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Phenotypic Plasticity and the Origins of Diversity: A Case Study on Horned Beetles

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Abstract

Phenotypic plasticity has long been proposed to play a major role in the origin and subsequent diversification of morphological and behavioral novelties. Polyphenism, as an extreme yet common case of phenotypic plasticity, is thought to be a particularly important facilitator of rapid evolution and diversification. In the first part of this chapter I review how phenotypic plasticity and polyphenic development are thought to contribute to and shape patterns of morphological and behavioral innovation and diversification in insects. I then apply these insights to a highly diverse and speciose group of insects: horned beetles. In horned beetles polyphenic development is involved in the production of alternative horned and hornless male morphologies which are used in the context of alternative reproductive tactics. It is in these species that phenotypic plasticity produces some of the most exaggerated and diverse secondary sexual traits known in insects. In the second part of this chapter I explore the roles of developmental and behavioral plasticity in the origins of diversity among horned beetles. I review the physiological and developmental mechanisms that regulate the expression of male phenotype and examine the ecological and behavioral context within which horn polyphenic beetles function. I then explore how ecology and behavior have shaped aspects of phenotypic plasticity in natural populations, and how plasticity in turn has contributed to and directed the evolutionary diversification of horn polyphenic beetles. I end by discussing how such insights, combined with recent novel approaches, can help in uncovering the evolutionary origins of phenotypic novelties and the causes and mechanisms of their subsequent diversification.

Introduction

Phenotypic plasticity is a universal property of all forms of life, from phages to higher multicellular organisms (e.g. Ptashne 1992, Sultan 1987, Nijhout 1999). It transcends taxonomic boundaries as well as organizational levels within individuals. Transcription, translation, cell proliferation, organ function, endocrine and neuronal regulation, mating behavior and so on—all are phenotypically plastic in one way or another, and being able to respond flexibly to changes in external conditions is an integral part of their proper functioning (West-Eberhard 2003). Even though its evolutionary implications were recognized early on, phenotypic plasticity has only recently regained attention from evolutionary biologists (Baldwin 1902, Schmalhausen 1949, Waddington 1953, West-Eberhard 1989, 1992, Stearns 1989, Sultan 1992, Nijhout 1999, Schlichting and Pigliucci 1998, Pigliucci 2001). Opinions on its contributions to evolutionary processes, however, vary widely (reviewed in West-Eberhard 2003). In this chapter, I will review the role of phenotypic plasticity in insect evolution. In particular, I will examine the importance of phenotypic plasticity for one of the most significant, and arguably most poorly understood, phenomena in evolutionary biology: the origin and diversification of phenotypic novelties. I will then apply these insights to a group of insects that has been among the focal taxa for studies on the evolution and development of phenotypic plasticity: horned beetles. Because much of the debate about the importance of phenotypic plasticity in evolution has at least in part been due to an inconsistent use of terminology I will begin this chapter with a few definitions.

Definitions

For the most part my definitions of key terms such as *phenotype* and *plasticity* follow those of West-Eberhard (2003). Accordingly, I use the term *phenotypic plasticity* in its broadest definition, that is, I consider the phenotype as including all traits of an organism, whether they are physiological, morphological, or behavioral. Furthermore, while many authors use separate terms in reference to adaptive and non-adaptive, active and passive, reversible and irreversible phenotypic plasticity, or plasticity that generates a continuous or discontinuous range of phenotypes, I consider all of the above different manifestations of the same fundamental property of an organism, namely its ability to respond to environmental stimuli via changing the expression of a phenotype. I will, however, use *polyphenism*

and *polyphenic development* to refer to organisms in which individuals are able to express two or more discretely different morphologies in response to external conditions. This is not meant to qualitatively separate polyphenism from plasticity, but rather to emphasize its extreme nature in many taxa. Furthermore, I will use terms such as *developmental* or *behavioral* plasticity to emphasize the context within which a particular plastic response is generated.

Phenotypic Plasticity and the Origins of Diversity

The main purpose of this section is to outline the mechanisms by which phenotypic plasticity can mediate the origin, exaggeration, and diversification of novel phenotypic traits in insects. However, to evaluate whether these mechanisms are likely to be of general importance in evolution or only relevant for special cases, and whether the phenotypic diversity generated by them is sufficient to mediate the evolution of complex traits, we must first understand the phenotypic and taxonomic range of phenotypic plasticity.

The Pervasiveness of Plasticity

Examples of phenotypic plasticity abound in the insects. They include extreme cases such as social castes in the Hymenoptera, termites and aphids (Wheeler and Nijhout 1983; Lüscher 1960, Stern and Foster 1996), seasonal polyphenisms in butterflies (Shapiro 1976), dispersal poyphenisms in a wide range of insects (Zera and Denno 1997), alternative asexual and sexual reproductive phases in aphids (Moran 1991), and alternative male morphologies in thrips (Crespi 1988) and beetles (Emlen 1994). In all these cases individuals have the ability to develop into one of two or more very different phenotypes, and decide based on genetic and environmental inputs which one to express. Many of these cases also involve the facultative expression of what in other contexts are considered important evolutionary transitions, such as the absence or presence of wings in ant castes (Abouheif and Wray 2002), the absence or presence of pattern elements on butterfly wings (Nijhout 1991), or the alternation between asexual and sexual modes of reproduction in aphids (Moran 1992). Distinct life stages are another form of extreme phenotypic plasticity. Here, each individual has the ability to consecutively express two or more discrete phenotypes. The holometabolous insects are thought to owe their evolutionary success in part to the extreme division of larval and adult stages (Yang 2001). The drastic differences in morphology, physiology, and behavior between the larval and adult stages

of groups such as the butterflies, bees or beetles attest to the remarkable phenotypic range that alternative phenotypes can accommodate (Gullan and Cranston 2000). By focusing on extreme cases of phenotypic plasticity it is, however, easy to overlook that phenotypic plasticity and alternative phenotypes are in reality far more widespread, if not ubiquitous. For example, many aspects of insect growth and development are influenced profoundly by external conditions such as temperature and nutrient availability (Allegret 1964, Beck 1971a, Blakley and Goodner 1978), and many insects can respond to changes in environmental conditions flexibly and adaptively by altering their behavior, physiology, and development (Beck 1971b, Shafiei et al. 2001). Alternative reproductive tactics, in which individuals switch facultatively between different behaviors to acquire mates, were once thought of as special cases that evolve only under rather unique conditions (e.g. Gadgil 1972), but have now been described in many insect orders and appear commonplace (Thornhill and Alcock 1983, Shuster and Wade 2003). Other types of behavior such as foraging, feeding or provisioning behavior, are similarly plastic in many insects (Mitchel 1975, Tanaka 1985, Field 1992, Sowig 1996, Moczek 1998, 1999). Phenotypic plasticity and alternative phenotypes are thus not only taxonomically widespread, they also occur at every part of the phenotype, whether behavioral, physiological, or developmental.

The processes that mediate such widespread plasticity themselves operate on a wide range of levels. For example, the expression of different castes in social insects involves differential gene expression (Evans and Wheeler 1999, 2001), differences in endocrine physiology (Wheeler and Nijhout 1983, 1984), differences in the regulation of appendage growth and development (Abouheif and Wray 2002), differential development of reproductive organs (Passera and Suzzoni 1979, Otto 1962), differences in behavioral repertoires (Wilson 1976) etc. The expression of seasonal morphs in butterflies involve differences in behavior (Brakefield and Reitsma 1991), endocrine physiology (Koch and Bückmann 1987, Roundtree and Nijhout 1995), pigment synthesis (Koch 1995), and so on. At each of these levels genetic and environmental inputs onto the already existing phenotype determine subsequent patterns of phenotype expression. As we will see, the range of levels at which plastic responses can be mediated, and the joint contributions of genetic and environmental factors in guiding phenotype expression, have important consequences for understanding the contributions of plasticity to patterns of phenotypic diversification.

Diversity without Specialization

Phenotypic plasticity allows individual genotypes to express multiple phenotypes as a function of environmental inputs (Nijhout 1999). How often a given phenotype is expressed then becomes a function of the frequency with which certain environmental conditions recur. Whenever a particular phenotype is expressed, it can be subject to selection and modification through subsequent generations. In the absence of the inducing environment, however, a given phenotype may disappear from a population and be replaced by an alternate phenotype, which itself becomes the subject of selection. Phenotypic plasticity thus provides the opportunity for the independent evolution and adaptation of different phenotypes to different sets of environmental circumstances (West-Eberhard 1989, 2003). Exactly how independent different evolutionary trajectories can be is likely to depend, among others, on the extent to which genetic and developmental regulatory mechanisms are shared among alternatives. That alternative phenotypes can, at least in some cases, evolve rather independently of one another is suggested by the often extreme physiological and morphological differences that exist between alternative morphs, e.g. between queens, workers and soldier in ants (Hölldobler and Wilson 1990). In such cases the evolution and elaboration of developmental switch mechanisms are thought to play a central role in mediating the diversification of alternative phenotypes (Nijhout 1999). For example, hormonally mediated threshold responses are an extremely widespread component of many developmental switch mechanisms in insects, e.g. in determining the timing of pupation in butterflies (Nijhout 1976), the expression of winged and wingless morphs in crickets (Cisper et al. 2000), the determination of castes in social insects (Wheeler and Nijhout 1983, 1984) or the expression of alternative male phenotypes in horned beetles (Moczek and Nijhout 2002a). In these cases, developmental switches permit the coordinated and integrated expression of a large number of phenotypic traits in response to changes in environmental conditions, and as such largely decouple two or more suites of phenotypic characters from each other (Nijhout 1999). This has several important implications for the evolution of alternative phenotypes. Once uncoupled by a developmental switch, alternatives can follow evolutionary trajectories that are less dependent on each other, which in turn can facilitate the further specialization and divergence between alternatives. At the same time, the switch mechanism itself can become a target of selection, opening up a previously unavailable avenue for phenotype evolution.

Switches as Targets of Selection

Switch mechanisms, whether behavioral, developmental or physiological, allow individuals to adjust the expression of a large suite of phenotypic traits in response to changes in external conditions. As such switches are potent modifiers of phenotype expression. Not surprisingly, even subtle evolutionary changes in exactly how a switch operates can have a profound impact on patterns of phenotype expression. Particularly illuminating examples come from studies of scaling relationships in polyphenic organisms (Emlen and Nijhout 2000). For example, body parts of castes in social insects, or body size and length of secondary sexual traits in males in numerous insects, often exhibit species-specific scaling relationships, or allometries (Emlen and Nijhout 2000). The exact shape of a given allometry is in part a product of the developmental switch mechanisms involved in the production of body parts during larval development (Wheeler 1991, Nijhout 1994, Nijhout and Wheeler 1996). In ants, caste determination occurs as early as during embryonic development in case of queens (Passera and Suzzoni 1979) or relatively early in larval development in case of worker and soldier castes (Wheeler and Nijhout 1983, 1984, Wheeler 1991). As a consequence, the developmental trajectories of different castes are decoupled while individuals have yet to undergo a significant portion of their growth. This relatively early onset of independent growth trajectories in different incipient castes allows the resulting adult phenotypes to be at times extremely discontinuous and scaling relationships of body parts to be non-overlapping (Fig. 1a; Wilson 1978, 1985, Moffett 1987, Wheeler 1991). In contrast, whether or not a male beetle develops into a horned, major morph or a hornless, minor morph is determined relatively late in larval development. Here the developmental switch involved in determining the subsequent fate of a male larva may occur as little as 72 h before pupation and thus after larvae have already completed almost all of their growth (Moczek and Nijhout 2002a). As a consequence, while major and minor morphs may differ dramatically in the degree of horn development, the remaining morphology is, for the most part, unaffected. Similarly, the resulting scaling relationships between horn length and body size may be highly non-linear, but typically remain continuous and with broad overlap between alternative morphs (Fig. 1b; Moczek 1998). The ontogenetic timing of a developmental switch thus can have important consequences for patterns of phenotype expression.

Changes in ontogenetic timing are not the only means by which evolutionary changes in developmental switches can contribute of phenotypic

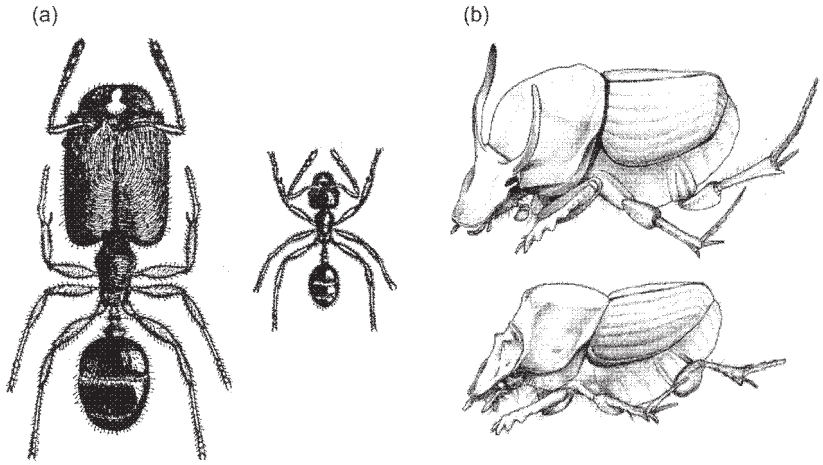

Fig. 1 Alternative phenotypes produced by developmental switches. **(a)** Different worker forms in the ant *Pheidole tepicana*. Developmental switches operating relatively early during larval development allow ants to produce highly divergent alternative phenotypes. Note relative sizes of head and alitrunk (fused thorax and first abdominal segment; after Wheeler 1910). **(b)** Alternative horned and hornless males in the beetle *Onthophagus taurus*. A developmental switch operating late during larval development causes adult males to differ dramatically in horn length while the remaining morphology is largely unaffected (drawings by Shane Richards).

diversity. Switches between alternative phenotypes typically employ a particular response threshold, e.g. to crowding (Denno et al. 1986), food quality (Moczek 1998) or photoperiod (Tauber and Tauber 1970), and usually generate a phenotypic transition of a certain well-defined magnitude and steepness. Threshold values, steepness and magnitude are all at least in part properties of the developmental switch itself (Nijhout and Wheeler 1996). Evolutionary changes in the developmental switch mechanism thus can modify one or more of these aspects of phenotype expression (Fig. 2). For example, size-dependent expression of alternative morphs in horned beetles is regulated at least in part via juvenile hormone titers present during certain sensitive periods (Emlen and Nijhout 1999). Experimental changes in JH titers (Emlen and Nijhout 1999), or evolutionary modifications in the sensitivity to JH (Moczek and Nijhout 2002, 2003), drastically change the body size at which adults switch between alternative morphs, which in turn results in substantial alterations of the average scaling relationship between body size and horn length (Moczek 2003, see also below). Developmental switches thus provide evolutionary mechanisms with an additional powerful set of targets by which phenotype expression can potentially be modified.

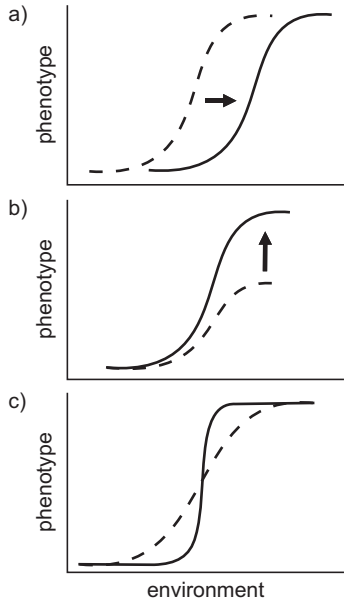

Fig. 2 Different aspects of developmental switches can become modified by selection. **(a)** Changes in the critical environment in which the switch between phenotypes occurs. **(b)** Changes in the magnitude of the phenotypic response to changes in environmental conditions. **(c)** Changes in the steepness of phenotypic transitions as environments change.

The next section highlights several important consequences of evolutionary changes in developmental switches.

