysis, we
233 performed Shapiro-Wilk tests of normality for all variables. Both mass of third instar and the log-
234 transformed mass of pupae were normally distributed and analyzed with linear mixed-effects
235 models; days to pupation and survival to adulthood were non-normally distributed and analysed
236 with generalised linear mixed-effects models. We used a Poisson distribution to analyze days to
237 pupation, after testing for overdispersion using the package *blmeo* (Korner-Nievergelt et al.,
238 2015).

239 All variables were analyzed with a model combining our two treatments and population as
240 fixed effects, along with all two-way interactions and three-way interaction between treatments
241 and population. The date on which breeding containers were set up ('batch') was included as a
242 random effect. Prior research suggests that environmental treatments used here affect both sexes
243 similarly in regards to our variables of interest (Schwab et al., 2017), therefore, to increase
244 statistical power, we excluded sex from our analysis. We removed non-significant three-way and
245 two-way interactions in a stepwise fashion (final models are in tables 1 and 2; intermediate models
246 are in Tables S3–S6 of the supplementary materials). For individuals accidentally injured due to

247 experimenter error, measures taken after injury were excluded from the analysis. Similarly,
248 experimenter-caused deaths were excluded from survival analysis.

249

250 **RESULTS**

251 *Larval and maternal niche construction individually affect offspring growth and development*
252 *regardless of population*

253 We first assessed whether larval and maternal niche construction affect offspring development
254 independently of each other and of beetle population. We found that peak larval mass, pupal mass
255 and adult body size did not differ significantly as a function of population, yet were similarly and
256 significantly reduced when either larval or maternal niche construction were experimentally
257 compromised (Table 1, Figure 1 and Supplemental Figures S1 and S2). These results support the
258 findings of Schwab et al. (2017), and suggest that our experimental approach adequately
259 manipulated developmentally and ecologically relevant components of niche construction.

260

261 **Table 1.** Linear mixed models comparing larval mass at day 10 of third instar, log of pupa mass and adult thorax width.

	Peak larval mass (day 10; mg)					Log of pupa mass (ln(mg))					Adult size (mm)				
	Est.	SE	df	<i>t</i>	<i>p</i>	Est.	SE	df	<i>t</i>	<i>p</i>	Est.	SE	df	<i>t</i>	<i>p</i>
Intercept	74.19	2.96	74.9	25.082	—	3.9677	0.0420	48.3	94.389	—	3.84	0.06	49.60	63.053	—
Population (WA)	-4.17	2.64	253.2	-1.579	0.1142	-0.0666	0.0438	131.0	-1.521	0.1307	-0.02	0.07	123.90	-0.281	0.7787
Larval niche constr. (NC[+])	16.48	2.31	340.5	7.108	<0.0001	0.1522	0.0394	192.0	3.863	0.0002	0.16	0.06	163.96	2.764	0.0057
Maternal niche constr. (Fluc.)	-8.64	2.41	320.6	-3.591	0.0003	-0.1791	0.0413	139.7	-4.333	<0.0001	-0.28	0.06	122.27	-4.728	<0.0001

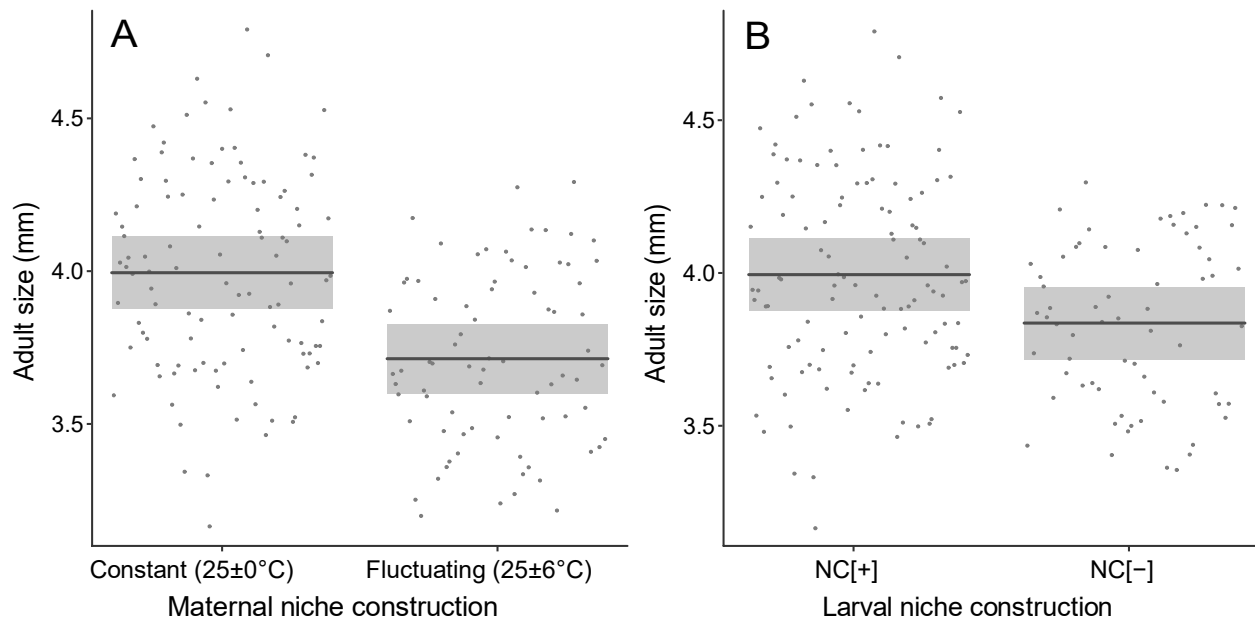
262 Note: Treatments, larval and maternal niche construction manipulations, and population were treated as fixed effects. Batch was treated as a random effect. Indicated in the table
 263 are the parameter estimates, the standard error (SE), the degrees of freedom (df), the *t* value of the test statistic, and the *p*-value for each factor in the model. Three- and two-way
 264 interactions between the different treatments and populations were non-significant, and thus removed from the final models.

