

Plasticity, symbionts and niche construction interact in shaping dung beetle development and evolution

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ABSTRACT

Developmental plasticity is an important product of evolutionary processes, allowing organisms to maintain high fitness in the face of environmental perturbations. Once evolved, plasticity also has the potential to influence subsequent evolutionary outcomes, for example, by shaping phenotypic variation visible to selection and facilitating the emergence of novel trait variants. Furthermore, organisms may not just respond to environmental conditions through plasticity but may also actively modify the abiotic and (sym)biotic environments to which they themselves respond, causing plasticity to interact in complex ways with niche construction. Here, we explore developmental mechanisms and evolutionary consequences of plasticity in horned dung beetles. First, we discuss how post-invasion evolution of plasticity in an introduced *Onthophagus* species facilitated rapid range expansion and concurrent local adaptation of life history and morphology to novel climatic conditions. Second, we discuss how, in addition to plastically responding to variation in nutritional conditions, dung beetles engage in behaviors that modify the environment that they themselves respond to during later development. We document that these environment-modifying behaviors mask heritable variation for life history traits within populations, thereby shielding genetic variants from selection. Such cryptic genetic variation may be released and become selectable when these behaviors are compromised. Together, this work documents the complex interactions between plasticity, symbionts and niche construction, and highlights the usefulness of an integrative Eco–Evo–Devo framework to study the varied mechanisms and consequences of plasticity in development and evolution.

KEY WORDS: Host–microbiome interactions, *Onthophagus* sp., Range expansion, Developmental plasticity, Allometric plasticity, Organism–environment interactions

Introduction

Developmental plasticity is ubiquitous across levels of biological organization and phylogeny (Pfennig, 2021; West-Eberhard, 2003). On its simplest level, plasticity emerges as a consequence of development's dependence on physical and biochemical circumstances, which themselves vary in time and space. As such, to develop is to respond to environmental conditions (Moczek, 2015). But developmental plasticity is more than just a product of organismal susceptibility to environmental conditions, it is also a

critical adaptation enabling organisms to adjust trait values and functions to suit changing circumstances, thereby maintaining high fitness in the face of environmental variability (DeWitt and Scheiner, 2004; Ghalambor et al., 2007). At the same time, developmental plasticity is not just a product of evolution shaped by natural selection; instead, once evolved, plasticity may feed back on subsequent evolution by biasing selectable variation in natural populations, providing new selective targets for diversification and by facilitating the emergence and elaboration of novel complex traits (reviewed in Moczek et al., 2011; Pfennig et al., 2010). Lastly, developmental plasticity interacts in important ways with another organismal property – niche construction – which occurs when organisms modify environmental conditions through their actions and choices, which feeds back to influence their own and/or their descendants' development, ecology, and more generally, fitness (Odling-Smee et al., 2003, 2013; Sultan, 2015). Nests, pupal cases, soil modification by earthworms or the creation of wetlands by beavers are prominent examples of niche construction. Yet, in many ways, such niche construction is intimately intertwined with plasticity: organisms sense and respond to environmental circumstances by modifying them, and the modified environment then impacts subsequent phenotype production differently from how it would have had earlier environment-modifying behaviors not been carried out. Put another way, organisms do not just respond to an external and otherwise independent environment, but frequently modify the environments they themselves respond to (for more in-depth discussions of the conceptual relationships between niche construction and other related concepts, including extended phenotypes, indirect genetic effects and developmental plasticity, see Dawkins, 2004; Laland et al., 2014, 2015; Matthews et al., 2014; Scott-Phillips et al., 2014).

Here, we summarize and review recent work aimed at characterizing the mechanisms and evolutionary consequences of developmental plasticity in horned dung beetles in the tribe Onthophagini in the contexts of range expansions and rapid adaptation to novel climatic conditions. In the second part, we then explore the complex interactions between developmental plasticity and environmental modifications arising from physical modifications of larval food niches and the vertical transmission and external cultivation of microbial symbionts. We further discuss the consequences these interactions can have for selectable phenotypic variation and, by extension, responses to selection. We begin with a brief overview of *Onthophagus* biology and the opportunities it provides for research into the varied mechanisms and consequences of developmental plasticity.

Horned dung beetles as models in the Eco–Evo–Devo of plasticity

Dung beetles consist of several beetle (sub)families that, apart from being coprophagous, differ widely in their ecology, life history and

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behavior (Hanski and Cambefort, 1991). Collectively, dung beetles provide vital ecosystem services, especially in agricultural grasslands (Losey and Vaughan, 2006). Most evolutionary and developmental studies have thus far focused on members of the genus *Onthophagus* and its close relatives (Hu et al., 2020; Kijimoto et al., 2013; Emlen et al., 2005; Simmons and Fitzpatrick, 2019), many of which colonize cow dung in nature and can be reared on it with ease in the laboratory. In contrast to the more familiar rollers (which carve out portions of dung pads above ground and roll them away for later burial), *Onthophagus* spp. dig tunnels directly underneath dung pads (Hanski and Cambefort, 1991). Once a sufficient depth has been reached, adults begin to move dung pieces underground, where females, sometimes assisted by males, construct a subterranean oval-shaped brood ball (as a very rough size reference, many *Onthophagus* adults are about the size of a coffee bean, may dig tunnels around 10–30 cm in depth, and construct brood balls roughly half the size of a golf ball). Each brood ball contains at its top end a brood chamber in which females place a small fecal pellet of their own, the so-called pedestal, onto which they then position a single egg (Fig. 1). The eclosing larva consumes the fecal pellet, and then proceeds to feed on the brood ball. All immature development including three larval stages, pupation, and eclosion of the young adult take place within the brood ball. After a few days, adults have hardened enough to be able to dig out of the brood ball and surrounding soil and emerge above ground (Fig. 1).

