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

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Abstract

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

KEYWORDS

Coleoptera, developmental stress, genotype–environment interaction, host–microbiome interaction, maternal effects, parental care

1 | INTRODUCTION

Understanding the mechanisms that facilitate persistence and local adaptation following colonization of novel habitats is a fundamental objective of evolutionary ecology and conservation biology. Recent work has begun to emphasize the potential significance of niche construction to this process, which occurs when organisms modify their environments in ways that alter the selective conditions that they or their descendants experience (Laland, Matthews, & Feldman, 2016; Matthews et al., 2014; Odling-Smee, Laland, & Feldman, 2003). Niche construction may result from modifications of biotic and

abiotic conditions, can be expressed through behavioral, physiological, and developmental processes, and can facilitate the inheritance of environmental states, (e.g., though the transmission of habitat conditions or symbionts). For example, when adult *Drosophila* colonize new food sources on which to feed and lay their eggs, the flies also vector yeasts (Begon, 1982). The larval generation then modifies the microbiotic conditions of the food source, dramatically affecting yeast densities and species composition, and thereby creating a predictable microbial environment conducive to larval development (Good & Tatar, 2001; Stamps, Yang, Morales, & Boundy-Mills, 2012). Furthermore, this microbial environment can be

shaped by the niche constructing activities of the yeast themselves: by preferentially engaging in fermentation despite the presence of oxygen, yeasts produce ethanol and heat, allowing them to defend sugar resources by generating an environment that is both toxic and too hot for many of their interspecific competitors (Goddard, 2008; Pfeiffer & Morley, 2014). Such niche construction is therefore of interest to evolutionary biologists because it offers additional sources of phenotypic variation and alternate routes to adaptation. Moreover, niche construction may contribute avenues for nongenetic inheritance in taxa in which modified environments are passed on to subsequent generations. Lastly, evolution in response to heritable environments is also thought to be faster than evolution to abiotic non-heritable environments (Drown & Wade, 2014). These same properties are also integral, though not as emphasized, in the overlapping frameworks of eco-evolutionary feedbacks (Hendry, 2016) and extended phenotypes (Dawkins, 1982).

Both the ubiquity and ecological relevance of niche construction are increasingly well established (Bateson & Gluckman, 2011; Laland, Odling-Smee, & Endler, 2017; Laland et al., 2016; Sultan, 2015). Similarly, explicit tests of niche construction and its effects on fitness-relevant traits are accumulating for diverse organisms and types of niche construction, and illustrate that niche construction is frequently adaptive, critical for normative development, and able to diverge among closely related species (e.g., arthropods: Bailey et al., 2009; Saltz & Foley, 2011; Schwab, Casasa, & Moczek, 2017; flatworms: Majdi, Boiché, Traunspurger, & Lecerf, 2014; Wilden, Majdi, Kuhlicke, Neu, & Traunspurger, 2019; plant-soil systems: Schweitzer et al., 2014; yeast: Goddard, 2008). Yet, whether niche construction also contributes to local adaptation among populations and in the face of novel or challenging environmental conditions is poorly understood. Moreover, many organisms engage in niche construction through diverse routes, yet the consequences of potential interactions among multiple forms of niche construction are essentially unexplored. Here, we investigate the relative contributions of, and interactions between, two forms of niche construction in the development and survival of *Onthophagus taurus* (Schreber, 1759) dung beetles from two rapidly diverging populations.

Onthophagus dung beetles are promising organisms to investigate the developmental, ecological, and evolutionary consequences of niche construction because individuals modify their environment during key phases of their life cycle and in ways that could impact both their own fitness and that of their descendants. For example, adult female *Onthophagus* dig tunnels underneath dung pats, and provision them with accumulations of dung (i.e., brood balls) that comprise all the food available for

larvae to complete development. Furthermore, within each brood ball, mothers provide their offspring with a maternal fecal pellet called a *pedestal* onto which they lay a single egg (Estes et al., 2013; Parker, Dury, & Moczek, 2019). Upon hatching, the larva consumes the pedestal, thereby obtaining maternal gut microbiota crucial for normal development (Schwab et al., 2017; Schwab, Riggs, Newton, & Moczek, 2016). Lastly, the depth at which mothers bury brood balls affects thermal conditions experienced by larvae: deeper brood balls ensure a more constant thermal environment, which enhances larval growth (Snell-Rood, Burger, Hutton, & Moczek, 2016). Mothers, therefore, construct important features of the developmental niche of their larval offspring.

