REVIEW

Developmental bias in horned dung beetles and its contributions to innovation, adaptation, and resilience

Yonggang Hu* | David M. Linz* | Erik S. Parker* | Daniel B. Schwab* | Sofia Casasa | Anna L. M. Macagno | Armin P. Moczek

Department of Biology, Indiana University, Bloomington, Indiana

Correspondence
Armin P. Moczek, Department of Biology, Indiana University, Bloomington, IN 47405.
Email: armin@indiana.edu

Abstract

Developmental processes transduce diverse influences during phenotype formation, thereby biasing and structuring amount and type of phenotypic variation available for evolutionary processes to act on. The causes, extent, and consequences of this bias are subject to significant debate. Here we explore the role of developmental bias in contributing to organisms' ability to innovate, to adapt to novel or stressful conditions, and to generate well integrated, resilient phenotypes in the face of perturbations. We focus our inquiry on one taxon, the horned dung beetle genus *Onthophagus*, and review the role developmental bias might play across several levels of biological organization: (a) gene regulatory networks that pattern specific body regions; (b) plastic developmental mechanisms that coordinate body wide responses to changing environments and; (c) developmental symbioses and niche construction that enable organisms to build teams and to actively modify their own selective environments. We posit that across all these levels developmental bias shapes the way living systems innovate, adapt, and withstand stress, in ways that can alternately limit, bias, or facilitate developmental evolution. We conclude that the structuring contribution of developmental bias in evolution deserves further study to better understand why and how developmental evolution unfolds the way it does.

KEYWORDS

developmental symbiosis, doublesex, genetic accommodation, homology, insulin signaling, niche construction, Onthophagus, orthodenticle

1 | INTRODUCTION

Organismal form and function are generated by the processes of development, with some variants arising more readily than others, a phenomenon known as developmental bias (Uller, Moczek, Watson, Brakefield, & Laland, 2018). Such bias then structures amount and type of phenotypic variation available for evolutionary processes to act on. This biasing capacity of development is uncontroversial, as is the potential of developmental bias to limit, or constrain, adaptive evolution by preventing phenotypic variation from arising that would otherwise be favored by selection (Alberch, 1989; Arthur, 2004). What is controversial, however, is the creative role developmental bias may play in evolution by facilitating the production of novel, potentially adaptive variation (Laland et al., 2015). Similarly, it is now broadly understood that developmental bias is itself

---

*Yonggang Hu, David M. Linz, Erik S. Parker, and Daniel B. Schwab contributed equally to this work.

a product of developmental evolution shaped by past rounds of selection; thus, exactly how developmental bias affects developmental evolution may itself change over evolutionary time. Yet controversy surrounds the position that developmental bias may evolve such as to preferentially increase phenotypic variability in the direction favored by past natural selection (Moczek, 2012; Uller et al., 2018).

Here we explore the degree to which developmental bias facilitates adaptive evolution and evolvability by focusing on three dimensions critical to the developmental evolution of all living systems: their ability to generate novel variation (innovation), their ability to enhance the fit between organism and environment (adaptation), and their ability to withstand stress and perturbations (resilience). We reason that if developmental bias facilitates innovation, adaptation, and/or resilience, then theories and approaches in evolutionary biology will benefit from more explicit incorporations of developmental bias as a structuring force shaping the evolution of organismal form and function.

In the sections that follow we focus our analysis on the horned dung beetle genus *Onthophagus*, a model system in *evo devo* and *eco evo devo* (Choi et al., 2010; Kijimoto, Pespeni, Beckers, & Moczek, 2012; Moczek, 2009). We begin by examining the potential role of developmental bias at the level of gene regulatory networks in the origin of novel complex traits and their resilient integration within established trait complexes. Specifically, we assess the role of developmental bias in the origin and diversification of *Onthophagus* horns— exaggerated and highly diversified secondary sexual traits used in male combat over access to females (Moczek, 2005). In the second part, we explore if developmental bias may manifest in evolutionarily significant ways through developmental plasticity, that is, organisms' ability to respond to changes in their environment by adjusting aspects of their phenotypes. In particular, we explore if ancestral plasticity may bias the direction and speed of exotic *Onthophagus* populations' adaptations to novel or stressful conditions during the colonization of new habitats (Moczek, 2010). Lastly, we examine the potential significance of developmental bias emerging through host–symbiont interactions and niche construction. Specifically, we explore the role of interactions between *Onthophagus* hosts and their gut microbial symbionts and the systematic modification of environmental states in ways that have the potential to influence host development and diversification (Schwab, Casasa, & Moczek, 2019). Throughout we highlight promising future avenues to further assess the role of developmental bias in innovation, adaptation, and resilience, in *Onthophagus* horned dung beetles and beyond.

2 | DEVELOPMENTAL BIAS THROUGH GENE REGULATORY NETWORKS

Gene regulatory networks consist of the interactions between DNA sequences and their mRNA and protein products in a sequential-hierarchical fashion across developmental space and time (Carroll, Grenier, & Weatherbee, 2005; Davidson & Erwin, 2006). These interactions play critical roles in guiding the production and functional integration of biological form during development (Levine & Davidson, 2005), while changes in these interactions contribute significantly to the emergence of novel traits and trait functions in evolution (Ciliberti, Martin, & Wagner, 2007; Prud'homme, Gompel & Carroll, 2007). At the same time, the behavior of gene regulatory networks is inherently responsive to context (von Dassow, Meir, Munro, & Odell, 2000; Wagner, 2005). As a result, gene regulatory networks also contribute to the resilience of developmental processes and outcomes to perturbations arising from internal and external environmental influences. Thus, gene regulatory networks may be key sources of bias in the development and evolution of functional, resilient, and novel phenotypes (Payne, Moore, & Wagner, 2014; Uller et al., 2018).

The relationship between developmental bias at the level of gene regulatory networks and innovation may perhaps be most easily seen when developmental evolution repurposes pre-existing and preassembled networks to scaffold innovations (Hu et al., 2018; Linz, Hu, & Moczek, 2019; Shubin, Tabin, & Carroll, 2009; Wagner, 2014). In such cases, the direction, type, and functional integration of incipient innovations are shaped by the pre-existing configuration and system properties of repurposed gene networks (Tomoyasu, Ohde, & Clark-Hachtel, 2017; Wagner, 2007, 2014). *Onthophagus* horned beetles offer several valuable opportunities to explore the potential significance of bias through repurposing. For example, a long-standing research program has explored the origin of head horns, exaggerated secondary sexual traits used in competition over mates. Head horns are not modified versions of ancestral outgrowths or appendages, and are positioned on the dorsal head where insects or noninsect arthropods ancestrally never developed any type of projection (Grimaldi & Engel, 2005). Head horns are therefore neither homologous to other insect appendages, nor homonomous to other structures along the animal's body, thus fulfilling even the most stringent of definitions of evolutionary novelty (Wagner, 2014). Yet even though head horns constitute a relatively recent evolutionary invention, they found ways to integrate successfully within the dorsal head, itself an ancient trait

complex in existence ever since the origin of insects >420 MYA and whose embryonic assembly is governed by a gene network ultraconserved across phyla (Posnien, Schinko, Kittelmann, & Bucher, 2010). Recent work, therefore, aimed to explore the degree to which the repurposing of pre-existing, ancestral embryonic head patterning mechanisms may have been redeployed to facilitate the seamless integration of novel horns within the adult head.

Generally, the gene network that patterns the adult insect head is not well known. However, the network that patterns the same region during embryonic development is deeply conserved across taxa and well studied. Because adult heads derive through metamorphosis from their larval and embryonic precursors this embryonic head patterning network is thus a prime candidate for having been repurposed for both patterning the adult head and the integration of novelty therein. However, the larval head produced by embryonic patterning undergoes massive remodeling during the larval to adult metamorphic transition, obfuscating developmental and morphological correlations between stages. So while embryonic head patterning gene network components have well established spatial and temporal patterns of expression, it was initially unknown how these regions corresponded to adult head structures and in particular those that give rise to horns.

Using a unique larval fate-mapping approach, Busey, Zattara, and Moczek (2016) ablated concise larval head regions and assessed developmental defects produced in the adult head. This study established specific locations along the ocular-clypeoplabral boundary in the anterior presegmental region of the larval head as the corresponding tissue regions that give rise to posterior head horns in adult beetles, the most common position of head horns across *Onthophagus* beetles (Figure 1a–c). Once the developmental fate of these and other head regions was understood, candidate genes acting within and across region boundaries could then be functionally explored to assess their role in constructing and patterning adult morphology. For example, two transcription factors, *sine oculis 3/optix* (*six3*) and *orthodenticle* (*otd*) are expressed in complementary domains at the clypeolabral-ocular boundary across metazoan phyla during embryonic development (Li et al., 1996; Posnien, Koniszewski, Hein, & Bucher, 2011; Figure 1d). These two genes were thus key targets for further, postembryonic functional analysis which established a major role for *otd* in the formation and positioning of horns across *Onthophagus* species (Zattara, Busey, Linz, Tomoyasu, & Moczek, 2016). Upon downregulation of

otd, horns were removed from typical horn-bearing regions, and instead formed ectopically in other normally non-horn-bearing regions (Figure 1e; Zattara et al., 2016). Importantly, this study also revealed that *otd* appears dormant, expressed but nonfunctional, in the dorsal heads of more basal hornless species such as *Tribolium,* while maintaining function during embryonic patterning. In contrast to *otd*, *six3/optix* was found to have no role in horn formation, even though it is critical for the embryonic head formation and must interact tightly with *otd* during this stage.