Onthophagus have garnered attention from plasticity researchers initially because of their often elaborate nutritional male

polyphenisms and associated alternative reproductive tactics (Emlen, 1996, 1997b; Hunt and Simmons, 2001; Moczek and Emlen, 2000). Adult males of many species compete with each other for access to breeding tunnels, often with the aid of horns and horn-like structures, which function as weapons during male combat. Combat is rarely injurious, and instead falls into the category of ‘trials of strength’, i.e. involves often prolonged pushing and jousting as one male tries to exclude the other from a tunnel (reviewed in Snell-Rood and Moczek, 2013), in the context of which the possession of long horns greatly improves fighting success (Moczek and Emlen, 2000). Horns, in turn, often exhibit extraordinary variability, not just among males of different species but also among conspecifics and in many species this variability has given rise to striking horn polyphenisms. Male larvae subject to optimal feeding conditions (e.g. a large brood ball of excellent dung quality located deep enough to shield developing larvae from aboveground temperature fluctuations) develop into adults that exceed a genetically determined critical size threshold and develop into ‘majors’, i.e. large males sporting a full set of horns (see Fig. 1E). In contrast, male larvae with access to only suboptimal feeding conditions develop into smaller adults, and if below said size threshold will metamorphose into ‘minors’ or smaller males with greatly reduced, rudimentary horns (see Fig. 1F). Majors and minors often differ strikingly in morphology but also in behavior and physiology. In *Onthophagus taurus*, majors will engage exclusively in aggressive male combat to secure matings, assist females extensively when no other males are around and invest little

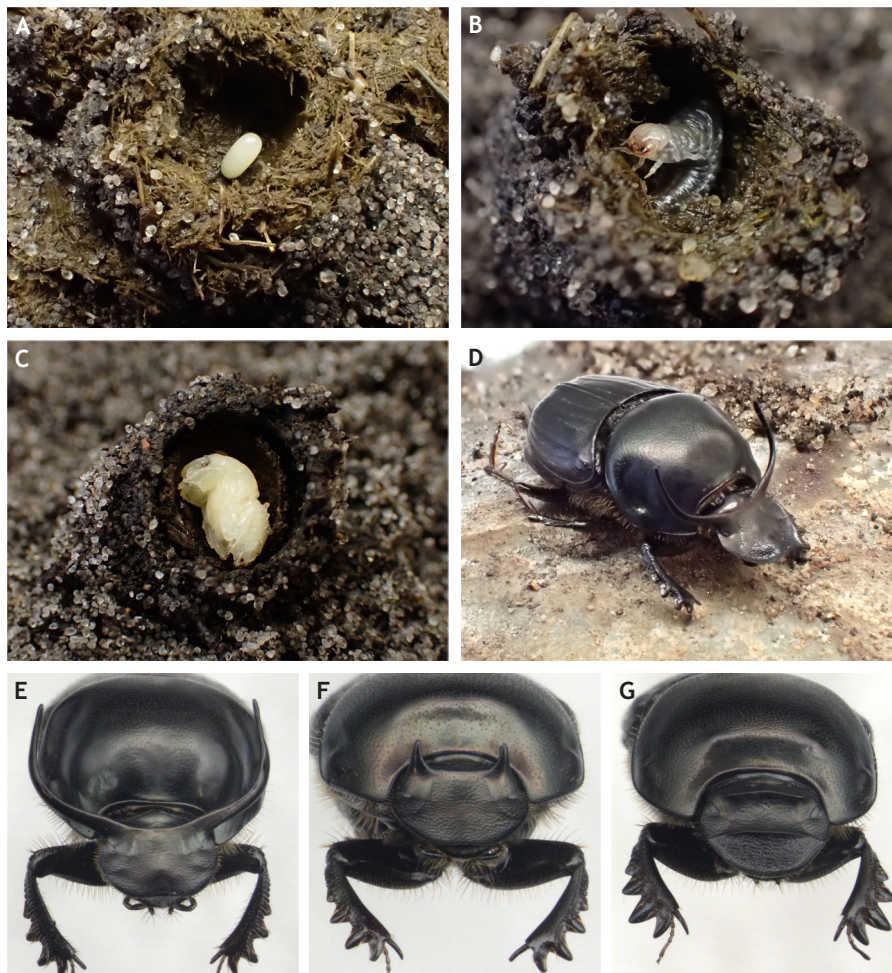


Fig. 1. Life cycle of *Onthophagus taurus*.

Reproductively active females construct underground ‘brood balls’ out of fresh cow dung. Within each brood ball, females construct a brood chamber within which they oviposit a single egg (A). Upon hatching, offspring begin feeding on the brood ball and complete their entire larval development within (B), ending in pupation inside a pupal chamber constructed out of larval gut content (C). After the pupal to adult molt and a brief period of hardening a new adult individual digs its way out of the soil (D). Adult males show alternative horn morphologies: large ‘major’ males develop a pair of large, curved horns used in male-male combat (E) while small ‘minor’ males develop greatly reduced, rudimentary horns (F). Females, in contrast, are always hornless regardless of size (G).

in sperm competition (Hunt and Simmons, 2001; Moczek and Emlen, 2000). In contrast, minors engage in aggressive fights when competing against size-matched opponents, but when encountering larger male rivals will employ a complex set of sneaking behaviors (including satellite behavior, digging horizontal intercept tunnels, and sustained courting of females aboveground), will assist females minimally even if no other males are around and invest heavily in testes formation and ejaculate volumes (Moczek, 1999; Simmons et al., 2007).