Developmental Divergence and Speciation

An important consequence of evolutionary changes in developmental plasticity involves its potential to initiate the evolution of reproductive isolation between developmentally divergent populations. Even though plasticity *per se* increases an organism's ability to respond to a range of environmental conditions, different sets of environments are likely to favor different kinds of plastic responses. For example, geographic comparisons and breeding experiments on a wide range on insect taxa have illustrated that response thresholds can vary heritably and adaptively among populations (e.g. Tauber and Tauber 1972, 1982, 1987, Harrison 1979, Hazel and West 1982, Denno et al. 1986, Semlitsch and Wilbur 1989, Semlitsch et al. 1990, Emlen 1996, Ahlroth et al. 1999). Such between-population divergence in plastic phenotype expression may have important consequences once divergent populations establish contact. Divergence in particular traits,

such as timing of pupation, eclosion and mating (Shafiei et al. 2001), or choice of host organism (Moran 1991) may alone be sufficient to reproductively isolate divergent populations spatially or temporally, even if they co-occur geographically. Even if premating isolation is incomplete or absent at first, between-population divergence in plastic phenotype expression may still facilitate speciation by reducing hybrid fitness. Hybrids may exhibit reduced fitness due to the expression of a sub-optimal phenotype (in this case a sub-optimal plastic response), which in turn may favor the spread of alleles that facilitate assortative mating within each parental population (Porter and Johnson 2002). While such a scenario should apply to all traits with important fitness consequences, there may be reasons to believe that phenotypically plastic trait expression, and especially developmental-switch mediated alternative phenotypes, might be particularly prone to initiate reproductive isolation through developmental divergence. Because developmental switches regulate the simultaneous expression of whole suites of phenotypic traits, between-population divergence in developmental switches instantly causes populations to diverge in a large number of phenotypic traits. Increasing the number of phenotypic traits involved in a divergence increases the probability that hybrids will express some type of sub-optimal phenotype for at least some traits under at least some conditions, which should intensify selection for premating or prezygotic isolation. While theoretical models (e.g. Porter and Johnson 2002) lend support to such scenarios, more comparative and experimental work is clearly needed to examine if and how plasticity contributes to the origins of reproductive isolation.

Loss and Recurrence of Alternatives

One important avenue through which phenotypic plasticity and polyphenic development can influence phenotypic diversity is through the loss of alternatives. For example, the loss of alternative phenotypes is thought to have played a major role in life cycle evolution of aphids (Moran and Whitham 1988, Moran 1991) and social evolution in halticid bees (Richards 1994). Loss of alternatives, though at first sight a reduction in phenotypic diversity, can nonetheless facilitate phenotypic diversification through a variety of very interesting mechanisms. First, the ability to produce alternative phenotypes may limit the kinds of genetic modifications that a polyphenic population can accommodate. Deletion of one or several alternative phenotypes would remove such constraints. The remaining phenotypes would then be free to incorporate genetic modifications previously selected

against, and as a consequence should become increasingly specialized to their particular environment (Moran 1988, West-Eberhard 1989, 2003). Similar to the argument presented in the last section, *differential* deletion of alternatives in different populations of the same species has the potential to instantly generate reproductive barriers and facilitate speciation (West-Eberhard 1989, 2003).

Loss of alternative phenotypes does, however, not have to be permanent. Instead, lost alternatives may reappear in temporarily monomorphic lineages, possibly leading to the subsequent fixation of the recurrent phenotype. Well-known examples of recurrent gain and loss of phenotypes include the paedomorphic and metamorphic populations of salamanders (Shaffer 1984), sword-bearing and sword-less species of swordfish (Schluter et al. 1997) and directly and indirectly developing sea urchins (Raff 1996). Among the insects, a remarkable example of recurrence is the repeated reappearance of wings among secondarily wingless stick insects (Whiting et al. 2003). Recent phylogenetic analyses of the Phasmatodea provide strong evidence that wings, and thus flight, have been re-gained as many as four times independently, reversing the earlier loss of wings in these insects prior to their diversification (Whiting et al. 2003). Interestingly, in two clades recurrence of wings appears to have then been followed again by loss of wings in a subset of taxa. While estimates of the exact number of gains and losses depend on the weighing of their respective probabilities, these data nonetheless provide striking support for the idea that loss of complex traits is reversible and need not be an evolutionary dead end. What determines whether the loss of a phenotype is permanent or potentially reversible? The answer to this question most likely lies in the developmental genetic basis of a given trait. The recurrence of wings among wingless stick insects would appear, at first, highly unlikely. Wings are complex traits whose development and function require the coordinated integration of wing tissue growth, muscle growth and attachment, innervation and so on (Dudley 2000a,b). Once wings are lost we would expect the requisite genetic regulators to accumulate mutations that should increasingly disrupt their function. However, if we look more carefully at how wing development is regulated, we may understand why in this case, maintenance of at least large portions of the machinery necessary for wing production might be feasible even when the final phenotype is not expressed. First, wing development in insects relies in large part on the same regulatory genes involved in patterning legs and other appendages (Campbell et al. 1993, Campbell and Tomlinson 1998, but see Kubota et al. 2003). Since the protein

products of these genes function in other important developmental contexts this should prevent the accumulation of mutations in at least the protein coding regions of these genes. Furthermore, in at least some insects, wing and leg primordia are derived from the same pool of embryonic cells (Cohen et al. 1993, Kim et al. 1996). In the early *Drosophila* embryo, interactions between two diffusible morphogens, *wingless* and *decapentaplegic*, determine which cells will develop into imaginal disks. This pool of cells subsequently undergoes a separation into wing and leg imaginal disk precursor cells. Leg and wing imaginal disks thus have a common origin in at least some insects (Cohen et al. 1993, Kim et al. 1996). One implication of these observations is that even though a complex phenotype may be absent in adults, large portions of the embryonic and larval developmental machinery required for its expression will remain intact because they are shared with, and integrated into, other developmental processes. If this perspective turns out to be correct, we would predict that phenotype recurrence should be more likely the greater the extent to which underlying developmental mechanisms are shared with and integrated into other developmental processes.

Phenotypic Plasticity and Diversity in Horned Beetles

The preceding sections outlined some of the major mechanisms through which phenotypic plasticity is thought to influence and contribute to phenotypic evolution. We are now in a position to apply these insights to horned beetles, a highly diverse and speciose group of insects that recently has been among the focal taxa for studies on the evolution and development of morphological diversity (Emlen 2000). The following sections introduce several important aspects of the biology of horned beetles. I begin by describing the most important patterns of morphological variation in horned beetles. I then summarize what is known about the proximate genetic and physiological mechanisms that generate this variation and review the behavioral and ecological context within which different morphologies function. In the following sections I draw heavily from work on the scarabaeid genus *Onthophagus*, where recent studies have accumulated the most extensive knowledge of the evolutionary ecology and physiology of any group of horned beetles. I include, also, studies on other beetle taxa and attempt to determine the extent to which patterns found in onthophagine beetles are likely to be true for horned beetles in general.

Morphological Diversity in Horned Beetles: within Species

Horns and horn-like structures in beetles have attracted the attention of biologists since Darwin's time (Wallace 1869, Darwin 1871, Arrow 1899). Early naturalists recognized not only the absolute size but also extraordinary variability in the expression of horns between and within species (Figs. 3 and 4; Arrow 1951). However, it was not until the 1990's that experimental evidence demonstrated the nature and source of some of this extreme variability (Emlen 1994; see below). Within species of horned beetles scientists noted two general patterns of morphological diversity: the relative absence of horns in females, and the often extraordinary variability of horns in males (Fig. 3; Paulian 1935). In the vast majority of species females show no or greatly reduced expression of horns compared to their male counterparts (von Reichenau 1881). If females do develop horns it is typically a similar horn *type* as in males, e.g. a paired head horn, or a single pronotal horn (Balthasar 1963). Both patterns extend across all groups of horned beetles, and exceptions are rare (Arrow 1951). One such exception is *Onthophagus sagittarius*, in which females not only develop relatively larger horns than males, but also a completely different type of horn (Fig. 3c). But in the vast majority of species horn expression is either restricted to, or much more pronounced, in males. Furthermore, it is also exquisitely variable. So much, in fact, that males with low and high levels of horn development were sometimes classified as belonging to separate species (Paulian 1935). Because of this extreme variability in trait expression, one common way of describing the morphology of a given species of horned beetle is by use of a static allometry, or scaling relationship, in which the horn length of individuals of different body sizes is graphed as a function of body size (Eberhard and Gutiérrez 1991, Emlen and Nijhout 2000, Moczek and Nijhout 2003). The shape of such scaling relationships can range from linear to broken and sigmoidal in different species of horned beetles (Fig. 5; Rasmussen 1994, Hunt and Simmons 1997, Moczek 2002). Because linear scaling relationship typically exhibit a slope >1, all three types of scaling relationships cause large males to not just be scaled-up, enlarged versions of their smaller conspecifics, but to develop a fundamentally different morphology (Emlen and Nijhout 2000). Interestingly, the exact shape of a given scaling relationship can vary subtly to dramatically between populations of the same species, suggesting that conspecific populations can differ in switch mechanisms and resulting developmental trajectories (Moczek and Nijhout 2003, Moczek 2003). The following sections therefore explore the relationship between intra- and interspecific patterns of variation among horned beetles.

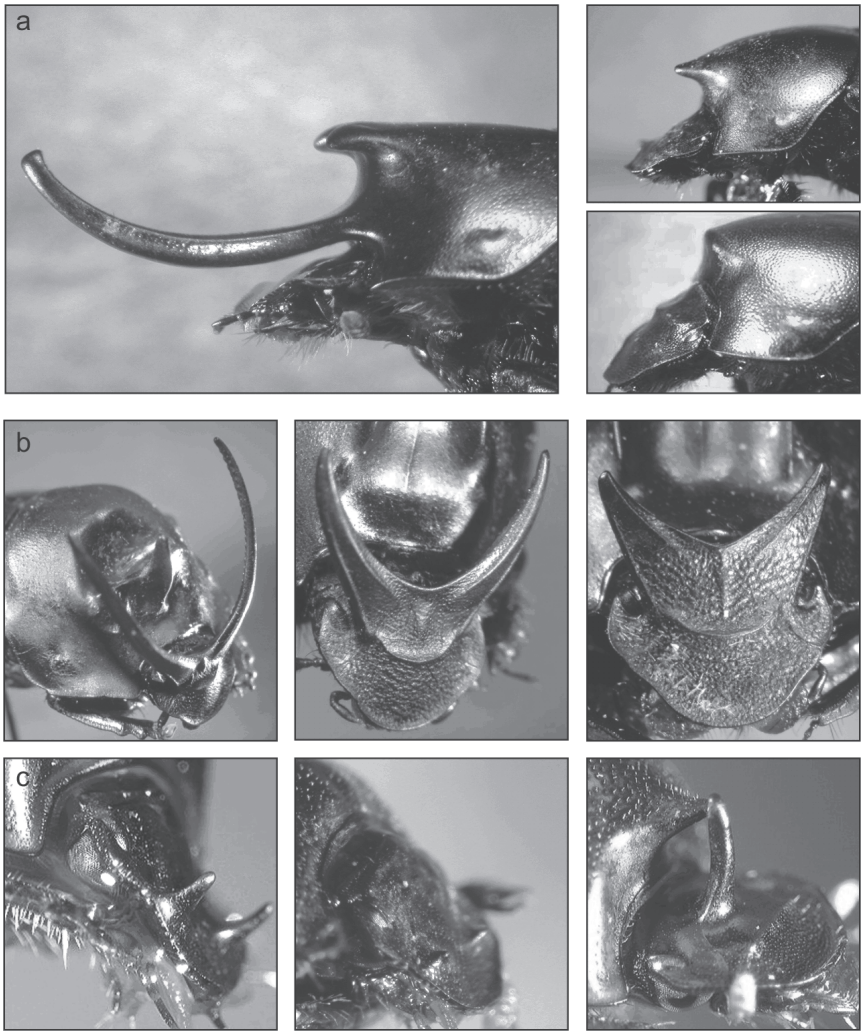

Fig. 3 Examples of intraspecific variation in horn development in *Onthophagus* beetles. **(a)** *Onthophagus nigriventris:* males above a certain body size develop a large, pronotal horn (left), whereas smaller males develop only a small, rudimentary horn (top right) and females remain entirely hornless regardless of body size (bottom right). **(b)** *O. watanabei:* Large (left) and small (center) males develop a pair of head horns, though horn development is relatively greater in large males. Large, but not small, males also express a central pronotal horn. Female *O. watanabei* develop a relatively small paired head horn and no pronotal horn. **(c)** *O. sagittarius*: This species is very unusual in that large (left) and small (right) males develop only a pair of minor head horns, while large females develop a single head and pronotal horn much larger in size than horns of males of similar body sizes.

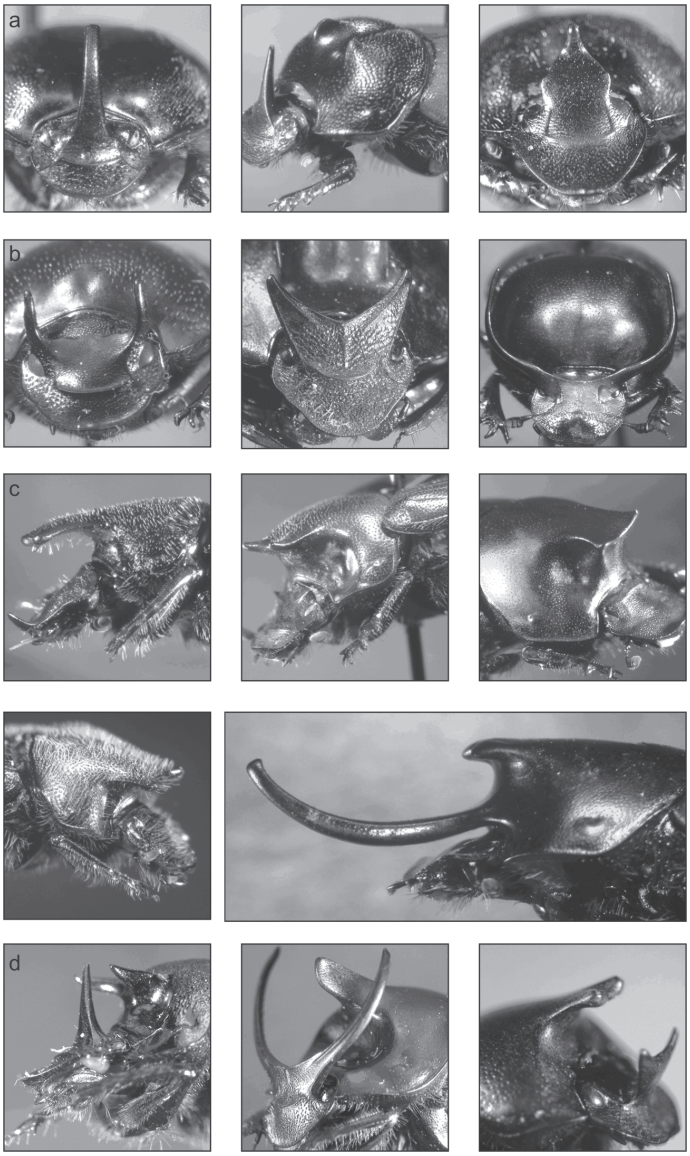

Fig. 4 Interspecific variation in size, shape, location, and number of horns in *Onthophagus* beetles. **(a)** Single head horns in (left to right): *O. spec* (unknown species; Vietnam), *O. insignis* (Malawi), *O. vacca* (India). **(b)** Paired head horns in *O. gazella* (S-Africa), *O. taurus* (U.S.A.), *O. watanabei* (Borneo). **(c)** Single pronotal horns in (top): *O. hecate* (U.S.A.), *O. turbatus* (U.S.A.), *O. binodis* (S-Africa); (bottom) *O. medorensis* (U.S.A.), *O. nigriventris* (Kenya). **(d)** Various combinations of head horns and pronotal horns in *O. ferox* (Australia), *O. atripennis* (Thailand), *O. lunatus* (Vietnam).

