ECOLOGY LETTERS

Ecology Letters, (2017) doi: 10.1111/ele.12830

LETTER

Evidence of developmental niche construction in dung beetles: effects on growth, scaling and reproductive success

Daniel B. Schwab,* Sofia Casasa and Armin P. Moczek

Department of Biology, Indiana University, Bloomington, IN 47405, USA

**Correspondence: E-mail: schwabd@indiana.edu*

Abstract

Niche construction occurs when organisms modify their environments and alter selective conditions through their physiology and behaviours. Such modifications can bias phenotypic variation and enhance organism–environment fit. Yet few studies exist that experimentally assess the degree to which environmental modifications shape developmental and fitness outcomes, how their influences may differ among species and identify the underlying proximate mechanisms. Here, we experimentally eliminate environmental modifications from the developmental environment of *Onthophagus* dung beetles. We show that these modifications (1) differentially influence growth among species, (2) consistently shape scaling relationships in fitness-related traits, (3) are necessary for the maintenance of sexual dimorphism, (4) influence reproductive success among females of at least one species and (5) implicate larval cultivation of an external rumen as a possible mechanism for environmental modification. Our results present evidence that *Onthophagus* larvae engage in niche construction, and that this is a fundamental component of beetle development and fitness.

Keywords

developmental plasticity, developmental symbiosis, ecological inheritance, horned beetles, *Onthophagus*.

Ecology Letters (2017)

INTRODUCTION

Understanding how organisms come to complement their environments to maintain or enhance their fitness has been a long-standing focus of evolutionary biology. For many organisms, this complementarity is achieved by modifying their traits either developmentally through phenotypic plasticity or alternatively through adaptive evolution (Zimmer & Emlen 2013). At the same time, rather than adjusting their traits to fit their selective environment, many organisms can physiologically or behaviourally modify their selective environment to better suit their traits through the process of *niche construction* (Odling-Smee *et al*. 2003; Laland *et al*. 2015). Niche construction is operationally defined as occurring when two criteria are fulfilled: (1) an organism significantly modifies environmental conditions, and (2) these organism-mediated environmental modifications influence selection pressures on a recipient of niche construction (Matthews *et al*. 2014). This has the potential to lead to evolution through niche construction when a third criterion is met, namely (3) that there is a detectable genetic change in the recipient population that alters the relationship between phenotype distribution and fitness variation (Matthews *et al*. 2014).

Niche construction theory (NCT) is not the first conceptual framework to emphasise the environment-modifying abilities of organisms, nor the potential feedbacks to selective pressures that arise from such interactions: for instance, the environment-constructing abilities of corals and earthworms were highlighted at least as early as Darwin (1842, 1881), and diverse other cases have since been described for many taxa (documented in Odling-Smee *et al*. 2003). Further, alteration of selective conditions due to environment-modifying activities are central to, and have been explored in depth both empirically and theoretically in, diverse research programs, such as density-dependent selection, sexual selection and social- and co-evolutionary theory, all of which are predicated on an understanding of how interactions between individuals, potential mates, social groups and other species influence the outcomes of selection (Scott-Phillips *et al*. 2014). In addition, the field of eco-evolutionary feedbacks has begun to demonstrate how organismal (e.g. predator–prey) interactions produce feedback loops between ecological and evolutionary dynamics, generating a conceptual framework that is independent of, though broadly overlapping with, NCT (Kylafis & Loreau 2008; Post & Palkovacs 2009; Odling-Smee *et al*. 2013; Matthews *et al*. 2014). Where NCT diverges from other frameworks, however, is in its explicit emphasis on environment-modifying abilities as sources of individual phenotypic variation, as an alternate route to adaptation and as an avenue for non-genetic inheritance in those cases in which modified environments are passed on to subsequent generations. Here, we use NCT as a framework for explicitly exploring the phenomenon of *developmental niche construction*, and evaluate whether environment-modifying behaviours of dung beetle larvae fulfil the two criteria needed to qualify as niche constructors.

Developmental niche construction manifests in diverse forms across an array of taxa, but is most often expressed when organisms alter ontogenetic environments via chemical excretions or through the construction of physical structures such as dams, burrows or pupal cases. One common function of these modifications is to buffer developing organisms against otherwise challenging environmental conditions. For instance, gall-flies induce gall formation on plants, providing the fly with nutrition as well as protection from parasitoids and avian predators (Abrahamson *et al*. 1989). In addition, organisms may depend on the physiological properties of symbionts to generate suitable developmental and nutritional environments, as in larval woodwasps that obtain their sole source of dietary sterols from maternally provisioned fungi (Thompson *et al.* 2013). In each of these cases, environmental modifications become fundamental to ensuring the normal development of the organisms that exert them (Laland *et al.* 2008).

While developmental niche construction is therefore a common characteristic of organismal development and has the potential to affect evolutionary outcomes, substantial empirical work remains necessary, for the following reasons. First, while extensive work exists documenting the ecological and evolutionary significance of soil-modifying properties of plants and their microbial partners (reviewed in Putten *et al.* 2013; Pii *et al.* 2015), few animal model systems have been developed where the mechanisms of developmental niche construction, and the environmental variables being modified, are well understood and experimentally manipulable. Understanding these mechanisms may elucidate not only how organisms modify their ontogenetic environment, but also how these mechanisms may be selected upon to influence evolutionary trajectories (Odling-Smee *et al.* 2003). Second, despite the fact that the outcomes of development are often highly environmentally contingent, whether and how niche constructed environments reciprocally shape norms of reaction across organismal traits are poorly understood. Finally, while it is generally assumed that niche constructing traits and their associated impacts on development and fitness exhibit genetic variation, much of the empirical literature has focused on traits that are largely functionally invariant among populations or species, such as the construction of termite mounds or bird nests (Saltz & Nuzhdin 2014). Thus, little is known about if and how individuals, populations and species vary in niche constructing traits, and how this variation shapes phenotypic variation and fitness in natural environments.

Larval dung beetles in the genus *Onthophagus* present a promising model system for addressing the study of niche construction. *Onthophagus* larvae spend the entirety of their development within a maternally constructed, subterranean brood (i.e. dung) ball, containing all the nutrition available to them until adulthood. To complete their development, larvae must extract nutrients from this food source, which is low in nutrition and primarily composed of recalcitrant plant polysaccharides such as cellulose (Muller 1980). In addition, larvae contend with diverse abiotic (e.g. below-ground temperature fluctuations) and biotic (e.g. parasitic mites, entomopathogenic fungi) stressors (Kotiaho & Simmons 2001; Snell-Rood *et al.* 2016). At the same time, *Onthophagus* larvae engage in putative niche constructing behaviours throughout their development. For instance, larvae defecate within the brood ball, thereby distributing maternally inherited microbes throughout the brood ball environment, and then re-feed on their own excrement (Schwab *et al.* 2016). Further, larvae mechanically manipulate surrounding dung to alter the physical composition of the brood ball throughout their growth period, eventually resulting in the construction of a pupation chamber prior to the metamorphic moult. Yet, if and how these brood ball modifications affect developmental and fitness outcomes, as well as the degree to which these effects may have diversified among *Onthophagus* species, is presently unclear.