265
 266 **Table 2.** Generalized linear mixed models for time to pupation and survival to adulthood

	Time to pupation (days)				Survival to adulthood			
	Estimate	SE	<i>Z</i>	<i>p</i>	Estimate	SE	<i>Z</i>	<i>p</i>
Intercept	2.978	0.037	80.30	—	-0.145	0.3448	-0.420	—
Population (WA)	0.190	0.056	3.39	<0.0001	-1.316	0.4350	-3.025	0.0025
Larval niche construction (NC[+])	-0.040	0.050	-0.81	0.5771	0.369	0.3856	0.957	0.3385
Maternal niche construction (Fluc.)	0.065	0.052	1.25	0.0031	-2.112	0.3734	-5.657	<0.0001
Pop. × NC (WA×NC[+])	0.047	0.078	0.59	0.3160	0.147	0.4858	0.303	0.7622
Pop. × Temp. (WA×Fluc.)	0.038	0.087	0.44	0.1187	-0.285	0.5085	-0.561	0.5750
NC × Temp. (NC[+]×Fluc.)	0.093	0.070	1.32	0.8726	1.380	0.4510	3.059	0.0022
Pop. × NC × Temp. (WA×NC[+]×Fluc.)	-0.229	0.117	-1.97	0.0493	—	—	—	—

267 Note: Treatments, larval and maternal niche construction manipulations, and population were treated as fixed effects. Batch was treated as a random effect. Indicated in the table
 268 are the parameter estimates, the standard error (SE), the degrees of freedom (df), the *Z* ratio of the test statistic, and the *p*-value for each factor and interaction in the model. In
 269 terms of survival, three-way interactions between the different treatments and populations was non-significant, and thus removed from the final model.

270
 271



272

273 **Figure 1.** Effects plots showing the influence of maternal and larval niche construction on variation in adult
 274 sizes of *Onthophagus taurus*. (A) Larvae exposed to constant rearing temperatures simulating intact
 275 maternal niche construction grew to significantly larger adult sizes than larvae exposed to fluctuating
 276 rearing temperatures simulating compromised maternal niche construction. (B) Larvae that developed in
 277 the same artificial brood ball (ABB) of dung grew to significantly larger adult sizes than larvae placed into
 278 new ABBs every 48 h, simulating compromised larval niche construction . Plots show the expected value
 279 (*dark line*), confidence interval (*pale band*) and partial residuals (*grey dots*) for the fixed effects displayed
 280 while controlling for all other variables in the model and the random effect of batch.

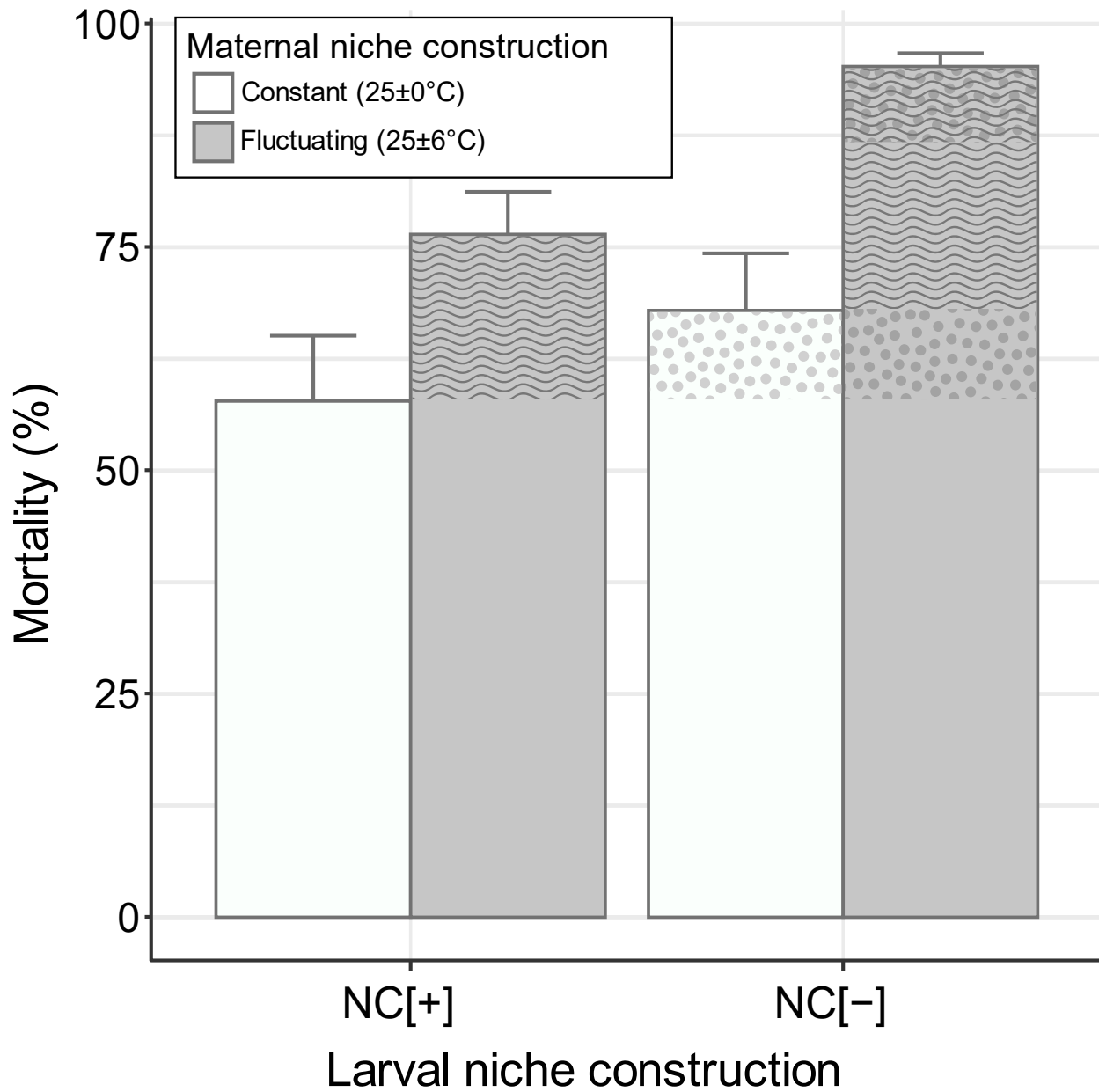
281

282 *Larval and maternal niche construction synergistically affect survival*

283 Our factorial design allowed us to test for possible interactions between larval and maternal niche
 284 construction. We failed to detect evidence for such interactions on any of the growth metrics we
 285 measured, but we found a significant interaction between larval and maternal niche construction
 286 with respect to survival (Figure 2). As predicted, mortality was lowest (57.77%) in beetles reared