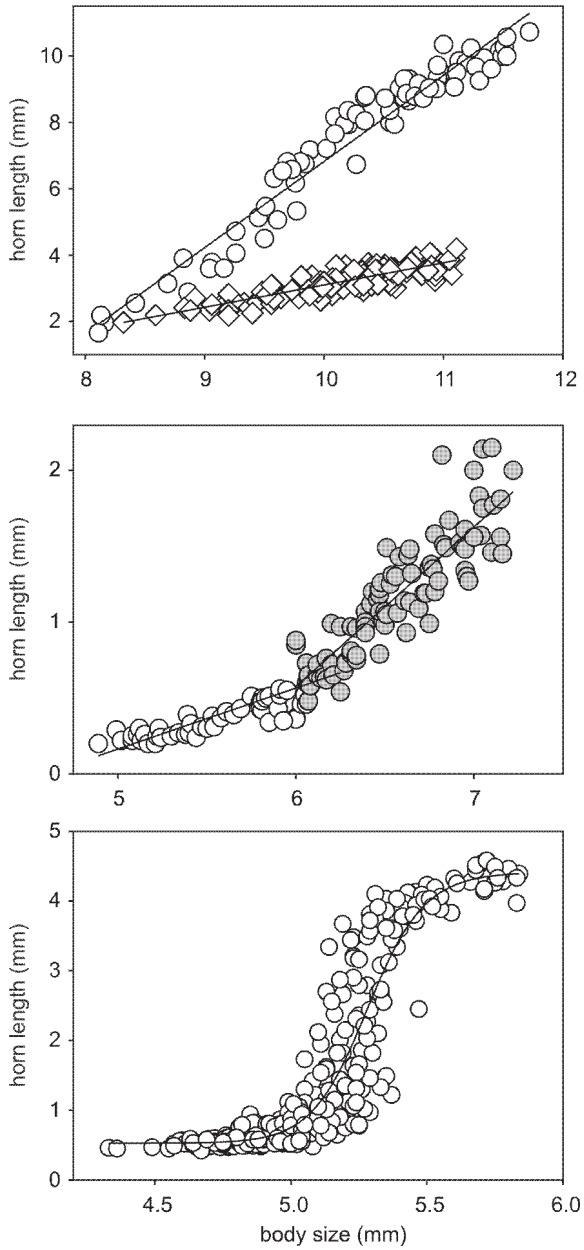

Fig. 5 Different types of horn length-body size scaling relationships in *Onthophagus* beetles. Top: linear allometries of paired head horns in male (open circles) and female (open diamonds) *O. watanabei*. Center: broken allometry of pronotal horn length in male *O. binodis*. Bottom: sigmoidal allometry of paired head horns in male *O. taurus* (after Moczek et al. 2004)

Morphological Diversity in Horned Beetles: between Species

Horns or horn-like structures have evolved independently in a number of beetle families, such as the Tenebrionidae (e.g. Pace 1967, Conner 1989), Staphilinidae (Darwin 1871), Passalidae (MacGown and MacGown 1996), Curculionidae (Eberhard and Garcia-C. 2000, Eberhard et al. 2000) or Chrysomelidae (Eberhard 1981, Windsor 1987). Beetle horns are, however, most extreme and most diverse in the chafers or scarab beetles (Scarabaeidae, e.g. Arrow 1951, Balthasar 1963, Matthews 1972). Horns or horn-like structures can develop from the clypeus (mouth plate), head, or thorax, horns may appear singly or paired, and different species may exhibit different combinations of single or paired horns produced by different regions of the body. In some cases such extreme variation in horn types may exist in a single genus, such as *Onthophagus* (Fig. 4; see also Balthasar 1963, Matthews 1972, Howden and Young 1981). Typically, however, closely related species exhibit similarities in horn development, allowing us to make inferences about the processes that have mediated this spectacular diversification. For example, closely related species usually express the same type of horn. What then distinguishes these species are differences in the exact *scaling* between horn length and body size. For example, species in the *Onthophagus incensus* group (Fig. 6; Emlen 1996) all develop paired head horns, yet differ widely in the range of horn lengths (*amplitude*), the body size at which the scaling relationship transitions rapidly from hornless to horned morphologies (*switch point* or *threshold*), and the steepness of the slope that characterizes this transition (*slope*). Differences in body size thresholds and slope have also been documented between species that otherwise cannot be distinguished by external characters alone. For example, *O. taurus* and *O. illyricus* are sympatric through large portions of the Mediterranean, and taxonomists have debated whether they should be classified as variants, subspecies, or true species (Balthasar 1963, Baraud 1992, Lohse and Lucht 1992). Currently, both are considered separate species, largely because of consistent differences in male genital morphology (Lohse and Lucht 1992). Apart from genital characters, however, both species are extremely difficult to distinguish. Males of either species develop very similar hornless and horned morphologies and transition from one to the other over a very narrow range of body sizes. Allometric analyses, however, revealed species-specific differences in slope and the exact location of body size thresholds (Fig. 7; Moczek and Nijhout 2003). Combined, these data suggest that changes in certain aspects of the scaling relationships between body size and horn length may constitute important avenues for phenotype diversification. However, to understand

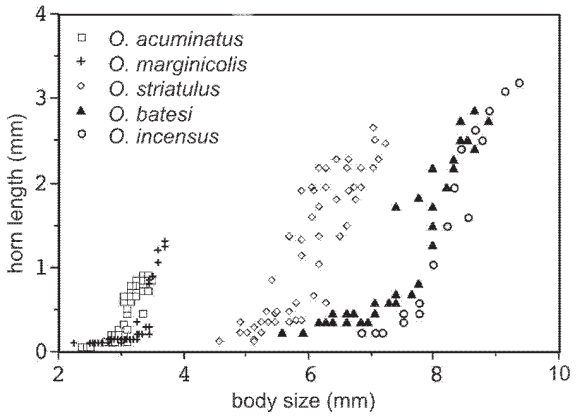

Fig. 6 Horn length-body size scaling relationships in the *Onthophagus acuminatus* group (after Emlen 1996, with permission).

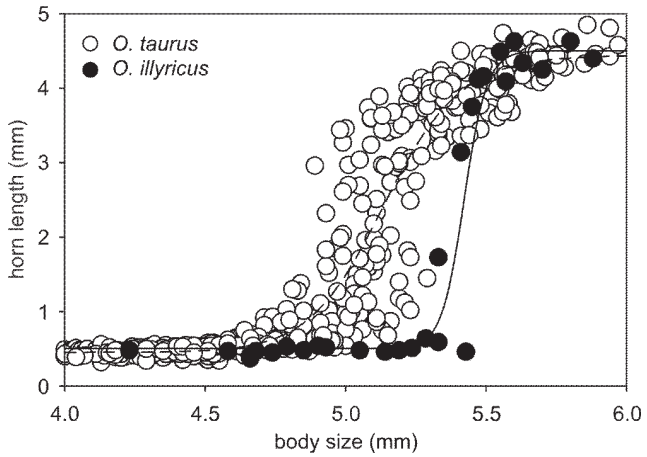

Fig. 7 Horn length-body size scaling relationships in *Onthophagus* sister species, *O. taurus* and *O. illyricus* (after Moczek and Nijhout 2002).

how and why scaling relationships might evolve on their own we first have to understand how and why beetles develop horns in the first place.

The Developmental Basis of Horns and Horn Dimorphisms

Whether or not a male beetle develops horns as an adult depends in large part on the nutritional conditions experienced during larval development (Emlen 1994, Hunt and Simmons 1997, Moczek 1998). This has been particularly well demonstrated in dung beetles where parents provision each egg with a discrete amount of dung, called a brood ball, in

underground tunnels. Brood balls can be weighed and manipulated and thus offer an excellent means of quantifying the effects of nutritional environment on adult beetle phenotype (Moczek 1998). Using experimental manipulation of brood ball mass in combination with a controlled breeding design Emlen (1994) showed in the horn polyphenic beetle *Onthophagus acuminatus* that male body size and horn length *per se* exhibit no significant heritable variation. However, experimental manipulation of brood ball mass had a profound effect on offspring horn phenotypes, and male larvae with access to large brood balls developed into large, horned males with few exceptions (Emlen 1994). A subsequent study (Moczek and Emlen 1999) on *O. taurus* explored the effects of natural variation in brood ball weights in combination with a controlled breeding design, with similar results (Fig. 8). While horn length and body size again exhibited no significant heritability, natural variation in brood ball weights explained 39% and 36% of variation in body size and horn length, respectively (Moczek and Emlen 1999). While brood ball weight affected body size in a continuous fashion, length of horns

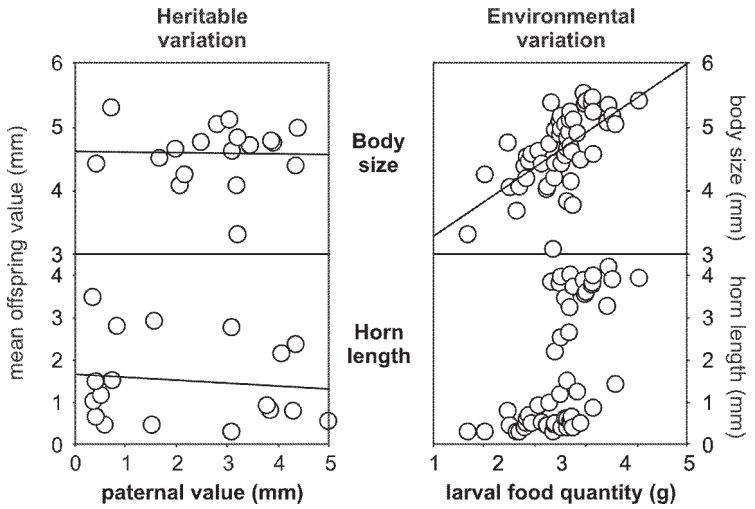

Fig. 8 Relative contributions of heritable (left) and environmental (right) factors to variation in male offspring phenotype in the horn polyphenic beetle *Onthophagus taurus*. Panels on the left show parent-offspring regressions of paternal (x-axis) body size (top) and horn length (bottom) against corresponding mean male offspring values (y-axis). Neither regression is significantly different from 0. Panels on the right show regressions of larval food quantity approximated as brood ball mass (x-axis) against individual male body size (top) and male horn length (bottom). Variation in brood ball mass explained 39% of the variation in male body size and 36% of the variation in male horn length. Note sigmoid distribution of data points in lower right panel. Modified after Moczek and Emlen (1999).

developed via a threshold response. Only males that exceeded a certain critical body size due to good nutrition developed horns, whereas males that developed to smaller sizes remained hornless (Moczek and Emlen 1999). Alternative horned and hornless morphologies are therefore not the manifestations of different genotypes. Instead, each individual male larva has the ability to develop into either morph. Horn dimorphisms are thus examples of polyphenic development, and as such are similar to caste determination in social Hymenoptera or seasonal polyphenism in butterflies. As we will see later on, individuals can, however, differ heritably in other aspects of horn development, with important consequences for patterns of phenotype diversification.

Horns themselves develop from imaginal disk-like tissues that undergo rapid and massive cell proliferation during the prepupal stage of late larval development (Emlen and Nijhout 1999). Because this growth occurs underneath the larval cuticle the resulting tissue cannot expand and instead undergoes massive folding underneath the larval cuticle (Fig. 9). Once the animal is ready to molt into a pupa and sheds its larval skin the folded-up horn tissue then becomes free to telescope outwards and to form the future adult horn. The timing and speed of horn development therefore resembles that of more conventional appendages such as legs, mouthparts and wings in holometabolous insects (Kim 1959, Schubiger 1971, Fristrom and Fristrom

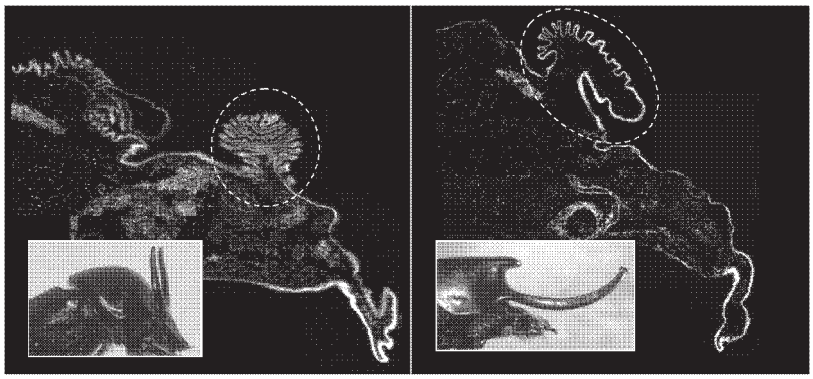

Fig. 9 Developmental basis of horn development. Horns develop during the prepupal stage at the end of larval development. Certain regions of the larval epidermis undergo rapid cell proliferation, which causes the resulting tissue to fold up underneath the larval cuticle. Once the animals molts into the pupa the horn tissue is free to expand into the pupal and subsequent adult horn. Shown are sagittal sections (DAPI stained to highlight nuclei) through head and thorax of incipient horned male *O. taurus* (left) and *O. nigriventris* (right). Future horns are highlighted by dashed line (after Moczek and Nagy 2005).

1993), which will become important later when we will explore the developmental origins of horns.

Recent research on *Onthophagus* beetles has identified some important components of the regulation of facultative, size-dependent expression of horns. *Onthophagus* larvae develop in underground brood balls, which are discrete and finite amounts of food provisioned for them by their parents (Halffter and Edmonds 1982). Larvae appear to use food availability as a cue to determine when to prepare for pupation. If larvae raised in artificial brood balls are removed from their food source at any time during the third and final instar they will initiate a stereotyped sequence of developmental transitions ultimately leading to pupation (Shafiei et al. 2001). Unlike numerous holometabolous insects, which require the attainment of a critical weight in order to pupate (e.g. Nijhout 1975), *Onthophagus* larvae can pupate at a wide range of body sizes and metamorphose into a wide range of adult sizes (Shafiei et al. 2001). This behavior appears adaptive since *Onthophagus* larvae do not have the option to locate additional food sources once their own brood ball is exhausted.

During development larvae are also somehow able to assess their own body size, predict their future adult body size, and adjust the subsequent development of horns accordingly (Emlen and Nijhout 2000). An important component of the regulatory mechanisms behind this appears to be juvenile hormone (JH), which is know to regulate a wide array of developmental processes in insects (Nijhout 1994, 1999). Several studies recently implicated juvenile hormone (JH) as an important endocrine regulator of horn development in beetles and changes in JH action as an important avenue for evolutionary diversification in phenotype expression (Emlen and Nijhout 1999, 2001; Moczek and Nijhout 2002, see below). In particular, earlier studies identified two brief sensitive periods in the last larval instar during which JH appears to determine the fate of developing larvae. During the first sensitive period, which occurs toward the end of the active feeding stage, application of the JH analogue methoprene causes larvae fated to develop into medium sized, horned males to suppress horn development and, instead, to develop into hornless males (Emlen and Nijhout 2001). During the second sensitive period around the gut purge and the onset of the prepupal stage, methoprene application has the opposite effect. Here, methoprene application to larvae fated to develop into small, hornless males causes them to develop into horned individuals instead (Moczek and Nijhout 2002). The presence or absence of sufficient JH titers during these two sensitive periods is therefore thought to determine which morph male larvae will develop into. Furthermore, the restriction of tissue sensitivity to

very brief periods, combined with the late, explosive growth of the presumptive horn tissue in the prepupal stages, is thought to allow horned beetles to generate the highly non-linear, broken or S-shaped allometries discussed earlier (Nijhout and Wheeler 1996, Emlen and Nijhout 2000, Moczek and Nijhout 2002). The notion that JH and the relative timing of sensitive and growth periods influence scaling relationships is further supported by the observation that populations that differ in patterns of morph expression also differ in the degree and timing of sensitivity to JH during the second sensitive period (Moczek and Nijhout 2002, 2003). We will return to this point when we explore the mechanisms of morphological diversification in horned beetles. But first we have to understand what, if anything, horns might be good for.

The Behavioral Ecology of Horned Beetles

Several hypotheses have been proposed to explain the evolution and potential adaptive significance of beetle horns (reviewed in Arrow 1951). Horns have been suggested to serve as indicators of male quality to choosy females (Darwin 1871), a hypothesis that recently has been re-examined without generating supporting evidence (Cook 1990, Kotiaho 2002). Alternatively, horns were thought to protect against predators (Wallace 1869, in Arrow 1951), serve as digging implements (Lameere 1904), or allow beetles to perforate and lacerate plants to feed on their sap (Doane 1913). Arrow (1951) himself suggested that beetle horns might be functionless, selectively neutral, and possibly the incidental outcome of selection towards larger body size. Eberhard was among the first to present substantial evidence that beetles in a range of families use their horns primarily in male-male competition (Eberhard 1978, 1979, 1981, 1982, 1987, Eberhard and Garcia-C. 2000, Eberhard et al. 2000). Many subsequent studies have since confirmed this conclusion (Rasmussen 1994, Otronen 1988, Siva-Jothy 1987, Windsor 1987, Cook 1990, Emlen 1997a, Moczek and Emlen 2000, Hunt and Simmons 2002). Regardless of the variation in sizes, location, and number of horns in different species of horned beetles, horns are used largely, if not entirely, as weapons in male-male combat over access to females. In species that fight inside tunnels fights typically occur head to head and are largely shoving contests (Palmer 1978, Emlen 1997a, Moczek and Emlen 2000). In these species horns appear to serve mainly as positioning devices which allow fighting males to deliver powerful blows with their heads and thorax, but also as means to prevent intruders from passing in tunnels (Moczek and Emlen 2000). In species that fight above ground, males often use their horns to grab, lift and throw opponents (e.g. Beebe 1944, Siva-Jothy 1987),

sometimes inflicting serious and occasionally fatal injuries. Using their horns male *Allomyrina dichotoma* may puncture the exoskeleton of their rivals and tear off elytra and hind wings. Throwing an opponent off a tree and onto the ground can result in the loss of appendages or massive cracks to the exoskeleton (Siva-Jothy 1987). A particularly interesting type of fight involving a particularly remarkable type of horn occurs in the weevil *Parisoschoenus expositus* (Eberhard and Garcia 2000, Eberhard et al. 2000). Here large males not only possess a pair of large, forward projecting, prothoracic horns, but also a forked tube or sheath that invaginates deep into the males prothoracic cuticle. During fights males interlock by inserting one of their horns into the sheath of his opponent. Males cannot use their own horns in fights unless they receive the other male's horn in their own sheath. Interlocked in this fashion males try to twist each other and lift each other from the substrate (Eberhard and Garcia 2000, Eberhard et al. 2000).