Combined, these data support the hypothesis that components of an ancient gene network already tasked with embryonic head development may have latent expression in adult head development, components of which can be reawakened and neofunctionalized to
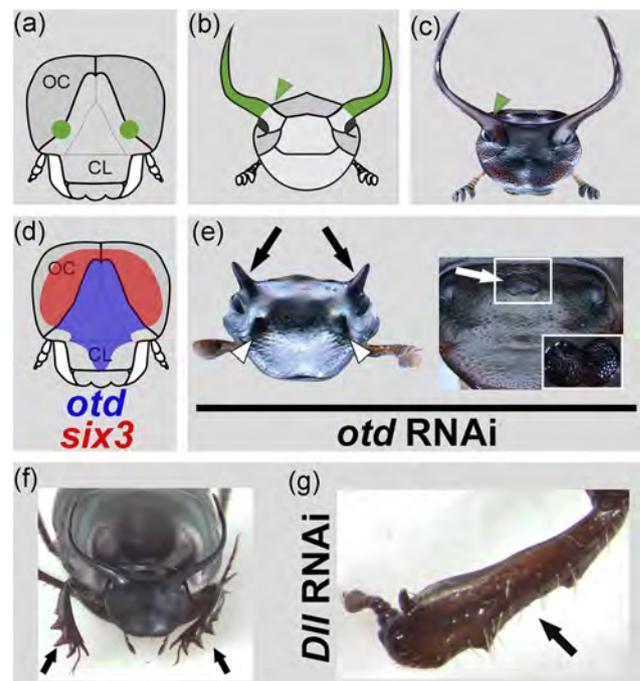

FIGURE 1 Developmental bias through gene regulatory networks exemplified by two classes of novelty in *Onthophagus* dung beetles. (a–c) Fate mapping approaches traced a specific location along the larval ocular-clypeolabral boundary (green dots in a) to adult posterior head horns (green region and green arrowheads in b and c). OC is the ocular region in the dark grey and CL is the clypeolabral region in light grey. (d) Two embryonic head patterning genes, *otd* (blue region) and *six3* (red region), have juxtaposed and mutually interdependent expression domains around the ocular-clypeolabral domain. (e) While *six3* RNAi causes no dorsal head defects, *otd* RNAi causes reduction of posterior horns (black arrows), induction of ectopic horns (white arrowheads) and induces a medial ectopic eye-like structure (white arrow and inset). (f) The tibial teeth of dung beetles (black arrows) are a modest novelty contained within the forelegs. (g) *Dll* RNAi causes severe disruptions in tibial teeth formation (black arrow) [Color figure can be viewed at wileyonlinelibrary.com]

integrate novelty within this region without compromising overall head patterning. Neofunctionalization can be accomplished in a way where the mutual interdependency of transcription factors at the embryonic stage can be shuffled or disengaged at the adult stage. The use of these genes, and the rewiring of the network they belong to, thus both facilitated novelty by providing customizable pre-existing mechanisms for spatial specification, but also biased positioning toward preferred locations, now reflected in the preponderance of posterior head horns across the *Onthophagus* phylogeny (Busey et al., 2016; Emlen, Corley Lavine, & Ewen-Campen, 2007).

Lastly, in an unexpected twist, *otd* downregulation, along with the major horn defects discussed above, also induced the formation of a medially-located ectopic eye-like structure, yet only in scarabaeid species (Figure 1e). In a second study, Zattara, Macagno, Busey, and Moczek (2017) further examined these eye-like structures revealing intact ommatidial lenses, crystalline cones, associated neural-like tissue within them as well as a transcriptomic landscape that mirrored that of regular compound eyes. In other words, these ectopic eyes appeared to be fully integrated morphologically and developmentally, yet their functionality was unknown. A behavioral assay was ultimately able to show that the ectopic eye was indeed photosensitive and fully functional, able to rescue a phototactic response in animals whose regular eyes had been surgically ablated. With the perturbation of horn formation and the simultaneous induction of functional eye-like structures, these results suggest that perturbing a gene network does not necessarily cause a region to disassemble. Instead knockdown of a single gene may allow the remaining gene network and associated developmental processes to reconfigure a morphological region in a functionally integrated manner, thereby highlighting how networks can contribute to resilience even when major network hubs are removed.

2.1 | Where does innovation start?

Beetle horns satisfy even the strictest definition of novelty —lacking homology or homonomy to other regions. At the same time, our understanding of the evolutionary process is rooted deeply within the notion of descent with modification—everything new must come from the old. Work on beetle horns (as well as evodevo generally) has now firmly established the significance of differential co-option and repurposing of gene networks as a common route to connect ancestral developmental features to novel morphological outcomes. Yet exactly how the former may yield the latter is far from understood: structures that fit strict novelty definitions (horns, eyes, butterfly eyespots, etc) are distinctly apomorphic, and as

such their study has so far offered little insight into the earliest phases of innovation. Here, the study of structures that do not fully fit within the most stringent novelty definition may provide some clues.

One such example is the tibial teeth of dung beetles (Figure 1f). Tibial teeth typically consist of four-pointed projections along the outer margin of the tibia of the forelegs, which play a critical role in enhancing beetles' ability to dig into compact soil (Linz et al., 2019). On one side tibial teeth thus conveyed significant adaptive potential and facilitated scarab beetles' radiation into a novel ecological niche. On the other side, tibial teeth are fully contained within the tibia, and thus a leg segment whose homology status is unambiguous. Combined, tibial teeth, therefore, embody what might be considered an early, modest innovation. Recent work examined the gene networks that help instruct the formation of tibial teeth, and found, perhaps expectedly, that reuse and repurposing of genes and pathways that are locally available (such as genes ancestrally tasked with establishing the proximo-distal axis of the leg) dominated the developmental evolution of tibial teeth (Linz et al., 2019). In fact, the precise function of several locally available genes was often found to be recapitulated in their novel role: for instance the gene *Distalless* (*Dll*) is critical for establishing the P-D axis during development of the leg, and was also observed to execute a similar function specifically during the formation of tibial teeth (Figure 1g). Importantly, however, tibial teeth formation also turned out to rely on genes whose ancestral functions lie well outside a leg formation context: specifically, at least two genes well studied for their roles in embryonic patterning emerged as critical for proper tibial teeth formation, having acquired a function well outside their ancestral spatial and temporal domains.

Our results may suggest a model for how developmental evolution scaffolds innovation: first through the reuse of genes whose products are locally already available and whose ancestral functions are preadapted to support key aspects of the development of a given novel trait, followed by genes whose products ancestrally function completely outside the context of a given novel trait, and thus have to evolve both novel domains of expression, as well as new functions within this domain. Such a scenario would suggest that early innovation may be both *facilitated* by locally available developmental-genetic building blocks, providing immediate opportunities for diversification with relatively modest genetic changes, but also *biased* by the functional repertoire of exactly what genes and pathways may be available for repurposing and the developmental degrees of freedom they may provide.

2.2 | *Old functions for novel traits*: The integration of doublesex and insulin signaling in the evolution of sex- and nutrition-dependent development of head horns

Repurposing and associated biases are not restricted to the developmental evolution of morphological novelties, but also factor prominently in the *functional* diversification of novel traits. For example, as is common in the genus, *Onthophagus taurus* possesses an intersexual and intrasexual dimorphism in head horn development. Only males well-nourished during the larval stage grow into large adults with fully developed horns, nearly 10-fold longer than those of smaller males raised in suboptimal nutrition (Moczek, 1998), while all adult females regardless of nutritional conditions experienced as larvae develop a shallow ridge in the same head location. While head horns represent an evolutionary novelty, an extensive body of work now shows that the developmental mechanisms underlying their sex- and nutrition responsive growth have been recruited and repurposed from a diverse, ancestral regulatory toolbox.

First hints emerged through transcriptomic screens which provided a first comprehensive list of candidate genes putatively underlying the evolution and diversification of beetle horns (Choi et al., 2010; Kijimoto, Costello, Tang, Moczek, & Andrews, 2009). Among the many identified candidates the transcriptional expression of the ortholog of *Drosophila doublesex* (*dsx*) stood out. In *Drosophila*, sex-specific *dsx* isoforms regulate sexually dimorphic differentiation (Saccone, Salvemini, Pane, & Polito, 2008; Sánchez, Gorfinkiel, & Guerrero, 2001; Tanaka, Barmina, Sanders, Arbeitman, & Kopp, 2011), and orthologous sequences showed significant differential expression across male body regions and nutritional conditions in *Onthophagus*, suggesting a potential role of *dsx* in patterning intersexual but also possibly intrasexual horn dimorphisms. Investigations of *dsx* gene structure identified one male-specific isoform and at least five female-specific isoforms, as well as one non-sex-specific but likely function-less isoform (Kijimoto, Moczek, & Andrews, 2012). Subsequent functional assessments of these transcripts implicated the male isoform in the nutrition-dependent promotion of horns, whereas the female isoform(s) inhibited horn formation in females. Sex-specific *dsx* isoforms have since been shown to also promote and inhibit head horns in males and females of the rhinoceros beetle *Trypoxylus dichotomus* (Ito et al., 2013), and enhance and hinder nutrition-responsive growth of mandible in males and females of the stag beetles *Cyclommatus metallifer*, respectively (Gotoh et al., 2014). Collectively, these results suggest that by providing

a pre-existing developmental switch mechanism responsive to somatic sex, the co-option of sex-specific *dsx* isoforms have repeatedly facilitated the sex-specific elaboration of horns and other weapons. However, how *dsx*-mediated horn expression became linked to nutrition was less clear. Here, recent work on the insulin signaling pathway has begun to provide important insights.