287 with both maternal and larval niche construction intact. Compromising either type of niche
288 construction individually significantly increased mortality: experimental removal of larval niche
289 construction alone increased mortality to 67.92%, whereas experimental removal of maternal niche
290 construction increased mortality to 76.44%. Simultaneously compromising both types of niche
291 construction further increased mortality 95.23%, a value in excess of the sum of the individual
292 effects of larval and maternal niche construction (Table 2; interaction: Z ratio = -1.97 ;
293 $p = 0.0493$). Overall mortality was also significantly higher for WA beetles (Z ratio = -3.025 ; $p =$
294 0.0025) than EUS beetles, a difference that was independent of either type of niche construction.
295 Overall, our finding of a significant interaction between larval and maternal niche construction
296 supports the hypothesis that both types of niche construction interact synergistically to enhance
297 larval survival.

298



299

300 **Figure 2.** Bar plot of percentage survival in each treatment, illustrating the effects of, and significant
 301 interaction between, maternal and larval niche construction, with the effects of batch and population
 302 removed. Larvae exposed to fluctuating rearing temperatures simulating compromised maternal niche
 303 construction have lower survival (*portion with wavy lines*), similarly larvae placed into new artificial brood
 304 balls every 48 h simulating compromised larval niche construction had lower survival (*portion with dots*),
 305 and there was a synergistic effect of both types of niche construction being compromised (*portion with*

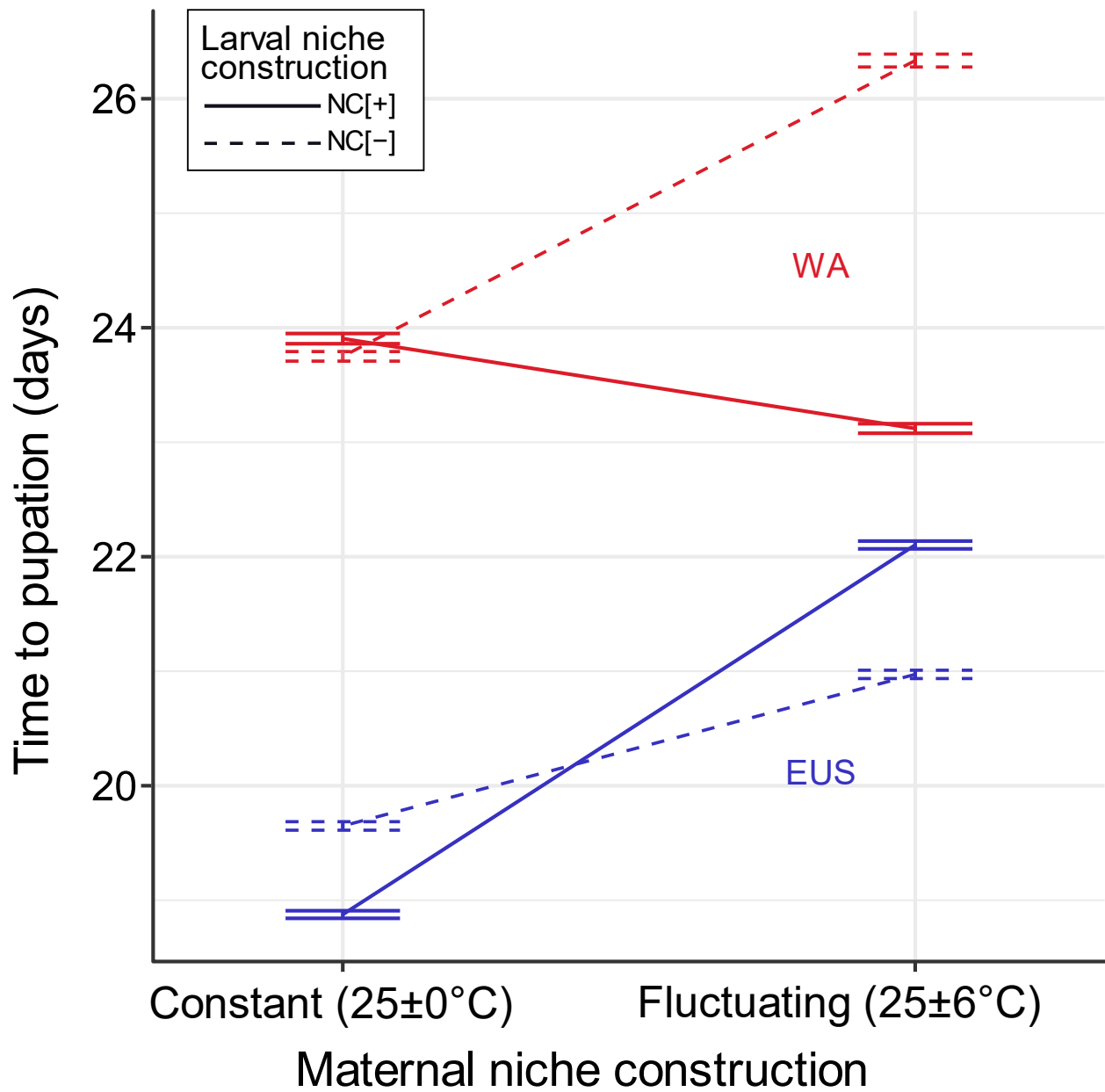
306 *wavy lines and dots superimposed*). Error bars represent standard errors and are symmetrical but only the
307 top half is shown for clarity.