Importantly, larvae engage in significant environment-modifying behaviors of their own. For example, throughout development larvae not only feed upon their brood ball, but also defecate within it, working their feces into the brood ball's remainder, then eat the resulting mix anew. Furthermore, recent work has documented diverse developmental and fitness consequences of larval niche construction across *Onthophagus* species, and proposed that larval modifications to the brood ball, in particular those involving larval fecal matter, help establish an external rumen (Schwab et al., 2017), as has been documented in other species (Costa, 2006; Swift, Heal, Anderson, & Anderson, 1979; Thompson, Grebenok, Behmer, & Gruner, 2013). Specifically, once established, this external rumen may pre-digest dung through the help of larval microbiota before ingestion, or re-ingestion, by larvae. In support of this hypothesis, the microbiota derived from artificial brood balls (ABBs) modified by larvae could digest a wider range of potential carbon sources and did so more substantially than microbiota derived from unmodified ABBs (Schwab et al., 2017). Evolutionary changes in these environment-modifying behaviors of larvae and their mothers could provide alternative routes to adaptation.

Lastly, many *Onthophagus* species have been introduced deliberately or accidentally to novel habitats, offering opportunities to investigate the role of niche construction in the colonization of and adaptation to novel ecological conditions. Here, we focus on *O. taurus*, a species native to the Palearctic (Ziani et al., 2015). In the 1970s, this species was deliberately introduced from the Mediterranean region to Western Australia (WA), and introduced accidentally to the Eastern United States (EUS; Hoebeke & Beucke, 1997; Tyndale-Biscoe, 1996). Since their establishment in both exotic locations, *O. taurus* populations have diverged heritably in diverse traits (e.g., genital morphology: Macagno et al., 2011; fecundity: Macagno, Beckers, & Moczek, 2015; hormone physiology: Moczek

& Nijhout, 2002; allometry: Moczek, Hunt, Emlen, & Simmons, 2002), including traits relevant to niche construction: WA females dig shallower breeding tunnels than EUS females, thereby exposing their offspring to higher and less stable temperatures (Macagno, Moczek, & Pizzo, 2016). Interestingly, larvae from WA but not EUS use developmental plasticity to adaptively respond to stressfully high temperatures (Macagno, Zattara, Ezeakudo, Moczek, & Ledón-Rettig, 2018).

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

2 | MATERIALS AND METHODS

2.1 | Beetle provenance and husbandry

Adult *O. taurus* were collected from two exotic ranges: EUS individuals were collected near Chapel Hill, North Carolina, USA (35°54'47.3"N, 79°3'20.9"W), whereas

individuals from WA were collected near Busselton (33°38'52"S, 115°20'45"E) and Serpentine (32°21'54"S, 115°58'51.6"E). These beetles, and their offspring, were used to establish laboratory colonies, maintained at 25°C in a sand/soil mixture at a 16 h:8 h light:dark cycle, and fed homogenized organic cow manure twice a week (as described in Moczek, 2006). In nature, WA populations are generally active from December to February, while EUS populations are active from approximately late May to August (Moczek, 2003). To execute our experiment at the same time for both populations we, therefore, used wild-caught EUS but lab-reared WA (F₁ and F₂) starter populations to rear larvae for our experiment, allowing us to experiment on both populations simultaneously. We opted for this approach rather than the alternative option of rearing the two populations separately to avoid introducing the confounding effects of timing and seasonal variation that are beyond our control. To rear larvae for experimentation, adult beetles were haphazardly selected from their respective colonies and placed into separate plastic breeding containers (26 cm tall × 20 cm diameter) filled ~75% with a packed moist sand and soil mixture, provided with ~0.5 L cow manure, and allowed to breed and produce brood balls for 5 days (as described in Moczek & Nagy, 2005). A total of 85 breeding containers (30 WA and 55 EUS) were set up between May and December 2017, generating 895 larvae used in this experiment. Sampling intentions and sample sizes for each metric can be found in Tables S1 and S2.