The insulin/insulin-like signaling pathway (IIS) is a highly conserved pathway well recognized for its role in regulating growth in response to nutrition across phyla (Barbieri, Bonafè, Franceschi, & Paolisso, 2003; Brogiolo et al., 2001). In insects, rich nutritional environments cause the insulin-producing cells (IPCs) in the brain to produce and secrete insulin-like peptides (ILPs) into the hemolymph. The ILPs bind to and activate the Insulin Receptor (InR) of the target tissues, which in turn activates a phosphokinase signal transduction cascade, thereby inducing cell growth and proliferation (Brogiolo et al., 2001; Géminard et al., 2006). Importantly, tissues differ in their sensitivity to IIS, resulting in different growth rates across tissues within an individual. For example, in *Drosophila*, wings and legs grow proportionally to body size in response to nutritional condition, while central nervous system and genitalia are much less sensitive to the nutritional state, resulting in minimal size variation even when nutritional conditions vary (Cheng et al., 2011; Koyama, Mendes, & Mirth, 2013; Shingleton, Das, Vinicius, & Stern, 2005; Tang, Smith-Caldas, Driscoll, Salhadar, & Shingleton, 2011). Studies on both rhinoceros beetles and *Onthophagus* horned beetles now also implicate the insulin signaling pathway as a critical transducer of nutritional conditions during the larval to pupal transition, including the relative growth of nutrition-sensitive horns and nutrition-insensitive genitalia (Casasa & Moczek, 2018a; Emlen, Warren, Johns, Dworkin, & Lavine, 2012). Importantly, both rhinoceros beetles (subfamily Dynastinae) and *Onthophagus* dung beetles (subfamily Scarabaeinae) are believed to represent independent inventions and radiations of sexually dimorphic and exaggerated horns (Emlen et al., 2007). While both lineages appear to have relied on the co-option of the IIS, data available to date suggest that key regulatory functions are carried out by different pathway members in the two subfamilies: In *Trypoxylus* rhinoceros beetles, downregulation of the insulin receptor InR reduces male horn length but leaves genitalia unaffected (Emlen et al., 2012). Conversely, in *Onthophagus*, the same manipulation has no effect on the body size—horn size allometry, but significantly reduces genitalia size relative to body size (Casasa & Moczek, 2018a). Here, however, knockdown of Fork head, subgroup O (Foxo, a growth suppressor downstream of the InR) greatly increases head horn length in small, low-nutrition males,

while modestly decreasing it in large, high-nutrition males, thereby linearizing the normally sigmodal body size—horn size allometry. At the same time, $Foxo^{RNAi}$ also increases nutrition sensitivity of genitalia. Most importantly, Casasa and Moczek (2018a) provided the first evidence suggesting a functional link between *dsx* expression (see above) and insulin signaling by demonstrating that *dsx* expression significantly decreases following knockdown of InR (Casasa & Moczek, 2018a). Taken together, these results suggest that by co-opting the IIS pathway horn formation acquired the ability to become exquisitely nutrition-responsive. Furthermore, by then linking *dsx* expression to IIS signaling, horns evolved the ability to exhibit nutrition responsive growth in a strictly sex-specific manner, thereby setting the stage for the dramatic radiation in sexual dimorphisms and male polyphenisms of this genus. More generally, these results suggest once again that morphological innovation and diversification are facilitated but also biased by the developmental opportunities and limits that emerge when a pre-existing developmental tool kit is reimplemented over and over again. Yet at the same time, by evolving novel interactions between pre-existing components of said toolkit, additional developmental degrees of freedom are generated with which evolution can subsequently tinker.

3 | DEVELOPMENTAL BIAS THROUGH DEVELOPMENTAL PLASTICITY

Developmental or *phenotypic plasticity* refers to a developing organism's ability to alter aspects of phenotype expression in response to changes in environmental conditions. Such responses may be subtle or dramatic, reversible or not, and can be shaped by either long periods of prior selection due to recurring or predictable environmental fluctuations, or alternatively, by conditions encountered for the very first time (Moczek, 2009). In all of these situations, developmental plasticity has the potential to exert developmental bias on variation in phenotype expression visible to selection, thereby shaping subsequent evolutionary trajectories (West-Eberhard, 2003).

For example, developmental plasticity is well established as a mechanism enabling organisms to maintain high fitness in the face of fluctuating environments, and in such cases may buffer the effects of diversifying selection, thereby limiting adaptive radiations (Schlichting & Pigliucci, 1998). In contrast, plasticity may facilitate rapid phenotypic divergences when populations colonize novel habitats or encounter major environmental

perturbations (Hendry, 2016; Yeh & Price, 2004). Such immediate plasticity-mediated responses in development may be further enhanced through the process of *phenotypic accommodation*, that is, the adaptive mutual adjustment of variable aspects of the phenotype during development, occurring without any genetic change (West-Eberhard, 1998). For example, when *Polypterus* fish are forced to develop in an environment in which they have to walk on their pectoral fins more than swim, fish develop a more efficient gait during their lifetimes, accompanied by bone structure and musculature changes more suited to a terrestrial, walking lifestyle (Standen, Du, & Larsson, 2014). All these phenotypic adjustments improve trait integration and performance within a novel, stressful environment. Furthermore, while these changes manifest within a single generation in the absence of genetic changes, they nevertheless parallel some of the same changes observed in the fossil record during the water-to-land transition of tetrapods.

One mechanism that may enable plastic responses to precede and bias subsequent genetic evolution is *genetic accommodation*. Genetic accommodation is broadly defined as a change in gene frequency due to selection on the regulation of an environmentally-induced response (West-Eberhard, 2003). As such it constitutes a mechanism whereby initially environmentally induced traits, including the products of phenotypic accommodation, may become genetically stabilized or canalized, for instance when plastic responses to environmental conditions make visible to selection cryptic genetic variation accumulated during previous generations (Paaby & Rockman, 2014). Evidence in support of genetic accommodation derived initially from environmental perturbation and artificial selection experiments (*Drosophila*: Dworkin, 2005; Rutherford & Lindquist, 1998; Waddington, 1953; *Manduca sexta*: Suzuki & Nijhout, 2006; *Caenorhabditis*: Sikkink, Reynolds, Ituarte, Cresko, & Phillips, 2014; *Arabidopsis*: Queitsch, Sangster, & Lindquist, 2002; fungi: Cowen & Lindquist, 2005; cyanobacteria: Walworth, Lee, Fu, Hutchins, & Webb, 2016). More recently, a growing number of studies have shown genetic accommodation in natural populations (spadefoot toad tadpoles: Gomez-Mestre & Buchholz, 2006; Ledón-Rettig, Pfennig, & Nascone-Yoder, 2008; Levis, Isdaner, & Pfennig, 2018; threespine sticklebacks: Robinson, 2013; Shaw, Scotti, & Foster, 2007; Wund, Baker, Clancy, Golub, & Foster, 2008; *Daphnia*: Scoville & Pfrender, 2010; house finches, Badyaev, 2009; Badyaev, Potticary, & Morrison, 2017; cavefish: Rohner et al., 2013). Collectively, this body of work demonstrates the feasibility and potential significance of genetic accommodation in evolution. Moreover, it highlights the potential for developmental bias, via environmentally

induced phenotypes, in the evolution of adaptive traits. Nonetheless, several critical dimensions remain to be addressed. For example, exactly how fast evolution by genetic accommodation may contribute to diversification, and the extent to which it actually does so in natural populations, remain largely unclear. Similarly, earlier work posited that because behavioral traits often exhibit both extreme plasticity and evolutionary lability, behavior may be more likely to evolve by genetic accommodation than other organismal features such as morphology (Allf, Durst, & Pfennig, 2016; West-Eberhard, 1986 &, 2003). However, little comparative work has addressed this issue thus far.

Onthophagus taurus has emerged as a promising study system to advance these and related questions, in part due to the existence of recently established and rapidly diverging exotic populations (reviewed in Casasa & Moczek, 2018b). While originally restricted to its native Mediterranean distribution, in the 1970s this species was introduced to Eastern and Western Australia to help control cow dung and dung-breeding flies (Tyndale-Biscoe, 1996) as well as to the Eastern United States by accident (Fincher & Woodruff, 1975). Since introduction, both Eastern US and Western Australian populations have diverged rapidly in diverse traits, both from each other and relative to their Mediterranean source population. This differentiation has likely been the product of differential adaptations to local dung beetle densities (very high in Western Australia, low in the Eastern US) and the resulting divergent intensity of mate and resource competition (Moczek, 2003; Figure 2a), as well as an expansion of the Eastern US population into a colder and more humid climatic niche (Silva, Vilela, Buzatto, Moczek, & Hortal, 2016). Trait differences between populations are maintained in common garden conditions, and include morphology (e.g., adult body size, allometric threshold for horn induction, male genitalia shape, female fore tibia shape), development and physiology (e.g., degree and timing of sensitivity to juvenile hormone, sensitivity to serotonin upregulation, duration of larval development, developmental responses to temperature stress), and behavioral and life-history traits (provisioning behavior and fitness; Beckers, Anderson, & Moczek, 2015; Macagno, Beckers, & Moczek, 2015; Macagno et al., 2011; Macagno, Moczek, & Pizzo, 2016; Macagno, Zattara, Ezeakudo, Moczek, & Ledón-Rettig, 2018; Moczek & Nijhout, 2002 &, 2003; Moczek, Hunt, Emlen, & Simmons, 2002; Newsom, Moczek, & Schwab, in review).

Considering six of these traits (body size and horn allometry threshold for morphology; brood ball mass and nesting depth for behavior; and brood ball number and eclosion success for life history), a recent study by Casasa and Moczek (2018b) examined the presence and direction of plasticity in response to variation in adult density in the Mediterranean source population. In controlled lab conditions, native beetles were subject to either very high (Western Australia-like) or very low (Eastern US-like) adult densities, and just 3 weeks of this treatment were sufficient to induce measurable plasticity in four of the six traits studied. Average responses matched the direction of canalized differences between descendent exotic populations in one morphological trait (adult body size) and one life-history trait (fecundity, as measured by the number of brood balls produced; Figure 2b). For these two traits, results are consistent with a "plasticity first" scenario, whereby plastic responses to environmental conditions unveil phenotypic variation that is later canalized by selection (Levis & Pfennig, 2016). Two other traits (one behavioral—the amount of food provisioned to offspring, and one life-history trait—eclosion success) exhibited plasticity in the direction *opposite* to that predicted based on the differences between exotic populations. However, by itself this observation does not reject the possibility of a "plasticity first" scenario: while plasticity in response to a novel environment is assumed to not be able to anticipate adaptive variation (Moczek, 2007), to *facilitate* adaptive evolution, it is only necessary that variation among ancestral reaction norms *encompasses at least some* novel variants that selection can promote (Casasa & Moczek, 2018b; Figure 2c).