308

309 *Recently diverged populations react differently to the reduction of niche construction*

310 Lastly, we assessed whether EUS and WA populations may have diverged in their dependence on
311 different types of niche construction. In support of our hypothesis, we recovered a significant
312 three-way interaction between population, larval niche construction, and maternal niche
313 construction on time to pupation. Specifically, with maternal niche construction intact, the
314 presence or absence of larval niche construction had little effect on time to pupation in either
315 population. Instead, WA beetles exhibited the standard 3-4 day extension in the length of their
316 larval developmental period compared to their EUS counterparts (as documented by previous
317 studies; see Beckers et al., 2015; Macagno et al., 2016; Moczek and Nijhout, 2003). However,
318 with maternal niche construction disrupted, among-population divergences emerged: whereas
319 EUS individuals showed an increase in development time regardless of the presence or absence of
320 larval niche construction, WA individuals showed a similar increase *only* in the absence of larval
321 niche construction (Figure 3 & Table 2). Therefore, larval niche construction may play a critical
322 role in buffering larvae against the generally low levels of maternal niche construction found in
323 the WA population, but not the EUS population. In contrast, we failed to detect significant three-
324 way interactions for any of the other traits measured. Collectively, these results support the
325 hypothesis that populations can diverge in their dependence on different types of niche
326 construction and their interactions, and do so on a trait-by-trait basis.

327



328

329 **Figure 3.** Time to pupation is significantly affected by the three-way interaction of maternal niche
 330 construction, larval niche construction, and beetle population. *Red (top)* represents beetles from Western
 331 Australia (WA) and *blue (bottom)* represents beetles from the Eastern United States (EUS). The two
 332 populations react differently to combinations of maternal and larval niche construction being compromised
 333 or intact. Values are extracted from the generalized linear mixed model (Table 2) and error bars represent
 334 the standard error of the fit.

335 **DISCUSSION**

336 Through the process of niche construction, organisms are capable of modifying their environments
337 in ways that alter how selection acts on themselves and their offspring (Laland et al., 2016).
338 However, the role of niche construction in shaping developmental and evolutionary trajectories,
339 as well as population divergence and local adaptation, remains unclear. In this study, we
340 manipulated the relative contributions of larval modifications to their natal brood ball (i.e. larval
341 niche construction), maternal influences on the thermal environment experienced by offspring (i.e.
342 maternal niche construction), and their interactions, on fitness-relevant traits in two rapidly
343 diverging populations of the bull-headed dung beetle, *Onthophagus taurus*. We find that both types
344 of niche construction enhance adult size, peak larval mass, and pupal mass, which when
345 compromised lead to a synergistic decrease in survival. Furthermore, for one measure, duration of
346 larval development (Figure 3), we find that both populations have diverged in their reliance on the
347 combination of niche construction types: larval niche construction appears to buffer against
348 compromised maternal niche construction in WA beetles only. Below we discuss the most
349 important implications of our results.

350

351 *Larval and maternal niche construction enhance offspring growth and synergistically increase*
352 *survival*

353 Consistent with our initial predictions, we found that compromising larval and maternal niche
354 construction led to statistically significant reductions in all growth metrics including peak larval
355 mass (Figure S1), pupal mass (Figure S2), adult body size (Figure 1), as well as survival to
356 adulthood (Figure 2). We further predicted that both types of niche construction interact
357 synergistically, and that their joint experimental reduction would result in fitness costs that exceed

358 the sum of their individual effects. We found a corresponding significant interaction with respect
359 to survival to adulthood, but not for any of the growth metrics we measured. Collectively, these
360 data support the hypothesis that both maternal and larval niche construction make significant
361 contributions to *Onthophagus* development and fitness.

362 Recent work has begun to identify the proximate mechanisms through which these
363 phenotypic and transgenerational effects of maternal and larval niche construction are transduced
364 (Macagno et al., 2018; Parker et al., 2019; Schwab et al., 2017; Snell-Rood et al., 2016). In
365 particular, vertically transmitted gut microbiota could link and facilitate both forms of niche
366 construction. For instance, when mothers construct brood balls, they deposit a fecal pedestal that
367 transmits gut microbiota to their offspring (Estes et al., 2013). This transmission of microbes can
368 be seen as a form of maternal niche construction. The transmitted microbes appear to be critical
369 for normal development: recent experimental work has demonstrated that pedestal microbes not
370 only increase developmental rate and adult body size under benign temperature conditions that
371 simulate high maternal investment, but that these benefits are disproportionately enhanced under
372 stressful temperature conditions that simulate low maternal investment (this study and Schwab et
373 al., 2016). Therefore, maternal transmission of gut microbes, alongside other forms of maternal
374 niche construction such as brood ball burial, play important and interactive roles in shaping the
375 outcomes of larval development.

376 At the same time, gut microbes also appear to play important roles in larval niche
377 construction. Following the consumption of the pedestal, larvae begin feeding on the dung that
378 comprises their brood ball environment, defecate throughout this environment, and then re-feed
379 on their own feces. Recent experimental work has demonstrated that brood balls modified via this
380 feeding and defecating behavior are enriched with microbial communities capable of breaking

381 down oligosaccharide components of plant and fungal cell walls, which larvae may otherwise be
382 unable to do on their own (Schwab et al., 2017). Thus, larval niche constructing behaviors may
383 establish an external rumen capable of pre-digesting otherwise recalcitrant dung and thereby
384 enhancing growth outcomes. Our results support this hypothesis as peak larval mass, pupal mass
385 and adult body size are all reduced when larval niche construction is disrupted.

386 Though key aspects of both maternal and larval niche construction appear to rely on
387 microbe-dependent mechanisms, others, such as the depth of brood ball burial, may function
388 independently of microbes. Soil reduces thermal fluctuations solely through its insulating
389 properties, thereby potentially buffering larval development, whereas larval niche construction
390 buffers development by enhancing larval nutrition. As a result, one form of niche construction may
391 be able to partly compensate for the loss or suppression of the other, while the joint reduction of
392 both forms of niche construction may result in synergistic decreases in fitness. Indeed, while the
393 independent loss of either maternal or larval niche construction significantly reduced multiple
394 indices of larval growth, simultaneously comprising both forms of niche construction led to a
395 synergistic reduction in survival in this study (Figure 2). While we found no significant differences
396 between the two populations in terms of growth or survival in the face of compromised niche
397 construction, we did find population differences in another metric.