Sexual dimorphisms are often similarly extreme (Emlen et al., 2005; Simmons and Fitzpatrick, 2019). With only few (but very interesting) exceptions (e.g. *Onthophagus sagittarius*; Watson and Simmons, 2010), females of most species are uniformly hornless (see Fig. 1G). In contrast to the nutritional determination of horn polyphenism, sexual dimorphism in horn development arises as a consequence of XX/XY sex determination, providing an important contrast for exploring alternative modes of conditional development. For example, male polyphenisms are almost always already discernible at the pupal stage, suggesting that differences in relative horn growth underpins the production of major and minors. In contrast, sexual dimorphism may result from (depending on species and horn type) differential growth, or differential resorption during the pupal stage (in this case both males and females grow a similar-sized horn primordium, which is then secondarily resorbed in females only) or a combination of the two (Moczek et al., 2006). Lastly, horns are broadly considered evolutionary novelties, that is, traits that lack obvious homology to other traits, and thus have received considerable attention in regard to their developmental genetics and evolutionary histories (Hu et al., 2019; Linz and Moczek, 2020), as well as how conditional development may serve as an important early step in the initiation, elaboration and diversification of novel traits (Kijimoto et al., 2010; Moczek et al., 2006).

More recent work has begun to examine not just the impact of external conditions on development but also how beetles actively shape the developmental environment to which they themselves or their descendants respond. For example, the construction of brood balls in subterranean tunnels does not just provide larvae with food but also shields them from thermal fluctuations, which, if compromised, reduces body size at emergence (Snell-Rood et al., 2016). Intriguingly, mothers also adjust burial depth plastically depending on ambient thermal conditions (Macagno et al., 2018). Most work, however, has focused on elucidating the significance of maternally inherited microbiota in larval development, as well as the physical niche construction larvae themselves undertake as they develop within their natal brood ball (see below).

Lastly, an important additional dimension that contributes to horned dung beetle versatility as model systems in the ecological and evolutionary developmental biology of plasticity is the abundance of recent introductions of many species across the globe, either accidentally or on purpose as part of biocontrol programs aimed at reducing dung accumulations, pasture fouling and nuisance flies (involving >20 successful introductions to parts of Australia alone; Hanski and Cambefort, 1991; Tyndale-Biscoe, 1996). The large number of introduced populations now provides researchers with the opportunity to investigate how plasticity shapes evolution on ecological timescales, and the roles of niche construction and host–microbiome interactions therein. Collectively, horned dung beetles are thus emerging as powerful model systems to investigate the micro- to macroevolution of, and through, plasticity. In the sections below we probe this power by investigating one particular invasion event, that of eastern North

America by the bull-headed dung beetle *Onthophagus taurus* and the role of plasticity in facilitating rapid range expansion and local adaptation, followed by a review of recent work on the interplay between plasticity, niche construction and symbioses in delineating evolutionary trajectories.

Evolution of plasticity in functional morphology and life history on ecological timescales

The evolution of plasticity is often challenging to study in nature because ancestral and derived forms of plasticity are usually not simultaneously accessible. In this context, the study of species introductions and subsequent range expansions emerged as a particularly useful tool (Ghalambor et al., 2007; Roux, 2021). In many such instances, the plastic responses found in the exotic range can be contrasted to patterns of plasticity found in the native range. By utilizing plasticity in the native range as an approximation for ancestral plasticity, such comparisons can reveal how plasticity interacts with local adaptation in the exotic range (Casasa and Moczek, 2018; Davidson et al., 2011; Yeh and Price, 2004).

Evolution of plasticity during rapid range expansion

The bull-headed dung beetle *O. taurus* was first introduced to North America in 1971 (Fincher and Woodruff, 1975), most likely accidentally (Fincher et al., 1983). The first individuals were found in northern Florida, although how the species got introduced and exactly where from its broad native Mediterranean distribution remains unclear. The exotic population grew rapidly and expanded its range northwards, reaching the Canadian border within just 40 years (Rounds and Floate, 2012), representing a maximum of 80–100 beetle generations. During this range expansion, exotic *O. taurus* successfully colonized much colder and more humid climatic conditions compared with their native range (Silva et al., 2016), including environments with a drastically shortened breeding season. Such a rapid range expansion thus promised an ideal opportunity to assess how populations may adapt to novel environmental conditions on ecological timescales.

Rohner and Moczek (2020) used the invasion of *O. taurus* in the USA to investigate the role of (ancestral) developmental plasticity and its evolution during adaptation to novel climatic conditions. To do so, they reared one population from the native Mediterranean region (as a proxy for an ancestral population) and four populations sampled along a 1600 km latitudinal cline ranging from northern Florida (29.8 deg latitude) to northern Michigan (44.3 deg latitude) in the exotic range. Wild-caught females were brought into the laboratory and allowed to reproduce. To control for maternal effects, larvae were removed from their natural brood balls and placed in standardized, artificial brood balls (Shafiei et al., 2001). The brood of each female was then evenly allocated to two different temperatures chosen to mimic the average soil temperatures at the southern (27°C) and northern (19°C) distribution edges.