Horns are not only used in the context of fights but also measurably improve a male's chances of winning a fight. For example, in *Onthophagus taurus* males fight in subterranean tunnels underneath dung pads (Fig. 10).

Fig. 10 Mating system and alternative reproductive tactics in males and females of the horn polyphenic beetle *Onthophagus taurus* (drawings by Barrett Klein). Big picture: Adults beetles colonize dung pads and dig tunnels into the soil underneath, creating a complex, interconnected tunnel system. Females pack dung into the blind ends of tunnels to provision food for their offspring in the form of brood balls. Each brood ball contains one egg only and constitutes the sole amount of food available for a developing larva. Males compete with each other for access to females during tunneling and brood ball production (see below). Once females stop producing brood balls males desert and females fill the remaining tunnel space with the previously excavated soil. **(a-d)** Alternative male reproductive tactics: Large, horned males try to monopolize access to breeding tunnels and females through aggressive fighting behavior. Males guard tunnel entrances and engage in head-to-head combat with other males that try to enter the tunnel using their horns as weapons. Small, hornless males employ alternative sneaking behaviors to gain access to females when confronted with a physically superior opponent. Sneaking behaviors include **(a)** passing guarding males engaged in fights and **(b)** waiting near tunnel exits for females that collect dung for brood balls and mating above ground with these females while guarding males remain inside tunnels. Hornless males are also able to access breeding tunnels and females underneath guarding males via **(c)** the use of tunnel interceptions created by the digging activity of breeding females and **(d)** actively digging horizontal side tunnels to intercept breeding tunnels. **(e)** Alternative female reproductive tactics: Females typically reproduce by provisioning dung for larvae in the form of brood balls at the end of tunnels, but will switch opportunistically to intraspecific kleoptoparasitic behavior when encountering a brood ball produced by another female (after Moczek 1996, 1998, 1999; Moczek and Emlen 2000; Moczek and Cochrane, in press).

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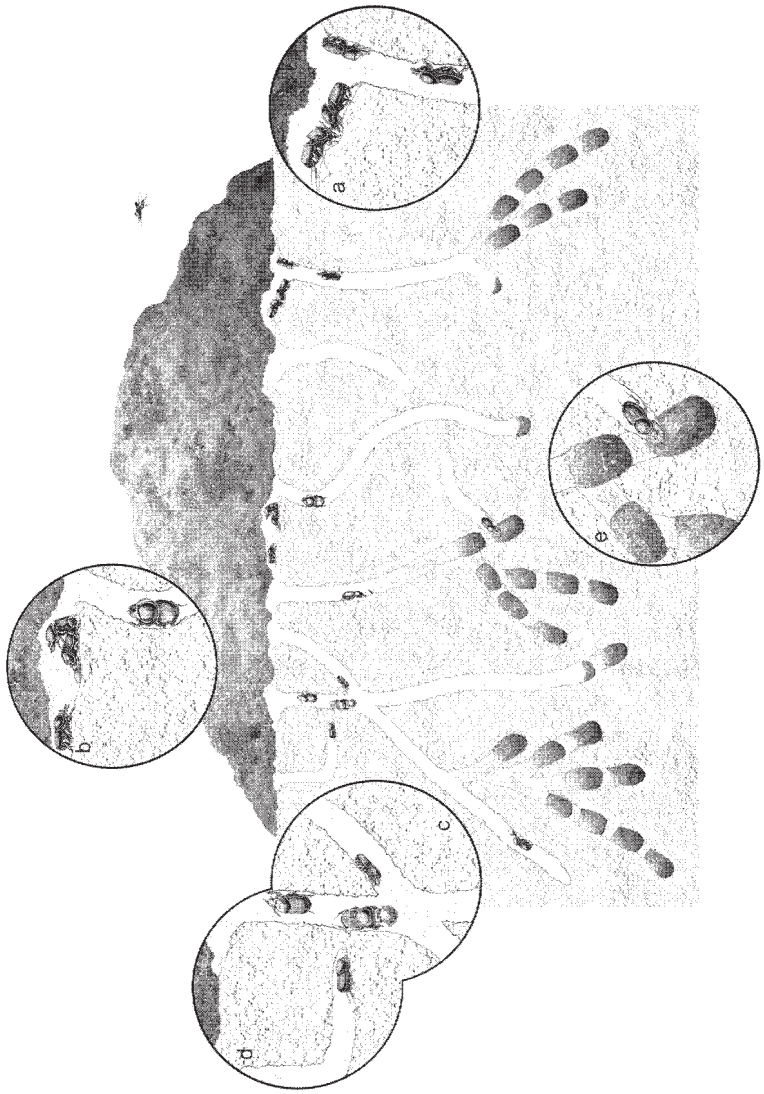

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Large males almost always win fights against smaller males. However, in fights between similar sizes males horn length is an excellent predictor of outcome and large-horned males almost always defeat their small-horned but otherwise equally-sized contestants (Moczek and Emlen 2000). However, not all males engage in fights to access females. In horn-dimorphic species, small hornless males commonly withdraw from fights with physically superior males and engage in non-aggressive sneaking behaviors to gain access to females. In dung-breeding, tunneling species such sneaking behaviors may include the use of naturally occurring tunnel intersections or the digging of a shallow, horizontal tunnel to intercept a breeding tunnel underneath a guarding male (Cook 1990, Emlen 1997a, Moczek 1999, Moczek and Emlen 2000). Sneaker males may also wait next to tunnel entrances for females searching for dung and mate with them above ground, or wait for the guarding male to emerge to help in brood provisioning, in which case sneaker males will quickly enter the breeding tunnel and mate with the female underground (Moczek 1999, Moczek and Emlen 2000). In one species, *Onthophagus taurus*, the absence of horns in small, sneaking males has been shown to significantly improve their agility and maneuverability inside tunnels, which in turn is thought to increase their chances of locating and fertilizing breeding females despite the presence of a guarding male (Moczek and Emlen 2000). While individual sneaker males may not be successful at circumventing large, guarding males, a group of sneaker males will eventually overwhelm a guarding male and at least one sneaker male will mate with the female (Hunt and Simmons 2002). It is important to note, however, that the presence of horns is not a prerequisite for fighting. In *O. taurus*, hornless males will fight for hours over access to females, provided the opponent is himself a hornless male. Such fights are indistinguishable from those of their horned counterparts, except of course, for the use of horns. Nonetheless, combined the available evidence to date suggests that beetle horns are adaptive in the context of male-male combat. It also suggests that fighting and sneaking behaviors and corresponding horned and hornless male morphologies reflect alternative solutions to the same problem: securing breeding opportunities in the presence of competing males.

In summary, three important conclusions can be drawn from the above: 1) Horns are expressed in response to environmental conditions, in particular larval feeding conditions. In all horn polyphenic species studied so far individual male larvae have the potential to develop into a horned and hornless adult, and "decide" during late larval development which morph to develop into. 2) The decision which morph to develop into is mediated in

part by JH, which acts during brief sensitive periods late in larval development. The dynamic interplay between JH mediated morph determination and the explosive, imaginal-disk like growth of future horns during prepupal development is thought to give rise to the species-specific, often highly non-linear scaling relationships observed in many species of horned beetles. 3) Alternative horned and hornless male morphs function in the context of alternative reproductive tactics. The success of a horned, fighting male has been shown to depend on his own body size relative to that of his opponents, the size of his horns, and the number of males he has to compete against at the same time. Hornless males in turn may benefit from the absence of horns through increased agility in locating females. We are now in a position to integrate the preceding chapters and to explore the consequences of polyphenic development for the origins of diversity among horned beetles.

The Consequences of Polyphenic Development in the Evolution of Horned Beetle Diversity

Scaling Relationships as Targets of Selection

Comparisons of horn length-body size scaling relationships between closely related species presented the first evidence that suggested that allometric parameters such as switch points or slopes might evolve independent of horns length *per se* (Kawano 1995, 1997, Emlen 1996). We now know from several studies that some of these components of scaling relationships indeed exhibit heritable variation and present important avenues for phenotypic diversification in horned beetles (Fig. 11). For example, artificial selection experiments (Emlen 1996), common garden breeding (Moczek et al. 2002) and large-scale geographic comparisons between isolated populations (Moczek 2003) have shown that switch points, or the critical body size at which males transition from hornless to horned morphologies, vary heritably between individuals and can evolve in different directions in different populations (Moczek and Nijhout 2003, Moczek 2003). The latter studies also indicated that such evolution can be extraordinarily rapid. Geographically isolated *Onthophagus taurus* populations have, since introduction to a new habitat less than 40 years ago, diverged in switch points to a degree typically only observed between species (Figs. 6, 7 and 11). Slight geographic differences were also detected in the steepness of the slope at the switch, though whether these differences are heritable remains to be investigated (Moczek and Nijhout 2003).

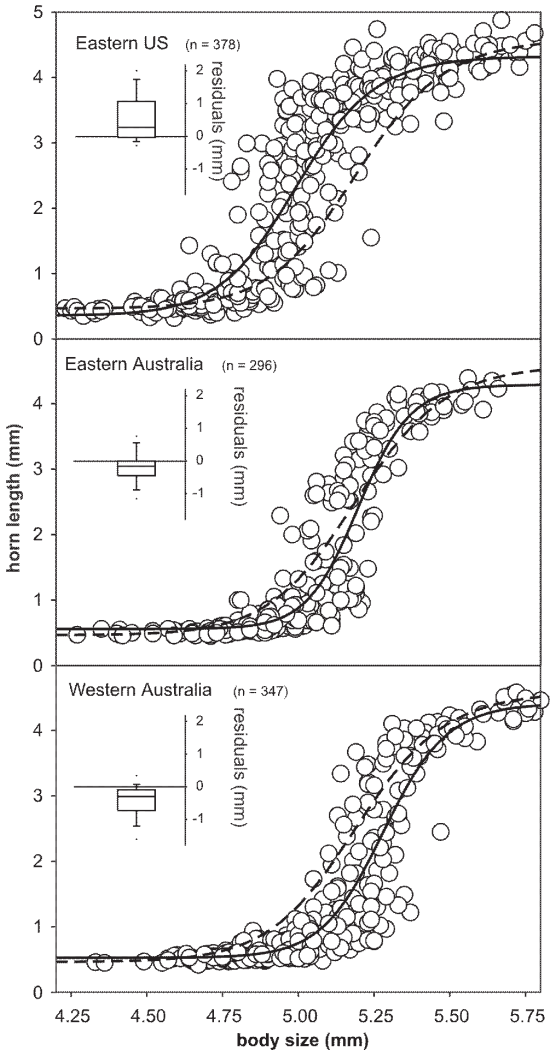

Fig. 11 Allometric divergence in exotic and geographically isolated populations of *O. taurus*. Plots show scaling relationship between horn length and body size for male *O. taurus* in the Eastern US (top), Eastern Australia (center) and Western Australia (bottom). For ease of interpretation also plotted is a common reference curve (dashed; identical in all three panels). This curve indicates a best-fit non-linear regression for all three ranges combined. This regression was used to calculate residual horn lengths (shown in inserts, see below). Solid curves indicate best-fit non-linear regressions calculated separately for each exotic range. Inserts: Box plot of horn length residuals (with 90/10% confidence intervals) for each exotic range based on best-fit non-linear regression for all three exotic ranges combined (dashed curve; after Moczek 2003).

The reason why body size thresholds may be particularly prone to rapid evolution is most likely due to the behavioral ecological context in which alternative male morphs function, and the factors that determine their relative success. Three alternative, but not mutually exclusive, hypotheses have been proposed and tested so far (Fig. 12). The *differential body size hypothesis* is derived from the observation that male fighting success is in part determined by male body size (Emlen 1996, Moczek and Emlen 2000). A male's overall competitive ability therefore can be considered a function of its own body size relative to the mean body size of males in the population within which he competes for mates. If the mean male body size in a population changes, e.g. via seasonal or geographic changes in larval feeding conditions, so should the competitive status of a given male, even if his absolute body size remains the same. In a population with a relatively small mean male body size genotypes would be favored that switch from the hornless to the horned morph at a relatively small threshold body size. Individuals in populations with relatively large mean male body size are instead predicted to delay the switch to a relatively larger body size. This hypothesis therefore predicts a positive correlation between male threshold body sizes and mean male body size in a population. This hypothesis received initial support in a study by Emlen (1996) on *Onthophagus acuminatus*, which showed a partial correlation between seasonal changes in mean male body size and body size thresholds. A more recent study on three exotic and highly threshold-divergent populations of *O. taurus* found no differences in average male body sizes in one comparison, and differences in the direction opposite to what was predicted by this hypothesis in two comparisons (Moczek 2003).

Alternatively, the *intraspecific competition hypothesis* argues that differences in the intensity of intraspecific competition for breeding opportunities has the potential to select for different threshold body sizes in different populations, via two different mechanisms. The first mechanism is derived from the observation that a horned male's ability to gain and maintain access to females through fighting decreases with an increase in the number of males with which he has to compete (Hunt and Simmons 2002). Under low density conditions, male-male encounter frequencies are likely to be low, and even medium-sized horned males may be able to deter rival males effectively enough to gain relatively higher fitness through fighting and the development of horns rather than through sneaking. Under such conditions, selection may favor a relatively low threshold body size. As male density increases, however, the likelihood that a guarding male will be challenged by one or more intruders at a given time increases as well. Under

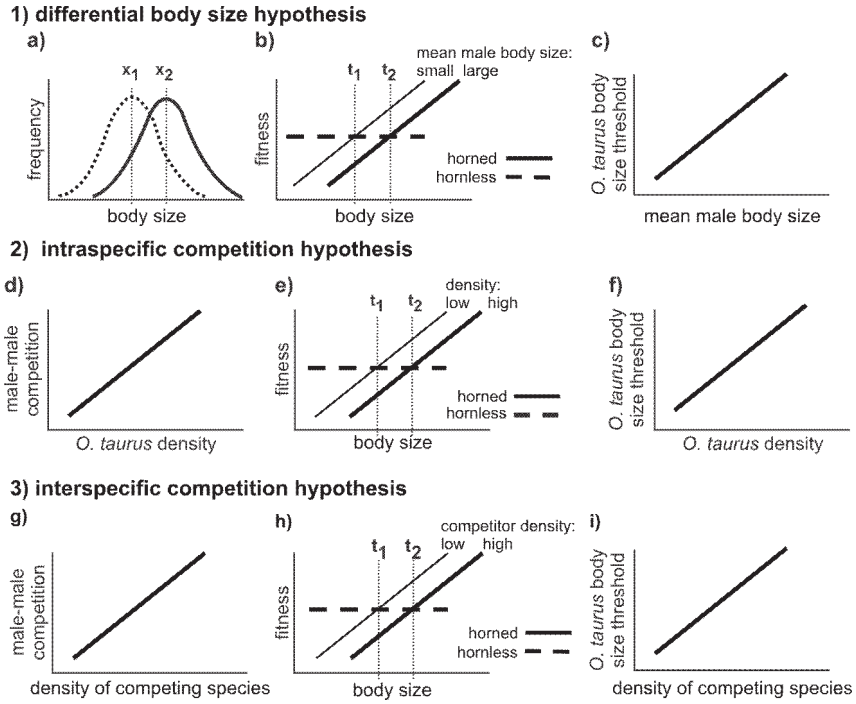

Fig. 12 Ecological mechanisms of threshold evolution in onthophagine beetles. **I:** Differential body size hypothesis: **(a)** Changes in the mean body size of competing males ($\mathbf{x_1}$ to $\mathbf{x_2}$) alter the average body size of males with which a given male has to compete for access to females. If the competitive status of a male is determined by its own body size relative to that of other males in the same population, then changes in mean male body size should alter the competitive status of a given male, even if his own body size remains the same. **(b)** In a population with a relatively small mean male body size, males of an intermediate body size—on an absolute scale—may maximize their fitness through fighting and the development of horns, whereas in a population with a relatively large mean male body size these same intermediate-sized males may fare better by remaining hornless and engaging in sneaking behaviors. Increases in the mean male body size in a population are therefore predicted to cause subsequent increases in the threshold body size that separates horned and hornless male morphs. **(c)** The differential body size hypothesis therefore predicts a positive correlation between male threshold body sizes and mean male body size in a population. **II:** Intraspecific competition hypothesis: **(d)** Increasing local densities intensify male-male competition via increasing male encounter rates inside dung pads. Increased local densities also result in an increase in the relative proportion of females that fail to secure breeding opportunities due to resource limitation. This in turn causes the ratio of competing males to breeding females to become more male biased and levels of male-male competition to intensify even further. **(e)** As local densities increase from low to high and male-male competition intensifies, sneaking behavior becomes more profitable than fighting behavior over a wider range of male body sizes, selecting for a shift of the critical threshold body size $\mathbf{t_1}$

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high-density conditions, medium-sized males may no longer be able to maintain access to females through fights. Instead, such males may maximize fitness by remaining hornless and engaging in sneaking behaviors. Under such conditions, selection may therefore favor a relatively high threshold body size. While this first mechanisms emphasizes changes in the nature of male-male interactions as a cause of threshold evolution, the second mechanism emphasizes that female-female interactions may be just as important, especially in species where females depend on patchy and ephemeral resources such as dung. It argues that under low-density conditions, most females will be able to secure enough resource to reproduce, resulting in a relatively even ratio of competing males to breeding females and relatively low levels of male-male competition. As the densities of competing females per resource patch increases, females will eventually become resource-limited, and a growing proportion of females will fail to secure enough resources to reproduce. Under such conditions a relatively large number of males will compete for access to a relatively small number of breeding females. As a consequence, the overall intensity of male-male competition will increase. As before, increased levels of male-male competition should in turn limit the profitability of fighting behavior to only but the largest males and favor a corresponding shift to a relatively high threshold body size. Combined, the intraspecific competition hypothesis predicts a positive correlation between male threshold body sizes and local population densities. Comparing three threshold divergent exotic *O. taurus* populations, Moczek (2003) found strong support for this hypothesis (Fig. 13).