Studies such as these suggest that developmental plasticity may bias evolution already at the very earliest stages of population differentiation, and possibly across trait categories, through the environment-responsive production of functionally integrated and potentially adaptive phenotypes. However, to determine whether these results are indeed generalizable will require the study of many more and diverse taxa, traits, and potentially inductive environmental contexts. Studies are also needed to better understand the molecular, genetic, and transcriptomic mechanisms of genetic accommodation. For example, recent work by Levis et al. (2018) examined the role of gene expression plasticity in spadefoot toads. In the genus *Spea,* a diet-induced polyphenism results in either omnivorous or carnivorous tadpole morphologies. Using a closely related nonpolyphenic species as a proxy for the ancestral condition, and exposing this species to a novel carnivorous diet, Levis et al. (2018) were able to document gene expression plasticity in genes associated with polyphenic development. This study marks an important effort to assess the mechanistic basis of genetic accommodation in the wild, but it does so only for relatively few genes. Yet, development of complex traits such as alternative feeding morphs (*Spea*) or male reproductive morphs (*Onthophagus*) is likely underpinned by hundreds or thousands of genes, which will ultimately necessitate a much broader genome- and transcriptome-
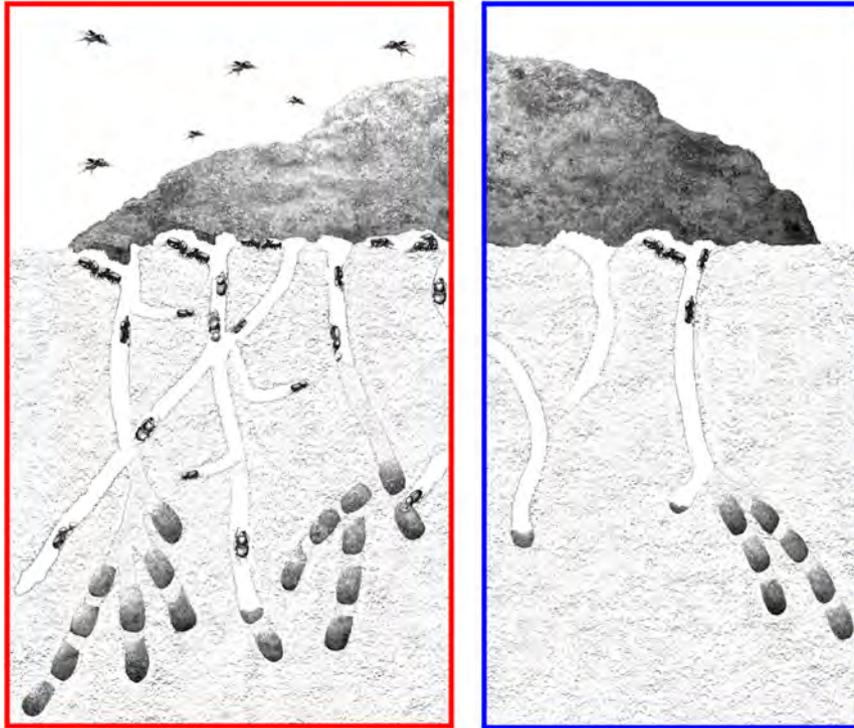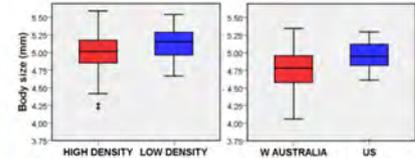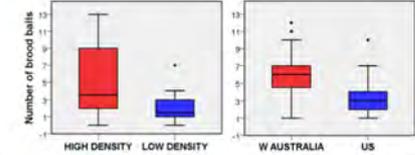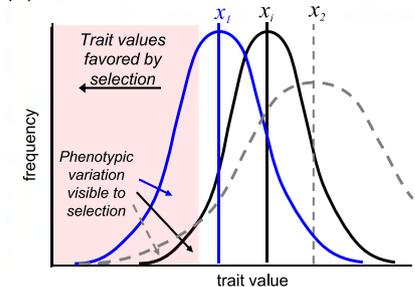
FIGURE 2 Developmental bias through developmental plasticity. (a) The horned dung beetle species *Onthophagus taurus* is subject to highly disparate ecological and social conditions in two exotic ranges. In Western Australia (WA, left, red frame) hundreds to thousands of individuals compete for breeding opportunities while in the Eastern United States (US, right, blue frame) local densities are up to three orders of magnitude lower and mate and resource competition are relaxed (drawing by Barret Klein). Since establishment WA and US populations have diverged heritably in a suite of behavioral, physiological, developmental, and morphological traits. (b) Developmental plasticity yields changes in phenotype expression that parallel canalized differences between populations. Plastic responses in beetles derived from an ancestral Mediterranean population to experimentally controlled high (red) or low (blue) densities yield significant phenotypic differences that parallel both direction and magnitude of evolved, canalized differences between high density Western Australian (WA, red) and low density Eastern US (US, blue) populations (data are from Beckers et al., 2015 and Casasa & Moczek, 2018b). (c) Plasticity biases evolution by altering type and frequency of phenotypic variation visible to selection. Plastic responses may bias adaptive evolution by shifting the initial mean (x_i) and frequency distribution of phenotypic variation in a direction favored by selection (x_1). Plasticity may bias adaptive evolution even in cases in which the mean phenotype shifts away from trait values favored by selection (x_2) as long as variation among ancestral reaction norms encompasses at least some novel variants that selection can promote [Color figure can be viewed at wileyonlinelibrary.com]

wide perspective (e.g., Ghalambor et al., 2015). A more comprehensive understanding of the mechanistic basis of genetic accommodation will likely enable key insights into how environmentally sensitive gene regulatory networks are rewired to produce integrated and functional phenotypes that have the potential to influence evolutionary trajectories.

4 | DEVELOPMENTAL BIAS THROUGH SYMBIOSES AND NICHE CONSTRUCTION

Traditionally, *evo devo* biologists have sought to explain biased patterns of phenotypic variability by interrogating the endogenous gene regulatory, physiological, and developmental mechanisms that regulate morphogenesis (Arthur, 2004; Uller et al., 2018). In the preceding sections, we focused on these same levels of biological organization, and explored how evolutionary processes may be biased towards deploying the same pre-existing and preassembled genes and gene networks in the advent of novel structures (Linz et al., 2019; Shubin et al., 2009), and discussed how environment-responsive development may be primed to generate well-integrated, functional, and sometimes adaptive variants in the face of ecological stressors (Casasa & Moczek, 2018b). Yet, bias may manifest at additional and even extra-organismal dimensions, for example when organisms actively modify their own selective environments through the process of *niche construction* or by engaging in *developmental symbioses* that can structure important functional variation. Here, we suggest that these

processes may both independently and synergistically play fundamental roles in promoting normal development in *Onthophagus* beetles and facilitate the formation of well-integrated, resilient phenotypes in the face of environmental perturbations.

4.1 | Niche construction, symbiosis, and the reciprocal nature of development

Niche construction occurs when organisms, via their physiology and behaviors, modify their own and each other's niches in systematic ways (Odling-Smee, Laland, & Feldman, 2003). When directly modifying developmental environments, the nature and scope of these modifications can range from the production of physical structures such as burrows or pupation chambers, to alterations of chemical states in the surrounding environment. One common and adaptive function of these modifications is to lend resilience to organisms developing under challenging environmental conditions. Among insects, some of the most prominent examples of this form of niche construction include gall-forming flies and tent building caterpillars, whose physical constructions buffer them from predators, parasites, and thermal fluctuations (Abrahamson, Sattler, McCrea, & Weis, 1989; Joos, Casey, Fitzgerald, & Buttemer, 1988). For these and many other organisms, niche construction is a characteristic feature of normal development, enhancing the fit between organism and environment (Laland, Odling-Smee & Gilbert, 2008; Schwab & Moczek, 2017). When scaled-up from an individual organism's development to the level of populations, evolutionary models suggest that niche construction can significantly alter the rate and direction of evolution and influence which genetic variants are maintained or lost (Kylafis & Loreau, 2008; Laland, Odling-Smee, & Feldman, 1999). Importantly, niche construction is fully consistent with phenomena that have been historically studied under alternative frameworks. For instance, parental effects may be considered a form of niche construction in which parents construct developmental environments such as nests or brood chambers for offspring, and ecosystem engineering may represent a form of niche construction expressed at the level of communities and beyond (Day, Laland, & Odling-Smee, 2003). In each case, niche construction represents a potent form of reciprocity between organism and environment that has the potential to shape patterns of phenotypic variation. Yet this reciprocity does not need to end at the boundaries of the individual organism, and a growing body of work illustrates that reciprocal niche construction between multiple organisms and their shared environmental domains can profoundly affect both development and evolution of phenotypic variation (Chiu & Gilbert, 2015).