Not unexpectedly, individuals reared at low temperatures – and regardless of origin, including the native population – generally took much longer to complete development, grew more slowly and emerged as smaller adults (Fig. 2; this is a response found in many insects; e.g. Khelifa et al., 2019). However, populations within the exotic range differed in the magnitude of their response to temperature. This was especially pronounced for northern populations, which regularly encounter low temperatures, and showed a reduced prolongation of development time when exposed to low temperatures compared with low-latitude populations (significant latitude×temperature interaction). This is likely to be an adaptive response to strong seasonal time constraints. Seasons in

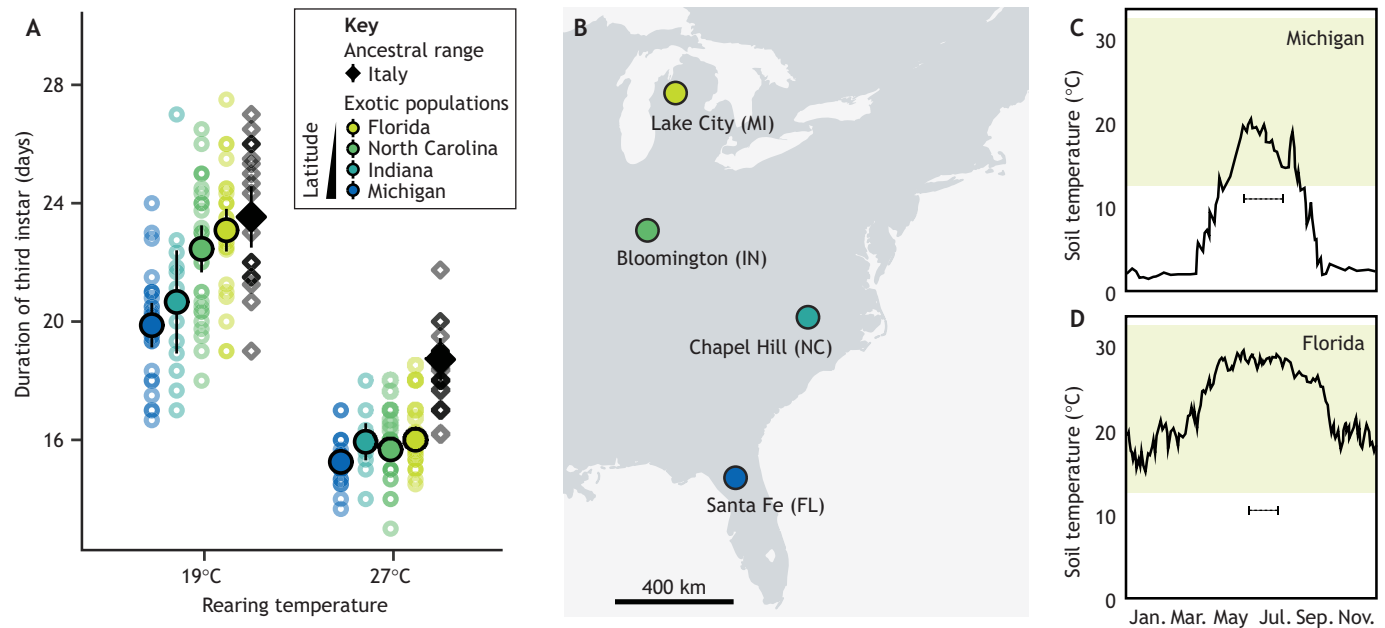


Fig. 2. Upon its introduction to Florida in the 1970s, *O. taurus* rapidly expanded its range in the USA. Range expansions went hand in hand with corresponding population differentiation in life history and functional morphology. For instance, low rearing temperatures generally prolong development time in all populations (A). However, this response was lessened in populations that experience increasingly shorter and cooler seasons at high latitudes (B). This is most likely an adaptive response to reduced season length in the north (C) compared with the southern populations (D). In C and D, the temperature range suitable for reproduction is indicated in green. The expected time required to develop from an egg into a sexually mature adult at the local average temperature is indicated with a dotted line (assuming 30 and 47 days egg-to-adult development time at constant average temperatures in Florida and Michigan, respectively; and 15 days of post-emergence maturation based on observations in the laboratory).

the north are so short that they barely accommodate a single generation (Fig. 2C). This is in stark contrast to the southern range edge where populations appear to be able to complete two or more generations a year. Natural selection is thus expected to favor genotypes that can complete an entire life cycle in a shorter time frame. The observed reduction in the response to cool temperatures in northern populations is thus consistent with adaptive countergradient variation (e.g. Blanckenhorn and Demont, 2004; Schultz et al., 1996; also referred to as ‘genetic compensation’, Grether, 2005). The observation that this reduction is more pronounced at low temperatures, while development at warmer temperatures did not diverge among populations, suggests that selection primarily led to the evolution of plasticity in development time, rather than mean development time, as local populations adapted to local seasonal time constraints. The evolution of developmental plasticity thus facilitated the rapid invasion and local adaptation of *O. taurus*.

