

Rapid evolution of a polyphenic threshold

Armin P. Moczek^{a,b,*} and H. Frederik Nijhout^a

^aDepartment of Biology, Duke University, Durham, NC 27708, USA

^bCenter for Insect Science, Departments of Molecular and Cellular Biology and of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA

*Author for correspondence (e-mail: arminmo@email.arizona.edu)

SUMMARY Polyphenisms are thought to play an important role in the evolution of phenotypic diversity and the origin of morphological and behavioral novelties. However, the extent to which polyphenic developmental mechanisms evolve in natural populations is unknown. Here we contrast patterns of male phenotype expression in native and exotic and ancestral and descendant populations of the horn polyphenic beetle, *Onthophagus taurus*. Males in this species express two alternative morphologies in response to larval feeding conditions. Favorable conditions cause males to grow larger than a threshold body size and to develop a pair of horns on their heads. Males that encounter relatively poor conditions do not reach this threshold size and remain hornless. We show that exotic and native populations of *O. taurus* differ significantly in the body size threshold that separates alternative male phenotypes. Comparison with archival museum collections and additional samples obtained from the native range of *O. taurus* suggests that allometric differences between exotic

and native populations do not reflect preexisting variation in the native range of this species. Instead, our data suggest that threshold divergences between exotic and native populations have evolved in less than 40 years since the introduction to a new habitat and have proceeded in opposite directions in two exotic ranges of this species. Finally, we show that the kind and magnitude of threshold divergence between native and exotic populations are similar to differences normally observed between species. Our results support the view that certain components of the developmental control mechanism that underlie polyphenic development can evolve rapidly in natural populations and may provide important avenues for phenotypic differentiation and diversification in nature. We discuss the role of developmental control mechanisms in the origin of allometric diversification and explore potential evolutionary mechanisms that could drive scaling relationship evolution in nature.

INTRODUCTION

Many organisms have developed the ability to adjust their phenotype to suit current or future environmental conditions, a phenomenon commonly referred to as adaptive phenotypic plasticity. An extreme yet common case of adaptive phenotypic plasticity is polyphenism: a single genotype's ability to express two or more discrete morphological variants in response to differences in environmental conditions (Nijhout 1999). Polyphenic development is taxonomically widespread and is involved in the production of a substantial portion of phenotypic diversity in nature (reviewed in Roff 1996). Polyphenic development is thought to play a pivotal role in speciation and the evolution of morphological and behavioral novelties (West-Eberhard 1989, 1992).

The proximate factors that determine which phenotype will develop are known for many polyphenisms (Denno et al. 1986; Wheeler 1986; Zera and Tiebel 1989), and the developmental and endocrine mechanisms that adjust developmental pathways to environmental conditions are at least in

part understood for some (reviewed in Nijhout 1999). How these mechanisms evolve in natural populations is, however, largely unknown. Theoretical models have provided a partial insight into the evolutionary dynamics of polyphenism evolution (Lively 1986a,b; Hazel et al. 1990; Moran 1992; Hazel and Smock 1993; Roff 1994; Gross 1996; Gross and Repka 1998), and geographic comparisons and breeding experiments have illustrated that at least one key component of polyphenic development, namely the response threshold, can vary heritably among populations (Tauber and Tauber 1972, 1982, 1987; Harrison 1979; Hazel and West 1982; Semlitsch and Wilbur 1989; Semlitsch et al. 1990; Denno et al. 1996; Emlen 1996; Ahlroth et al. 1999). However, insights from natural population remain scarce, and the extent to which polyphenic developmental mechanisms evolve in natural populations is unclear. Likewise, the ecological factors that shape the evolution of polyphenism in natural populations are unexplored for most polyphenisms, as are the consequences of such modifications for patterns of morphological diversity.

Here we explore a case of rapid polyphenism evolution in the horn-polyphenic dung beetle *Onthophagus taurus*. Male *O. taurus* vary continuously in body size as a function of larval feeding conditions. Only males that exceed a critical body size threshold develop a pair of long curved horns on their heads, whereas males below this threshold remain hornless or develop only rudimentary horns (Fig. 1) (Hunt and Simmons 1997, 1998; Moczek 1998; Emlen and Nijhout 1999; Moczek and Emlen 1999). As a consequence of this threshold, natural populations of *O. taurus* are composed of two relatively discrete male phenotypes: horned and hornless (Paulian 1935; Hunt and Simmons 1997, 1998; Emlen and Nijhout 1999; Moczek and Emlen 1999). Male horn dimorphism coincides with a behavioral dimorphism because different horn phenotypes use strikingly different reproductive tactics to acquire mating opportunities (Emlen 1997; Moczek 1999; Moczek and Emlen 2000).