To standardize larval age, we only used brood balls that contained an egg upon inspection. Approximately 900 brood balls in total were used for the experiment, which were inspected daily until each egg was observed to have hatched. Freshly hatched larvae were left in their native brood ball for 24–48 h before transfer to an ABB, as explained below. This allowed larvae to consume the pedestal made of maternal excrement that serves as the main conduit for maternal to larval microbiome transmission (Estes et al., 2013).

2.2 | Rearing of experimental animals

Larvae for all treatments were transferred into 12-well tissue culture plates containing standardized ABBs following Shafiei, Moczek, and Nijhout (2001). To standardize the quality of ABBs, the dung used to generate them was collected from a single organic farm (Marble Hill Farm, Bloomington, IN, USA, 39°3'8"N, 86°36'12" W), homogenized on-site, frozen, thawed and homogenized again in two sets, thereby ensuring that dung used in the experiment was of equivalent quality both within and among treatment groups.

2.3 | Manipulation of niche construction

To assess the respective roles of maternal and larval niche construction in *Onthophagus* development, we experimentally simulated different levels of maternal investment by imposing alternate temperature regimes on developing larvae, while simultaneously manipulating the ability for larvae to modify their own brood ball environment. To assess possible interactions between both forms of niche construction on larval performance, we executed this experiment in a fully factorial manner. Lastly, to assess the potential contributions of each form of niche construction and their interactions to population divergence, we replicated this effort across both EUS and WA populations. Experimental manipulations of maternal and larval niche construction followed previously established protocols (Schwab et al., 2016, 2017; Snell-Rood et al., 2016) as detailed next.

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

Manipulating larval niche construction involved experimentally manipulating the amount of time larvae had available to modify their own brood ball. Larval niche construction significantly enhances larval growth and survival, and may do so by altering the microbial community

that establishes in the brood ball environment. This interaction can be studied in the laboratory by altering the residence time of larvae in their individual brood balls. Following a previously established protocol (Schwab et al., 2017) we experimentally reduced larval niche construction (NC[−]) by relocating larvae into a new ABB every 48 h, whereas larvae subject to normal levels of niche construction (NC[+]) were allowed to modify the same ABB throughout their entire development. To control for the potential effects of handling, larvae belonging to this second group were removed from their brood ball every 48 h for approximately 3s, approximating the time it takes to transfer NC[−] larvae to a new ABB, but were instead returned to the same, original ABB.

2.4 | Developmental measures

To assess the developmental consequences of compromising larval and maternal niche construction, we collected data on a number of metrics throughout ontogeny. To evaluate if and to what extent larval and maternal niche construction affect the speed of development, the lengths of time (in days) to reach both the third (final) instar and pupal stages were recorded. To assess the effects of both forms of niche construction on mass gain, we weighed larvae at their approximate peak mass (i.e., 10 days into the third instar; Moczek & Nijhout, 2002) and on Day 3 of the pupal stage. All individuals were monitored for survival at 48 h intervals until adulthood, and all weights were collected using a scientific balance with 0.0001 g readability (Mettler Toledo AL54; Mettler-Toledo Inc., Columbus, OH, USA). Lastly, we used thorax width as a proxy for adult body size to evaluate the effect of both forms of niche construction on overall growth. Adults were preserved in 70% ethanol after their cuticle had hardened for a minimum of 10 days, and were measured using a Leica MZ6 dissecting microscope (Leica, Heerbrugg, Switzerland), a Scion digital camera (Scion Corp., Frederick, MD, USA), and ImageJ v1.44p software as described in Moczek (2006).