In addition to life history traits (i.e. age and size at maturation), northern populations also evolved differences in functional morphology. For instance, northern populations evolved disproportionately larger wings relative to body size compared to southern populations (Rohner and Moczek, 2020). This latitudinal cline was in the same direction as thermal plasticity (larger wings at low temperatures). In flying insects, relative wing size is associated with dispersal capacity in the cold (Dudley, 2002; Neve and Hall, 2016) and similar plastic and genetic patterns have been found in other insects, both across and within species (Azevedo et al., 1998; Rohner et al., 2018, 2019). Similarly, changes in multivariate wing shape caused by temperature in the ancestral range are more closely aligned with the evolved differences between southern and northern populations in the exotic range as expected by chance (Rohner and Moczek, 2020). These findings are similar to those observed for

body size, where the plastic response in the ancestral range (being smaller at low temperatures) is in the same direction as the genetic response in the exotic range (smaller at high latitudes). Collectively, these findings suggest that ancestral plasticity may be useful in predicting population responses to novel environments (also see: Johansson et al., 2023; Stamp and Hadfield, 2020). More generally, this work highlights the role of divergence in plasticity, and the role of ancestral plasticity in facilitating rapid population differentiation and local adaptation on ecological timescales.

When plasticity is itself dependent on the environment

Developmental systems are exposed to – and evolve in – complex natural environments. Development is thus often sensitive to multiple and interacting environmental variables. That is, plastic responses to a given environmental variable may themselves vary in magnitude or direction depending on other environmental conditions (Rodrigues and Beldade, 2020; Westneat et al., 2019). This has implications for adaptation to novel environments where the combination of environmental variables that initiate adaptive plastic responses in the ancestral range may be absent or altered.

Recall that the pronounced horn length polyphenism in *O. taurus* is associated with alternative reproductive tactics. Major males engage in male–male competition and dyadic fights over breeding opportunities while hornless minor males are less likely to fight but primarily engage in post-copulatory competition and sneaking tactics (e.g. by intercepting breeding tunnels and copulating with the female while an unsuspecting major male defends the tunnel entrance). Such alternative reproductive tactics have been suggested to be driven by status-dependent selection (SDS) (Gross, 1996). This model predicts that individuals will employ the tactic (i.e. fighting or sneaking) that maximizes their fitness given the relative status of the competitors. That is, selection should favor genotypes

that switch from minor to major morphologies at the body size at which the fitness associated with sneaking starts to be outweighed by the fitness gained from fighting. In agreement with this model, Hunt and Simmons (2001) showed that the inflection point of the sigmoidal body size horn length allometry – or the body size at which males transition from minor to major morphology – coincides with the body size at which morph-specific fitness functions intersect. This model further explains why Australian populations, which have very high population densities, exhibit a much larger threshold body size compared with North American populations where densities are generally low (Moczek, 2003). These findings are similar to other systems where developmental thresholds or switches evolve in response to selection (e.g. Emlen, 1996; Schmidt et al., 2005; Tomkins and Brown, 2004).

Testing the predictions of the status-dependent selection model in novel environments

If status-dependent selection drives horn polyphenism, the threshold separating majors from minors is further predicted to track population-wide changes in body size ranges. For example, an evolutionary reduction in body size would cause all males to emerge as minors. This should in turn favor the evolution of reduced threshold sizes to maintain both morphs in the population. Based on this prediction, Rohner and Moczek (2023a) tested whether the reduction in body size due to thermal plasticity or genetic population differentiation along the longitudinal cline in the exotic range leads to corresponding plastic or genetic changes in the threshold. Both rearing temperature and the population of origin indeed affect threshold body sizes, yet the directions of effects are counter to the predictions based on the SDS model (Fig. 3A). For example, when rearing the most southern population (collected in Florida) at local temperatures (27°C), most individuals emerged as major males (proportion of major males: 0.81 [0.67,0.90] 95% CI; Fig. 3B). In contrast, almost all individuals from the most northern population (Michigan) developed into hornless minor males when developing at 19°C (proportion of major males: 0.11 [0.04,0.25]; Fig. 3B). This suggests that the frequency of the two morphs changes drastically, and systematically, with latitude, in a manner that conflicts with the predictions from status-dependent selection models.

Why do morph frequencies change so much? The shift in morph frequencies was driven by a genetic shift towards higher threshold sizes in the north, coupled with an effect of temperature on the polyphenism itself. Specifically, low temperatures, which decrease body size, simultaneously increase the threshold size across populations, collectively further decreasing the proportion of major males in cold habitats (Fig. 3). Such ‘allometric plasticity’, that is the dependence of nutritional responses on environmental conditions (Emlen, 1997a; Rhebergen et al., 2022), was unexpected, yet the magnitude of its effect on threshold size is comparable to evolutionary shifts documented across populations (e.g. Macagno et al., 2021; Moczek and Nijhout, 2003; Rohner et al., 2020). The environment in which (nutritionally) plastic responses occur thus plays a considerable role in shaping phenotypic variation. In this and possibly other cases, such environment-dependent plasticity, probably also affects fitness as shifts in morph frequencies are predicted to affect status-dependent reproductive success. However, the precise location-dependent changes in the behavioral ecology of this species remain to be investigated. Taken together, these studies indicate that allometric plasticity and its evolution may impact population’s responses to environmental changes in ways that are unexpected and inconsistent with a standard model of status-dependent selection. More broadly, this work highlights the complex ways by which environment×environment interactions influence developmental outcomes and the need for future work to better understand their ecological and evolutionary consequences (also see: Rodrigues and Beldade, 2020; Westneat et al., 2019).