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to a larger body size t_2. **(f)** The intraspecific competition hypothesis therefore predicts a positive correlation between male threshold body sizes and *O. taurus* densities. **III:** Interspecific competition hypothesis: **(g)** Increasing competition from other species that compete for the same ephemeral resource crucial for reproduction (dung) indirectly intensifies male-male competition by increasing the proportion of females that are unable to breed due to resource limitation. This in turn causes males to compete for relatively fewer breeding females and levels of male-male competition to increase. Consequently, as interspecific competitor densities increase from low to high, male-male competition intensifies. **(h)** This in turn limits the profitability of fighting behavior to only but the largest males, causes sneaking behaviors to become more profitable over a wider range of body sizes, which in turn selects for a shift of the critical threshold body size to larger body sizes. **(i)** The interspecific competition hypothesis therefore predicts a positive correlation between threshold body sizes of male *O. taurus* and the densities of competing dung beetle species (modified after Moczek 2003).

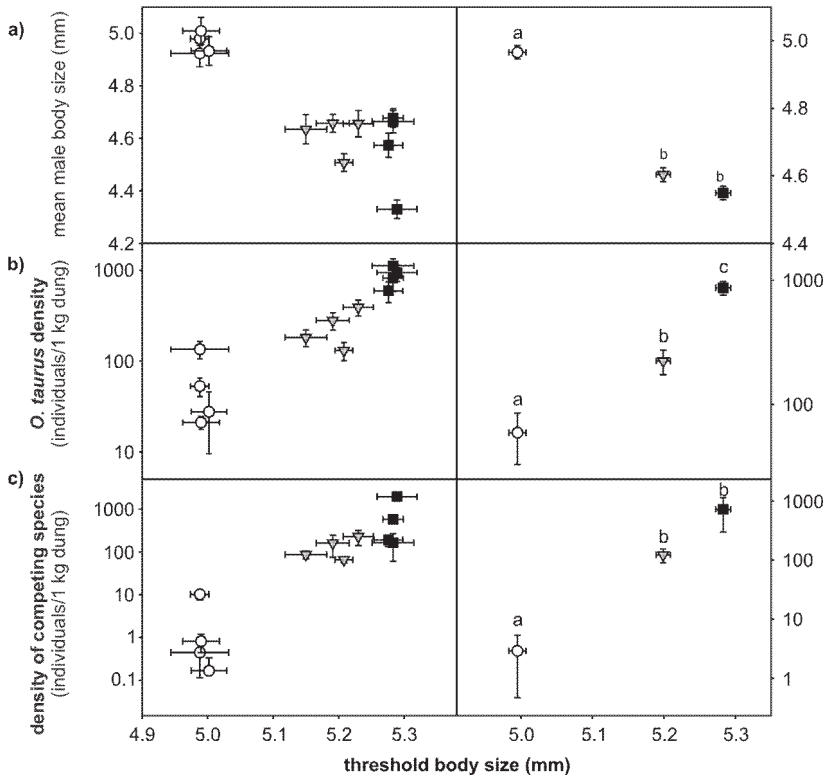

Fig. 13 Relationship between threshold body size (x-axis) and **(a)** mean male body size, **(b)** *O. taurus* density, and **(c)** competitor density within and between exotic ranges of the horn polyphenic beetle *Onthophagus taurus*. Left: sites within each exotic range. Right: means for each exotic range (open circles: Eastern US, gray triangle: Eastern Australia, solid squares: Western Australia). All three exotic ranges expressed highly significantly different threshold body sizes. Samples collected from different sites within each exotic range did not differ significantly in threshold body sizes, even though some sites differed considerably in densities or male body sizes. Different letters in the exponent denote significant differences in range-wide mean male body sizes, *O. taurus* densities, or competitor densities, respectively. Note that densities are plotted on a logarithmic scale (modified after Moczek 2003).

Lastly, the *interspecific competition hypothesis* rests on the observation that many species compete with other species over access to patchy and ephemeral breeding opportunities (e.g. Ridsdill-Smith 1993, Giller and Doube 1989). Low levels of interspecific competition for breeding opportunities should allow a relatively large portion of females to secure the resources necessary to breed. In such a population males will compete for access to a relatively large number of breeding females, resulting in relatively

low levels of male-male competition. As before, under such conditions selection is predicted to favor a relatively low threshold body size. As levels of interspecific competition increase, however, an increasing proportion of females will be denied the opportunity to breed. Under such conditions males will compete for access to a relatively small number of breeding females, causing levels of male-male competition to increase. As before, increased levels of male-male competition should in turn limit the profitability of fighting behavior to only the largest males and favor a shift of the threshold body size to relatively larger body sizes. The interspecific competition hypothesis argues that increased competition from other species that compete for breeding resources can intensify male-male competition indirectly by increasing the proportion of females that are unable to breed due to resource limitation. The interspecific competition hypothesis thus predicts a positive correlation between male threshold body sizes and the densities of competing species. So far this hypothesis has received partial support through the comparison of exotic, threshold divergent populations of *O. taurus*. While some populations differed highly significantly in competitor densities as predicted by this hypothesis, others exhibited at least a tendency in the expected direction (Moczek 2003).

Combined, the available evidence supports the hypothesis that differences in the degree of intra- and interspecific competition may indeed be able to drive threshold divergences between geographically isolated populations. However, so far the available evidence is entirely correlational and experimental results are strongly needed. In particular, quantification of fitness ratios of horned and hornless males of identical body sizes under a range of external conditions are necessary to allow for a more rigorous examination of these and other hypotheses. If the intra- and interspecific competition hypotheses receive further support by future studies, this would have important implications for our understanding the relative ease with which divergences in scaling relationships could be initiated. For example, the degree of intra- and interspecific competition present at a given site is in likely to depend on a variety of ecological factors, such as resource availability, predation pressure, or parasite density, which are themselves likely to differ in intensity from one local to another (Giller and Doube 1989, Ridsdill-Smith 1991, 1993). As a consequence, different populations of horn polyphenic beetles are bound to differ in at least some of these factors, and thus between-population divergences in threshold body sizes may be far more widespread than currently appreciated. As we will see next, threshold divergences driven by different ecologies may also have additional more subtle, but possibly more far-reaching consequences than just a change in

threshold, due to nature of the developmental mechanisms that regulate threshold responses in horned beetles.

The Physiological Basis of Threshold Evolution and the Integration of Development

The preceding sections discussed the role of juvenile hormone as an important regulator of threshold responses in polyphenic beetles. The implication of JH in the regulation of beetle horn development raises the possibility that changes in JH metabolism and physiology may provide an important avenue for the evolution of allometries. Results of a recent study support this notion. Investigating the same populations of *O. taurus* that were used to study the behavioral ecology of threshold evolution, Moczek and Nijhout (2002) explored whether evolved changes in how JH regulates morph expression during the second sensitive period in late larval development may have contributed to the divergence in body size thresholds present between these populations. As mentioned before, during this second sensitive period artificial JH application induces horn expression in males otherwise fated to develop into the hornless morph (Emlen and Nijhout 1999). Based on these findings, Emlen and Nijhout (1999) developed a model of the endocrine control of horn expression during this period, which suggests that male larvae differ in their JH titers depending on their body mass (Fig. 14a). According to the model, small male larvae exhibit JH titers below a certain threshold concentration during a well-defined sensitive period, and consequently develop into the hornless morph. Larger male larvae express JH titers above this threshold and develop into the horned morph (Emlen and Nijhout 1999). This model suggests at least two major developmental avenues for threshold evolution. First, changes in the *sensitivity* to JH could alter the location of the body size threshold (Fig. 14b). Reduced sensitivity, for example, would cause males that would have expressed JH titers just above the threshold to now develop into the hornless, instead of the horned, morph. Second, changes in the *timing of sensitivity* to JH relative to the temporal pattern of JH secretion could also result in a modification of the body size threshold (Fig. 14c). For example, if the JH sensitive period normally occurs during a high but falling phase of JH titers, then a delay in the sensitive period could now cause it to coincide with JH titers that fall below the threshold required to induce horn growth. As a consequence, males who previously expressed JH titers just above the threshold now fall below the threshold and consequently, will express the hornless male morph. At the level of a population, both mechanisms would be manifest as a shift of the body size threshold to larger

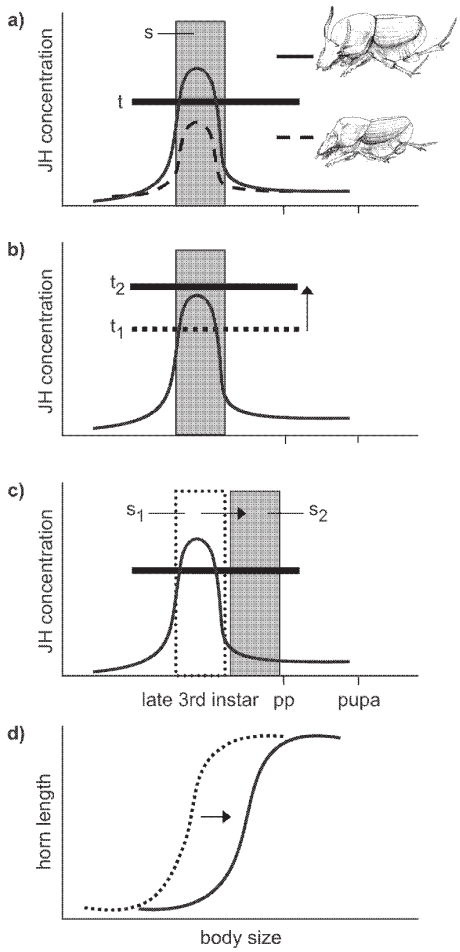

Fig. 14 Potential developmental mechanisms that mediate threshold evolution in exotic populations of *O. taurus.* **(a)** Endocrine control of male horn dimorphism (modified after Emlen and Nijhout 1999). Males are assumed to differ in juvenile hormone (JH) titers depending on their body size. Only large males express JH-titers above a threshold (**t**) during a certain sensitive period (**s**), and will develop horns as adults, whereas smaller males with JH-titers below the threshold will remain hornless. **(b)** Elevation in the JH-threshold (**t_1** to **t_2**) causes a medium-sized male larva to express JH-titers below the threshold necessary for horn development and to express the hornless instead of horned morph as adults. **(c)** A delay in the JH sensitive period (**s_1** to **s_2**) relative to JH-secretion results in JH titers of medium-sized male larvae to fall below the JH-threshold necessary for horn development before the horn-developing tissue acquires JH-sensitivity, causing these males to express the hornless instead of horned morph as adults (pp = prepupal stage). **(d)** On the level of a population both developmental modifications would be manifest in a shift of the critical threshold body size to larger body sizes. Modified after Moczek and Nijhout (2002).

body sizes (Fig. 14d). Contrasting population specific responses to the JH analogue methoprene, Moczek and Nijhout (2002) found support for both hypotheses. Males derived from a population with a high body size threshold, i.e. in which only very large males develop horns, were less sensitive to JH and exhibited their sensitive period later in larval development, compared to males derived from a population with a low body-size threshold. Strain-specific differences in the sensitivity to JH have previously been suggested to be responsible for differences in morph expression patterns in hemipterans (Dingle and Winchell 1997), suggesting that evolutionary modification of JH sensitivity may be a common mechanism that mediates the evolution of novel response thresholds in insects. However, strain specific differences in timing of tissue sensitivity have so far not been reported in any insect, but may possibly play an equally important role in the diversification of response thresholds. Combined, these results also suggest that relatively simple and subtle changes in the regulation of a threshold response can have profound consequence for patterns of phenotype expression.

Interestingly, threshold evolution via changes in JH regulation may bring about a number of correlated responses in other developmental and life history events. High-threshold populations in *O. taurus* did not only exhibit reduced and delayed sensitivity to JH, they also required more time to complete larval development and exhibited delayed pupation, metamorphosis, and eclosion. This may not be surprising as JH is involved in the regulation of numerous larval developmental events, and plays a central role in the coordination of molting, pupation, and metamorphosis (Nijhout 1994, 1999). For example, pupation generally requires the *absence* of JH during a particular sensitive period during late larval development (Nijhout 1994, 1999). In *O. taurus* this latter period is preceded by the sensitive period for horn induction. Here, JH has to be *present* above a certain concentration to induce horn expression (Emlen and Nijhout 1999). A delay in the sensitive period for JH-mediated horn expression, as is the case in high-threshold *O. taurus* populations, may cause a correlated delay in subsequent JH-sensitive periods, such as the one involved in regulating pupation, which in turn would result in an extension of the larval stage. If this hypothesis is correct, delayed pupation and an extended larval stage would reflect correlated responses to an evolutionary modification of the threshold response that mediates horn expression. Interestingly, a delay in pupation was also seen in hornless males and females of high threshold populations, even though neither express horns. This indicates that, while the evolutionary alteration of the developmental threshold for horns has changed the morphology of

only large males, the underlying developmental modifications required to achieve this alteration may have had consequences for all members of the population. A close genetic or developmental correlation between morph expression and other developmental and life-history events has also been implicated in earlier studies on wing-polyphenic crickets and hemipterans (Zera and Zhang 1995, Dingle and Winchell 1997, Roff et al. 1997, 1999).

The amount of developmental differentiation that accumulates between horn polyphenic populations may become important once populations re-establish contact and hybridize. Hybrids may express intermediate thresholds suboptimal for competition within either parental population. Furthermore, hybrids may have to contend with reduced developmental integration as their ontogeny is now controlled by developmental mechanisms derived from two developmentally divergent parental strains. Consequently, hybrids may suffer reduced fitness, which may favor the spread of alleles that facilitate assortative mating among members of both parental populations. This, in turn, may then lead to the subsequent evolution of reproductive isolation between these populations, an outcome also observed in recent theoretical models (Porter and Johnston 2002). While this scenario is, at this point, largely speculative, it provides a plausible and experimentally testable avenue for how divergent social regimes can cause geographically isolated populations to diverge rapidly in certain developmental and morphological properties, which in turn can foster the evolution of reproductive isolation once these populations come into secondary contact.

Body Size Thresholds are Themselves Phenotypically Plastic

An interesting aspect of body size thresholds in horn polyphenic beetles is that they themselves exhibit a certain degree of phenotypic plasticity. Emlen (1997b) showed that *Onthophagus acuminatus* reared on artificially low diet switched to the horned morph at slightly but significantly smaller body sizes compared to animals reared on regular diet. Mean body size of males in the low quality food treatment was also smaller, and Emlen interpreted this diet-induced threshold plasticity as an adaptive mechanism by which developing larvae adjust the optimal body size threshold to the range of body sizes likely to be present in the adult generation within which they have to compete, analogous to the differential body-size hypothesis outlined above. A more recent study used natural variation in feeding conditions in *O. taurus* and, too, observed that threshold body sizes covaried slightly but significantly with feeding conditions in laboratory reared as well as natural populations (Moczek 2002). Some, but not all of the populations tested also

exhibited corresponding differences in mean male body size. This study, however, emphasized an alternative explanation for this phenomenon. A certain degree of diet induced plasticity in body size thresholds should be expected simply because of how adult horn size and body size are determined during larval development, independent of whether such plasticity may be adaptive or not. In particular, diet induced plasticity may emerge simply because whether or not a male larva will develop horns as an adult is determined *before* the animal ceases to accumulate body mass. As a consequence male larvae will still increase in body mass even after their future horn phenotype has been specified. Exactly how much larvae change body mass after morph determination should depend on larval feeding conditions. A larvae with access to good feeding conditions will gain relatively more weight than a larvae restricted to poorer conditions. However, if both of these larvae exceed the critical larval weight required for horn expression at the time of morph determination, they will both develop horns as adults. Because their post-morph determination mass gain is different, however, they will differ in the final weight with which they will pupate, the body size at which they will eclose as adults, and therefore the lengths of horns relative to their body size. At the level of a population this alone may be sufficient to bring about a change in the critical body size threshold that separates alternative male morphologies, causing populations with access to relatively poor conditions to initiate horn expression at relatively small body sizes (Moczek 2002). The main implication of this alternative explanation is, however, not about the adaptiveness of threshold-plasticity. True, it is plausible that plasticity in body size thresholds could be adaptive under certain conditions, e.g. in the context of population-wide changes in larval food quality or availability (Edwards 1991, Emlen 1997b), in which case this alternative explanation may illustrate the physiological means by which such an adaptive response could be achieved. Instead, the main implication of this alternative explanation is to illustrate another example for the increasingly common observation that plasticity may emerge initially simply as a by-product of development, without requiring any initial changes in genotypes and gene frequencies (West-Eberhard 2003). Even though non-heritable at first, if the change in phenotype expression happens to be in a direction favored by selection, subsequent genetic changes would then have the opportunity to assimilate and stabilize this new response. It is intriguing to speculate whether the dramatic divergences in body size threshold observed in different exotic *O. taurus* populations discussed above might have been initiated though population-wide changes in larval feeding conditions, e.g. through changes in food quality (Edwards 1991) or via changes

in the intensity of resource competition between provisioning females (Hirschberger 1999).