Here, we use a novel method for suppressing all known larval modifications to the brood ball environment to evaluate (1) whether larvae indeed engage in niche construction and (2) the extent to which environmental modifications are central to normal development across three *Onthophagus* species. We first test the prediction that these modifications enhance larval growth, larval survival and adult reproductive success. Next, we investigate whether larval modifications alter morphological scaling relationships, including the nature and degree of sexual dimorphism, in three environmentally responsive traits. Then, to evaluate if and to what extent the effect of environmental modifications on growth outcomes has diverged among closely related species, we compare their effect sizes among three *Onthophagus* species. Finally, to better characterise the known mechanisms by which larvae modify their brood ball environment, we generate a community-level physiological profile of the faecal microbiota that larvae spread throughout the brood ball, testing the prediction that this community is enriched for potentially beneficial microbes capable of metabolising recalcitrant, dung-associated carbon substrates (e.g. cellobiose). In combination, our study demonstrates that larval brood ball modifications are (1) a normal, fitness-determining component of dung beetle development necessary for (2) the maintenance and expression of sexual dimorphism, and whose (3) effects on growth and development are variable among species, thereby providing evidence that *Onthophagus* larvae engage in niche construction.

MATERIALS AND METHODS

Beetle husbandry and manipulation of brood ball modifications

Brood balls from *O. taurus*, *O. gazella* and *O. sagittarius* were generated as described previously (Shafiei *et al.* 2001; but also see supplementary materials and methods in the Supporting Information). Larvae were maintained within their natal brood ball for *c.* 24 h, allowing them to inoculate their guts with brood ball dung containing maternally inherited microbiota previously shown to be critical for larval growth and development (Schwab *et al.* 2016). To assess the significance, if any, of larval modifications to the brood ball environment, larvae were transferred into individual *artificial brood balls* (ABBs) provisioned with dung *ad libitum* within 12-well tissue culture plates and haphazardly assigned to one of two treatments. In one treatment, larval modifications to the ABB were eliminated periodically (i.e. NC[−]) by relocating larvae into a novel ABB of fresh dung every 48 h. This 48-h period was selected because it allowed ABB modifications to be disrupted without inducing the substantial stress of daily manipulations. In the second (control) treatment, larvae persisted in and modified the same ABB throughout development (i.e. NC[+]), allowing any modifications to accrue within the ABB throughout development. Importantly, all dung was homogenised, divided evenly into ABBs and frozen into 12-well plates prior to the experiment, ensuring that dung within and among treatment groups was nearly identical. To control for the potential stress of relocation in the NC[−] treatment, NC[+] larvae were removed from their ABB in parallel, but placed back after 3 s, approximating the time that it takes to transfer larvae across wells.

Additionally, we sought to control for the possible differential effects of dung age across our treatments. Specifically, if older dung would, for whatever reasons, be more easily digested by larvae, then NC[+] larvae would be predicted to outperform NC[−] larvae, yet this performance would be due to dung age rather than brood ball modifications. Thus, in order to validate that any positive phenotypic effects of the NC[+] treatment were not simply due to an enhanced larval performance on older dung, we haphazardly transferred day-old (as above) *O. taurus* larvae into 12-well plates containing dung that either (1) had been maintained at 24 °C for 8 days, or (2) was freshly prepared shortly before larval transfer, akin to our experimental treatments outlined above. In this additional experiment, individuals from each treatment were allowed to feed for 4 days, corresponding to the period before larvae are large enough to make substantial brood ball modifications, and weighed daily to assess growth responses.

Assessing the effect of larval brood ball modifications on adult reproductive success

To assess whether larval modifications influence reproductive success in the resulting adults, we reared larval female *O. gazella* to adulthood under NC[+] and NC[−] conditions. Upon eclosion, adult females were transferred to treatment-specific colonies containing males at a 1 male : 2 female ratio, and allowed to mate for 21 days. Adult females were then allowed to breed individually in cylindrical, light-impermeable containers (27 cm $H \times 7.2$ cm D) filled to a height of 20 cm with soil and provided ~ 200 g of homogenised cow manure (as in Macagno *et al.* 2015). Females were allowed to construct brood balls for 5 days, and at the end of this period all brood balls were collected, checked for offspring, counted to determine number of offspring produced and weighed to the closest 0.01 g using a Mettler Toledo (AL 54 Ohio, USA) scale to determine investment into each brood ball.

Community-level physiological profiling

To assess whether the brood ball environment may be modified by microbiota that are spread throughout the brood ball environment via larval defecation, we exposed sterilised (see Schwab *et al.* 2016 for details) ABBs to one of two treatments. In one treatment (i.e. Larva[+]), surface-sterilised 3rd (= final) instar *O. gazella* larvae were haphazardly assigned to ABBs ($N = 5$) and allowed to feed on and putatively modify their brood ball environment. In another treatment (i.e. Larva [−]), ABBs ($N = 5$) were not occupied by any larvae.

Following a 1-week incubation, we collected *c.* 0.1 g of the brood ball environment from each sample, homogenised and diluted these samples in phosphate-buffered saline and tested the ability of the microbial community to metabolise diverse carbon substrates *in vitro* using Ecoplates™ (Biolog: Hayward, CA, USA). Ecoplates™ contain independent aliquots of 31 of the most prevalent carbon substrates found in soil communities, including the reduced form of sugars (e.g. D-cellobiose, D-xylose, D-galacturonic acid, etc.) of many complex organic macromolecules common in dung, such as cellulose, hemicellulose, chitin, pectin and lignin (Muller 1980; see Table S1 for full list of Ecoplate™ substrates and supplementary materials for details on data analysis and interpretation).

Data collection and morphometrics

To assess the effects of brood ball modifications on growth outcomes, we collected the following developmental data: approximate larval peak mass (*O. taurus*: day 13; *O. gazella*, *O. sagittarius*: day 9), time to pupation (i.e. developmental rate), survival to adulthood and adult body size (measured as pronotum width, following Emlen 1994). To assess changes in morphological scaling relationships, we used a standard two-dimensional morphometric setup and ImageJ v1.44p software to measure the following traits: (1) eye area, a sexually monomorphic trait in *Onthophagus* that exhibits modest sensitivity to environmental conditions (Schwab & Moczek 2014, 2016); (2) the size of the foretibia, the main digging tool for tunnelling scarab beetles, which exhibits moderate (*O. taurus*, *O sagittarius*) to more pronounced (*O. gazella*) sexual dimorphism and (3) the length of head horns, a highly sexually dimorphic trait expressed in all three species studied here: in *O. taurus* and *O. gazella* only males express horns, and do so in a highly nutrition sensitive manner. In contrast, *O. sagittarius* males express only rudimentary horns, whereas females express greatly enlarged head horns. Details on measurements and statistical analyses can be found in the supplementary material.

RESULTS

Brood ball modifications differentially affect larval growth among species and reproductive success

We first sought to test whether brood ball modifications influenced growth, rate of development and mortality in larvae exposed to NC[+] and NC[−] conditions. In partial support of our predictions, NC[+] larvae had a higher peak mass during larval development than their NC[−] counterparts for *O. taurus* (24.7% larger) and *O. gazella* (16.3% larger), but not *O. sagittarius* (Fig. 1a, Table 1). These treatment effects persisted into adulthood (*O. taurus*: 13.6% larger, *O. gazella*: 2.3% larger), and did not differ among the sexes of each species. However, we recovered no effect on any species for both time to pupation (Fig. 1a, Table 1), and survival to adulthood (Table 1). At the same time, we were able to reject the alternative hypothesis that the positive growth effects of NC[+] conditions may be attributable to enhanced larval performance on older dung: newly hatched larvae, which are incapable of making major modifications to the brood ball environment, failed to grow more rapidly when reared on old dung in comparison with fresh dung (Fig. S1).