398

399 *Dung beetle populations may diverge rapidly in their reliance on niche construction*

400 Eastern US (EUS) and Western Australia (WA) populations of *O. taurus* differed in how larval
401 and maternal niche construction affected the length of time that larvae required to reach the pupal
402 stage (Figure 3; Table 2). When maternal niche construction was disrupted, EUS beetles took

403 longer to develop regardless of whether larval niche construction was also disrupted or not. In
404 contrast, WA beetles took longer to develop *only* when both types of niche construction were
405 compromised, but not when maternal niche construction was compromised in isolation, suggesting
406 that larval niche construction buffers against compromised maternal niche construction.

407 Importantly, the rate of larval development may substantially influence the ability of adults
408 to secure mates and produce offspring during the relatively short reproductive season, and has been
409 shown to be fitness-relevant in other systems (Kingsolver and Huey, 2008); however, further
410 studies are needed to confirm the direct relevance of this metric to dung beetle fitness and
411 reproductive success. The observation that WA larvae require approximately three days longer to
412 complete larval development has been documented previously and associated with population-
413 specific differences in the degree and timing of larval male sensitivity to juvenile hormone (less
414 sensitive and delayed in WA males; Moczek and Nijhout, 2002), ovarian maturation and
415 investment (earlier and enhanced in WA females; Macagno et al., 2015) and fecundity (enhanced
416 in WA females; Beckers et al., 2015). However, if and how these endocrine, developmental, and
417 fecundity traits are functionally connected remains to be explored. Furthermore, it is unclear how
418 differences in larval developmental duration could explain why the two populations would respond
419 differently to compromised niche construction.

420 Identifying the evolutionary causes underlying these population divergences is also a
421 critical area for further studies. Recall that both EUS and WA populations were introduced from
422 ancestral European populations in the early 1970s by accident (EUS) and as part of a biocontrol
423 program intended to control dung breeding flies and enhance pasture quality (WA; Fincher and
424 Woodruff, 1975; Tyndale-Biscoe, 1996). While the population introduced to the EUS is unknown,
425 the deliberate AUS introduction drew predominantly from animals collected from Spain, Greece,

426 and Turkey (Moczek and Nijhout, 2003). Population divergences documented here could thus
427 simply reflect differences in pre-existing variation within the native range. For example, native
428 Italian *O. taurus* females bury their brood balls deeper than introduced WA females, but less deep
429 than their EUS counterparts (Macagno et al., 2016), however, clearly much more work is needed
430 to address this hypothesis further.

431 Alternatively, population divergence in reliance on niche construction may reflect recent
432 adaptations to divergent ecological and social conditions that populations encountered in their
433 respective exotic ranges. For instance, following establishment, the WA introduction yielded
434 population densities 2–3 orders of magnitude higher than those typically observed in EUS
435 populations, resulting in corresponding differences in the intensity of inter- and intra-specific
436 competition for breeding opportunities (Moczek, 2003). These ecological differences in turn have
437 been hypothesized to have driven heritable divergences in various morphological, physiological,
438 and behavioral traits (reviewed in Casasa and Moczek, 2018; Macagno et al., 2018) across EUS
439 and WA populations, including the depth at which adult females bury their brood balls and the
440 shape of adult front tibiae, the main appendage used for excavating tunnels (Macagno et al., 2016).
441 Deeper burial ensures a more isothermic developmental environment for offspring but requires
442 significantly more time and energy from mothers (Snell-Rood et al., 2016). Because of this trade-
443 off, deep burial may only be advantageous when competition for breeding opportunities is low.

444 Consistent with this notion, adult females from low competition EUS populations do, in
445 fact, bury their brood balls significantly deeper compared to adult females from high competition
446 WA populations (Macagno et al., 2016). This raises the possibility that WA larvae may have
447 experienced a history of more shallow burial over many generations, exposing larvae to more
448 stressful, fluctuating temperatures during development, and thus favoring the evolution of larval

449 compensatory mechanisms such as enhanced brood ball modifications. Our results provide partial
450 support for this hypothesis by showing that larval niche construction in WA larvae, but not EUS
451 larvae, fully compensates for compromised maternal niche construction. These results partially
452 mirror recent findings in the burying beetle *Nicrophorus vespilloides* Herbst, 1783: varying the
453 amount of parental care in experimentally evolving populations led to the evolution of larger
454 mandibles in larvae that did not receive direct parental care (Jarrett et al., 2018). Though the study
455 was not conducted in a niche construction framework, larger mandibles are thought to allow larvae
456 to more effectively self-feed, potentially compensating for the lack of parental feeding. Together
457 with our results, these findings raise the possibility that populations may adapt to local ecologies
458 through changes in their reliance on one or more forms of niche construction, yielding potentially
459 rapid divergences in the process.

460

461 *Conclusions*

462 In this study, we have experimentally demonstrated that both larval and maternal niche
463 construction can generate environmental feedbacks that positively shape growth, development
464 time, and survival in *O. taurus*. Furthermore, we have shown that these different forms of niche
465 construction do not act in isolation, nor are they static features on *Onthophagus* development:
466 together, both larval and maternal niche construction have the potential to synergistically influence
467 developmental outcomes, and these effects may diverge heritably across recently established
468 populations. Previous work documented that WA beetles bury their brood balls closer to the
469 surface (Macagno et al., 2016), possibly as a consequence of the much higher above-ground
470 competition for dung they face compared to their EUS counterparts. Here, we show that WA larvae
471 lessen the impact of elevated temperature fluctuations resulting from shallow burial by their

472 mothers through increased reliance on larval niche construction. Yet, important questions
473 regarding the causes and consequences of niche construction in *Onthophagus* remain to be
474 explored. For instance, recent work documents a partial divergence of microbiota between EUS,
475 WA, and native *O. taurus* populations (Parker et al., 2020) raising the possibility that a
476 corresponding divergence in microbial functions may underlie divergences in larval niche
477 construction. Cross-fostering experiments (as in Parker et al., 2019; Parker and Moczek, in review)
478 coupled with quantitative genetic breeding designs may permit quantification of the relative
479 contributions of maternal, microbial, and host genetic effects to variation in fitness within and
480 across populations.