Developmental Trade Offs as a Source of Phenotypic Diversity

An additional and possibly very important mechanism contributing to phenotypic diversification in horned beetles may arise from resource allocation trade offs during the growth of horns. Allocation trade offs during development may arise when two or more structures compete for a shared and limited pool of resources necessary to sustain their growth. A shared limiting resource could be nutrient that is used up during the physical growth of a tissue, or a hormone, growth factor, or morphogen that is sequestered by competing binding sites in different tissues (Kawamura et al. 1999, Gibson and Schubiger 2000, Oldham et al. 2000, Brogiolo et al. 2001, Nijhout and Grunert 2002). Such limiting resources may thus constrain the absolute sizes to which a structure can grow, and the presence of absence of a growing structure may therefore, theoretically, influence the size to which other structures are able to develop (Nijhout and Wheeler 1996). Recent work on butterfly wings and beetle horns lend support to this hypothesis (Kawano 1995, 1997; Klingenberg and Nijhout 1998, Nijhout and Emlen 1998). Studying giant rhinoceros beetles (*Chalcosoma* and *Dynastes* species) Kawano (1995) was the first to describe a negative correlation between relative horn size and wing area in males, i.e. males that developed disproportionally large horns also expressed relatively smaller wings. Kawano (1997) found similar results when studying a large number of stag beetle species in which males with disproportinally large mandibles developed relatively smaller wings, and suggested that resource allocation trade offs during development between mandibles and wings and horns and wings might account for these results (Kawano 1997). In *Onthophagus* beetles Nijhout and Emlen (1998) and Emlen (2002) showed that males that develop relatively long horns also develop relatively smaller antennae, eyes, or wings compared to their hornless female counterparts. Interestingly, exactly which structures participated in this interaction appeared to depend on exactly where horns developed. For example, individuals with large head horns developed relative smaller eyes without antennae or wings being affected (Emlen 2001). In one species, *Onthophagus acuminatus*, artificial selection for relatively long horns resulted in a correlated response in the expression of relatively small eyes, demonstrating that evolutionary changes in one trait, horns, can bring about evolutionary changes in another, eye size in this case (Nijhout and Emlen 1998). Since eyes,

antennae, and wings are functionally very important traits, reduction in their size might carry certain costs with it, which in turn may limit the extent to which horn growth can be enlarged at certain locations (Emlen 2001). Similarly, this might bias which types of horns evolve in a given taxon depending on its ecology. For example, species that rely heavily on flight might be biased to evolve head rather than thoracic horns as the former are more likely not to negatively affect wing size (Emlen 2001). Trade offs between competing structures could also influence selection on other systems. In the above case, evolution of head horns and, consequently, reduced eye size might cause such a population to rely increasingly on pheromones rather than vision in mate location. Trade offs between competing structures thus have the potential to cause populations with different phenotypes to follow very different evolutionary trajectories.

Evaluating the significance of developmental trade offs in horned beetles faces several challenges. Emlen (2001) used females as controls to establish whether a given negative correlation between the relative sizes of horns and other structures is indeed due to the presence of horns in males. In most species females do not express horns but develop the same morphological landmarks, which can be used to obtain morphological measurements. While this is a logical approach in theory, in practice it poses a challenge due to the extremely short relative horn length measurements in females and the correspondingly large effects of measurement errors. This in turn may obscure horn growth-independent phenotypic correlations in females, especially given the moderate sample sizes used in this study (Emlen 2001). Furthermore, not all species examined so far showed the predicted trade offs. For example, large *O. nigriventris* males develop one of the relatively largest thoracic horns of any species in the genus (see 4c), yet without any corresponding reduction in relative wing size (Emlen, pers. communication). Most importantly, however, evaluating the evolutionary significance of resource allocation trade offs requires a solid understanding of the fitness consequences, if any, that reductions in the sizes of eyes, antennae, and wings might bring with them. To date no such data are available, and it may therefore be premature to label the relatively moderate reductions in the relative sizes of antennae, eyes and wings that accompany the development of horns as functional costs of horn expression (Emlen 2001).

Interestingly, at this point no mechanism has been identified that could account for why primarily neighboring structures should trade off during development. So far, nutrients, hormones, and growth factors have been shown to limit the growth of imaginal-disk derived structures, yet all these factors can circulate freely in the haemolymph and it is unclear why

competition for them should be restricted to adjacent structures (Kawamura et al. 1999, Oldham et al. 2000, Brogiolo et al. 2001, Nijhout and Grunert 2002). This is not to suggest that such tradeoffs do not exist, but that proximity may not be the most important determinant of trade off intensity. To test whether distant structures can, in fact, engage in resource allocation tradeoffs, Moczek and Nijhout (2004) examined interactions between head horns and genitalia in *O. taurus*. Both structures develop on opposite ends of the animal. This study not only uncovered evidence in support of significant resource allocation tradeoffs between these distant structures, but also showed that trade off intensity depended on exactly when one of the structures was removed from the competition. The more the growth periods of genitalia and head horns overlapped, the stronger was the tradeoff. This suggests that timing of growth and resource consumption might be significant in determining whether or not two structures will exhibit resource allocation tradeoffs. An important implication of these findings is that changes in the relative timing of growth periods may provide an important avenue for morphological evolution to escape potentially constraining developmental tradeoffs.

Fixation and Recurrence of Alternative Phenotypes

An important avenue by which phenotypic plasticity is thought to contribute to phenotypic diversity is through the temporary fixation of one of several alternative phenotypes, followed by rapid specialization and, under certain circumstances, the subsequent recurrence of lost alternatives. Whether these mechanisms have contributed to phenotypic diversity and speciation in horned beetles is an open question. Recent phylogenetic analyses of horned beetle taxa, however, are beginning to lend tentative support to the notion that the ability to express horns may have been gained and lost repeatedly and independently in certain groups of horned beetles. For example, if patterns of horn expression are mapped onto a recent molecular phylogeny of Iberian dung beetles, including members of the tribe Onthophagini (Villalba et al. 2002), single head horns appear to either have evolved four times (Fig. 15a) or twice independently followed by 3 independent losses (Fig. 15b). The latter scenario would require one additional independent event, which, depending on how losses and gains are weighed, presents a credible possibility. Interestingly, the same phylogeny provides relatively strong evidence that paired head horns evolved independently at least twice in this clade (Fig. 15c). A single origin of paired head horns would require at least four independent, subsequent losses to explain present differentiation patterns, which appears considerably less probable

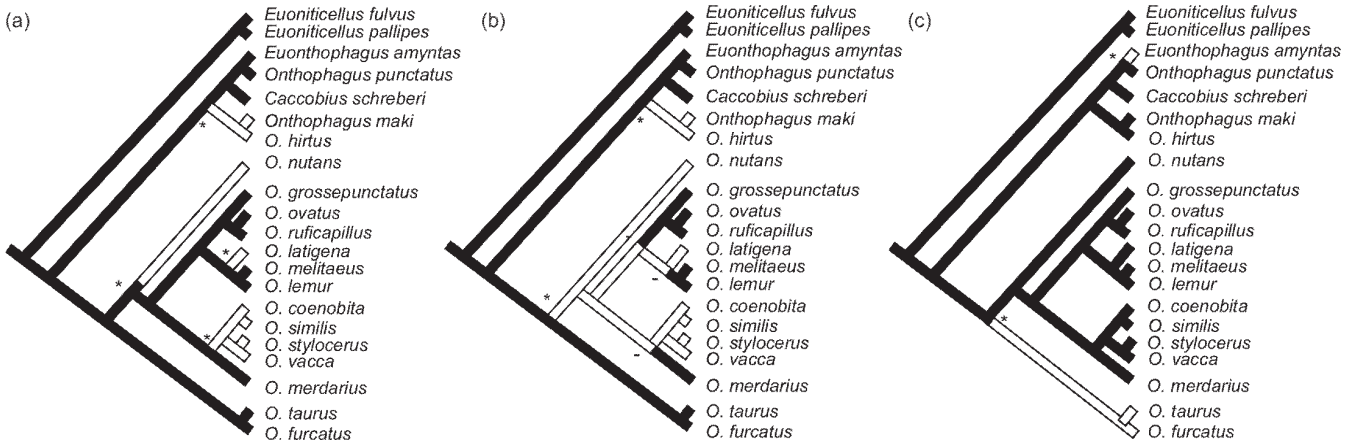

Fig. 15 Alternative phylogenetic scenarios for the evolution of single and paired head horns in *Onthophagus* beetles. Stars indicate gains of horns, whereas (-) symbols indicate possible losses. **(a)** Independent gains of a single head horns in four lineages; **(b)** two independent gains of single head horns followed by three independent losses. **(c)** Independent gains of paired head horns in two lineages. Species descriptions were obtained from Balthasar (1963) and Zardoya (pers. communication). Phylogeny after Villalba et al. 2002, with permission from R. Zardoya.

(Fig. 15c). Clearly, more detailed phylogenetic analyses of horned beetles are strongly needed to get a better insight into patterns of horn evolution and the rates of gains, losses, and possibly recurrence of horn morphologies. Ideally, such studies should be accompanied by behavioral and biogeographic studies to learn more about the ecological factors that might facilitate such transitions. Given the environmental determination of male horn dimorphism, population-wide losses of especially the horned morph appear feasible, even without any initial genetic changes. For example, changes in population-wide larval feeding conditions (Edwards 1991), or changes in climatic conditions (hotter, dryer conditions result in larger portions of brood balls drying out and cause adults to emerge at smaller body sizes; Moczek unpublished) could result in temporary loss of the horned morph, without requiring any changes in gene frequencies. Subsequent genetic changes could then stabilize and assimilate this initially environmental monomorphism. Ideally, such studies should also be accompanied by developmental genetic approaches which would be ideal for detecting developmental remnants of lost alternative phenotypes (Abouheif and Wray 2002, and see below).

The Origin(s) of Horns

The preceding sections have dealt primarily with mechanisms of diversification, rather than origin, of horns and horn-like structures in beetles. In this last sections I will explore how horns and horn polyphenisms originated, highlight some of the most interesting, open questions, and point out promising new approaches that have the potential to address them.

A Horn is a Horn is a Horn?

Horns are not a prerequisite for fights. Head to head shoving contests are a common form of male-male combat in many non-horned beetles. In horn polyphenic species, hornless males fight just as intensely against other males as their horned counterparts, provided their opponent is themselves hornless (Moczek 1999, Moczek and Emlen 2000). Beetle horns are therefore likely to be an example of a trait where the evolution of a behavior prepared the way for the evolution of a corresponding, adaptive morphology.

In many species, horns develop in places where hornless females and males develop ridges or bumps (Balthasar 1963). In fact, in some species large males differ from small males just by slight elevations of the corners of ridges that run across the head (Balthasar 1963). Developmentally, ridges

and bumps originate through simple folds in the epidermis during the prepupal stage prior to the secretion of the pupal and subsequent adult cuticle (Moczek and Nagy 2005). As such, ridges are qualitatively not too different from horns, which originate as massive folds of the epidermis during the prepupal stage. Horns might therefore have originated from ridges, via the localized addition of epidermal folds during prepupal development. It is conceivable that even an initially minor increase in ridge hight might already have been sufficient to improve performance in aggressive encounters. Such a scenario is supported by results from staged fights between males of identical body size but different horn lengths, which have shown that even horn length differences as small as 0.5–1 mm significantly improve a male's chances of winning a fight (Moczek and Emlen 2000). Interestingly, for at least two species there is anecdotal evidence that pointy outgrowths can appear spontaneously in some individuals even though members of the species normally do not express horns in that location (Carpaneto and Piatella 1988, Ziani 1994).

Other aspects of horn development, its timing and speed, are reminiscent of the development of regular appendages such as legs, antennae and mouthparts in holometabolous insects (Kim 1959, Schubiger 1971, Fristrom and Fristrom 1993). In fact, incipient horns first become discernible around the same time as mouthparts and antennae during the larvae-prepupal transition. Preliminary results suggest that some of the same regulatory genes involved in the development of traditional appendages are also associated with the development of horns. For example, the transcription factor *Distalless* has been shown to play a central role in patterning the distal portion of arthropod appendages, and *Distalless* protein is also expressed in the distal portion of future beetle horns (Moczek and Nagy 2005). Beetle horns thus offer an exciting possibility to explore how regulatory genes used in a traditional developmental process such as appendage formation became redeployed and reorganized in a novel developmental and evolutionary context. Modern developmental genetic approaches provide all the tools necessary for such an exploration.

Not all horns, however, need to be created equally. Different types of horns may have evolved independently, and the same type of horn may have evolved more than once in a given clade (Fig. 15b,c). Thus, it is conceivable that horns develop by different means in different species. Preliminary evidence in favor of this hypothesis comes from comparative studies of pupal and adult morphologies in species with different horn types (Fig. 16). Many *Onthophagus* species develop a thoracic horn as pupae. In *O. taurus*, the epidermis that produced this horn recedes during the pupal stage before

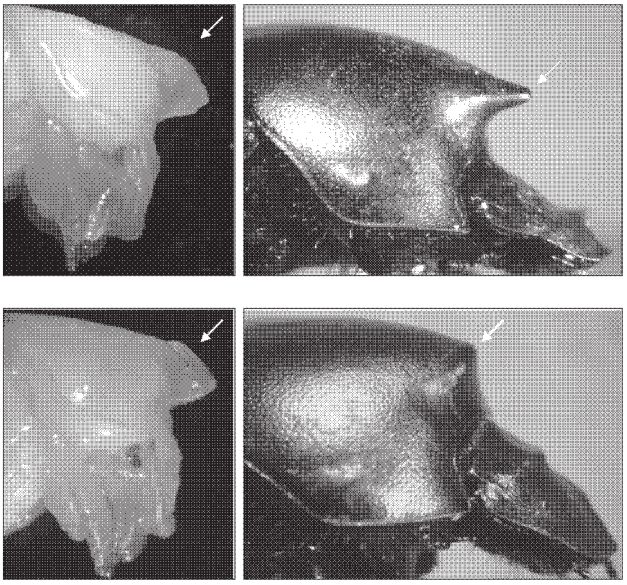

Fig. 16 Regulation of horn development during the pupal stage in *O. nigriventris.* Pupae in minor males (top left) and females (bottom left) both express a single pronotal outgrowth (arrow). In the males, the pupal epidermis underneath this outgrowth stays in place prior to depositing the adult cuticle, and these males also express a corresponding pronotal horn as adults (top right). In females, the pupal epidermis underneath the outgrowth retracts before the adult cuticle is deposited and adult females do not develop a corresponding pronotal horn (bottom right).

it produces the adult cuticle. As a consequence, neither male nor female *O. taurus* develop a thoracic horn as an adult. In *O. nigriventris*, pupae of both sexes also develop a thoracic horn. In fact, large male pupae develop an extra-large, down curved and coiled thoracic horn, which later gives rise to the large thoracic horn present in the adult. Smaller males and females develop regular-sized thoracic horns as pupae much like *O. taurus*, however, only in females *O. nigriventris* does the thoracic epidermis recede and give rise to a completely hornless adult (Fig. 16). In small to medium-sized male *O. nigriventris* this epidermis instead stays in place and gives rise to a significant, pointy thoracic horn in the adult. This modulation of horn growth is quite different from the regulation of head horns in *O. taurus*. Here, future horns grow through the prepupal stage only. Once the animal pupates no further major modulation of horn size takes place. Combined, this suggests that different processes, operating at different times during development, may regulate when and where a horn develops (Moczek 2005).