When organisms modify the environment to which they themselves respond

Diverse organisms modify the environments that they or their descendants encounter (Clutton-Brock, 1991; Donohue, 2005; Duarte et al., 2021; Sultan, 2015). In *Onthophagus* dung beetles and their relatives, adult size and morphology are strongly dependent on maternal provisioning and especially the nutritional conditions that larvae encounter during development (Buzatto et al., 2012; Emlen, 1997a; Hunt and Simmons, 2000; Macagno et al., 2018; Moczek, 1998). Larvae grow faster and become larger adults when they

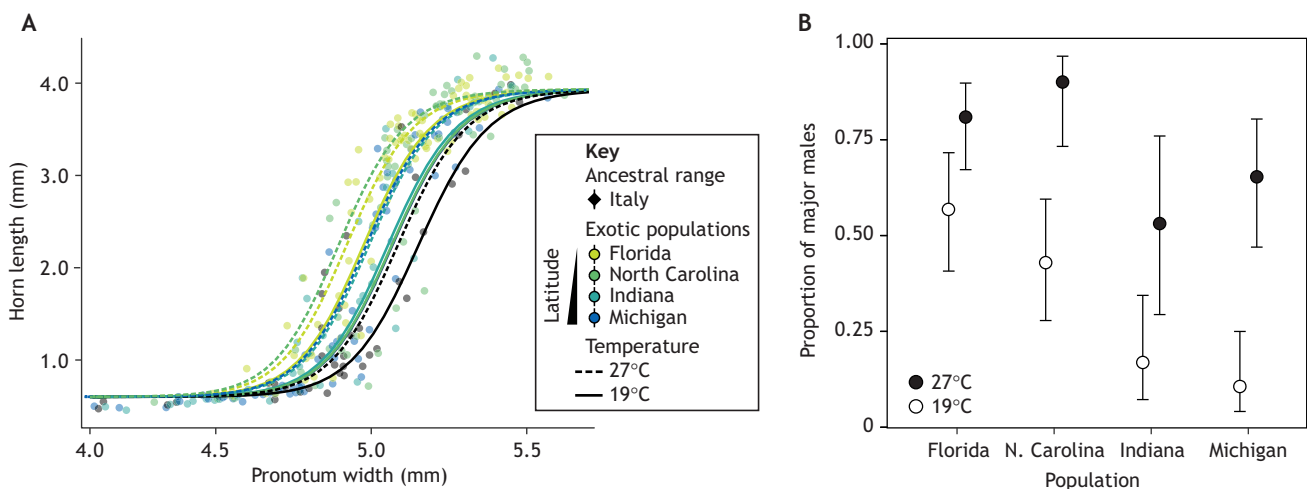


Fig. 3. In *O. taurus*, horn length has a sigmoidal scaling relationship separating hornless ‘minor’ males from horned ‘major’ males. The position of the body size threshold at which developing larvae switch from one morph to the other evolved across populations and is itself environmentally plastic (A). In contrast to the predictions based on status-dependent selection, plastic and genetic population differences lead to systematic variation in morph frequency across the latitudinal cline (B). This likely affects the behavioral ecology of local populations. (B shows proportions and associated 95% binomial confidence limits.)

develop in large brood balls and are fed a high-quality diet (Moczek, 1998). To mitigate the environmental stress their offspring experience, females have evolved a range of environmentally plastic behaviors. Females, for instance, will produce larger brood balls when they construct brood balls out of low-quality dung (Moczek, 1998) and when encountering hot temperatures, mothers bury their brood balls deeper in the soil, thereby shielding their offspring from hot temperatures (Macagno et al., 2018). Larvae, on the other hand, also have evolved plastic responses to deal with suboptimal ontogenetic environments. For instance, larvae will develop a larger midgut and digest more slowly when provided dung from hay-fed cows compared with more nutritious dung of cows that fed on grass, probably to improve nutrient extraction (Rohner and Moczek, 2021). Parental (mostly maternal) provisioning and larval plastic responses thus play major roles in shaping developmental outputs. However, recent work shows that dung beetle larvae are not just passive respondents to environmental circumstances, but actively modify their environment in multiple and interacting ways.

Dealing with a crappy diet: eat, excrete, re-eat and repeat

Extracting nutrients from a cellulose-rich diet, such as grass and hay, is challenging for most animals. Ruminants largely rely on their specialized multi-chambered guts, the repeated mechanical chewing of their food, and their symbiotic gut microbiome to digest their recalcitrant plant-based diet (Mackie, 2002; Weimer, 2022). Dung beetles feeding on cow dung face the additional challenge of feeding on what remains after a very effective ruminant digested its food and have evolved a suite of environment-modifying behaviors that shape their nutritional ecology. One important aspect is the mechanical manipulation of the ontogenetic environment. As soon as larvae hatch from their egg, they start to feed on and restructure the contents of their brood ball. Larvae masticate and digest the fibrous brood ball contents with their well-developed and heavily sclerotized mandibles, excrete back into their brood ball, and re-eat the resulting mixture. Throughout development, larvae work themselves through the entire dung mass multiple times until they pupate (Estes et al., 2013; Schwab et al., 2017). What is left of the brood ball at that stage is a heavily modified and much finer mixture compared to the fresh cow dung with which mothers initially constructed the brood ball. Preventing larvae from physically manipulating their environment leads to smaller body size, prolonged development time and significantly smaller secondary sexual traits (Schwab et al., 2017). For instance, preventing larvae of the gazelle dung beetle (*Digitonthophagus gazella*) from physically manipulating their environment reduces the proportion of major males in the population from 0.92 [0.86, 0.96] to 0.52 [0.42, 0.62] (Rohner and Moczek, 2023b). The developmental and functional ecological consequences of these behaviors are thus considerable. Interestingly, both species (Schwab et al., 2017) and populations (Dury et al., 2020) have diverged heritably in the phenotypic and fitness consequences caused by the experimental manipulation of maternal and larval niche construction, suggesting that the reliance on (or capacity for) these environment-modifying behaviors can evolve.