481 At the same time, the natural history of dung beetles provides exciting opportunities to
482 investigate the role of niche construction in both micro- and macroevolutionary processes. For
483 example, recent work has documented that exotic populations of *O. taurus* have rapidly expanded
484 into novel climatic niches (Silva et al., 2016), which may have been facilitated by the niche
485 constructing behaviors addressed here (Parker and Moczek, in review). Further, dung beetles
486 inhabit a wide array of ecological niches and engage in disparate levels of parental niche
487 constructing behaviors across diverse genera (Costa, 2006; Hanski and Cambefort, 2014). For
488 instance, while tunneling dung beetles such as *Onthophagus* provide offspring with intermediate
489 levels of parental care by generating brood balls deep underground (Hunt and Simmons, 2002a,
490 2002b), other taxa provide both higher and significantly lower levels of care, respectively. Female
491 *Copris* spp., for instance, provision and clean their larvae throughout development (Klemperer,
492 1982; Tyndale-Biscoe, 1984), whereas diverse *Aphodius* spp. generally provide no further care
493 after laying directly in or under dung pats (Hanski and Cambefort, 2014). Addressing whether and
494 to what extent the evolution of, and interactions between, maternal and larval niche construction

495 may have facilitated the diversification of dung beetles may be key to fully understanding the
496 ecological and evolutionary success of dung beetles.

497

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508

509 **CONFLICT OF INTERESTS**

510 The authors declare that there are no conflict of interests.

511

512 **DATA AVAILABILITY STATEMENT**

513 The data generated for this study, as well as the R code used to analyse them, are available in the
514 supplementary materials and on GitHub (<https://doi.org/10.5281/zenodo.3976037>).

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651

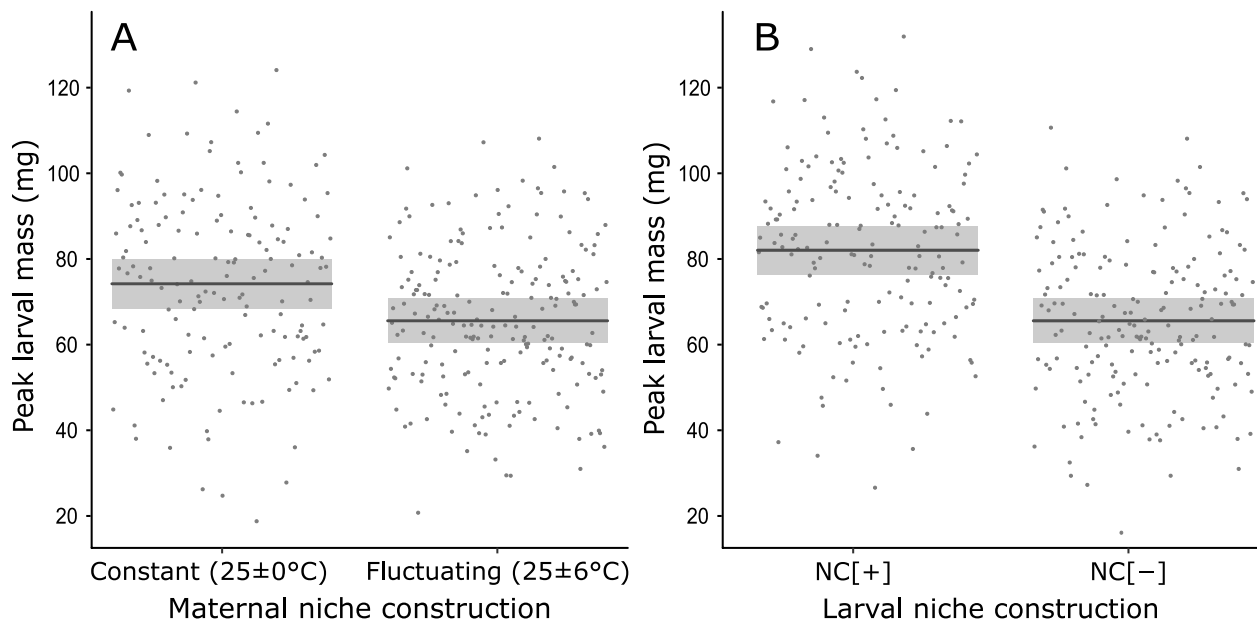
652 **SUPPLEMENTARY METHODS**

653 *Sampling intentions*

654 Because sampling intentions affect resulting *p*-values (Greenland, 2019; Kruschke, 2013), we
655 report that we intended to collect 20 adults from each of the eight treatments. Despite rearing 895
656 larvae in total, low survival prevented us from reaching 20 adults in three of eight treatments—in
657 those, we reached 17, 14 and 2 adults, despite respectively allocating 142, 60 and 141 larvae to
658 those treatments. Final sample sizes for each metric can be found in the supplementary tables S1
659 and S2.