The significance of reciprocal niche construction in developmental evolution is perhaps best illustrated by the rapidly growing work on host–microbe interactions. Recent technological advances in the ability to taxonomically characterize and evaluate the potential functions of microbial communities of eukaryotic hosts has resulted in a far greater appreciation of the importance of microbes for virtually all aspects of host biology, including in the regulation of the normal development of their hosts (M. McFall-Ngai et al., 2013). Indeed, the influence of microbial symbionts can be observed across all stages of animal development. For instance, species of obligate intracellular bacteria promote germline proliferation in nematodes (*Wolbachia*: Foray, Pérez-Jiménez, Fattouh, & Landmann, 2018), and protect embryos against pathogenic infections in arthropods (*Wolbachia* and *Spiroplasma*: Jaenike, Unckless, Cockburn, Boelio, & Perlman, 2010; Teixeira, Ferreira, & Ashburner, 2008). During postembryonic development, microbial symbionts have been implicated in instructing the completion of digestive (e.g., guts of mice: Hooper & Gordon, 2001; Sommer & Bäckhed, 2013; and zebrafish: Bates et al., 2006; Rawls, Samuel, & Gordon, 2004) and immune system development (reviewed in Gilbert, Bosch, & Ledón-Rettig, 2015) across vertebrate taxa. Furthermore, microbial symbionts have been linked to transitions between developmental stages, producing signals that induce metamorphosis in a suite of marine invertebrate taxa such as tubeworms, corals, and sponges (Shikuma et al., 2014; Sneed, Sharp, Ritchie, & Paul, 2014; Whalan & Webster, 2014). These developmental symbioses also have the potential to be highly reciprocal. For example, bacterially-mediated induction of light organ formation in the bobtail squid, in turn, activates gene expression changes in the inducing bacteria, causing the bacteria to express the bioluminescent properties that are characteristic of the new organ (M. J. McFall-Ngai, 2014). In many instances, these developmentally significant symbionts are passed down or selectively acquired from host environments during development, ensuring the maintenance of their functions across host generations. Alongside niche construction, developmental symbioses thus present another avenue through which organisms reciprocally interact to facilitate each others' as well as their own development (see Gilbert, 2019, this issue, for additional examples).

Although niche construction and developmental symbiosis have emerged from disparate conceptual frameworks and empirical investigations, these processes share a common potential to bias the outcomes of development and developmental evolution (Laland et al., 2015). For instance, developmental bias is an inherent feature of niche construction, in which organisms engage with and predictably alter their environments in ways that may better suit their traits (Schwab & Moczek, 2017). In so

doing, organisms bias their selective environment while simultaneously channeling the expression of developmental variation toward particular states. The latter is best demonstrated when niche constructors plastically respond to the environments that they themselves have generated (see below; Schwab, Casasa, & Moczek, 2017). These modifications can lead to further, transgenerational bias when altered environmental states are inherited, including via the inheritance of microbial symbionts that are necessary for normal development. In developmental symbiosis, bias is expressed not only through the effects that host–microbe interactions have on the production of functional variation, but also in how these interactions can bias or facilitate innovation in the face of novel environments. Yet, while it is true that both niche construction and developmental symbioses present important sources of developmental bias, few systems have been leveraged to experimentally evaluate the potential individual and synergistic contributions of each process to phenotypic and evolutionary outcomes. For instance, although niche construction is thought to play important roles in the development and evolution of niche constructors, their descendants, and even other species, few experimentally tractable model systems have been developed in which the mechanisms of niche construction (a) are well understood, (b) can be experimentally manipulated, and (c) produce effects that can be rigorously quantified. Conversely, while the experimental study of the causes and consequences host–microbe interactions have a long and productive history, additional systems are needed to fully evaluate the role of developmental symbiosis in e.g., rapid adaptation to local environments, ecological radiations, or speciation.

4.2 | Developmental symbiosis: A characteristic feature of *Onthophagus* life history, growth, and survival

The life cycle of *Onthophagus* dung beetles provides a promising, experimentally tractable model system in which to address these questions as both niche construction and symbiosis play critical roles in facilitating normal development (Figure 3a). These contributions first begin when mothers engage in niche construction by digging deep tunnels underneath cow dung pats, within which they construct brood balls. When mothers invest in burying brood balls deep underground, this tunneling behavior provides developing offspring with a stable thermal niche (Snell-Rood, Burger, Hutton, & Moczek, 2016) and increased access to oxygen (Schwab, Flores, Linz, Moczek, & Tennessen, in prep), while brood ball construction provides each larva with all the food needed to complete development and metamorphosis. Moreover, each brood ball is further endowed with a maternal fecal

pedestal onto which a single egg is oviposited (Estes et al., 2013). Upon hatching, larvae immediately consume this pedestal, thereby inoculating themselves with maternal gut microbiota (Schwab, Riggs, Newton, & Moczek, 2016).
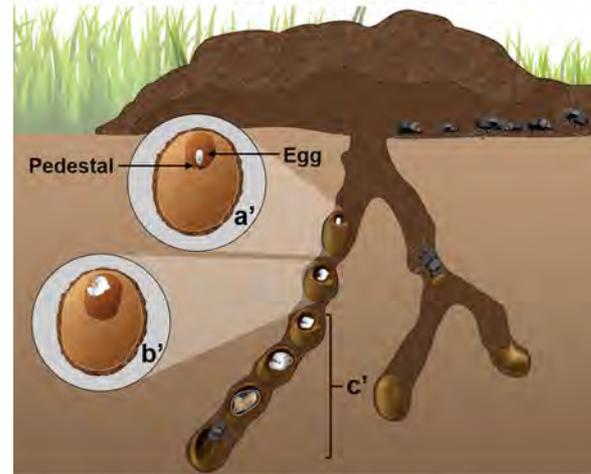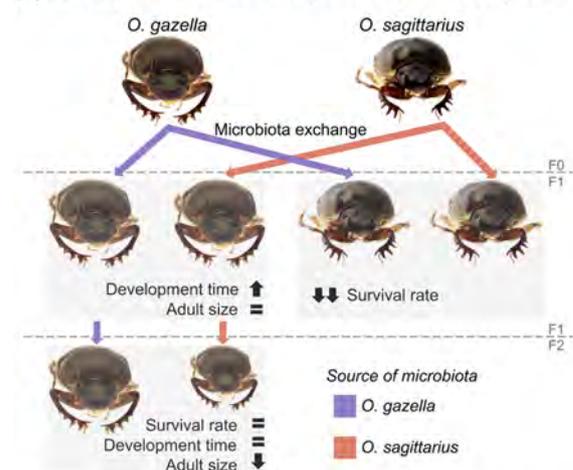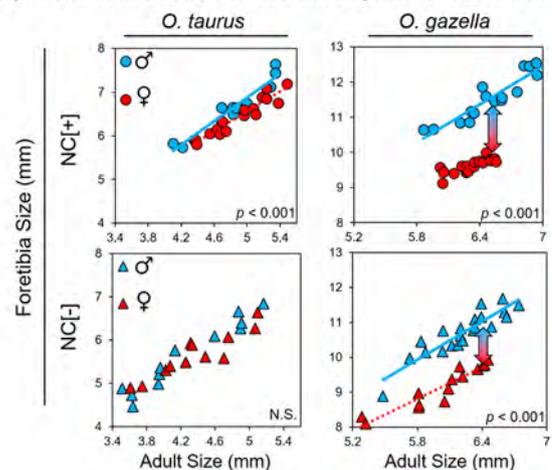

FIGURE 3 Continued

Microbes have long been hypothesized to play a critical role in enabling both juvenile and adult *Onthophagus* to subsist and diversify upon dung, which is primarily composed of complex polysaccharides such as cellulose and relatively low in amino acids (Flachowsky & Hennig, 1990; Frank, Brückner, Hilpert, Heethoff, & Blüthgen, 2017; Halffter & Edmonds, 1982; Holter, 2016; Muller, 1980). Recent work on *O. taurus* and the closely related genus *Euoniticellus* now shows that pedestal microbiota are enriched for genes implicated in cellulose degradation and nitrogen fixation (Estes et al., 2013; Shukla, Sanders, Byrne, & Pierce, 2016). Additional experimental support for this hypothesis derives from the demonstration that *Onthophagus* larvae forced to develop without their pedestal microbiota require more time to complete development and metamorphose into smaller adults compared to larvae that are provided with their pedestal microbes. Importantly, these disparities are further exaggerated under ecologically relevant temperature and desiccation stressors (Schwab et al., 2016). Furthermore, pedestal microbiota conveys resilience against dung-associated pathogens: larval mortality in the presence of the entomopathogenic fungus, *Metarhizium anisopliae*, increases by 20–40% when reared in the absence of pedestal microbes (Schwab et al., in prep). Lastly, host species appear to have specialized onto nonequivalent sets of microbial partners. Specifically, recent work shows that the exchange of pedestals

between two dung beetle species results in pronounced negative survival outcomes for one host species, while the other species demonstrates modest developmental delays with no significant effect on survival (Figure 3b). These findings provide the first experimental evidence that different *Onthophagus* host species may diverge in the extent to which they rely on gut microbiota to support normal development (Parker et al., 2018).

4.3 | Niche construction as a critical and evolvable feature of normal development

Shortly after feeding on the maternally-provisioned pedestal, larvae begin expressing a range of putative niche constructing behaviors that continue throughout their development. For instance, larvae mechanically manipulate surrounding dung to alter the physical composition of the brood ball throughout their growth period, repairing the brood ball where maternal construction is inadequate, and eventually constructing a complex pupation chamber from dung fibers and the beetle's own feces shortly before the metamorphic molt. Throughout this time, larvae defecate throughout their brood ball, thereby distributing pedestal-derived microbiota across the brood ball microenvironment, and then refeed on their own excrement until metamorphosis (Schwab et al., 2017). Recent experimental studies suggest that these collective modifications may further bias or promote particular developmental and fitness outcomes. For instance, experimentally suppressing the extent to which larvae can directly modify their brood ball environment decreases growth and common proxies of fitness (i.e., brood ball size and number produced) in multiple species of *Onthophagus*. Furthermore, suppressing niche construction alters scaling relationships in a number of key morphological traits, and even eliminates or substantially diminishes the degree of sexual dimorphism between male and female tibiae (Figure 3c; Schwab et al., 2017). Although the contribution of individual larval behaviors to these niche construction phenotypes is still unclear, preliminary findings suggest that the spreading of larval feces throughout the brood ball may generate a symbiont-mediated external rumen that aids in the chemical breakdown of chitin, lignin, and cellulose, thus promoting larval growth by making more readily digestible carbohydrates available to larvae (Schwab et al., 2017). Intriguingly, the developmental consequences of larval niche construction may coevolve with those of maternal niche construction: females derived from the Eastern US population of *O. taurus*, which engage in high levels of maternal care as measured by their deep brood ball burial depth, produce offspring