The Origins of Horn Polyphenisms

Two alternative hypotheses have been proposed to explain the evolutionary origin of horn polyphenism (Emlen and Nijhout 2000). Polyphenic development in horned beetles may have evolved directly from uniformly hornless ancestors, that is, genotypes acquired the ability to "turn on" horn development once a certain body size could be attained (Fig. 17). In this scenario hornlessness would be the ancestral state for all males, and facultative expression of horns in large males would constitute the more derived state. Alternatively, the facultative, polyphenic expression of horns may have evolved from obligately horned ancestors, in which all males expressed horns proportional to their body size. Genotypes then acquired the ability to suppress horn development below a certain body size threshold (Fig. 17). In this case, facultative expression of horns could have evolved much later than horns themselves. In this scenario, the secondarily hornless, small males would constitute the more derived state. The presence of rudimentary and possibly functionless horn remnants in small males of many horn polyphenic species could be interpreted as evidence in favor of this second scenario. Both hypotheses thus differ with respect to which morphology they predict to be the more derived state, which could be used to distinguish between them through comparative developmental genetic studies. For example, if hornlessness in small males of horn polyphenic species constitutes a secondary, derived state, certain components of the ancestral gene network involved in horn expression should still be detectable in small males during larval and pupal development, even through they do not express horns as adults. Recent elegant studies on the evolution of wing loss in ants illustrate the power of such an approach (Abouheif and Wray 2002).

Conclusions

Over the past decade, horned beetles have been the focus of a variety of research approaches designed to explore the behavioral ecology, endocrine physiology or evolutionary biology of beetle horns and horn polyphenisms. The recurring theme that emerges from all these studies is one of dramatic developmental plasticity and phenotypic flexibility in all aspects of the beetles' life. At the same time horned beetles have been known for a long time to be among the most speciose taxa in the insects, producing some of the most exaggerated and diverse secondary sexual traits in existence. Here I have attempted to explore how ecology and behavior have shaped aspects of

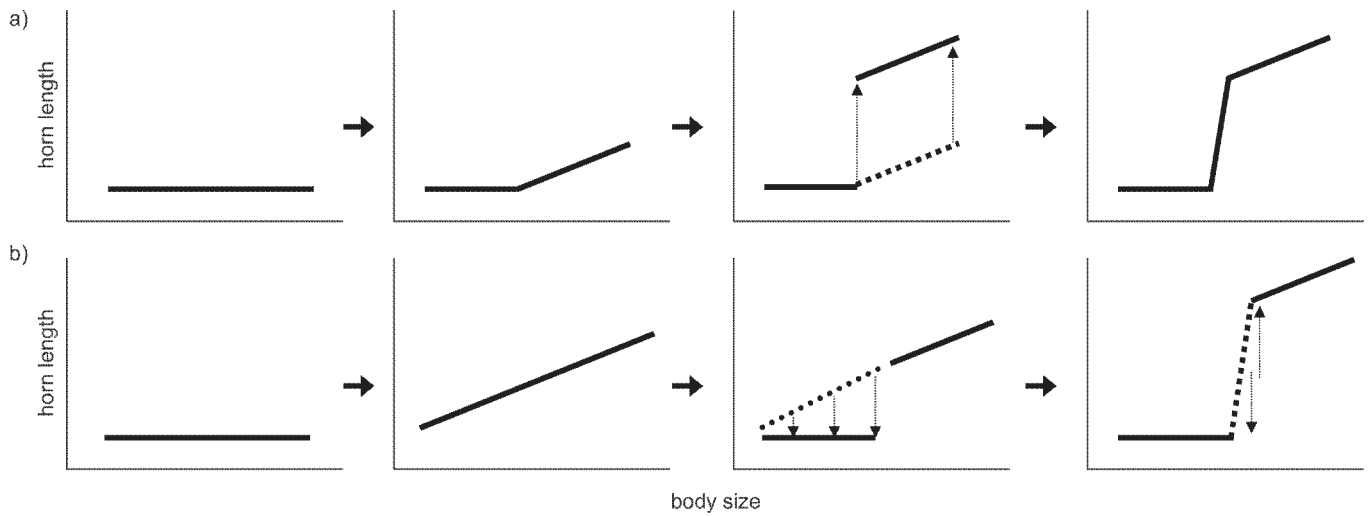

Fig. 17 Alternative origins of horn polyphenic development. **(a)** Horn polyphenic development originated from uniformly hornless ancestors. Genotypes initially expressed no horns regardless of body size, but subsequently evolved the ability to express horns in phenotypes above a certain body size. The ability to induce horns above a certain size threshold then becomes accentuated over time. **(b)** As before, horn polyphenic development originated from uniformly hornless ancestors, however, genotypes first evolved the ability to express horns as a linear function of body size, i.e. small males ancestrally represented a small version of larger males. Genotypes subsequently evolved the ability to repress horn development in males below a certain body size threshold. The ability to repress horns below and induce horns above a certain threshold body size then becomes accentuated over time.

developmental and behavioral plasticity in horned beetles, such as the evolution of flexible timing of pupation to accommodate unpredictable larval feeding conditions. At the same time I have tried to highlight how developmental and behavioral plasticity in turn have contributed to and directed the evolutionary diversification of horned beetles, for example by enabling simple ecological factors to shape patterns of morph expression through evolutionary modifications of response thresholds. Horned beetles emerge as an outstanding opportunity for integration, not only of genetics, physiology, ecology and behavior, but also of external conditions and their role in shaping phenotypes and the environment in which they function. Many of the interactions between phenotypic plasticity and evolution presented here are likely not to be unique to horned beetles, but may be relevant to the numerous taxa in which alternative morphologies rely on alternative reproductive tactics, and whose performances themselves depend on external, social, and ecological conditions. As horned beetles demonstrate, integrating the role of phenotypic plasticity and environment in the evolution of phenotypes makes our understanding of the origins of diversity not only more complete, but also by far more interesting.

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References

Abouheif, E., and G. A. Wray. 2002. Evolution of the gene network underlying wing polyphenism in ants. Science, 297: 249–252.

Abu-Shaar, M. and R. S. Mann. 1998. Generation of multiple antagonistic domains along the proximodistal axis during *Drosophila* leg development. Development, 125: 3821–3830.

Allegret, P. 1964. Interrelationship of larval development, metamorphosis and age in a pyralid lepidopteran, *Galleria melloneella* (L), under the influence of dietetic factors. Experimental Gerontology, 1: 49–66.

Ahlroth, P., Alatalo, R. V., Hyvärinen, E., and J. Suhonen. 1999. Geographical variation in wing polymorphism in the water strider *Aquarius najas* (Heteroptera: Gerridae). Journal of Evolutionary Biology, 12: 156–160.

Arrow, G. H. 1899. On sexual dimorphism in beetles of the family Rutelidae. Transaction of the Entomological Society of London, 1899: 255–269.

Arrow, G. H. 1951. Horned Beetles. Junk Publishers, The Hague.

Baldwin, J. M. 1902. Development and Evolution. Macmillan, New York.

Balthasar, V. 1963. Monographie der Scarabaeidae und Aphodiidae der palaearktischen und orientalischen Region (Coleoptera: Lamellicornia). Band 2, Coprinae. Verlag der tschechoslowakischen Akademie der Wissenschaften, Prag.

Baraud, J. 1992. Coléoptères scarabaeiodea d'Europe. Fédéracion Française des Sociétiés des Sciences Naturelles, Paris.

Beck, S. D. 1971a. Growth and retrogression in larvae of *Trogoderma glabrum* (Coleoptera: Dermestidae). 1. Characteristics under feeding and starvation. Annals of the Entomological Society of America, 64: 149–155.

Beck, S. D. 1971b. Growth and retrogression in larvae of *Trogoderma glabrum* (Coleoptera: Dermestidae). 2. Factors influencing pupation. Annals of the Entomological Society of America, 64: 946–949.

Blakley, N. and S. R. Goodner. 1978. Size-dependent timing of metamorphosis in milkweed bugs (*Oncopeltus*) and its life history implications. Biological Bulletin, 155: 499–510.

Brakefiel, P. M. and Reitsma, N. 1991. Phenotypic plasticity, seasonal climate and the population biology of *Bycyclus* butterflies (Satyridae) in Malawi. Ecological Entomology, 16: 291–303.

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

Campbell, G. and A. Tomlinson. 1998. The roles of the homeobox genes *aristaless* and *Distal-less* in patterning the legs and wings of *Drosophila*. Development, 125: 4483–4493.

Campbell, G., Weaver, T., and A. Tomlinson. 1993. Axis specification in the developing *Drosophila* appendage: the role of *wingless*, *decapentaplegic*, and the homeobox gene *aristaless*. Cell, 74: 1113–1123.

Carpaneto, G. M. and E. P. Piatella. 1988. Due casi di teratologia bilaterale simmetrica in coleotteri scabeoidei. Bollettino dell'Associazione Romana di Entomologia 43: 29–32.

Cisper, G., Zera, A. J., and D. W. Borst. 2000. Juvenile hormone titer and morph-specific reproduction in the wing-polymorphic cricket, *Gryllus firmus*. Journal of Insect Physiology, 46: 585–596.

Cohen, B., Simcox, A. A., and S. M. Cohen. 1993. Allocation of the thoracic imaginal primordia in the *Drosophila* embryo. Development, 117: 597–608.

Conner, J. 1989. Density dependent sexual selection in the fungus beetle, *Bolitotherus cornutus*. Evolution, 43: 1378–1386.

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

Crespi, B. J. 1988. Adaptation, compromise and constraint: the development, morphometrics, and behavioral basis of a fighter-flier polymorphism in male *Hoplothrips karni* (Insecta: Thysanoptera). Behavioral Ecology and Sociobiology, 23: 93–104.

Darwin, C. 1871. The descent of man and selection in relation to sex. The Modern Library, Random House 1959. Toronto, Canada.

Denno, R. F., Douglass, L. W., and D. Jacobs. 1986. Effects of crowding and host plant nutrition on a wing-dimorphic planthopper. Ecology, 67: 116–123.

Dingle, H. and R. Winchell. 1997. Juvenile hormone as a mediator of plasticity in insect life histories. Archives of Insect Biochemistry and Physiology, 35: 359–373.

Doane, R. W. 1913. The rhinoceros beetle *Oryctes rhinoceros* in Samoa. Journal of Economic Entomology, 6: 437–442.

Dudley, R. 2000a. The evolutionary physiology of animal flight: paleobiological and present perspectives. Annual Review of Physiology, 62: 135–155.

Dudley, R. 2000b. The Biomechanics of Insect Flight. Princeton University Press. Princeton, NJ.

Eberhard, W. G. 1978. Fighting behavior of male *Golofa porteri* beetles (Scarabaeidae: Dynastinae). Psyche, 83: 292–298.

Eberhard, W. G. 1979. The functions of horns in *Podischnus agenor* Dynastinae and other beetles, pp. 231–258. *In* M. S. Blum and N. A. Blum [eds] 1979. Sexual Selection and Reproductive Competition in Insects. Academic Press, New York.

Eberhard, W. G. 1981. The natural history of *Doryphora* sp. (Coleoptera: Chrysomelidae) and the function of its sternal horn. Annals of the Entomological Society of America, 74: 445–448

Eberhard, W. G. 1982. Beetle horn dimorphism: making the best of a bad lot. American Naturalist, 119: 420–26.

Eberhard, W. G. 1987. Use of horns in fights by the dimorphic males of *Ageopsis nigricollis* (Coleoptera, Scarabaeidae, Dynastinae). Journal of the Kansas Entomological Society, 60: 504–509.

Eberhard, W. G. and E. Gutierrez. 1991. Male dimorphisms in beetles and earwigs and the question of developmental constraints. Evolution, 45: 18–28.

Eberhard, W. G. and J. M. Garcia-C. 2000. Ritual jousting by horned *Parisoschoenus expositus* weevils (Coleoptera, Curculionidae, Baridinae). Psyche, 103: 55–84.

Eberhard W. G., Garcia, J. M., and J. Lobo 2000. Size-specific defensive structures in a horned weevil confirm a classic battle plan: avoid fights with larger opponents. Proceedings of the Royal Society of London Series B, 267: 1129–1134.

Edwards, P. 1991. Seasonal variation in the dung of African grazing mammals, and its consequences for coprophagous insects. Functional Ecology, 5: 617–628.

Emlen D. J. 1994. Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). Proceedings of the Royal Society of London Series B, 256: 131–136.

Emlen, D. J. 1996. Artificial selection on horn length-body size allometry in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). Evolution, 50: 1219–1230.

Emlen, D. J. 1997a. Alternative reproductive tactics and male-dimorphism in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). Behavioral Ecology and Sociobiology, l41: 335–341.

Emlen, D. J. 1997b. Diet alters male horn allometry in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). Proceedings of the Royal Society of London Series B, 264: 567–574.

Emlen, D. J. 2000. Integrating development with evolution: a case study with beetle horns. Bioscience, 50: 403–418.

Emlen, D. J. 2001. Costs and the diversification of exaggerated animal structures. Science, 291: 1534–1536.

Emlen, D. J., and H. F. Nijhout. 1999. Hormonal control of male horn length dimorphism in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). Journal of Insect Physiology, 45: 45–53.

Emlen, D. J., and H. F. Nijhout. 2000. The development and evolution of exaggerated morphologies in insects. Annual Review of Entomology, 45: 661–708.

Emlen, D. J., and H. F. Nijhout. 2001. Hormonal control of male horn length dimophism in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae): a second critical period of sensitivity to juvenile hormone. Journal of Insect Physiology, 47: 1045–1054.

Evans, J. D. and D. E. Wheeler. 1999. Differential gene expression between developing queens and workers in the honey bee, *Apis mellifera*. Proceedings of the National Academy of Science, 96: 5575–5580.

Evans, J. D. and D. E. Wheeler. 2001. Expression profiles during honeybee caste determination. Genome Biology 2: Research 0001.

Field, J. 1992. Intraspecific parasitism as an alternative reproductive tactic in nestbuilding wasps and bees. Biological Reviews, 67: 79–126.

Fristrom, D. and J. W. Fristrom. 1993. The metamorphic development of the adult epidermis, pp. 843–897. *In* Bate, M. and A. M. Arias. [eds] 1993. The Development of *Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, New York.

Gadgil, M. 1972. Male dimorphism as a consequence of natural selection. American Naturalist, 106: 574–580.

Gibson, M. C. and G. Schubiger. 2000. Peripodial cells regulate proliferation and patterning of *Drosophila* imaginal discs. Cell, 103: 343–350.

Giller, P. S. and B. M. Doube. 1989. Experimental analysis of inter- and intraspecific competition in dung beetle communities. Journal of Animal Ecology, 58: 129–144.

Gullan, P. J. and P. S. Cranston. 2000. The Insects. An Outline of Entomology. Blackwell Science, Oxford, UK.

Halffter, G. and W. G. Edmonds, 1982. The nesting behavior of dung beetles (Scarabaeidae): an ecological and evolutive approach. Instituto de Ecologica, Mexico, FD, Mexico.

Harrison, R. G. 1979. Flight polymorphism in the field cricket *Gryllus pennsylvanicus.* Oecologia, 40: 125–132.

Hazel, W. N. and D. A. West. 1982. Pupal colour dimorphism in swallowtail butterflies as a threshold trait: selection in *Eurytides marcellus* (Cramer). Heredity, 49: 295–301.

Hirschberger, P. 1999. Larval population density affects female weight and fecundity in the dung beetle *Aphodius ater*. Ecological Entomology, 24: 316–322.

Howden, H. F. and O. P. Young. 1981. Panamanian Scarabaeinae: taxonomy, distribution, and habits (Coleoptera: Scarabaeidae). Contributions of the American Entomological Institute, 18: 1–204.

Hunt, J. and L. W. Simmons. 1997. Patterns of fluctuating asymmetry in beetle horns: an experimental examination of the honest signalling hypothesis. Behavioral Ecology and Sociobiology, 41: 109–114.

Hunt, J. and L. W. Simmons. 2002. Confidence of paternity and paternal care: covariation revealed through experimental manipulation of a mating system in the beetle *Onthophagus taurus*. Journal of Evolutionary Biology, 15: 784–795.

Hölldobler, B. and E. O. Wilson. 1990. The Ants. Springer Verlag, Berlin.

Kawamura, K. Shibata, T., Saget, O., Peel, D., and P. J. Bryant. 1999. A new family of growth factors produced by the fat body and active on *Drosophila* imaginal disk cells. Development, 126: 211–219.

Kawano, K. 1995. Horn and wing allometry and male dimorphism in giant rhinozeros beetles (Coleoptera: Scarabaeidae) of tropical Asia and America. Annals of the Entomological Society of America, 88: 92–99.