2.5 | Statistical analyses

Data analyses were performed in R (R Core Team, 2017) using RStudio (RStudio Team, 2016) and packages *lme4* (Bates, Mächler, Bolker, & Walker, 2015), *lmerTest* (Kuznetsova, Brockhoff, & Christensen, 2017), *dplyr* (Wickham, Francois, Henry, & Müller, 2020), and *alr4* (Weisberg, 2014). To quantify the effect of the treatments on the different variables, we used linear and generalized linear mixed-effects models. Before analysis, we performed Shapiro-Wilk tests of normality for all variables.

Both mass of third instar and the log-transformed mass of pupae were normally distributed and analyzed with linear mixed-effects models; days to pupation and survival to adulthood were non-normally distributed and analyzed with generalized linear mixed-effects models. We used a Poisson distribution to analyze days to pupation, after testing for overdispersion using the package *blmecc* (Korner-Nievergelt et al., 2015).

All variables were analyzed with a model combining our two treatments and population as fixed effects, along with all two- and three-way interactions between treatments and population. The date on which breeding containers were set up (“batch”) was included as a random effect. Prior research suggests that environmental treatments used here affect both sexes similarly in regard to our variables of interest (Schwab et al., 2017), therefore, to increase statistical power, we excluded sex from our analysis. We removed nonsignificant three-way and two-way interactions in a stepwise fashion (final models are in Tables 1 and 2 and intermediate models are in Tables S3–S6). For individuals accidentally injured due to experimenter error, measures taken after injury were excluded from the analysis. Similarly, experimenter-caused deaths were excluded from survival analysis.

3 | RESULTS

3.1 | Larval and maternal niche construction individually affect offspring growth and development regardless of population

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

3.2 | Larval and maternal niche construction synergistically affect survival

Our factorial design allowed us to test for possible interactions between larval and maternal niche construction. We failed to detect evidence for such interactions on

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	t	df	t	p	Est.	SE	df	t	p					
Intercept	74.19	2.96	25.082	74.9	25.082	–	3.9677	0.0420	48.3	94.389	–	63.053	–			
Population (WA)	–4.17	2.64	–1.579	253.2	–1.579	.1142	–0.0666	0.0438	131.0	–1.521	.1307	–0.02	0.07	123.90	–0.281	.7787
Larval niche constr. (NC[+])	16.48	2.31	7.108	340.5	7.108	<.0001	0.1522	0.0394	192.0	3.863	.0002	0.16	0.06	163.96	2.764	.0057
Maternal niche constr. (Fluc.)	–8.64	2.41	–3.591	320.6	–3.591	.0003	–0.1791	0.0413	139.7	–4.333	<.0001	–0.28	0.06	122.27	–4.728	<.0001

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 are the parameter estimates, the 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 (*p* < 0.05) are in bold. Three- and two-way interactions between the different treatments and populations were nonsignificant, and thus removed from the final models.

TABLE 2 Generalized linear mixed models for time to pupation and survival to adulthood

	Time to pupation (days)				Survival to adulthood			
	Estimate	SE	Z	p	Estimate	SE	Z	p
Intercept	2.978	0.037	80.30	–	–0.145	0.3448	–0.420	–
Population (WA)	0.190	0.056	3.39	<.0001	–1.316	0.4350	–3.025	.0025
Larval niche construction (NC[+])	–0.040	0.050	–0.81	.5771	0.369	0.3856	0.957	.3385
Maternal niche construction (Fluc.)	0.065	0.052	1.25	.0031	–2.112	0.3734	–5.657	<.0001
Pop. × NC (WA × NC[+])	0.047	0.078	0.59	.3160	0.147	0.4858	0.303	.7622
Pop. × Temp. (WA × Fluc.)	0.038	0.087	0.44	.1187	–0.285	0.5085	–0.561	.5750
NC × Temp. (NC[+] × Fluc.)	0.093	0.070	1.32	.8726	1.380	0.4510	3.059	.0022
Pop. × NC × Temp. (WA × NC[+] × Fluc.)	–0.229	0.117	–1.97	.0493	–	–	–	–