FIGURE 3 Developmental bias through symbiosis and niche construction. (a) *Onthophagus* mothers engage in niche construction by creating subterranean brood balls made of dung. In addition to providing all the dung that offspring will need to complete their development, mothers deposit a fecal secretion containing gut microbiota known as the pedestal, upon which they lay a single egg (a'). Immediately following hatching, larvae consume the pedestal and begin engaging in niche constructing behaviors (b'), which will continue through pupation and into adulthood (c') (image modified after Estes et al., 2013). (b) The exchange of the maternally-transmitted pedestal microbiota between two dung beetle species results in developmental delay and increased mortality compared with beetles receiving their own microbiota (Parker, Dury, & Moczek, 2018). The magnitude of the negative effects uncovered through pedestal exchange differed between species, and in the case of one species, these effects were inherited across generations. (c) Larvae that engage in niche construction during normal development (NC[+]) express a significant sexual dimorphism in traits such as the foretibia, with males of *O. taurus* and *O. gazella* developing longer foretibia than females. When niche construction is experimentally inhibited (NC[−]), this dimorphism is either eliminated (*O. taurus*) or significantly reduced in magnitude (*O. gazella*; image modified after Schwab et al., 2017) [Color figure can be viewed at wileyonlinelibrary.com]

that exhibit a pronounced increase in development time when this maternal niche construction is inhibited, doing so regardless of the presence or absence of larval niche construction. However, in the relatively low maternal care Western Australia population, development time increases *only* when larval niche construction is inhibited, doing so regardless of the presence or absence of maternal niche construction. These results suggest that larvae from the Western Australia population may have undergone selection to compensate for low maternal niche construction by increasing investment in, and thereby their reliance upon, larval niche construction (Dury, Moczek, & Schwab, in review). Altogether, these early experimental findings suggest that niche construction is a critical *and evolvable* component of environmentally-responsive development in *Onthophagus*.

Although studies of *Onthophagus* niche construction and developmental symbiosis are only in their early stages, the findings presented here suggest that both of these highly reciprocal processes play fundamental roles in supporting normal development. It is clear, for instance, that both processes are capable of biasing the nature of phenotypic variation that results from ontogeny, that they lend resilience to development in the presence of ecological challenges, and that their effects are evolvable at the level of populations and species. Yet much remains to be explored. Of particular interest is determining what role, if any, the microbiota plays in the adaptation of *Onthophagus* beetles to novel environments. As discussed above, diverse *Onthophagus* species have been introduced around the world, and some of these introductions, such as that of *O. taurus* into the Eastern United States, have resulted in remarkable climatic niche expansions (Silva et al., 2016). Work exploring how developmental bias may have enabled such range expansions, whether acting through host-symbiont interactions, niche construction, or developmental plasticity, is currently ongoing. More generally, experimental studies of niche construction must expand to additional model systems beyond *Onthophagus* to garner a more complete understanding of the developmental and evolutionary consequences of this process in natural populations.

5 | CURRENT FRONTIERS IN THE STUDY OF DEVELOPMENTAL BIAS

In this review, we sought to explore the role of developmental bias across diverse levels of biological organization in the genesis of novel, adaptive, and resilient phenotypes within a single taxon, the horned dung beetle genus *Onthophagus*. We find developmental bias to be pervasive, able to shape patterns of phenotypic variation across diverse

traits, and able to bias evolutionary changes over both macro- and micro-evolutionary time scales. Our findings thus add to a growing call to investigate the role of developmental bias in evolution more systematically and across a broader array of taxa, traits, and environmental contexts.

Our discussion also highlights several areas of particular significance. For example, because developmental bias is itself a product of evolution shaped by past rounds of selection, how developmental bias affects evolution may change over evolutionary time. The studies highlighted here broadly support this notion, and do so across disparate evolutionary time scales. For example, the deeply conserved head-patterning mechanisms that evolved in pre-Cambrian times now bias head innovations in derived insect lineages, while host–microbiome interactions that most likely originated when scarab beetles first evolved a dung-feeding life style (perhaps as recent as 50 MYA: Sole & Scholtz, 2010) now shape divergences among recently evolved *Onthophagus* species. Yet exactly how fast developmental bias can evolve, and the direction of this evolution, remain understudied. An especially contentious perspective derives from the hypothesis that developmental bias may evolve such as to preferentially modify phenotypic variability in the direction favored by past natural selection (Uller et al., 2018). The studies reviewed here suggest that developmental biases resulting from ancestral plasticity, developmental symbioses, and niche construction may well evolve in ways consistent with such a scenario, but more direct tests are needed to evaluate this hypothesis. Another poorly explored frontier concerns the existence and possible consequences of *interactions* among different types of developmental bias. Work on *Onthophagus* has begun to hint at a possibly significant synergism between developmental symbioses and niche construction (Schwab et al., 2017). Yet much more work is needed to evaluate if and how different types of developmental bias, operating on different levels of biological organization, interact in ways that may either restrain, combine, or synergize their impact on developmental evolution. Combined, such investigations into the role of developmental bias in evolution have the potential to significantly enhance our understanding of why and how organismal evolution unfolds the way it does.

CONFLICT OF INTERESTS

ORCID

Yonggang Hu http://orcid.org/0000-0002-3438-7296
David M. Linz http://orcid.org/0000-0003-2096-3958
Erik S. Parker http://orcid.org/0000-0002-5537-7900
Daniel B. Schwab http://orcid.org/0000-0001-5094-2598
Sofia Casasa http://orcid.org/0000-0002-5910-3739
Anna L. M. Macagno http://orcid.org/0000-0003-4133-2098

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.

Alberch, P. (1989). The logic of monsters: Evidence for internal constraint in development and evolution. *Geobios, 22*, 21–57.

Allf, B. C., Durst, P. A. P., & Pfennig, D. W. (2016). Behavioral plasticity and the origins of novelty: The evolution of the rattlesnake rattle. *The American Naturalist, 188*, 475–483.

Arthur, W. (2004). The effect of development on the direction of evolution: Toward a twenty-first century consensus. *Evolution & Development, 6*, 282–288.

Badyaev, A. V. (2009). Evolutionary significance of phenotypic accommodation in novel environments: An empirical test of the Baldwin effect. *Philosophical Transactions of the Royal Society, B: Biological Sciences, 364*, 1125–1141.

Badyaev, A. V., Potticary, A. L., & Morrison, E. S. (2017). Most colorful example of genetic assimilation? Exploring the evolutionary destiny of recurrent phenotypic accommodation. *The American Naturalist, 190*, 266–280.

Barbieri, M., Bonafè, M., Franceschi, C., & Paolisso, G. (2003). Insulin/IGF-I-signaling pathway: An evolutionarily conserved mechanism of longevity from yeast to humans. *American Journal of Physiology-Endocrinology and Metabolism, 285*, E1064–E1071.

Bates, J. M., Mittge, E., Kuhlman, J., Baden, K. N., Cheesman, S. E., & Guillemin, K. (2006). Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. *Developmental Biology, 297*, 374–386.

Beckers, O. M., Anderson, W., & Moczek, A. P. (2015). A combination of developmental plasticity, parental effects, and genetic differentiation mediates divergences in life history traits between dung beetle populations. *Evolution & Development, 17*, 148–159.

Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., & Hafen, E. (2001). An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Current Biology, 11*, 213–221.

Busey, H. A., Zattara, E. E., & Moczek, A. P. (2016). Conservation, innovation, and bias: Embryonic segment boundaries position posterior, but not anterior, head horns in adult beetles. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution, 326*, 271–279.

Carroll, S. B., Grenier, J. K., & Weatherbee, S. D. (2005). *From DNA to diversity: Molecular genetics and the evolution of animal design.* Oxford, MA: Blackwell Science Ltd.

Casasa, S., & Moczek, A. P. (2018a). Insulin signalling's role in mediating tissue-specific nutritional plasticity and robustness in the horn-polyphenic beetle Onthophagus taurus. *Proceedings of the Royal Society of London, Series B: Biological Sciences, 285*, 20181631.

Casasa, S., & Moczek, A. P. (2018b). The role of ancestral phenotypic plasticity in evolutionary diversification: Population density effects in horned beetles. *Animal Behaviour, 137*, 53–61.

Cheng, L. Y., Bailey, A. P., Leevers, S. J., Ragan, T. J., Driscoll, P. C., & Gould, A. P. (2011). Anaplastic lymphoma kinase spares organ growth during nutrient restriction in *Drosophila. Cell, 146*, 435–447.

Chiu, L., & Gilbert, S. F. (2015). The birth of the holobiont: Multi-species birthing through mutual scaffolding and niche construction. *Biosemiotics, 8*, 191–210.

Choi, J.-H., Kijimoto, T., Snell-Rood, E., Tae, H., Yang, Y., Moczek, A. P., & Andrews, J. (2010). Gene discovery in the horned beetle *Onthophagus taurus. BMC Genomics, 11*, 703.

Ciliberti, S., Martin, O. C., & Wagner, A. (2007). Innovation and robustness in complex regulatory gene networks. *Proceedings of the National Academy of Sciences United States of America, 104*, 13591–13596.

Cowen, L. E. (2005). Hsp90 potentiates the rapid evolution of new traits: Drug resistance in diverse fungi. *Science, 309*, 2185–2189.

Davidson, E. H. (2006). Gene regulatory networks and the evolution of animal body plans. *Science, 311*, 796–800.

Day, R. L., Laland, K. N., & Odling-Smee, F. J. (2003). Rethinking adaptation: The niche-construction perspective. *Perspectives in Biology and Medicine, 46*, 80–95.

Dury, G. J., Moczek, A. P., & Schwab, D. B. (in review). Maternal and larval niche construction interact to shape development, survival, and population divergence in the dung beetle, *Onthophagus taurus.*

Dworkin, I. (2005). A study of canalization and developmental stability in the sternopleural bristle system of *Drosophila melanogaster. Evolution, 59*, 1500–1509.

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.

Emlen, D. J., Corley Lavine, L., & Ewen-Campen, B. (2007). On the origin and evolutionary diversification of beetle horns. *Proceedings of the National Academy of Sciences, 104*, 8661–8668.