Next, we assessed for one of our three focal species whether environment-modifying behaviours engaged in during the larval stage affect the reproductive success of the resulting adults by measuring the number and mass of brood balls produced by adult female *O. gazella* that were reared as larvae under NC[+] or NC[−] conditions. We found that NC[+] females (5.6% larger than NC[−] in this experiment; $t = 4.15$,

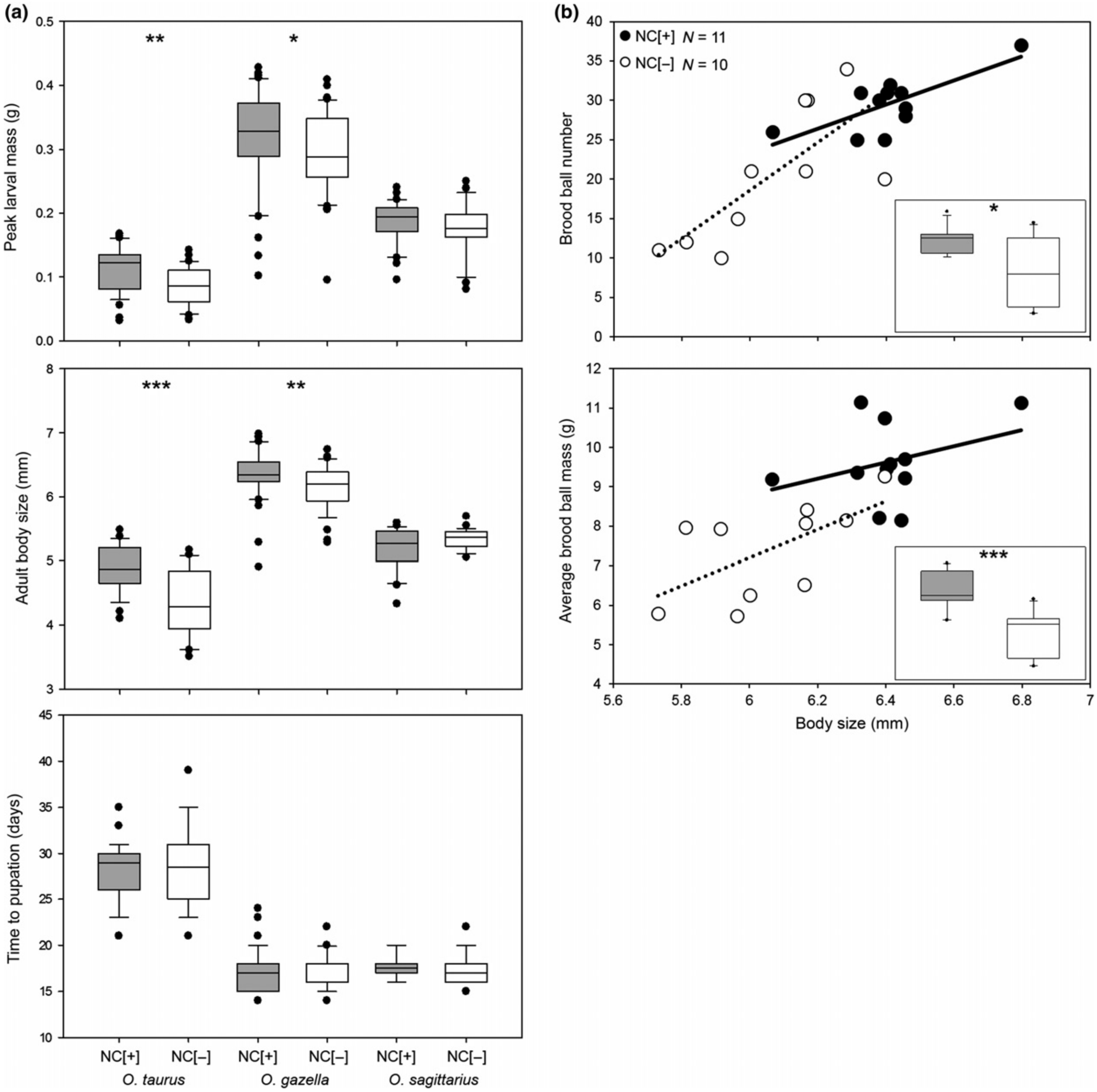


Figure 1 Developmental and fitness consequences of the experimental removal of larval niche construction (NC) behaviors. (a) Under NC[+] conditions, *Onthophagus taurus* and *O. gazella*, but not *O. sagittarius*, exhibited higher peak larval masses and adult body sizes relative to NC[−] individuals. There was no effect of treatment on time to pupation for any species. (b) Adult female *O. gazella* reared as larvae under NC[+] conditions produce more and larger brood balls relative to NC[−] individuals. Responses of NC[+] and NC[−] individuals are shown in dark grey and white boxes respectively. Asterisks indicate statistical significance ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Scatterplots include solid line of best fit for the NC[+] treatment and dotted lines for the NC[−] treatment.

$P < 0.001$) produced significantly more ($U = 21.00$, $P = 0.018$) and larger brood balls on average ($t = 4.50$, $P < 0.001$) than NC[−] females, and recovered a significant positive correlation between adult female body size and brood ball number ($R^2 = 0.982$, $P < 0.001$) and mass ($R^2 = 0.858$, $P < 0.001$) across both treatments (Fig. 1b). In combination, our results demonstrate that *Onthophagus* larvae modify their brood ball to a degree that has the potential to affect adult reproductive success, and that the phenotypic consequences of such modifications differ among species as well as traits.

Brood ball modifications differentially affect morphological scaling relationships among sexes and species

We next investigated the degree to which the growth and scaling of morphological traits are dependent on brood ball modifications. Specifically, we tested whether species that grew to larger body sizes under NC[+] conditions (i.e. *O. taurus*, *O. gazella*) also allocate relatively more resources to the growth of the foretibia, the eyes and the horns. Once again, we recovered partial support for our predictions.

Table 1 Mann–Whitney *U* tests, *t*-tests and chi-square tests comparing the effect of NC[+] and NC[−] treatments on peak larval mass, adult body size, time to pupation and survival to adulthood in *Onthophagus taurus*, *O. gazella* and *O. sagittarius*

	Peak larval mass – *O. taurus*				Adult body size – *O. taurus*				Time to pupation – *O. taurus*				Survival to adulthood – *O. taurus*			
	N	Median	*U*	*P*	*N*	Median	*U*	*P*	*N*	Mean	*T*	*P*	Survived	Died	χ^2	*P*
NC[+]	35	0.122	345.5	0.001	29	4.867	146.0	< 0.001	29	27.97	−0.971	0.336	29	11	1.944	0.163
NC[−]	36	0.0978			24	4.285			30	28.97			25	20		

	Peak larval mass – *O. gazella*				Adult body size – *O. gazella*				Time to pupation – *O. gazella*				Survival to adulthood – *O. gazella*			
	N	Median	*U*	*P*	*N*	Median	*U*	*P*	*N*	Median	*U*	*P*	Survived	Died	χ^2	*P*
NC[+]	40	0.329	583.0	0.025	40	6.345	474.0	0.007	40	17.0	723.0	0.455	40	7	0.079	0.778
NC[−]	41	0.283			37	6.201			40	18.0			39	8		

	Peak larval mass – *O. sagittarius*				Adult body size – *O. sagittarius*				Time to pupation – *O. sagittarius*				Survival to adulthood – *O. sagittarius*			
	N	Median	*U*	*P*	*N*	Mean	*U*	*P*	*N*	Median	*U*	*P*	Survived	Died	χ^2	*P*
NC[+]	31	0.194	367.0	0.053	26	5.274	271.0	0.109	28	17.5	381.0	0.687	27	9	0.077	0.781
NC[−]	33	0.175			28	5.370			29	17.0			28	8		

For nearly all species, peak larval mass, adult body size, time to pupation and differences were assessed using the Mann–Whitney *U* test. For *O. taurus*, time to pupation was assessed using a *t*-test. Indicated is the sample size (*N*), median (or mean) value of each treatment, the *U*-value (or *t*-value) of the test statistic and the *P*-value. For survival to adulthood, chi-square tests were used to assess differences in mortality among treatments. Included is the contingency table, the chi-square value and the *P*-value.