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 are the parameter estimates, the 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. Significant effects ($p < .05$) are in bold. In terms of survival, three-way interactions between the different treatments and populations were nonsignificant, and thus removed from the final model.

any of the growth metrics we measured, but we found a significant interaction between larval and maternal niche construction with respect to survival (Figure 2). As predicted, mortality was lowest (57.77%) in beetles reared with both maternal and larval niche construction intact. Compromising either type of niche construction individually significantly increased mortality: experimental removal of larval niche construction alone increased mortality to 67.92%, whereas experimental removal of maternal niche construction increased mortality to

76.44%. Simultaneously compromising both types of niche construction further increased mortality 95.23%, a value in excess of the sum of the individual effects of larval and maternal niche construction (Table 2; interaction: Z ratio = -1.97 ; $p = .0493$). Overall mortality was also significantly higher for WA beetles (Z ratio = -3.025 ; $p = .0025$) than EUS beetles, a difference that was independent of either type of niche construction. Overall, our finding of a significant interaction between larval and maternal niche construction supports the hypothesis that

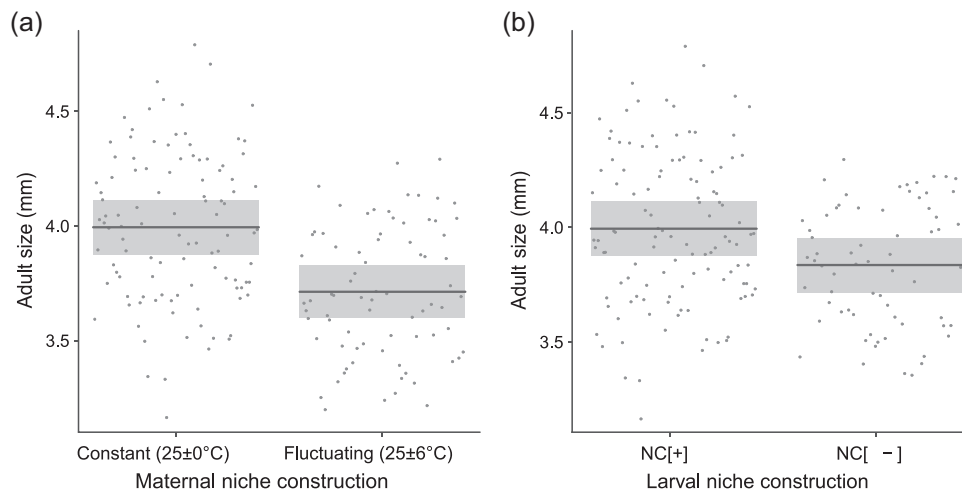


FIGURE 1 Effects plots showing the influence of maternal and larval niche construction on variation in adult sizes of *Onthophagus taurus*. (a) Larvae exposed to constant rearing temperatures simulating intact maternal niche construction grew to significantly larger adult sizes than larvae exposed to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