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

Fincher, G. T., & Woodruff, R. E. (1975). A European dung beetle, *Onthophagus taurus* Schreber, new to the U.S. (Coleoptera: Scarabaeidae). *Coleopterists Bulletin, 29*, 349–350.

Flachowsky, G., & Hennig, A. (1990). Composition and digestibility of untreated and chemically treated animal excreta for ruminants-A review. *Biological Wastes, 31*, 17–36.

Foray, V., Pérez-Jiménez, M. M., Fattouh, N., & Landmann, F. (2018). *Wolbachia* control stem cell behavior and stimulate

germline proliferation in filarial nematodes. *Developmental Cell*, *45*, 198–211.e3.

Frank, K., Brückner, A., Hilpert, A., Heethoff, M., & Blüthgen, N. (2017). Nutrient quality of vertebrate dung as a diet for dung beetles. *Scientific Reports*, *7*, 1–12.

Geminard, C., Arquier, N., Layalle, S., Bourouis, M., Slaidina, M., Delanoue, R., ... Leopold, P. (2006). Control of metabolism and growth through insulin-like peptides in *Drosophila*. *Diabetes*, *55*, S5–S8.

Ghalambor, C. K., Hoke, K. L., Ruell, E. W., Fischer, E. K., Reznick, D. N., & Hughes, K. A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature*, *525*, 372–375.

Gilbert, S. F. (2019). Developmental symbiosis facilitates the multiple origins of herbivory. *Evolution & Development*, 1–11. https://doi.org/10.1111/ede.12291

Gilbert, S. F., Bosch, T. C. G., & Ledón-Rettig, C. (2015). Eco-Evo-Devo: Developmental symbiosis and developmental plasticity as evolutionary agents. *Nature Reviews Genetics*, *16*, 611–622.

Gomez-Mestre, I., & Buchholz, D. R. (2006). Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *Proceedings of the National Academy of Sciences*, *103*, 19021–19026.

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

Grimaldi, D., & Engel, M. S. (2005). *Evolution of the insects*. Cambridge, UK: Cambridge University Press.

Halffter, G., & Edmonds, W. D. (1982). The nesting behavior of dung beetles (Scarabaeinae). An ecological and evolutive approach (pp. 139–143). Mexico City, Mexico: Instituto de Ecologia.

Hendry, A. P. (2016). Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *Journal of Heredity*, *107*, 25–41.

Holter, P. (2016). Herbivore dung as food for dung beetles: Elementary coprology for entomologists. *Ecological Entomology*, *41*, 367–377.

Hooper, L. V. (2001). Commensal host-bacterial relationships in the gut. *Science*, *292*, 1115–1118.

Hu, Y., Schmitt-Engel, C., Schwirz, J., Stroehlein, N., Richter, T., Majumdar, U., & Bucher, G. (2018). A morphological novelty evolved by co-option of a reduced gene regulatory network and gene recruitment in a beetle. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *285*, 20181373.

Ito, Y., Harigai, A., Nakata, M., Hosoya, T., Araya, K., Oba, Y., ... Niimi, T. (2013). The role of *doublesex* in the evolution of exaggerated horns in the Japanese rhinoceros beetle. *EMBO Reports*, *14*, 561–567.

Jaenike, J., Unckless, R., Cockburn, S. N., Boelio, L. M., & Perlman, S. J. (2010). Adaptation via symbiosis: Recent spread of a *Drosophila* defensive symbiont. *Science*, *329*, 212–215.

Joos, B., Casey, T. M., Fitzgerald, T. D., & Buttemer, W. A. (1988). Roles of the tent in behavioral thermoregulation of eastern tent caterpillars. *Ecology*, *69*, 2004–2011.

Kijimoto, T., Costello, J., Tang, Z., Moczek, A. P., & Andrews, J. (2009). EST and microarray analysis of horn development in *Onthophagus* beetles. *BMC Genomics*, *10*, 504.

Kijimoto, T., Moczek, A. P., & Andrews, J. (2012). Diversification of *doublesex* function underlies morph-, sex-, and species-specific development of beetle horns. *Proceedings of the National Academy of Sciences United States of America*, *109*, 20526–20531.

Kijimoto, T., Pespeni, M., Beckers, O., & Moczek, A. P. (2012). Beetle horns and horned beetles: Emerging models in developmental evolution and ecology. *Wiley Interdisciplinary Reviews: Developmental Biology*, *2*, 405–418.

Koyama, T., Mendes, C. C., & Mirth, C. K. (2013). Mechanisms regulating nutrition-dependent developmental plasticity through organ-specific effects in insects. *Frontiers in Physiology*, *4*, 263.

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

Laland, K. N., Odling-Smee, F. J., & Feldman, M. W. (1999). Evolutionary consequences of niche construction and their implications for ecology. *Proceedings of the National Academy of Sciences United States of America*, *96*, 10242–10247.

Laland, K. N., Odling-Smee, J., & Gilbert, S. F. (2008). EvoDevo and niche construction: Building bridges. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *310*, 549–566.

Laland, K. N., Uller, T., Feldman, M., Sterelny, K., Müller, G. B., Moczek, A. P., ... Odling-Smee, J. (2015). The extended evolutionary synthesis: its structure, assumptions, and predictions. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *282*, 20151019.

Ledón-Rettig, C. C., Pfennig, D. W., & Nascone-Yoder, N. (2008). Ancestral variation and the potential for genetic accommodation in larval amphibians: Implications for the evolution of novel feeding strategies. *Evolution & Development*, *10*, 316–325.

Levine, M., & Davidson, E. H. (2005). Gene regulatory networks for development. *Proceedings of the National Academy of Sciences United States of America*, *102*, 4936–4942.

Levis, N. A., Isdaner, A. J., & Pfennig, D. W. (2018). Morphological novelty emerges from pre-existing phenotypic plasticity. *Nature Ecology & Evolution*, *2*, 1289–1297.

Levis, N. A., & Pfennig, D. W. (2016). Evaluating 'plasticity-first' evolution in nature: Key criteria and empirical approaches. *Trends in Ecology & Evolution*, *31*, 563–574.

Li, Y., Brown, S. J., Hausdorf, B., Tautz, D., Denell, R. E., & Finkelstein, R. (1996). Two *orthodenticle*-related genes in the short-germ beetle *Tribolium castaneum*. *Development Genes and Evolution*, *206*, 35–45.

Linz, D. M., Hu, Y., & Moczek, A. P. (2019). The origins of novelty from within the confines of homology: the developmental evolution of the digging tibia of dung beetles. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *286*, 20182427.

Macagno, A. L. M., Beckers, O. M., & Moczek, A. P. (2015). Differentiation of ovarian development and the evolution of fecundity in rapidly diverging exotic beetle populations. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *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. *The American Naturalist*, *187*, E143–E151.

Macagno, A. L. M., Pizzo, A., Parzer, H. F., Palestrini, C., Rolando, A., & Moczek, A. P. (2011). Shape—but not size—codivergence between male and female copulatory structures in *Onthophagus* beetles. *PLOS One*, 6, e28893.

Macagno, A. L. M., Zattara, E. E., Ezeakudo, O., Moczek, A. P., & Ledón-Rettig, C. C. (2018). Adaptive maternal behavioral plasticity and developmental programming mitigate the transgenerational effects of temperature in dung beetles. *Oikos*, 127, 1319–1329.

McFall-Ngai, M. J. (2014). The importance of microbes in animal development: Lessons from the squid-vibrio symbiosis. *Annual Review of Microbiology*, 68, 177–194.

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C. G., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., ... Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences United States of America*, 110, 3229–3236.

Moczek, A. P. (1998). Horn polyphenism in the beetle *Onthophagus taurus*: Larval diet quality and plasticity in parental investment determine adult body size and male horn morphology. *Behavioral Ecology*, 9, 636–641.

Moczek, A. P. (2003). The behavioral ecology of threshold evolution in a polyphenic beetle. *Behavioral Ecology*, 14, 841–854.

Moczek, A. P. (2005). The evolution and development of novel traits, or how beetles got their horns. *BioScience*, 11, 935–951.

Moczek, A. P. (2007). Developmental capacitance, genetic accommodation, and adaptive evolution. *Evolution & Development*, 9, 299–305.

Moczek, A. P. (2009). Developmental plasticity and the origins of diversity: A case study on horned beetles. In T. N. Ananthakrishnan, & D. Whitman (Eds.), *Phenotypic plasticity in insects: mechanisms and consequences* (pp. 81–134). Plymouth, UK: Science Publishers, Inc.

Moczek, A. P. (2010). Phenotypic plasticity and diversity in insects. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 365, 593–603.

Moczek, A. P. (2012). The nature of nurture and the future of evodevo: Toward a theory of developmental evolution. *Integrative and Comparative Biology*, 52, 108–119.

Moczek, A. P., & Nijhout, H. F. (2002). Developmental mechanisms of threshold evolution in a polyphenic beetle. *Evolution & Development*, 4, 252–264.

Moczek, A. P., & Nijhout, H. F. (2003). Rapid evolution of a polyphenic threshold. *Evolution & Development*, 5, 259–268.

Moczek, A. P., Hunt, J., Emlen, D. J., & Simmons, L. W. (2002). Threshold evolution in exotic populations of a polyphenic beetle. *Evolutionary Ecology Research*, 4, 587–601.

Muller, Z. O. (1980). Feed from animal wastes: State of knowledge, *FAO Animal Production and Health Paper 18*. Rome, Italy: Food and Agriculture Organization of the United Nations. http://www.fao.org/3/X6518E/X6518E00.htm

Odling-Smee, F. J., Laland, K. N., & Feldman, M. W. (2003). *Niche construction: The neglected process in evolution*. Princeton, NJ: Princeton University Press.

Paaby, A. B., & Rockman, M. V. (2014). Cryptic genetic variation: Evolution's hidden substrate. *Nature Reviews Genetics*, 15, 247–258.