First, for foretibiae, we found that male *O. taurus* and *O. gazella* invested significantly more into this trait under NC[+] conditions, whereas male *O. sagittarius* unexpectedly invested more under NC[−] conditions (Fig. 2, Table S2). In addition, we found that female *O. taurus* responded similarly to males, investing significantly more into the foretibia under NC[+] conditions, whereas females of *O. gazella* and *O. sagittarius* exhibited significantly higher slopes under NC[−] (Fig. 2, Table S2). Second, for eyes, we found that male *O. taurus* and *O. sagittarius*, but not *O. gazella*, exhibited similar responses as in the foretibiae, investing significantly more into eyes under NC[+] and NC[−] conditions respectively (Fig. 2, Table S2). In contrast, for female *O. gazella* and *O. sagittarius*, but not *O. taurus*, NC[+] beetles exhibited significantly steeper slopes (Fig. 2, Table S2). Finally, for horns, we found that residual horn sizes were significantly larger for NC[+] *O. taurus* but not *O. gazella* in comparison with NC[−] beetles (Fig. S2, Table S2). We additionally found no effect of treatment on horn investment for female *O. sagittarius* (Fig. S2, Table S2). Collectively, these results demonstrate that brood ball modifications influence not only adult body size, but also exert subtle but significant effects on the scaling of morphological traits.

Brood ball modification alters the nature and degree of sexual dimorphism

We next investigated whether the effects of larval brood ball modification extended beyond scaling relationships within sexes to also alter the nature (i.e. presence/absence) and degree of sexual dimorphism. We focused primarily on the foretibiae, because this trait is both sexually dimorphic (unlike eyes) and present in both sexes (unlike horns). Under NC[+] conditions, we recovered the well-established sexual dimorphisms in foretibia size for all species, which manifested in a significant difference in intercept between male and female *O. taurus* (3.0–5.6% longer in males) and *O. sagittarius* (2.2–3.4% longer in females), and a significant difference in slope for *O. gazella* (Fig. 3, Table 2). In addition to the difference in slope, the foretibiae of male *O. gazella* were overall substantially longer (14.5–18.5%) than those of size-matched females from across the female body size range. Under NC[−] conditions, sexual dimorphisms in tibial size were eliminated for *O. taurus* and *O. sagittarius* but not *O. gazella* (Fig. 3, Table 2). Instead, in this species the degree of tibial sexual dimorphism was lessened, with male foretibiae only 12.4–13.6% larger than those of size-matched females from across the female body size range. Therefore, modifications to the brood ball environment appear critical for promoting and maintaining sexual dimorphisms that arise during normal development in *Onthophagus*.

Onthophagus faecal microbiota utilise diverse carbon substrates within dung

Finally, we sought to identify potential mechanisms by which larvae may modify the brood ball environment to enhance developmental outcomes. Given that larvae have been observed to defecate throughout the brood ball environment, we hypothesised that larval defecation may enable faecal microbiota to colonise brood ball dung and aid larval digestion by externally breaking down the complex macromolecules that are present in dung, akin to the usage of an external rumen documented for other insect taxa (e.g. Thompson *et al.* 2013). Specifically, we predicted that dung microbial communities cultivated through the presence of a larva (Larva[+]

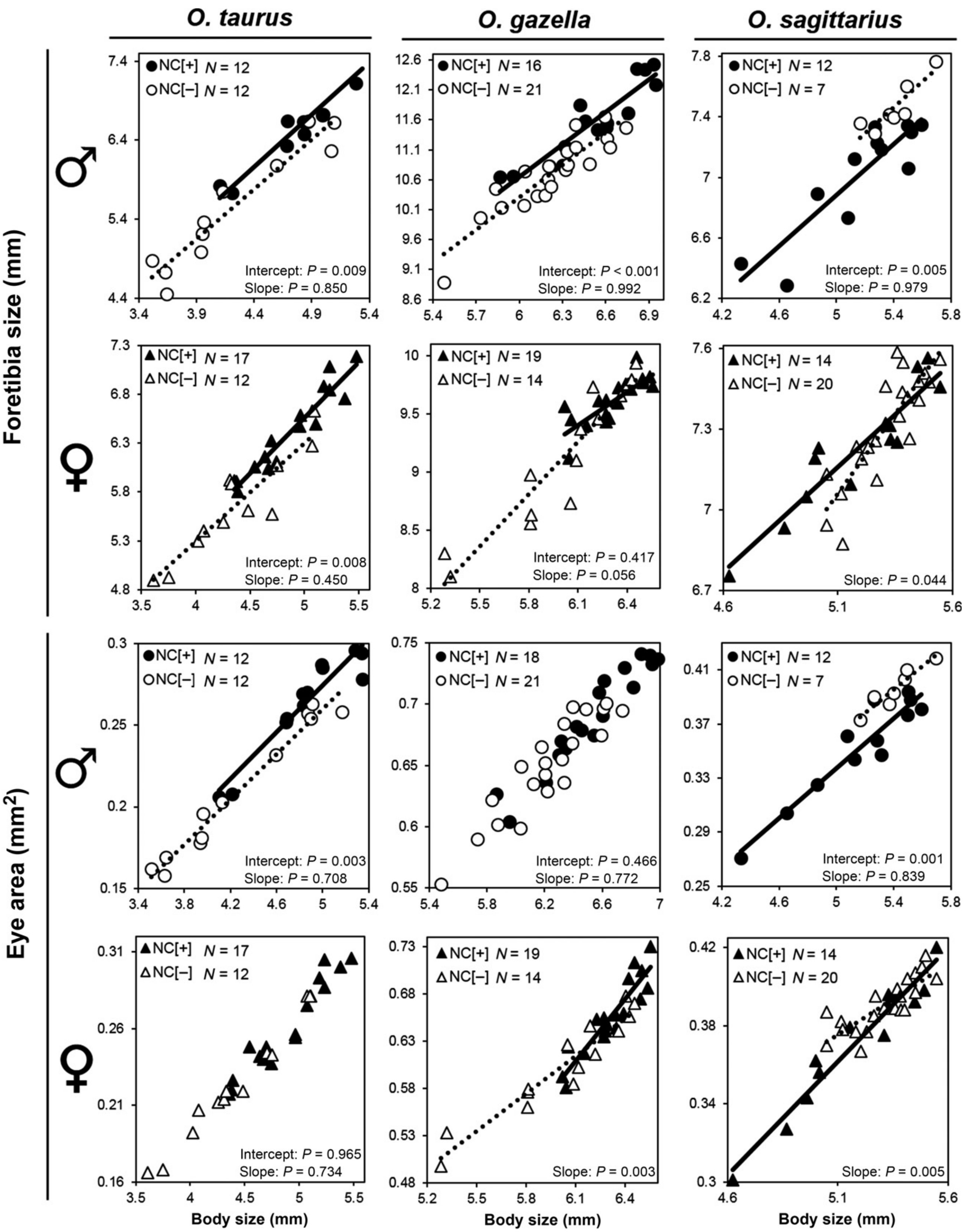


Figure 2 Scaling relationship between body size (*x*-axis) and foretibia and eye size (*y*-axis) for male and female *Onthophagus taurus*, *O. gazella* and *O. sagittarius*. Rearing under NC[−] conditions tends to decrease the intercept (but see *O. sagittarius* males for both traits) or alter the slope of the allometry relative to NC[+]. Graphs include line of best fit (solid for NC[+], dotted for NC[−]) when significant main effects of treatment or interactions exist.