660

661 **SUPPLEMENTARY FIGURES**

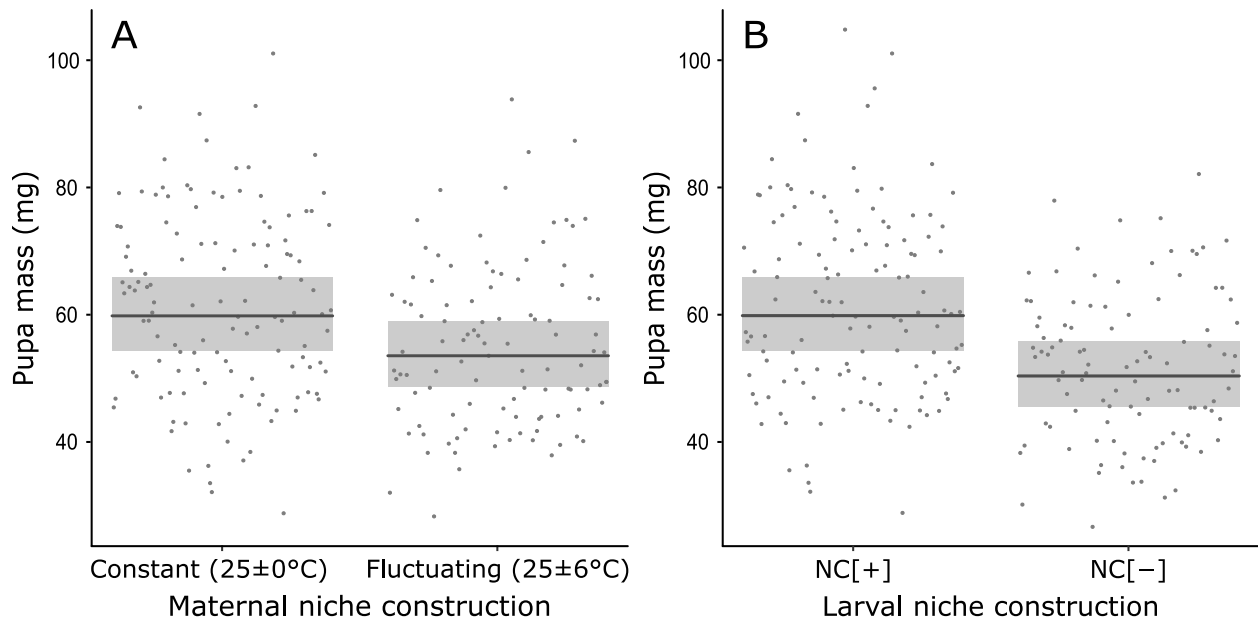


662

663 **Figure S1.** Effects plots showing the significant regressors on peak larval mass: (A) maternal niche
664 construction and (B) larval niche construction. Larvae were weighed 10 days into the third (i.e., final)
665 instar. Plots show the expected value (*dark grey line*), confidence interval (*pale grey band*) and partial residuals
666 (*grey dots*) for the fixed effects displayed while controlling for all other variables in the model and the
667 random effect of batch.

668

669



670

671 **Figure S2.** Effects plots showing the significant regressors on pupa mass: (A) maternal niche construction
 672 and (B) larval niche construction. Pupae were weighed on the third day after pupation. Plots show the
 673 expected value (*dark grey line*), confidence interval (*pale grey band*) and partial residuals (*grey dots*) for
 674 the fixed effects displayed while controlling for all other variables in the model and the random effect of
 675 batch.

676

677

678 **SUPPLEMENTARY TABLES**

679 **Table S1.** Percent survival, mortality rates, number of individuals that survived to adulthood, number of individuals that died before
680 reaching adulthood and sample size.

Larval NC	Maternal NC	Population	Survival (%)	Mortality (%)	Survived	Died	Sample size
[-]	Cons.	EUS	42.25	57.75	30	41	71
[+]	Cons.	EUS	54.76	45.24	46	38	84
[-]	Fluc.	EUS	13.44	86.56	25	161	186
[+]	Fluc.	EUS	42.11	57.89	40	55	95
[-]	Cons.	WA	26.79	73.21	15	41	56
[+]	Cons.	WA	39.58	60.42	19	29	48
[-]	Fluc.	WA	3.01	96.99	4	129	133
[+]	Fluc.	WA	14.75	85.25	18	104	122

681 Abbreviations: Niche construction (NC); constant temperature (Cons.); fluctuating temperature (Fluc.); Eastern United States (EUS); Western Australia (WA).

682
683 **Table S2.** Mean, median, standard error and sample sizes for peak larval mass, pupal mass, time to pupation and adult size.

Larval NC	Maternal NC	Population	Peak larval mass (day 10; mg)				Pupal mass (day 3; mg)				Time to pupation (days)				Adult size (mm)			
			Mean	Median	SE	N	Mean	Median	SE	N	Mean	Median	SE	N	Mean	Median	SE	N
[-]	Cons.	EUS	74.32	76.80	3.30	39	54.76	54.25	2.21	32	19.65	18	0.70	37	3.81	3.83	0.05	29
[+]	Cons.	EUS	92.55	95.40	3.14	54	64.13	61.00	2.58	41	18.88	18	0.46	48	4.04	4.00	0.05	43
[-]	Fluc.	EUS	67.08	67.90	2.24	77	48.66	45.80	2.82	27	20.97	20	0.59	36	3.61	3.64	0.08	22
[+]	Fluc.	EUS	84.37	84.40	2.58	45	53.13	50.65	2.08	36	22.10	22	0.46	39	3.76	3.75	0.05	36
[-]	Cons.	WA	73.39	75.70	4.19	33	53.95	54.05	2.86	24	23.75	23	0.97	24	3.88	3.93	0.08	14
[+]	Cons.	WA	89.58	88.40	4.91	28	61.75	66.50	4.72	18	23.90	24	0.80	21	3.99	4.02	0.11	19
[-]	Fluc.	WA	63.89	62.00	2.71	38	40.09	35.65	4.01	10	26.33	25	1.77	12	3.64	3.64	0.28	2
[+]	Fluc.	WA	72.46	78.10	3.23	41	47.65	44.80	2.62	22	23.12	22	0.63	25	3.57	3.49	0.08	17

684 Abbreviations: Niche construction (NC); constant temperature (Cons.); fluctuating temperature (Fluc.); Eastern United States (EUS); Western Australia (WA); Sample size (N);
685 Standard error (SE).

686 **Table S3.** Intermediate linear mixed models for peak larval mass with non-significant levels of interaction included.

	Peak larval mass (day 10; mg)					Peak larval mass (day 10; mg)				
	Estimate	SE	df	<i>t</i>	<i>p</i>	Estimate	SE	df	<i>t</i>	<i>p</i>
Intercept	73.365	3.88	148.1	18.896	—	71.8125	3.72	131.4	19.327	—
Population (WA)	-4.778	5.02	341.4	-0.952	0.3419	-1.2938	4.46	322.5	-0.290	0.7720
Larval niche construction (NC[+])	16.222	4.69	330.9	3.459	0.0006	19.4496	4.16	326.3	4.678	<0.0001
Maternal niche construction (Fluc.)	-7.865	4.23	338.2	-1.859	0.0639	-5.2292	3.84	334.0	-1.363	0.1740
Pop. × NC (WA×NC[+])	6.857	6.99	346.8	0.981	0.3275	-0.8946	4.77	333.9	-0.188	0.8510
Pop. × Temp. (WA×Fluc.)	2.596	6.60	336.3	0.394	0.6941	-4.0173	4.91	328.6	-0.818	0.4140
NC × Temp. (NC[+]×Fluc.)	1.884	6.01	343.9	0.313	0.7541	-3.8103	4.65	343.3	-0.819	0.4140
Pop. × NC × Temp. (WA×NC[+]×Fluc.)	-14.012	9.34	345.7	-1.500	0.1346	—	—	—	—	—

687 Note: Treatments, larval and maternal niche construction manipulations, and population were treated as fixed effects. Batch was treated as a random effect. Indicated in the table
 688 are the parameter estimates, the standard error (SE), the degrees of freedom (df), the *t* value of the test statistic, and the *p*-value for each factor in the model. Significant effects
 689 (*p*<0.05) are in bold. In terms of larval mass, neither three-way interactions (left) nor two-way interactions (right) between the different treatments and populations were non-
 690 significant, and thus were removed from the final model and presented here.