Parker, E. S., Dury, G. J., & Moczek, A. P. (2018). Transgenerational developmental effects of species-specific, maternally transmitted microbiota in *Onthophagus* dung beetles. *Ecological Entomology*, 44, 274–282.

Payne, J. L., Moore, J. H., & Wagner, A. (2014). Robustness, evolvability, and the logic of genetic regulation. *Artificial Life*, 20, 111–126.

Posnien, N., Koniszewski, N. D. B., Hein, H. J., & Bucher, G. (2011). Candidate gene screen in the red flour beetle *Tribolium* reveals *six3* as ancient regulator of anterior median head and central complex development. *PLOS Genetics*, 7, e1002416.

Posnien, N., Schinko, J. B., Kittelmann, S., & Bucher, G. (2010). Genetics, development and composition of the insect head—A beetle's view. *Arthropod structure & Development*, 39, 399–410.

Prud'homme, B., Gompel, N., & Carroll, S. B. (2007). Emerging principles of regulatory evolution. *Proceedings of the National Academy of Sciences United States of America*, 104, 8605–8612.

Queitsch, C., Sangster, T. A., & Lindquist, S. (2002). Hsp90 as a capacitor of phenotypic variation. *Nature*, 417, 618–624.

Rawls, J. F., Samuel, B. S., & Gordon, J. I. (2004). From the cover: Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proceedings of the National Academy of Sciences United States of America*, 101, 4596–4601.

Robinson, B. W. (2013). Evolution of growth by genetic accommodation in Icelandic freshwater stickleback. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 280, 20132197.

Rohner, N., Jarosz, D. F., Kowalko, J. E., Yoshizawa, M., Jeffery, W. R., Borowsky, R. L., ... Tabin, C. J. (2013). Cryptic variation in morphological evolution: HSP90 as a capacitor for loss of eyes in cavefish. *Science*, 342, 1372–1375.

Rutherford, S. L., & Lindquist, S. (1998). Hsp90 as a capacitor for morphological evolution. *Nature*, 396, 336–342.

Saccone, G., Salvemini, M., Pane, A., & Polito, L. C. (2008). Masculinization of XX *Drosophila* transgenic flies expressing the *Ceratitis capitata* DoublesexM isoform. *The International Journal of Developmental Biology*, 52, 1051–1057.

Sánchez, L., Gorfinkiel, N., & Guerrero, I. (2001). Sex determination genes control the development of the *Drosophila* genital disc, modulating the response to *Hedgehog*, *Wingless* and *Decapentaplegic* signals. *Development*, 128, 1033–1043.

Schlichting, C. D., & Pigliucci, M. (1998). *Phenotypic evolution: A reaction norm perspective*. Sunderland, MA: Sinauer Associates Inc.

Schwab, D. B., & Moczek, A. P. (2017). Evo devo and niche construction. In Nuño de la Rosa, L., & Müller, G. B. (Eds.), *Evolutionary developmental biology—A reference guide* (pp. 1–14). Basel: Springer International Publishing.

Schwab, D. B., Casasa, S., & Moczek, A. P. (2017). Evidence of developmental niche construction in dung beetles: Effects on growth, scaling and reproductive success. *Ecology Letters*, 20, 1353–1363.

Schwab, D. B., Casasa, S., & Moczek, A. P. (2019). On the reciprocally causal and constructive nature of developmental plasticity and robustness. *Frontiers in Genetics*, 9, 735.

Schwab, D. B., Flores, B. J., Linz, D., Moczek, A. P., & Tennessen, J. M. (In preparation). Characterizing the developmental and metabolic response to hypoxia in dung beetles.

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

Scoville, A. G., & Pfrender, M. E. (2010). Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators.

Proceedings of the National Academy of Sciences United States of America, *107*, 4260–4263.

Shaw, K. A., Scotti, M. L., & Foster, S. A. (2007). Ancestral plasticity and the evolutionary diversification of courtship behaviour in threespine sticklebacks. *Animal Behaviour*, *73*, 415–422.

Shikuma, N. J., Pilhofer, M., Weiss, G. L., Hadfield, M. G., Jensen, G. J., & Newman, D. K. (2014). Marine tubeworm metamorphosis induced by arrays of bacterial phage tail-like structures. *Science*, *343*, 529–533.

Shingleton, A. W., Das, J., Vinicius, L., & Stern, D. L. (2005). The temporal requirements for insulin signaling during development in *Drosophila*. *PLOS Biology*, *3*, e289.

Shubin, N., Tabin, C., & Carroll, S. (2009). Deep homology and the origins of evolutionary novelty. *Nature*, *457*, 818–823.

Shukla, S. P., Sanders, J. G., Byrne, M. J., & Pierce, N. E. (2016). Gut microbiota of dung beetles correspond to dietary specializations of adults and larvae. *Molecular Ecology*, *25*, 6092–6106.

Sikkink, K. L., Reynolds, R. M., Ituarte, C. M., Cresko, W. A., & Phillips, P. C. (2014). Rapid evolution of phenotypic plasticity and shifting thresholds of genetic assimilation in the nematode *Caenorhabditis remanei*. *G3: Genes Genomes Genetics*, *4*, 1103–1112.

Silva, D. P., Vilela, B., Buzatto, B. A., Moczek, A. P., & Hortal, J. (2016). Contextualized niche shifts upon independent invasions by the dung beetle *Onthophagus taurus*. *Biological Invasions*, *18*, 3137–3148.

Sneed, J. M., Sharp, K. H., Ritchie, K. B., & Paul, V. J. (2014). The chemical cue tetrabromopyrrole from a biofilm bacterium induces settlement of multiple Caribbean corals. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *281*, 1–9.

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. *Evolutionary Ecology*, *30*, 251–265.

Sole, C. L., & Scholtz, C. H. (2010). Did dung beetles arise in Africa? A phylogenetic hypothesis based on five gene regions. *Molecular Phylogenetics and Evolution*, *56*, 631–641.

Sommer, F., & Bäckhed, F. (2013). The gut microbiota-masters of host development and physiology. *Nature Reviews Microbiology*, *11*, 227–238.

Standen, E. M., Du, T. Y., & Larsson, H. C. E. (2014). Developmental plasticity and the origin of tetrapods. *Nature*, *513*, 54–58.

Suzuki, Y., & Nijhout, H. F. (2006). Evolution of a polyphenism by genetic accommodation. *Science*, *311*, 650–652.

Tanaka, K., Barmina, O., Sanders, L. E., Arbeitman, M. N., & Kopp, A. (2011). Evolution of sex-specific traits through changes in HOX-dependent *doublesex* expression. *PLOS Biology*, *9*, e1001131.

Tang, H. Y., Smith-Caldas, M. S. B., Driscoll, M. V., Salhadar, S., & Shingleton, A. W. (2011). *FOXO* regulates organ-specific phenotypic plasticity in *Drosophila*. *PLOS Genetics*, *7*, e1002373.

Teixeira, L., Ferreira, Á., & Ashburner, M. (2008). The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLOS Biology*, *6*, e1000002.

Tomoyasu, Y., Ohde, T., & Clark-Hachtel, C. (2017). What serial homologs can tell us about the origin of insect wings. *F1000Research*, *6*, 268.

Tyndale-Biscoe, M. (1996). *Australia's introduced dung beetles: original releases and redistributions*. Technical Report 62. Canberra, ACT: CSIRO, Division of Entomology.

Uller, T., Moczek, A. P., Watson, R. A., Brakefield, P. M., & Laland, K. N. (2018). Developmental bias and evolution: A regulatory network perspective. *Genetics*, *209*, 949–966.

von Dassow, G., Meir, E., Munro, E. M., & Odell, G. M. (2000). The segment polarity network is a robust developmental module. *Nature*, *406*, 188–192.

Waddington, C. H. (1953). Genetic assimilation of an acquired character. *Evolution*, *7*, 118–126.

Wagner, A. (2005). *Robustness and evolvability in living systems*. Princeton, NJ: Princeton University Press.

Wagner, G. P. (2007). The developmental genetics of homology. *Nature Reviews Genetics*, *8*, 473–479.

Wagner, G. P. (2014). *Homology, Genes, and Evolutionary Innovation*. Princeton, NJ: Princeton University Press.

Walworth, N. G., Lee, M. D., Fu, F.-X., Hutchins, D. A., & Webb, E. A. (2016). Molecular and physiological evidence of genetic assimilation to high CO_2 in the marine nitrogen fixer *Trichodesmium*. *Proceedings of the National Academy of Sciences United States of America*, *113*, E7367–E7374.

West-Eberhard, M. J. (1986). Alternative adaptations, speciation, and phylogeny (A Review). *Proceedings of the National Academy of Sciences United States of America*, *83*, 1388–1392.

West-Eberhard, M. J. (1998). Evolution in the light of developmental and cell biology, and vice versa. *Proceedings of the National Academy of Sciences United States of America*, *95*, 8417–8419.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. New York, NY: Oxford University Press.

Whalan, S., & Webster, N. S. (2014). Sponge larval settlement cues: The role of microbial biofilms in a warming ocean. *Scientific Reports*, *4*, 28–32.

Wund, M. A., Baker, J. A., Clancy, B., Golub, J. L., & Foster, S. A. (2008). A test of the "flexible stem" model of evolution: Ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. *The American Naturalist*, *172*, 449–462.

Yeh, P. J., & Price, T. D. (2004). Adaptive phenotypic plasticity and the successful colonization of a novel environment. *The American Naturalist*, *164*, 531–542.

Zattara, E. E., Busey, H. A., Linz, D. M., Tomoyasu, Y., & Moczek, A. P. (2016). Neofunctionalization of embryonic head patterning genes facilitates the positioning of novel traits on the dorsal head of adult beetles. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *283*, 20160824.

Zattara, E. E., Macagno, A. L. M., Busey, H. A., & Moczek, A. P. (2017). Development of functional ectopic compound eyes in scarabaeid beetles by knockdown of *orthodenticle*. *Proceedings of the National Academy of Sciences United States of America*, *114*, 12021–12026.