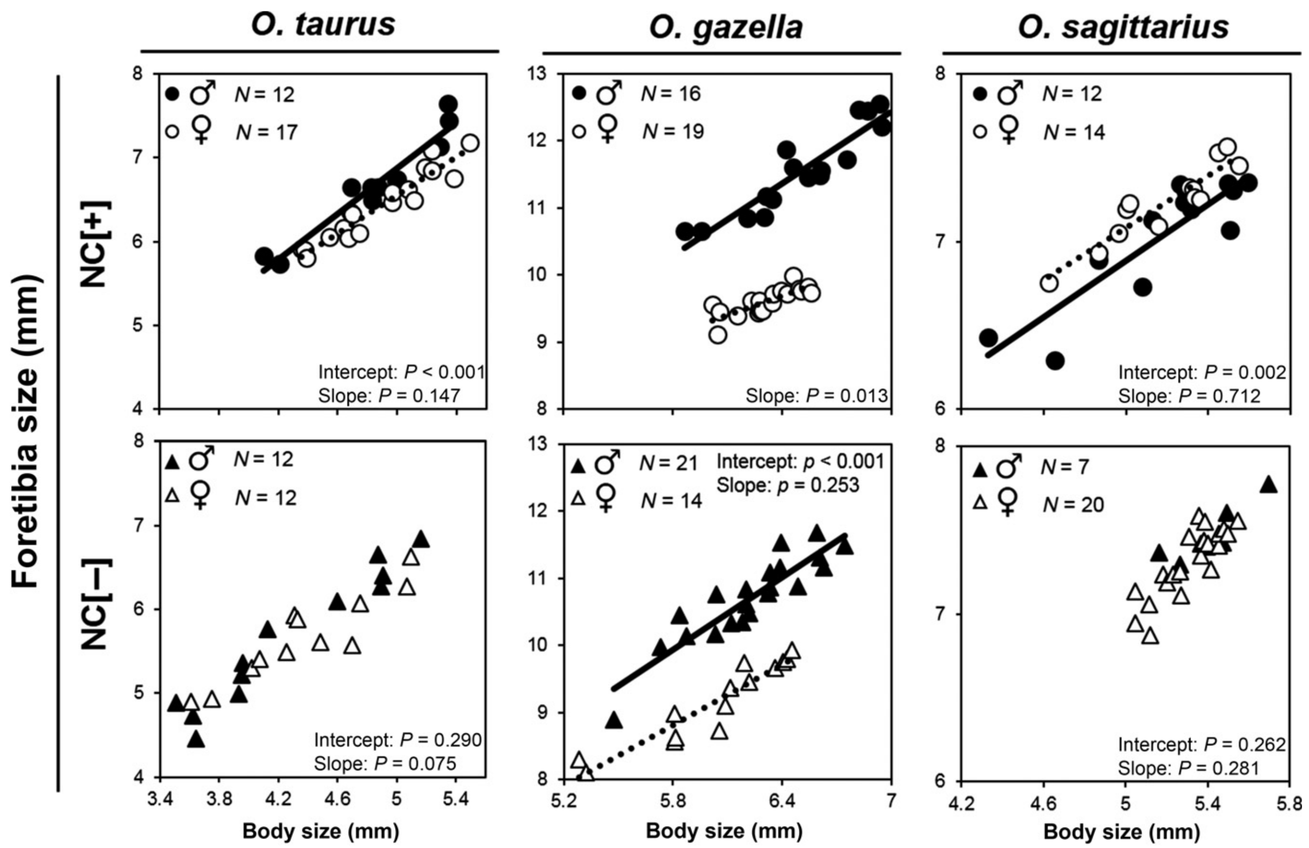


Figure 3 The relationship between brood ball modification and sexual dimorphism in foretibia size. Rearing under NC[+] conditions maintains the characteristic sexual dimorphisms in foretibia size of *Onthophagus taurus*, *O. gazella* and *O. sagittarius*. Conversely, rearing under NC[−] conditions eliminates (*O. taurus*, *O. sagittarius*) or significantly reduces the degree (*O. gazella*) of sexual dimorphism. Graphs include line of best fit (solid for NC[+], dotted for NC[−]) when significant main effects of treatment or interactions exist.

treatment) would (1) utilise a greater number of carbon substrates than Larva[−] communities, and that these substrates would be (2) utilised more intensively and (3) enriched for substrates that are components of plant and fungal cell walls, such as cellobiose (found in cellulose), D-galacturonic acid (found in pectin) and *N*-acetyl-D-glucosamine (found in chitin).

Under Larva[+] conditions, we found that brood ball microbiota significantly utilised 10 substrates relative to the water only internal control. Among these, the most highly utilised substrates included those we hypothesised would be more intensively used, including the carbohydrates D-cellobiose, D-galacturonic acid and *N*-acetyl-D-glucosamine. Also highly significantly utilised was D-galactonic acid γ-lactone, a metabolite of the monosaccharide sugar galactose, as well as carboxylic acids including D-glucosaminic acid (Fig. 4, see Table S1 for full list of substrates and significance values). Conversely, under Larva[−] conditions, the remnant dung microbial community significantly utilised only D-galactonic acid γ-lactone and the amino acid L-asparagine; however, both of these substrates were utilised significantly more under Larva[+] conditions (D-galactonic acid γ-lactone: $t = 5.64$, $P < 0.001$, L-asparagine: $U = 0.0$, $P = 0.008$, Fig. 4, Table S1). Therefore, our preliminary analysis of the metabolic properties of the brood ball microbiota suggests that these microbes may break down the plant and fungal cell wall polysaccharides that comprise the majority of dung biomass (Muller 1980).

DISCUSSION

Biologists have long been fascinated by the diverse routes through which organisms engage in – and are affected by – modifications to their physical and social environments. From the construction of dams, nests, burrows and webs by diverse organisms, to the creation of superorganisms by social insects, to the farms and cities created by humans, organisms display profound abilities to modify environments and alter the selective conditions that they themselves are exposed to, thereby shaping the evolutionary trajectories of the lineages they belong to (Odling-Smee *et al*. 2003; Kendal *et al*. 2011). Further, each of these and similar phenomena has inspired partly independent but often broadly overlapping conceptual frameworks, including co-evolutionary, sexual selection and parental effects theory (e.g. Trivers 1972; Mousseau & Fox 1998), the frameworks of eco-evolutionary feedbacks and ecosystem engineering (Jones *et al*. 1994; Post & Palkovacs 2009), and the concept of the extended phenotype (Dawkins 1982; Matthews *et al*. 2014). All of these frameworks are further fully congruent with NCT, a framework that has engendered at times passionate debate about its novelty and distinctiveness

Table 2 Analysis of covariance comparing male and female foretibia size in *Onthophagus taurus*, *O. gazella* and *O. sagittarius*

	Foretibia size – NC[+] *O. taurus*				Foretibia size – NC[−] *O. taurus*			
	Estimate	SE	*T*	*P*	Estimate	SE	*T*	*P*
Intercept	0.372	0.371	1.004	0.325	0.417	0.394	1.057	0.303
Body size	1.240	0.076	16.41	< 0.001	1.200	0.089	13.49	< 0.001
Sex	0.286	0.056	5.126	< 0.001	0.099	0.091	1.086	0.290
Body size × Sex	0.222	0.148	1.495	0.147	0.323	0.172	1.879	0.075

	Foretibia size – NC[+] *O. gazella*				Foretibia size – NC[−] *O. gazella*			
	Estimate	SE	*T*	*P*	Estimate	SE	*T*	*P*
Intercept	3.556	1.768	2.011	0.053	−0.867	0.789	−1.099	0.280
Body size	0.958	0.280	3.42	0.001	1.662	0.130	12.75	< 0.001
Sex	−3.734	2.031	−1.838	0.076	1.226	0.092	13.26	< 0.001
Body size × Sex	0.846	0.319	2.648	0.013	0.302	0.260	1.166	0.253