Recent work demonstrates that as larvae manipulate the physical aspects of their environment, they also alter its biotic components and, in particular, the microbial community present in the brood ball. During oviposition, mothers place their offspring on a pedestal, which is a maternally derived fecal pellet containing microbial symbionts whose presence promotes normative development (Estes et al., 2013; Shukla et al., 2016). Depriving larvae of these

symbionts by experimentally removing the pedestal causes larvae to take longer to develop and emerge as smaller adults compared with larvae that received a maternal pedestal (Parker et al., 2021; Schwab et al., 2016). In addition, the presence of inherited microbiota helps to mitigate the effects of environmental stress, such as temperature fluctuations or desiccation (Schwab et al., 2016). The vertically inherited symbionts thus shape host performance and fitness (as has been shown in other systems: Moran, 2007; Moran et al., 2019; Müller et al., 2016). Recent evidence further suggests that such host–symbiont interactions are not only ecologically important but may also diverge readily across different evolutionary timescales. For example, when pedestals were reciprocally exchanged between *Digitonthophagus gazella* and *Onthophagus sagittarius* (members of two genera that diverged ~36 mya; Breeschoten et al., 2016), both species were negatively affected. *Digitonthophagus gazella* larvae experienced prolonged development and reduced pupal mass whereas cross-inoculated *O. sagittarius* offspring suffered from increased mortality (Parker et al., 2019). A similar experiment swapped pedestals between two more closely related sister species, *O. vacca* and *O. medius*, which diverged ~8.7 mya (Roy et al., 2016) and showed that beetles developing with their conspecific pedestal survive better compared with those who received the other species' pedestal or none at all (Parker et al., 2021). Similar negative effects were also found for development time and body size. Dung beetle host–microbiome interactions thus diverge even between closely related, ecologically similar host species (Parker et al., 2021).

Contrasting microbial community composition across different life stages and beetle populations is beginning to provide further insights into possible mechanisms driving host–microbe divergences (also see: Estes et al., 2013; Shukla et al., 2016; Suarez-Moo et al., 2020). For example, Parker and Moczek (2020) compared microbiome compositions across native and introduced populations of *O. taurus* to each other as well as those of other dung beetle species native to a given location. This study found that the microbiomes of introduced populations are composed of microbes found in both the ancestral host population as well as beetle species native to each exotic range (Parker and Moczek, 2020). This suggests that microbiome composition is influenced by both host ancestry and the presence of other microbes in the local environment. Taken together, this work demonstrates that maternally acquired microbiota can have significant effects on dung beetle development, that beetle–microbe interactions diverge across distant and closely related beetles, and that microbiota composition is influenced by both evolutionary and ecological forces, results that broadly parallel findings in a growing number of taxa (hominids: Brooks et al., 2017; aphids: McLean et al., 2019; *Nasonia* wasps: Opstal and Bordenstein, 2019). Future work in the system should aim to elucidate the specific components of host–microbe interactions, such as determining the fidelity of microbiome composition across generations, identifying which microbial taxa affect which developmental outcomes and the mechanisms through which microbes influence beetle evolution and ecology.

Intriguingly, the action of maternally inherited microbiomes does not appear to be limited to the larval gut. As detailed above, larvae work their own fecal matter into their surrounding brood ball and repeatedly re-eat the resulting and increasingly modified composite. In so doing, larvae distribute their inherited symbionts throughout their entire brood ball environment. Notably, microbiota in brood balls modified by larvae have higher potential to break down diverse complex carbon sources, such as those associated with herbivore

dung, than microbes in unmodified dung (Schwab et al., 2017). This may create an external rumen in which larvae are able to increase their access to nutrients by taking advantage of microbial digestion of brood ball material outside the confines of the larval gut. The cultivation of maternally derived gut microbiota and the physical modifications in which larvae engage therefore interact as they modify their brood ball to shape the developmental environment. This interdependence becomes especially obvious when both aspects are experimentally manipulated simultaneously: both the removal of a pedestal or preventing larvae to physically manipulate their brood ball prolong development time in female *D. gazella*, yet these effects are especially strong when females are deprived of both the pedestal and the ability to modify their brood ball (Fig. 4). More generally, findings such as these underscore the importance of environment-modifying behaviors, particularly the interaction with symbionts, in structuring selective environments experienced in the wild.

Role of symbionts and environment-modifying behaviors in microevolution

The findings that both species and populations diverge in their host–microbiome relationships and in their dependence on physical niche construction suggests that such interactions may evolve on both macro- and microevolutionary scales, with the potential to impact subsequent evolutionary trajectories (Donohue, 2005; Duarte et al., 2021; Moran, 2007; Macagno and Moczek, 2023). Using a full-sib/half-sib design (P.T.R. and A.P.M., unpublished data; also see Rohner and Moczek, 2023b) we tested whether the ability of larvae to manipulate biotic and abiotic components of their ontogenetic environment impacts standing genetic variation for life history traits. Experimentally reducing individuals' ability to structure their biotic and abiotic environment generally led to an increase in additive genetic variance in development time, both in absolute