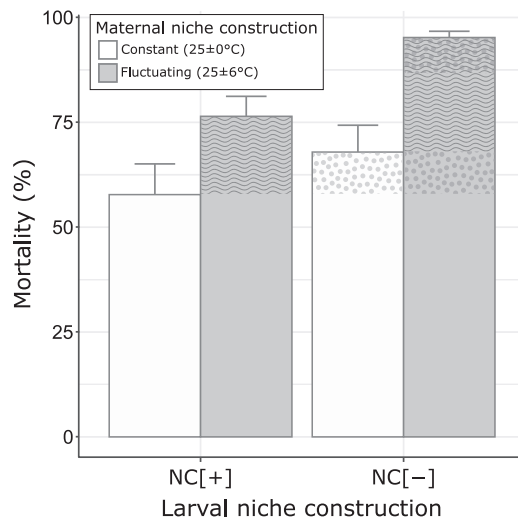


FIGURE 2 Bar plot of percentage survival in each treatment, illustrating the effects of, and significant interaction between, maternal and larval niche construction, with the effects of batch and population removed. Larvae exposed to fluctuating rearing temperatures simulating compromised maternal niche construction have lower survival (*portion with wavy lines*), similarly, larvae placed into new artificial brood balls every 48 h simulating compromised larval niche construction had lower survival (*portion with dots*), and there was a synergistic effect of both types of niche construction being compromised (*portion with wavy lines and dots superimposed*). Error bars represent standard errors and are symmetrical but only the top half is shown for clarity

both types of niche construction interact synergistically to enhance larval survival.

3.3 | Recently diverged populations react differently to the reduction of niche construction

Lastly, we assessed whether EUS and WA populations may have diverged in their dependence on different types of niche construction. In support of our hypothesis, we recovered a significant three-way interaction between population, larval niche construction, and maternal niche construction on time to pupation. Specifically, with maternal niche construction intact, the presence or absence of larval niche construction had little effect on time to pupation in either population. Instead, WA beetles exhibited the standard 3–4-day extension in the length of their larval developmental period compared to their EUS counterparts (as documented by previous studies; see Beckers, Anderson, & Moczek, 2015; Macagno et al., 2016; Moczek & Nijhout, 2003). However, with maternal niche construction disrupted, among-population divergences emerged:

whereas EUS individuals showed an increase in development time regardless of the presence or absence of larval niche construction, WA individuals showed a similar increase *only* in the absence of larval niche construction (Figure 3 and Table 2). Therefore, larval niche construction may play a critical role in buffering larvae against the generally low levels of maternal niche construction found in the WA population, but not the EUS population. In contrast, we failed to detect significant three-way interactions for any of the other traits measured. Collectively, these results support the hypothesis that populations can diverge in their dependence on different types of niche construction and their interactions, and do so on a trait-by-trait basis.

4 | DISCUSSION

Through the process of niche construction, organisms are capable of modifying their environments in ways that alter how selection acts on themselves and their offspring (Laland et al., 2016). However, the role of niche construction in shaping developmental and evolutionary trajectories, as well as population divergence and local adaptation, remains unclear. In this study, we manipulated the relative contributions of larval modifications to

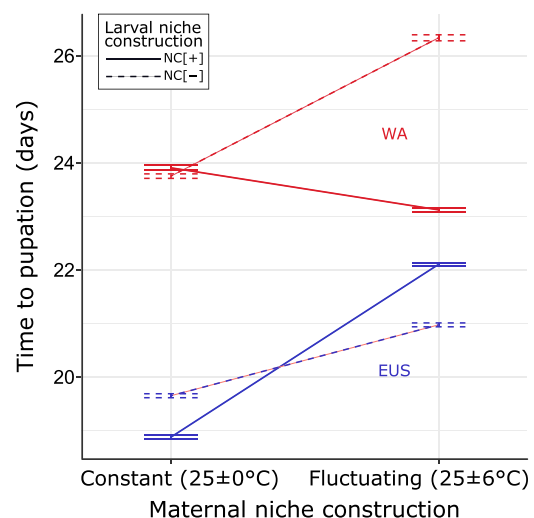


FIGURE 3 Time to pupation is significantly affected by the three-way interaction of maternal niche construction, larval niche construction, and beetle population. *Red (top)* represents beetles from Western Australia (WA) and *blue (bottom)* represents beetles from the Eastern United States (EUS). The two populations react differently to combinations of maternal and larval niche construction being compromised or intact. Values are extracted from the generalized linear mixed model (Table 2) and error bars represent the standard error of the fit. [Color figure can be viewed at wileyonlinelibrary.com]

their natal brood ball (i.e., larval niche construction), maternal influences on the thermal environment experienced by offspring (i.e., maternal niche construction), and their interactions, on fitness-relevant traits in two rapidly diverging populations of the bull-headed dung beetle, *Onthophagus taurus*. We find that both types of niche construction enhance adult size, peak larval mass, and pupal mass, which when compromised lead to a synergistic decrease in survival. Furthermore, for one measure, duration of larval development (Figure 3), we find that both populations have diverged in their reliance on the combination of niche construction types: larval niche construction appears to buffer against compromised maternal niche construction in WA beetles only. Below we discuss the most important implications of our results.