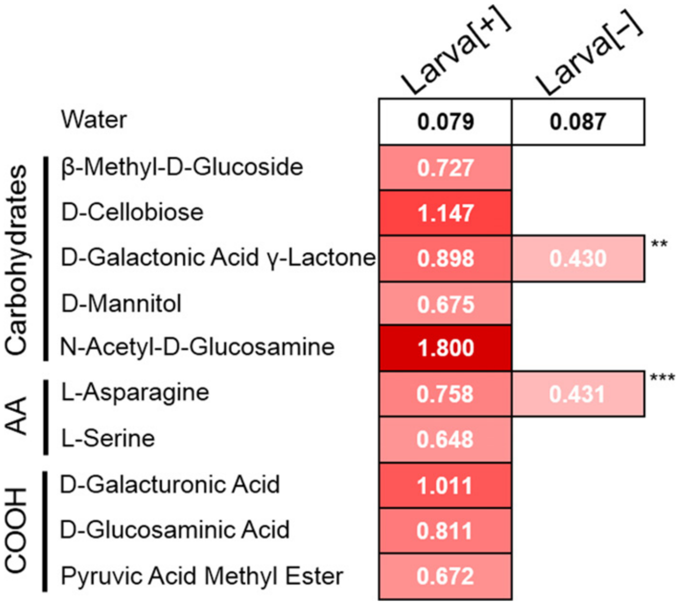
	Foretibia size – NC[+] *O. sagittarius*				Foretibia size – NC[−] *O. sagittarius*			
	Estimate	SE	*T*	*P*	Estimate	SE	*T*	*P*
Intercept	2.977	0.431	6.904	< 0.001	1.476	0.730	2.023	0.054
Body size	0.819	0.082	9.890	< 0.001	1.098	0.137	8.004	< 0.001
Sex	−0.186	0.052	−3.581	0.002	0.055	0.048	1.148	0.262
Body size × Sex	0.066	0.176	0.374	0.712	−0.345	0.300	−1.152	0.261

Sex was treated as a fixed effect and body size as a covariate. Indicated are the parameter estimate, the standard error, the *t*-value of the test statistic and the *P*-value for each factor and interaction in the model. Where interaction terms are non-significant, values for the main effects and intercept are derived from a model with the interaction term removed.

relative to the concepts and frameworks listed above (Laland *et al.* 2014; Scott-Phillips *et al.* 2014). Ongoing debates notwithstanding, we place our study and findings in the context of NCT in general, and *developmental* niche construction in particular, because of NCTs specific emphasis on environment-modifying behaviours as a potential source of phenotypic variation and an alternate route to adaptation and inheritance. We did so by investigating the environment-modifying abilities of dung beetle larvae to assess their phenotypic and fitness consequences, as well as a potential proximate mechanism mediating these effects. We chose this focus because although the ecological and evolutionary implications of niche construction may be striking, many critical assumptions and predictions inherent in this framework remain to be broadly evaluated, due in large part to the paucity of study systems in which environment-modifying behaviours and their products can be experimentally manipulated and their effects on trait variation and fitness assessed (Matthews *et al.* 2014).

Previous observations established that *Onthophagus* larvae substantially modify the physical structure of their natal brood ball throughout their development, qualifying these beetles as candidate niche constructors by fulfilling the first criterion for niche construction (see above; Estes *et al.* 2013). Although we failed to detect any effect of these modifications on time or survival to adulthood, we recovered significant effects on adult body size. Specifically, in *O. taurus* and *O. gazella*, we found that NC[−] treated animals grew to smaller sizes than those reared under NC[+]. In numerous insect species, even marginally smaller adult body sizes are associated with reduced reproductive success (e.g. lower fecundity; Brown *et al.* 1993; Kingsolver & Huey 2008). Indeed, previous studies in *Onthophagus* have demonstrated significant positive relationships between (1) maternal body size and the size of brood masses, which heavily influence offspring size (Hunt & Simmons 2000); (2) maternal body size and offspring number (Hunt & Simmons 2002) and (3) male body size and the probability of winning fights and remaining with females during oviposition (Moczek & Emlen 2000). Here, we extended these observations to the environmental modifications of larvae, demonstrating that larger *O. gazella* females from the NC[+] treatment produce more offspring and invest more in each brood ball than NC[−] females, two key metrics of reproductive success. Our results are thus at least partially consistent with the second criterion for niche construction (see above), and suggest that environment modification may be an adaptive component of dung beetle development that enhances developmental and fitness outcomes. Our results further raise the question as to whether and which niche constructing behaviours also qualify as extended phenotypes, that is, constitute genetic adaptations expressed outside the body of individuals (Laland *et al.* 2016), or generate ecosystem engineering effects by contributing unique modifications to soil ecosystems.

Phenotypic plasticity, the ability of organisms to adjust their phenotype in response to their environment, is increasingly understood to be a characteristic feature of organismal traits and development more generally (Gilbert & Epel 2015; Sultan 2015). One key hypothesis of NCT is that environment-modifying organisms are developmentally responsive to the environments that they, themselves, have generated (Saltz & Nuzhdin 2014). We tested this hypothesis by assessing



		Larva[+]	Larva[–]	
	Water	0.079	0.087	
Carbohydrates	β-Methyl-D-Glucoside	0.727		
	D-Cellobiose	1.147		
	D-Galactonic Acid γ-Lactone	0.898	0.430	**
	D-Mannitol	0.675		
	N-Acetyl-D-Glucosamine	1.800		
AA	L-Asparagine	0.758	0.431	***
	L-Serine	0.648		
COOH	D-Galacturonic Acid	1.011		
	D-Glucosaminic Acid	0.811		
	Pyruvic Acid Methyl Ester	0.672		

Figure 4 Community-level physiological profiling on dung microbial communities incubated with (Larva[+]) and without (Larva[−]) larvae. Under Larva[+] conditions, the dung microbial community established via larval NC behaviours significantly utilises 10 carbon substrates, including five unique carbohydrates, two amino acids (i.e. AA) and three carboxylic acids (COOH). Conversely, under Larva[−] conditions, the dung microbial community significantly utilises only two of these substrates, and to a significantly lesser degree. Numbers within boxes represent the median spectrophotomer reading of the five samples for each substrate; darker red indicates higher median reading. Asterisks indicate statistically significant differences *among* treatments for those substrates found to be significantly utilised *within* treatments. See Table S1 for a full list of substrates and significance values (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

whether and to what extent the scaling relationships of adult foretibiae, eyes and horns are altered in the presence or absence of larval brood ball modifications. For each trait and in both sexes, we generally found that rearing under NC[−] conditions either altered allometric slope or broadly decreased trait investment, with the sole exception of male *O. sagittarius* which, unexpectedly, exhibited *increased* investment under NC[−] conditions. Although the causes underlying this difference are presently unclear, one possibility is that brood ball modification may affect trait investment in a similar manner across species in nature, but that our laboratory conditions were too benign to observe this pattern for *O. sagittarius* alone.

We used the same approaches to assess whether brood ball modification influences the nature and degree of sexual dimorphism, with particular focus on the sexually dimorphic foretibiae, used as the primary digging tool in tunnelling scarabs (Macagno *et al.* 2016) as well as in mating behaviours (Cook 1990). We found that sexual dimorphism in foretibiae was either eliminated or severely reduced in all three species of *Onthophagus* under NC[−] conditions. Sexual dimorphisms, including those of *Onthophagus* beetles, have long been known to be highly condition dependent (Moczek 2006; Bonduriansky 2007). While the transcriptomic (Kijimoto *et al.* 2014; Ledón-Rettig & Moczek 2016), cellular (Moczek 2006; Kijimoto *et al.* 2010) and endocrine factors (Emlen *et al.* 2012; Gotoh *et al.* 2014) that underlie condition-dependent sexual dimorphism in beetles are increasingly well understood, our study is among the first to demonstrate the contingency of sexual dimorphism on modifications to the external environment by larvae.