terms as well as relative to the total phenotypic variance and mean – as indicated by an increase in heritability and evolvability. This is consistent with the hypothesis that larvae's ability to modify their own ontogenetic environment promotes developmental stability not just in the face of environmental perturbations, but also with respect to genetic differences among individuals. Because intact niche construction may shield genetic differences among individuals from being expressed at the phenotypic level, such otherwise heritable differences become invisible to selection, and thus accumulate as cryptic genetic variation (CGV). However, as soon as larvae's ability to modify their environment is curtailed, this previously cryptic genetic variation may become phenotypically expressed and selectable. This is similar to recent findings in fruit flies, where the presence of symbionts was found to buffer genetic effects in stressful environments (Ma et al., 2019). The construction of (symbiotic) environments may thus constitute an underappreciated source of genetic capacitance. On one hand, these findings add to a growing body of work that demonstrates the ubiquity and importance of CGV (Ledon-Rettig et al., 2014; Paaby and Rockman, 2014; Snell-Rood et al., 2010, 2016), while at the same time providing one of the first demonstrations of niche construction as a mechanism facilitating its maintenance and possibly its accumulation.

Interestingly, our study also indicated genetic variation for the response to the elimination of environmental modifications, that is genotypes differed in their dependence on the ability to manipulate their environment. However, the precise causes of these genetic effects are unclear. There might, for instance, be a genetic component to the reliance on microbial members, which are, in part, vertically inherited. Taken together, this work highlights that the ability of organisms to actively manipulate their developmental environment may affect the potential of populations to evolve, especially when populations encounter novel, stressful conditions.

To develop is to interact with the environment; to evolve is to change the nature of these interactions in a heritable manner

The studies reviewed in this paper add important evidence to the growing body of work that supports plasticity's role in shaping evolutionary trajectories as they manifest during range expansion over ecological timescales. In the process, developmental plasticity emerges as an important factor during the evolution of adaptations as populations encounter novel environments (also see Pfennig, 2021; Sultan, 2015). At the same time, this work also highlights the capacity of plasticity to yield maladaptive developmental outcomes, especially when multiple environmental factors are incorporated into the analysis: colder temperatures delay development time despite organisms facing a drastically curtailed season (Rohner and Moczek, 2020), and nutrition-responsive growth and temperature interact in ways likely to be non-adaptive to shape morph ratios in populations at the edge of an invasion (Rohner and Moczek, 2023a).

The work reviewed here, however, also expands our perspective on the ecological and evolutionary implications of plasticity beyond the traditional conceptual boundaries of the field. First, niche construction emerges as an important – and likely ubiquitous – means by which organisms modify and bias their immediate environments in ways that then shape their own, subsequent developmental responses. Viewed this way, environments experienced during development do not exist separately from the organisms responding to them. Instead, developmental environments and responses to them emerge as cause and effect

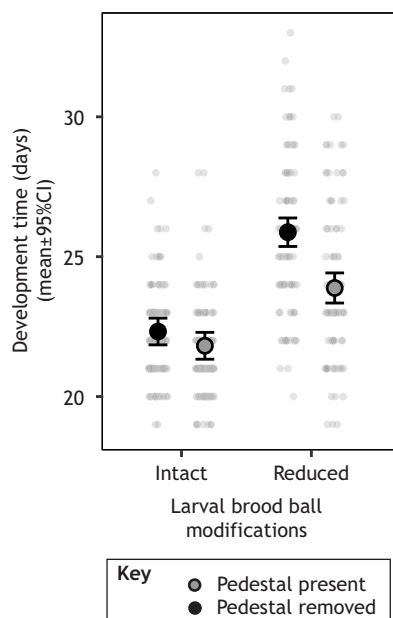


Fig. 4. Egg-to-adult development time in days of female *D. gazella* depends on the presence of a maternal microbial inoculate (the 'pedestal') and whether larvae were able to physically manipulate their ontogenetic environment. Females take especially long to complete their juvenile development time when larvae are unable to manipulate their environment and do not have access to maternal microbiota. (Estimated marginal means and corresponding 95% confidence limits.)

of each other. Second, because niche construction in dung beetles is tightly linked to a maternally inherited microbiome, plasticity in niche construction and developmental responses to constructed environments inevitably create opportunities for non-genetic inheritance and indirect genetic effects (Baud et al., 2021; De Lisle et al., 2022) and for contributions to evolutionarily meaningful heritable variation residing outside the beetle genome. Here, much work remains to be done: for instance, we are only beginning to understand the degree of fidelity by which microbes are passed throughout the *Onthophagus* life cycle and across *Onthophagus* generations. However, it appears likely that larval and adult microbiota are shaped by both maternally and environmentally acquired taxa, including taxa that may transition from one mode to the other depending on circumstances. If so, this creates constant opportunities for the acquisition and possible transmission of novel microbial partners, which during range expansions and confrontations with novel environments could be a powerful source of variation fueling rapid diversification and local adaptation. More generally, integrating developmental, ecological and evolutionary perspectives in the study of plasticity in horned dung beetles underscores the significance of viewing the environment as more than merely an external matrix, but also in relation to what organisms do to it and with it. By extension, this calls for conceptualizing developmental plasticity and its evolution not just through reaction norms but to also consider the organism as the key level of biological organization that interprets and responds to environmental conditions it itself shapes (Nadolski and Moczek, 2023). As such, this work documents the power and versatility of horned dung beetles as a study system with which to explore the varied mechanisms and consequences of plasticity in development and evolution.

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Competing interests

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Special Issue

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