4.1 | Larval and maternal niche construction enhance offspring growth and synergistically increase survival

Consistent with our initial predictions, we found that compromising larval and maternal niche construction led to statistically significant reductions in all growth metrics including peak larval mass (Figure S1), pupal mass (Figure S2), adult body size (Figure 1), as well as survival to adulthood (Figure 2). We further predicted that both types of niche construction interact synergistically and that their joint experimental reduction would result in fitness costs that exceed the sum of their individual effects. We found a corresponding significant interaction with respect to survival to adulthood, but not for any of the growth metrics we measured. Collectively, these data support the hypothesis that both maternal and larval niche construction make significant contributions to *Onthophagus* development and fitness.

Recent work has begun to identify the proximate mechanisms through which these phenotypic and transgenerational effects of maternal and larval niche construction are transduced (Macagno et al., 2018; Parker et al., 2019; Schwab et al., 2017; Snell-Rood et al., 2016). In particular, vertically transmitted gut microbiota could link and facilitate both forms of niche construction. For instance, when mothers construct brood balls, they deposit a fecal pedestal that transmits gut microbiota to their offspring (Estes et al., 2013). This transmission of microbes can be seen as a form of maternal niche construction. The transmitted microbes appear to be critical for normal development: recent experimental work has demonstrated that pedestal microbes not only increase developmental rate and adult body size under benign temperature conditions that simulate high maternal

investment, but that these benefits are disproportionately enhanced under stressful temperature conditions that simulate low maternal investment (this study and Schwab et al., 2016). Therefore, maternal transmission of gut microbes, alongside other forms of maternal niche construction such as brood ball burial, play important and interactive roles in shaping the outcomes of larval development.

At the same time, gut microbes also appear to play important roles in larval niche construction. Following the consumption of the pedestal, larvae begin feeding on the dung that comprises their brood ball environment, defecate throughout this environment, and then refeed on their own feces. Recent experimental work has demonstrated that brood balls modified via this feeding and defecating behavior are enriched with microbial communities capable of breaking down oligosaccharide components of plant and fungal cell walls, which larvae may otherwise be unable to do on their own (Schwab et al., 2017). Thus, larval niche constructing behaviors may establish an external rumen capable of pre-digesting otherwise recalcitrant dung and thereby enhancing growth outcomes. Our results support this hypothesis as peak larval mass, pupal mass, and adult body size are all reduced when larval niche construction is disrupted.

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

4.2 | Dung beetle populations may diverge rapidly in their reliance on niche construction

EUS and WA populations of *O. taurus* differed in how larval and maternal niche construction affected the

length of time that larvae required to reach the pupal stage (Figure 3 and Table 2). When maternal niche construction was disrupted, EUS beetles took longer to develop regardless of whether larval niche construction was also disrupted or not. In contrast, WA beetles took longer to develop *only* when both types of niche construction were compromised, but not when maternal niche construction was compromised in isolation, suggesting that larval niche construction buffers against compromised maternal niche construction.