Organisms' modifications to their environments manifest in remarkably diverse ways, from the webs of spiders to the nests of social hymenoptera and birds to the wetlands constructed by beavers (Odling-Smee *et al.* 2003). Such diversity may not be unexpected, however, given the enormous taxonomic distances between these organisms. Much less is known, however, about the degree to which the developmental and fitness *consequences*, and not simply the products, of environment-modifying behaviours diversify among closely related taxa. In this study, the effect of brood ball modifications on body size and scaling relationships was unexpectedly variable among all three *Onthophagus* species. Specifically, the phenotypic response to rearing under NC[−] conditions was substantial for *O. taurus*, yet only slightly apparent in the scaling relationships of *O. sagittarius*, even though both species shared a common ancestor as little as 5 MYA (Emlen *et al.* 2005). In contrast, the basal *O. gazella* (separation time ~ 40 MYA) demonstrated intermediate responsiveness. These results raise the possibility that at least moderate brood ball modification may be an ancestral feature of *Onthophagus* development that was secondarily elaborated in *O. taurus* but lost in *O. sagittarius*. If correct, this raises questions as to whether such interspecific differences are consistent across replicate populations, whether these differences are underlain by genetic variation that may arise at the population level and what the selective conditions are that shape this variation in nature.

For many species of insects that persist on nutritionally incomplete, plant-based diets that are similar to dung, symbiotic gut microbes have been shown to provision their hosts with essential nutrients and contribute to the degradation of polysaccharides, thereby facilitating host growth and development (Douglas 2009). However, such processes need not occur exclusively in the gut, but can occur outside of the organism as part of an external rumen such as the fungus gardens of termites and woodwasps (Swift *et al.* 1979; Thompson *et al.* 2013). In this study, we hypothesised that larvae establish an external rumen by spreading maternally provisioned microbiota throughout the brood ball environment via defecation. In support of this hypothesis, we found that larva-modified brood balls were highly significantly enriched for bacterial communities capable of utilising oligosaccharide components of plant and fungal cell walls relative to a sterile control. However, we stress that additional studies are needed to address key questions regarding the external rumen hypothesis and its role in larval niche construction. First, our *in vitro* assay may not fully reflect processes occurring *in vivo*, and future studies should investigate the specific microbiota added to the brood ball environment by larvae, and how these microbes and their metabolites differ from those found in the larval gut and in dung. Second, results demonstrating that these communities significantly metabolise substrates such as D-cellobiose should not necessarily be extrapolated to larger polymers such as cellulose, since the enzymes required for the hydrolysis of these molecules differ. Finally, external microbial processes are capable of providing other benefits for their hosts (e.g. protection from pathogens) beyond those assessed here.

In this study, we have experimentally shown that larval brood ball modifications are capable of generating environmental feedbacks that shape growth, scaling relationships, sexual dimorphism and reproductive success, and have attempted to evaluate the extent to which dung beetles qualify as niche constructors. We stress that, although our results are consistent with the first two criteria for niche construction (see Introduction), much work remains to be done. For instance, while it is clear that dung beetle larvae substantially modify their physical environment (criterion 1), the function of each behaviour and modification is presently poorly characterised. Furthermore, although the presence of these modifications bears positive developmental and fitness consequences (criterion 2), measures of selection on individual traits are lacking. Despite these limitations, we suggest that dung beetles provide fertile ground for assessing assumptions and predictions of NCT, and present promising opportunities for exploring the potential role of niche construction in the ecological radiation of dung beetles.

ACKNOWLEDGEMENTS

We thank Tim Coulson and three anonymous reviewers for constructive comments that substantially improved the manuscript. Bonnie Dietrich and Bruno Buzatto collected beetles for this study, and Irene Newton provided critical support and advice on Biolog Ecoplate™ usage, data analysis and helpful comments on earlier drafts. This study was carried out while DBS was supported by a National Science Foundation Graduate Research Fellowship. Additional support was provided by the National Science Foundation IOS grants 1256689 and 1120209 to APM and through a grant from the John Templeton Foundation. The opinions expressed in this publication are those of the authors and do not necessarily reflect the views of the National Science Foundation or the John Templeton Foundation.

AUTHOR CONTRIBUTIONS

DBS, SC and APM designed the research; DBS and SC performed the research; DBS performed all statistical analyses; DBS, SC and APM wrote the article.

DATA ACCESSIBILITY STATEMENT

All data are available from the Dryad Digital Repository: https://doi.org/http://dx.doi.org/10.5061/dryad.nn1rk.

REFERENCES

Abrahamson, W.G., Sattler, J.F., McCrea, K.D. & Weis, A.E. (1989). Variation in selection pressures on the goldenrod gall fly and the competitive interactions of its natural enemies. *Oecologia*, 79, 15–22.

Bonduriansky, R. (2007). The evolution of condition-dependent sexual dimorphism. *Am. Nat.*, 169, 9–19.

Brown, J.H., Marquet, P.A. & Taper, M.L. (1993). Evolution of body size: consequences of an energetic definition of fitness. *Am. Nat.*, 142, 573–584.

Cook, D.F. (1990). Differences in courtship, mating and postcopulatory behaviour between male morphs of the dung beetle *Onthophagus binodis* Thunberg (Coleoptera: Scarabaeidae). *Anim. Behav.*, 40, 428–436.

Darwin, C. (1842). *The Structure and Distribution of Coral Reefs*. Reprinted in 2013. Cambridge University Press, London: UK.

Darwin, C. (1881). *The Formation of Vegetable Mould, Through the Action of Worms, with Observations of Their Habits*. Murray, London.

Dawkins, R. (1982). *The Extended Phenotype*. Oxford University Press, Oxford: UK.

Douglas, A.E. (2009). The microbial dimension in insect nutritional ecology. *Funct. Ecol.*, 23, 38–47.

Emlen, D.J. (1994). Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proc. R. Soc. Lond. B Biol. Sci.*, 256, 131–136.

Emlen, D.J., Marangelo, J., Ball, B. & Cunningham, C.W. (2005). Diversity in the weapons of sexual selection: horn evolution in the beetle genus *Onthophagus* (Coleoptera: Scarabaeidae). *Evolution*, 59, 1060–1084.

Emlen, D.J., Warren, I.A., Johns, A., Dworkin, I. & Lavine, L.C. (2012). A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science*, 337, 860–864.

Estes, A.M., Hearn, D.J., Snell-Rood, E.C., Feindler, M., Feeser, K., Abebe, T. *et al.* (2013). Brood ball-mediated transmission of microbiome members in the dung beetle, *Onthophagus taurus* (Coleoptera: Scarabaeidae). *PLoS ONE*, 8, e79061.

Gilbert, S.F. & Epel, D. (2015). *Ecological Developmental Biology: The Environmental Regulation of Development, Health, and Evolution*. Sinauer Associates, Incorporated, Sunderland: MA.

Gotoh, H., Miyakawa, H., Ishikawa, A., Ishikawa, Y., Sugime, Y., Emlen, D.J. *et al.* (2014). Developmental link between sex and nutrition; *doublesex* regulates sex-specific mandible growth via juvenile hormone signaling in stag beetles. *PLoS Genet.*, 10, e1004098.

Hunt, J. & Simmons, L.W. (2000). Maternal and paternal effects on offspring phenotype in the dung beetle *Onthophagus taurus*. *Evolution*, 54, 936–941.