Kawano, K. 1997. Cost of evolving exaggerated mandibles in stag beetles (Coleoptera: Lucanidae). Annals of the Entomological Society of America, 90: 453–461.

Kubota, K., Goto, S., and Hayashi, S. 2003. The role of Wg signaling in the patterning of embryonic leg primordium in *Drosophila*. Developmental Biology, 257: 117–126.

Kim, C. W. 1959. The differentiation centre inducing the development from larval to adult leg in *Pieris brassicae* (Lepidoptera). Journal of Embryology and Experimental Morphology, 7: 172–582.

Kim, J., Sebring, A., Esch, J. J., Kraus, M. E., Vorwerk, K., Magee, J., and S. B. Carroll. 1996. Integration of positional signals and regulation of wing formation and identity by *Drosophila* vestigial gene. Nature, 382: 133–138.

Klingenberg, C. P. and H. F. Nijhout. 1998. Competition among growing organs and the developmental control of morphological asymmetry. Proceedings of the Royal Society of London, Series B, 265: 1135–1139.

Koch, P. B. 1995. Color pattern specific melanin synthesis is controlled by ecdysteroids via dopa dicarboxylase activity in a butterfly wing of *Precis coenia* Hubner. Insect Biochemistry and Molecular Biology, 25: 73–82.

Koch, P. B. and D. Bückmann. 1987. Hormonal control of seasonal morphs by the timing of ecdysteroid release in *Araschnia levana* (Nymphalidae: Lepidoptera). Journal of Insect Physiology, 33: 823–829.

Kotiaho, J. S. 2002. Sexual selection and condition dependence of courtship display in three species of horned dung beetles. Behavioral Ecology, 13: 791–799.

Lameere, A. 1904. L'Evolution des ornaments sexuels. Bulletin Academe Belgique, 1904: 1327–1364.

Lohse, G. A. and W. H. Lucht. 1992. Die Käfer Mitteleuropas. Volume 13. Goecke and Evers, Krefeld, Germany.

Lüscher, M. 1960. Hormonal control of caste differentiation in termites. Annals of the New York Academy of Science, 89: 549–563.

MacGown, J. and M. MacGown. 1996. Observation of a nuptial flight of the horned *Passalus* beetle, *Odontotaenius disjunctus* (Illiger) (Coleoptera: Passalidae). Coleopterist Bulletin, 50: 201–203.

Matthews, E. G. 1972. A revision of the Scarabaeinae dung beetles of Australia. I. Tribe Onthophagini. Australian Journal of Zoology Supplemental Series, 9: 1–330.

Mitchel, R. 1975. The evolution of oviposition tactics in the bean weevil, *Callosobruchus maculatus* (F.). Ecology, 56: 696–702.

Moczek, A. P. 1996. Male dimorphism in the scarab beetle *Onthophagus taurus* Schreber, 1759 (Scarabaeidae, Onthophagini): Evolution and plasticity in a variable environment. Ms-Thesis, Julius-Maximilians-University Würzburg, Germany.

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. 1999. Facultative paternal investment in the polyphenic beetle *Onthophagus taurus*: the role of male morphology and social context. Behavioral Ecology, 10: 641–647.

Moczek, A. P. 2002. Allometric plasticity in a polyphenic beetle. Ecological Entomology, 27: 58–67.

Moczek, A. P. 2003. The behavioral ecology of threshold evolution in a polyphenic beetle. Behavioral Ecology, 14: 831–854.

Moczek, A. P. 2005. The evolution and development of novel traits, or how beetles got their horns. BioScience, 11: 935-951.

Moczek, A. P. and L. M. Nagy. 2005. Diverse developmental mechanisms contribute to different levels of diversity in horned beetles. Evolution and Development, 7: 175–185

Moczek, A. P. and D. J. Emlen. 1999. Proximate determination of male horn dimorphism in the beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). Journal of Evolutionary Biology, 12: 27–37.

Moczek, A. P. and D. J. Emlen. 2000. Male horn dimorphism in the scarab beetle *Onthophagus taurus*: do alternative reproductive tactics favor alternative phenotypes? Animal Behavior, 59: 459–466.

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

Moczek, A. P. and H. F. Nijhout. 2002b. A method for sexing third instar larvae of the genus *Onthophagus* Latreille (Coleoptera: Scarabaeidae). Coleopterist Bulletin, 56: 279–284.

Moczek, A. P. and H. F. Nijhout. 2003. Rapid evolution of a polyphenic threshold. Evolution and Development, 5: 259–268.

Moczek, A. P. and H. F. Nijhout. 2004. Trade offs during the development of primary and secondary sexual traits in a horn dimorphic beetle. American Naturalist, 163: 184–191.

Moczek, A. P., Krell, F. T., and Bruehl, C. 2004. Gradual and threshold-dependent expression of secondary sexual traits in the same individual: insights from a horned beetle. Biological Journal of the Linnean Society, 83: 473–480.

Moczek, A. P. and J. Cochrane. Intraspecific female brood parasitism in the dung beetle *Onthophagus taurus*. Ecological Entomology. (In Press)

Moczek, A. P., Hunt, J., Emlen, D. J. and L. W. Simmons. 2002. Evolution of a developmental threshold in exotic populations of a polyphenic beetle. Evolutionary Ecology Research, 4: 587–601.

Moffett, M. W. 1987. Division of labor and diet in the extremely polymorphic ant *Pheidologeton diversus*. National Geographic Research, 3: 282–304.

Moran, N. A. 1991. Phenotype fixation and genotypic diversity in the complex life cycle of the aphid *Pemphigus betae*. Evolution, 45: 957–970.

Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. American Naturalist, 139: 971–989.

Moran, N. A. and T. G. Whitham. 1988. Evolutionary reduction of complex life cycles: loss of host alternation in *Pemphigus* (Homoptera: Aphididae). Evolution, 42: 717–728.

Nijhout, H. F. 1975. A threshold size for metamorphosis in the tabacco hornworm, *Manduca sexta*. Biological Bullettin, 149: 214–225.

Nijhout H. F. 1976. The role of Ecdysone in pupaption of Manduca sexta. Journal of Insect Physiology, 22: 453-463.

Nijhout, H. F. 1991. The Development and Evolution of Butterfly Wing Patterns. Smithonian Institution Press, Washington, USA.

Nijhout, H. F. 1994. Insect Hormones. Princeton: Princeton University Press, USA.

Nijhout, H. F. 1999. Control mechanisms of polyphenic development in insects. Bioscience, 49: 181–192.

Nijhout, H. F. and D. E. Wheeler. 1996. Growth models of complex allometries in holometabolous insects. American Naturalist, 148: 40–56.

Nijhout, H. F. and D. J. Emlen. 1998. Competition among body parts in the development and evolution of insect morphology. Proceedings of the National Academy of Science, 95: 3685–3689.

Nijhout, H. F. and L. W. Grunert. 2002. Bombyxin is a growth factor for wing imaginal disks in Lepidoptera. Proceedings of the National Academy of Science, 99: 15446–15450.

Oldham, S., Böhni, R., Stocker, H., Broglio, W., and E. Hafen. 2000. Genetic control of size in *Drosophila*. Philosophical Transaction of the Royal Society, London, Series B, 355: 945–952.

Otronen, M. 1988. Intra- and intersexual interactions at breeding burrows in the horned beetle, *Coprophaneus ensifer*. Animal Behavior, 36: 741–748.

Otto, D. 1962. Die roten Waldameisen. A. Ziemsen Verlag, Wittenberg, Germany.

Pace, A. 1967. Life history and behavior of a fungus beetle, *Bolitotherus cornutus* (Tenebrionidae). Occasional papers of the Museum of Zoology at the University of Michigan, 653: 1–15.

Palmer, T. J. 1978. A horned beetle which fights. Nature, 274: 583–584.

Panganiban, G., Nagy, L., and S. B. Carroll. 1994. The role of the Distal-less gene in the development and evolution of insect limbs. Current Biology, 4: 671–675.

Passera, M. P. and J. P. Suzzoni. 1979. Le rôle de la reine de *Pheidole pallidula* (Nyl.) (Hymenoptera: Formicidae) dans la sexualization du couvain après traitement par l'hormone juvénile. Insectes Sociaux, 26: 343–353.

Paulian, R. 1935. Le polymorphisme des males de coléopteres. In: Exposés de biométrie et statistique biologique IV, pp. 1–33. *In* G. Tessier [ed.] 1935.Actualités scientifiques et industrielles 255. Hermann and Cie., Paris, France.

Pigliucci, M. 2001. Phenotypic Plasticity. John Hopkins University Press, Maryland, USA.

Porter, A. H. and N. A. Johnson. 2002. Speciation despite gene flow when developmental pasthways diverge. Evolution, 56: 2103–2111.

Ptashne, M. 1992. Genetic Switch: Phage Lambda and Higher Organisms (2nd ed.). Blackwell Sciences, San Diego, CA, USA.

Raff, R. 1996. The Shape of Life: Genes Development, and the Evolution of Animal Form. University of Chicago Press, Chicago, USA.

Rasmussen, J. L. 1994. The influence of horn and body size on the reproductive behavior of the horned rainbow scarab beetle *Phanaeus difformis* (Coleoptera: Scarabaeidae). Journal of Insect Behavior, 7: 67–82.

Reichenau, W. von. 1881. Üeber den Ursprung der secundären männlichen Geschlechtscharakteren, insbesondere bei den Blatthornkäfern. Kosmos, 10: 172–194.

Richards, M. H. 1994. Social evolution in the genus *Halictus*: a phylogenetic approach. Insectes Sociaux, 41: 315–325.

Ridsdill-Smith, T. J. 1991. Competition in dung-breeding insects, pp. 264–292. *In* W. J. Baily and T. J. Ridsdill-Smith [eds] 1991. Reproductive Behaviour of Insects. Chapman & Hall, London, UK.

Ridsdill-Smith, T. J. 1993. Asymmetric competition in cattle dung between two species of *Onthophagus* dung beetle and the bush fly, *Musca vetustissima*. Ecological Entomology, 18: 241–246.

Roff, D. E., Stirling, G., and D. J. Fairbairn. 1997. The evolution of threshold traits: a quantitative genetic analysis of the physiological and life-history correlates of wing dimorphism in the sand cricket. Evolution, 51: 1910–1919.

Roff, D. E., Tucker, J., Stirling, G., and D. J. Fairbairn. 1999. The evolution of threshold traits: effects of selection on fecundity and correlated response in wing dimorphism in the sand cricket. Journal of Evolutionary Biology, 12: 535–546.

Roundtree, D. B. and H. F. Nijhout. 1995. Hormonal control of a seasonal polyphenism in *Precis coenia* (Lepidoptera: Nymphalidae). Journal of Insect Physiology, 41: 987–992.

Shaffer, H. B. 1984. Evolution in a paedomorphic lineage I. An electrophoretic analysis of the Mexican ambystomatid salamanders. Evolution, 38: 1194–1206.

Schlichting, C. and M. Pigliucci. 1998. Phenotypic Evolution: A Reaction Norm Perspective. Sinauer Associates, Sunderland, MA.

Schluter, D., Price, T. D., Mooers, A. Ø., and D. Ludwig. 1997. Likelihood of ancestor states in adaptive radiations. Evolution, 51: 1699–1711.

Schmalhausen II. 1949. *Factors of Evolution*. University of Chicago Press, Chicago.

Schubiger, G. 1971. Regeneration, duplication and transdetermination in fragments of the leg disk of *Drosophila melanogaster*. Developmental Biology, 26: 277–295.

Semlitsch, R. D., Harris, R. N., and H. M. Wilbur. 1990. Paedomorphosis in *Ambystoma talpoideum*: maintenance of population variation and alternative life-history pathways. Evolution, 44: 1604–1613.

Semlitsch, R. D. and H. M. Wilbur. 1989. Artificial selection for paedomorphosis in the salamander *Ambystoma talypoideum*. Evolution, 43: 105–112.

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

Shapiro, A. M. 1976. Seasonal polyphenism. Evolutionary Biology, 9: 259–333.

Shuster, S. M. and M. J. Wade. 2003. Mating Systems and Strategies. Princeton University Press. Princeton, NJ, USA.

Siva-Jothy, M. T. 1987. Mate securing tactics and the cost of fighting in the Japanese horned beetle, *Allomyrina dichotoma* L. (Scarabaeidae). JEthol, 5: 165-172.

Sowig, P. 1996a. Duration and benefits of biparental brood care in the dung beetle *Onthophagus vacca* (Coleoptera: Scarabaeidae). EcolEntomol, 21: 81-86.

Stearns, S. C. 1989. The evolutionary significance of phenotypic plasticity. Bioscience, 39: 436–445.

Stern, D. L. and W. A. Foster. 1996. The evolution of soldiers in aphids. Biological Reviews, 71: 27–79.

Sultan, S. E. 1987. Evolutionary implications of phenotypic plasticity in plants. Evolutionary Biology, 20: 127–178.

Sultan, S. E. 1992. Phenotypic plasticity and the neo-Darwinian legacy. Evolutionary Trends in Plants, 6: 61–71.

Tanaka, Y. 1985. Alternative manners of prey carrying in the fossorial wasp, *Oxybelus strandi* Yasumatsu (Hymenoptera: Sphecoidae). Kontyû, 53: 277–283.

Tauber, M. J. and C. A. Tauber. 1970. Photoperiodic induction and termination of diapause in an insect: Response to changing day lengths. Science, 167: 170.

Tauber, M. J. and C. A. Tauber. 1972. Geographic variation in critical photoperiod and in diapause intensity of *Chrysopa carnea* (Neuroptera). Journal of Insect Physiology, 18: 25–29.

Tauber, C. A. and M. J. Tauber. 1982. Evolution of seasonal adaptations and life history traits in Chrysopa: response to diverse selective pressures, pp. 51–72. *In* H. Dingle and J. P. Hegmann [eds] 1982. Evolution and Genetics of Life Histories. Springer, New York, USA.

Tauber, C. A. and M. J. Tauber. 1987. Inheritance of seasonal cycles in *Chrysoperla* (Insecta: Neuroptera). Genetical Research Cambridge, 49: 215–223.

Thornhill, R. and J. Alcock. 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, Mass., USA.

Villalba, S., Lobo, J. M., Martin-Piera, F., and R. Zardoya. 2002. Phylogenetic relationships of Iberian dung beetles (Coleoptera: Scarabaeinae): insights on the evolution of nesting behavior. Journal of Molecular Evolution, 55: 116–126.

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

Wallace, A. R. 1869. The Malay Archipelago. Dover Publications 1962, New York, USA.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics, 20: 249–278.

West-Eberhard, M. J. 1992. Behavior and evolution, pp. 57–75. *In* P. R. Grant and H. S. Grant [eds] 1992. Molds, Molecules and Metazoa: Growing Points in Evolutionary Biology. Princeton Univ Press, Princeton, NJ, USA.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

Wheeler, W. M. 1910. Ants: their structure, development and behavior. Columbia University Press, NY, USA.

Wheeler, D. E. 1991. Developmental basis of worker caste polymorphism in ants. American Naturalist, 138: 1218–1238.

Wheeler, D. E. and H. F. Nijhout. 1983. Soldier determination in *Pheidole bicarinata*: effect of methophrene on caste and size within castes. Journal of Insect Physiology, 29: 847–854.

Wheeler, D. E. and H. F. Nijhout. 1984. Soldier determination in the ant *Pheidole bicarinata*: inhibition by adult soldiers. Journal of Insect Physiology, 30: 127–135.

Whiting, M. F., Bradler, S., and T. Maxwell. 2003. Loss and recovery of wings in stick insects. Nature, 421: 264–267.

Wilson, E. O. 1976. Behavioral discretization and the number of castes in an ant species. Behavioral Ecology and Sociobiology, 1: 141–154.

Wilson, E. O. 1978. Division of labor in fire ants based on physical castes (Hymenoptera: Fomicidae: *Solenopsis*). Journal of the Kansas Entomological Society, 51: 615–636.

Wilson, E. O. 1985. The sociogenesis of insect colonies. Science, 228: 1489–1495.

Windsor, D. M. 1987. Natural history of a subsocial tortoise beetle, *Acromis sparsa* Boheman (Chrysomelidae, Cassidinae) in Panama. Psyche, 94: 127–150.

Yang, A. S. 2001. Modularity, evolvability, and adaptive radiations: a comparison of the hemi- and holometabolous insects. Evolution and Development, 3: 59–72.

Zera, A. J. and R. F. Denno, 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology, 42: 207–231.

Zera, A. J. and C. Zhang. 1995. Evolutionary endocrinology of juvenile hormone esterase in *Gyllus assimilis*: direct and correlated responses to selection. Genetics, 141: 1125–1134.

Ziani, S. 1994. Un interessante caso di teraologia simmetrica in *Onthophagus (Paleonthophagus) fracticornis* (Coleoptera, Scarabaeidae). Bollettino dell'Associazione Romana di Entomologia, 49: 165–167.