692 **Table S4.** Intermediate linear mixed models for log of pupa mass with non-significant levels of interaction included.

	Log of pupa mass (ln(mg))					Log of pupa mass (ln(mg))				
	Estimate	SE	df	<i>t</i>	<i>p</i>	Estimate	SE	df	<i>t</i>	<i>p</i>
Intercept	3.9320	0.0564	73.5	69.760	—	3.9360	0.0543	71.3	72.430	—
Population (WA)	0.0003	0.0768	186.3	0.003	0.9970	-0.0090	0.0707	169.7	-0.127	0.8993
Larval niche construction (NC[+])	0.1730	0.0704	158.5	2.459	0.0150	0.1645	0.0646	162.3	2.547	0.0118
Maternal niche construction (Fluc.)	-0.1078	0.0779	136.5	-1.384	0.1690	-0.1180	0.0706	138.2	-1.671	0.0971
Pop. × NC (WA×NC[+])	0.0020	0.1102	197.8	0.018	0.9850	0.0249	0.0836	195.9	0.298	0.7657
Pop. × Temp. (WA×Fluc.)	-0.1710	0.1256	199.6	-1.362	0.1750	-0.1424	0.0862	180.6	-1.652	0.1003
NC × Temp. (NC[+]×Fluc.)	-0.0367	0.0996	173.9	-0.368	0.7130	-0.0182	0.0807	183.4	-0.225	0.8221
Pop. × NC × Temp. (WA×NC[+]×Fluc.)	0.0525	0.1654	200.5	0.317	0.7510	—	—	—	—	—

693 Note: Treatments, larval and maternal niche construction manipulations, and population were treated as fixed effects. Batch was treated as a random effect. Indicated in the table
 694 are the parameter estimates, the standard error (SE), the degrees of freedom (df), the *t* value of the test statistic, and the *p*-value for each factor in the model. Significant effects
 695 (*p*<0.05) are in bold. In terms of pupa mass, neither three-way interactions (left) nor two-way interactions (right) between the different treatments and populations were non-
 696 significant, and thus were removed from the final model and presented here.

697 **Table S5.** Intermediate linear mixed models for adult size with non-significant levels of interaction included.

	Adult size (mm)					Adult size (mm)				
	Estimate	SE	df	<i>t</i>	<i>p</i>	Estimate	SE	df	<i>t</i>	<i>p</i>
Intercept	3.78	0.08	68.95	49.117	—	3.78	0.08	65.56	49.926	—
Population (WA)	0.08	0.12	171.13	0.694	0.4887	0.10	0.11	167.36	0.903	0.3679
Larval niche construction (NC[+])	0.21	0.09	148.31	2.348	0.0202	0.23	0.09	146.05	2.567	0.0113
Maternal niche construction (Fluc.)	-0.20	0.11	123.14	-1.877	0.0628	-0.19	0.10	116.29	-1.821	0.0711
Pop. × NC (WA×NC[+])	-0.07	0.15	174.00	-0.436	0.6637	-0.10	0.13	173.73	-0.744	0.4580
Pop. × Temp. (WA×Fluc.)	-0.00	0.28	170.88	-0.013	0.9898	-0.12	0.13	164.49	-0.924	0.3570
NC × Temp. (NC[+]×Fluc.)	-0.05	0.13	164.83	-0.377	0.7069	-0.07	0.12	163.56	-0.622	0.5346
Pop. × NC × Temp. (WA×NC[+]×Fluc.)	-0.15	0.31	173.02	-0.478	0.6332	—	—	—	—	—

698 Note: Treatments, larval and maternal niche construction manipulations, and population were treated as fixed effects. Batch was treated as a random effect. Indicated in the table
 699 are the parameter estimates, the standard error (SE), the degrees of freedom (df), the *t* value of the test statistic, and the *p*-value for each factor and interaction in the model.

700 Significant effects (*p*<0.05) are in bold. In terms of adult size, neither three-way interactions (left) nor two-way interactions (right) between the different treatments and
 701 populations were non-significant, and thus were removed from the final model and presented here.

703 **Table S6.** Intermediate generalized linear mixed models for survival to adulthood with non-significant levels of interaction included.

	Survival to adulthood			
	Estimate	SE	<i>Z</i>	<i>p</i>
Intercept	-0.087	0.3554	-0.245	—
Population (WA)	-1.456	0.4778	-3.047	0.0023
Larval niche construction (NC[+])	0.243	0.4216	0.577	0.5641
Maternal niche construction (Fluc.)	-2.226	0.4065	-5.476	<0.0001
Pop. × NC (WA×NC[+])	0.459	0.6438	0.713	0.4761
Pop. × Temp. (WA×Fluc.)	0.137	0.7441	0.183	0.8544
NC × Temp. (NC[+]×Fluc.)	1.608	0.5486	2.932	0.0034
Pop. × NC × Temp. (WA×NC[+]×Fluc.)	-0.711	0.9521	-0.747	0.4550

704 Note: Treatments, larval and maternal niche construction manipulations, and population were treated as fixed effects. Batch was treated as a random effect. Indicated in the table
 705 are the parameter estimates, the standard error (SE), the degrees of freedom (df), the *Z* ratio of the test statistic, and the *p*-value for each factor and interaction in the model.

706 Significant effects (*p*<0.05) are in bold. In terms of survival, three-way interactions between the different treatments and populations was non-significant, and thus presented here
 707 but removed from the final model.

708 **LITERATURE CITED IN SUPPLEMENTARY MATERIALS**

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