Hunt, J. & Simmons, L.W. (2002). The genetics of maternal care: direct and indirect genetic effects on phenotype in the dung beetle *Onthophagus taurus*. *Proc. Natl Acad. Sci.*, 99, 6828–6832.

Jones, C.G., Lawton, J.H. & Shachak, M. (1994). Organisms as ecosystem engineers. In: *Ecosystem Management* (ed Samson, F.B. and Knopf, F.L.). Springer, New York, NY, pp. 130–147.

Kendal, J., Tehrani, J.J. & Odling-Smee, J. (2011). Human niche construction in interdisciplinary focus. *Philos. Trans. R. Soc. B: Biol. Sci.*, 366, 785–792.

Kijimoto, T., Andrews, J. & Moczek, A.P. (2010). Programed cell death shapes the expression of horns within and between species of horned beetles. *Evol. Dev.*, 12, 449–458.

Kijimoto, T., Snell-Rood, E.C., Pespeni, M.H., Rocha, G., Kafadar, K. & Moczek, A.P. (2014). The nutritionally responsive transcriptome of the polyphenic beetle *Onthophagus taurus* and the importance of sexual dimorphism and body region. *Proc. R. Soc. Lond. B Biol. Sci.*, 281, 20142084.

Kingsolver, J.G. & Huey, R.B. (2008). Size, temperature, and fitness: three rules. *Evol. Ecol. Res.*, 10, 251–268.

Kotiaho, J.S. & Simmons, L.W. (2001). Effects of *Macrocheles* mites on longevity of males of the dimorphic dung beetle *Onthophagus binodis*. *J. Zool.*, 254, 441–445.

Kylafis, G. & Loreau, M. (2008). Ecological and evolutionary consequences of niche construction for its agent. *Ecol. Lett.*, 11, 1072–1081.

Laland, K.N., Odling-Smee, J. & Gilbert, S.F. (2008). EvoDevo and niche construction: building bridges. *J. Exp. Zool. B Mol. Dev. Evol.*, 310, 549–566.

Laland, K., Wray, G.A. & Hoekstra, H.E. (2014). Does evolutionary theory need a rethink? *Nature*, 514, 161.

Laland, K.N., Uller, T., Feldman, M.W., Sterelny, K., Müller, G.B., Moczek, A. *et al.* (2015). The extended evolutionary synthesis: its structure, assumptions and predictions. *Proc. R. Soc. B*, 282, 2015 1019.

Laland, K., Matthews, B. & Feldman, M.W. (2016). An introduction to niche construction theory. *Evol. Ecol.*, 30, 191–202.

Ledón-Rettig, C.C. & Moczek, A.P. (2016). The transcriptomic basis of tissue-and nutrition-dependent sexual dimorphism in the beetle *Onthophagus taurus*. *Ecol. Evol.*, 6, 1601–1613.

Macagno, A.L.M., Beckers, M. & Moczek, A.P. (2015). Differentiation of early ovarian development and the evolution of fecundity in rapidly diverging exotic beetle populations. *J. Exp. Zool. A Ecol. Genet. Physiol.*, 323, 679–688.

Macagno, A.L.M., Moczek, A.P. & Pizzo, A. (2016). Rapid divergence of nesting depth and digging appendages among tunneling dung beetle populations and species. *Am. Nat.*, 187, E143–E151.

Matthews, B., De Meester, L., Jones, C.G., Ibelings, B.W., Bouma, T.J., Nuutinen, V. *et al.* (2014). Under niche construction: an operational bridge between ecology, evolution, and ecosystem science. *Ecol. Monogr.*, 84, 245–263.

Moczek, A.P. (2006). Pupal remodeling and the development and evolution of sexual dimorphism in horned beetles. *Am. Nat.*, 168, 711–729.

Moczek, A.P. & Emlen, D.J. (2000). Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do alternative reproductive tactics favour alternative phenotypes? *Anim. Behav.*, 59, 459–466.

Mousseau, T.A. & Fox, C.W. (1998). The adaptive significance of maternal effects. *Trends Ecol. Evol.*, 13, 403–407.

Muller, Z.O. (1980). Feed from animal wastes: state of knowledge. *FAO Anim. Prod. Health*, 18, 190.

Odling-Smee, F.J., Laland, K.N. & Feldman, M.W. (2003). *Niche Construction: The Neglected Process in Evolution*. Princeton University Press, Princeton: NJ.

Odling-Smee, J., Erwin, D.H., Palkovacs, E.P., Feldman, M.W. & Laland, K.N. (2013). Niche construction theory: a practical guide for ecologists. *Q. Rev. Biol.*, 88, 3–28.

Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S. & Crecchio, C. (2015). Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biol. Fertil. Soils*, 51, 403–415.

Post, D.M. & Palkovacs, E.P. (2009). Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 364, 1629–1640.

Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T. *et al.* (2013). Plant–soil feedbacks: the past, the present and future challenges. *J. Ecol.*, 101, 265–276.

Saltz, J.B. & Nuzhdin, S.V. (2014). Genetic variation in niche construction: implications for development and evolutionary genetics. *Trends Ecol. Evol.*, 29, 8–14.

Schwab, D.B. & Moczek, A.P. (2014). Resource allocation during ontogeny is influenced by genetic, developmental and ecological factors in the horned beetle, *Onthophagus taurus*. *Proc. R. Soc. Lond. B Biol. Sci.*, 281, 20141625.

Schwab, D.B. & Moczek, A.P. (2016). Nutrient stress during ontogeny alters patterns of resource allocation in two species of horned beetles. *J. Exp. Zool. Part A Ecol. Genet. Physiol.*, 325, 481–490.

Schwab, D.B., Riggs, H.E., Newton, I.L. & Moczek, A.P. (2016). Developmental and ecological benefits of the maternally transmitted microbiota in a dung beetle. *Am. Nat.*, 188, 679–692.

Scott-Phillips, T.C., Laland, K.N., Shuker, D.M., Dickins, T.E. & West, S.A. (2014). The niche construction perspective: a critical appraisal. *Evolution*, 68, 1231–1243.

Shafiei, M., Moczek, A.P. & Nijhout, H.F. (2001). Food availability controls the onset of metamorphosis in the dung beetle *Onthophagus taurus* (Coleoptera:Scarabaeidae). *Physiol. Entomol.*, 26, 173–180.

Snell-Rood, E.C., Burger, M., Hutton, Q. & Moczek, A.P. (2016). Effects of parental care on the accumulation and release of cryptic genetic variation: review of mechanisms and a case study of dung beetles. *Evol. Ecol.*, 30, 251–265.

Sultan, S.E. (2015). *Organism and Environment: Ecological Development, Niche Construction, and Adaptation*. Oxford University Press, New York: NY.

Swift, M.J., Heal, O.W. & Anderson, J.M. (1979). *Decomposition in Terrestrial Ecosystems* (Vol. 5). University of California Press, Berkeley: CA.

Thompson, B.M., Grebenok, R.J., Behmer, S.T. & Gruner, D.S. (2013). Microbial symbionts shape the sterol profile of the xylem-feeding woodwasp, *Sirex noctilio*. *J. Chem. Ecol.*, 39, 129–139.

Trivers, R.L. (1972). Parental investment and sexual selection. In B. Campbell (Ed.), *Sexual selection and the descent of man: The Darwinian pivot*. (pp. 136–179). Chicago, IL,Aldine.

Zimmer, C. & Emlen, D.J. (2013). *Evolution: Making Sense of Life*. Roberts, Greenwood Village, CO.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Editor, Tim Coulson
Manuscript received 20 July 2017
First decision made 24 July 2017
Manuscript accepted 1 August 2017