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

Identifying the evolutionary causes underlying these population divergences is also a critical area for further studies. Recall that both EUS and WA populations were introduced from ancestral European populations in the early 1970s by accident (EUS) and as part of a biocontrol program intended to control dung breeding flies and enhance pasture quality (WA; Fincher & Woodruff, 1975; Tyndale-Biscoe, 1996). While the population introduced to the EUS is unknown, the deliberate AUS introduction drew predominantly from animals collected from Spain, Greece, and Turkey (Moczek & Nijhout, 2003). Population divergences documented here could thus simply reflect differences in pre-existing variation within the native range. For example, native Italian *O. taurus* females bury their brood balls deeper than introduced WA females, but less deep than their EUS counterparts (Macagno et al., 2016), however, clearly much more work is needed to address this hypothesis further.

Alternatively, population divergence in reliance on niche construction may reflect recent adaptations to divergent ecological and social conditions that populations

encountered in their respective exotic ranges. For instance, following establishment, the WA introduction yielded population densities 2–3 orders of magnitude higher than those typically observed in EUS populations, resulting in corresponding differences in the intensity of inter- and intraspecific competition for breeding opportunities (Moczek, 2003). These ecological differences, in turn, have been hypothesized to have driven heritable divergences in various morphological, physiological, and behavioral traits (reviewed in Casasa & Moczek, 2018; Macagno et al., 2018) across EUS and WA populations, including the depth at which adult females bury their brood balls and the shape of adult front tibiae, the main appendage used for excavating tunnels (Macagno et al., 2016). Deeper burial ensures a more isothermic developmental environment for offspring but requires significantly more time and energy from mothers (Snell-Rood et al., 2016). Because of this trade-off, deep burial may only be advantageous when competition for breeding opportunities is low.

Consistent with this notion, adult females from low competition EUS populations do, in fact, bury their brood balls significantly deeper compared to adult females from high competition WA populations (Macagno et al., 2016). This raises the possibility that WA larvae may have experienced a history of more shallow burial over many generations, exposing larvae to more stressful, fluctuating temperatures during development, and thus favoring the evolution of larval compensatory mechanisms such as enhanced brood ball modifications. Our results provide partial support for this hypothesis by showing that larval niche construction in WA larvae, but not EUS larvae, fully compensates for compromised maternal niche construction. These results partially mirror recent findings in the burying beetle *Nicrophorus vespilloides* Herbst, 1783: varying the amount of parental care in experimentally evolving populations led to the evolution of larger mandibles in larvae that did not receive direct parental care (Jarrett et al., 2018). Though the study was not conducted in a niche construction framework, larger mandibles are thought to allow larvae to more effectively self-feed, potentially compensating for the lack of parental feeding. Together with our results, these findings raise the possibility that populations may adapt to local ecologies through changes in their reliance on one or more forms of niche construction, yielding potentially rapid divergences in the process.

5 | CONCLUSIONS

In this study, we have experimentally demonstrated that both larval and maternal niche construction can generate

environmental feedbacks that positively shape growth, development time, and survival in *O. taurus*. Furthermore, we have shown that these different forms of niche construction do not act in isolation, nor are they static features on *Onthophagus* development: together, both larval and maternal niche construction have the potential to synergistically influence developmental outcomes, and these effects may diverge heritably across recently established populations. Previous work documented that WA beetles bury their brood balls closer to the surface (Macagno et al., 2016), possibly as a consequence of the much higher above-ground competition for dung they face compared to their EUS counterparts. Here, we show that WA larvae lessen the impact of elevated temperature fluctuations resulting from shallow burial by their mothers through increased reliance on larval niche construction. Yet, important questions regarding the causes and consequences of niche construction in *Onthophagus* remain to be explored. For instance, recent work documents a partial divergence of microbiota between EUS, WA, and native *O. taurus* populations (Parker, Newton, & Moczek, 2020) raising the possibility that a corresponding divergence in microbial functions may underlie divergences in larval niche construction. Cross-fostering experiments (as in Parker et al., 2019; Parker & Moczek, in review) coupled with quantitative genetic breeding designs may permit quantification of the relative contributions of maternal, microbial, and host genetic effects to variation in fitness within and across populations.

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

and larval niche construction may have facilitated the diversification of dung beetles may be key to fully understanding the ecological and evolutionary success of dung beetles.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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