Onthophagus taurus originally exhibited a circum-Mediterranean distribution (Balthasar 1963). As part of a dung beetle release program under the authority of the Commonwealth Scientific Research Organization (CSIRO) of Australia, *O. taurus* was introduced to several Australian states, including Western Australia (AMRC Workshop Report 1982; Tyndale-Biscoe

1996). At least 36 releases with 500–1800 individuals per release took place between 1969 and 1983 (Tyndale-Biscoe 1996). All beetles released were the direct descendants of parents collected in the native range of *O. taurus*. Parent beetles were collected in Spain, Greece, and Turkey, and a subset of specimens belonging to the original parent populations has been preserved in entomological collections (Tyndale-Biscoe 1996).

In the late 1960s *O. taurus* also became introduced to what is now its second major exotic range: the eastern United States. Unlike the deliberate release of *O. taurus* into Western Australia, the American introduction appears to have been accidental (Fincher and Woodruff 1975). *Onthophagus taurus* was first recorded in 1971 in St. Rosa County, Florida (Fincher and Woodruff 1975) and has since spread as far north as New York and as far west as Indiana (Hoebeke and Beucke 1997; Smith 1997). In North Carolina *O. taurus* has massively altered the native dung beetle fauna (Davis 1958) and now constitutes the dominant onthophagine species in many regions of the state (Moczek 1996, 2002a). The origin of the population that gave rise to the invasion of *O. taurus* of the eastern United States is unknown.

Recent comparisons of Western Australian and North American populations of *O. taurus* revealed that both popula-

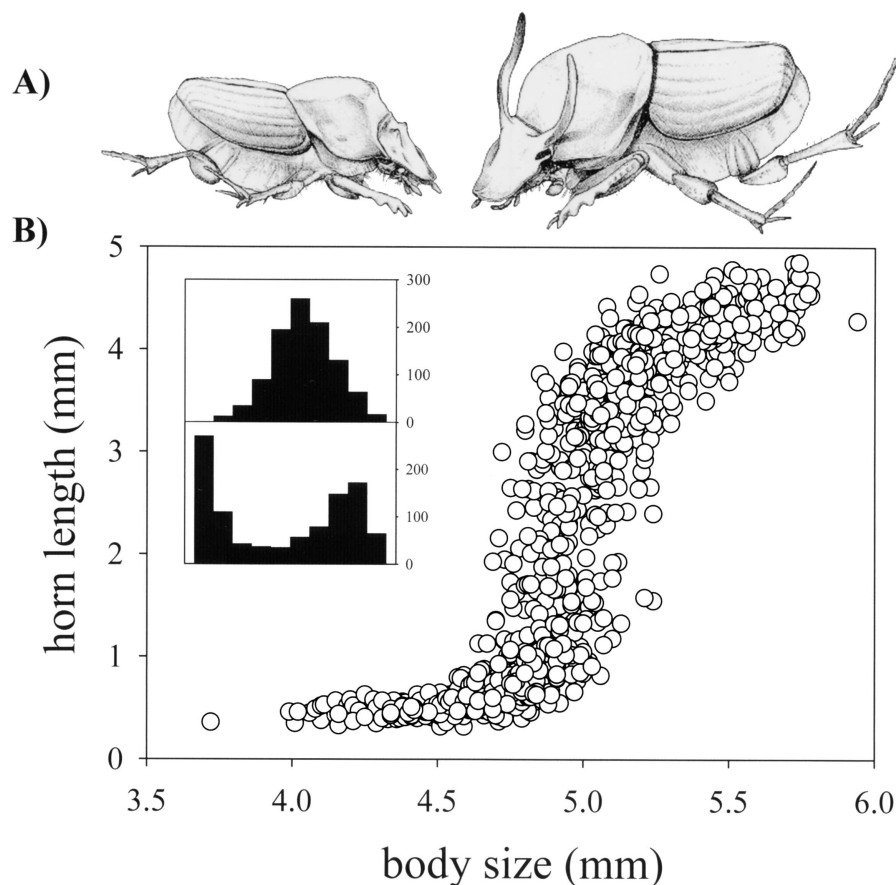


Fig. 1. (A) Typical morphology of hornless and horned male *O. taurus*. (Drawings by Shane Richards.) (B) Typical sigmoidal scaling relationship between horn length and body size of male *O. taurus*. Males were collected in Durham and Orange County, North Carolina ($n = 1016$). Inserts show frequency distributions for body size (top) and horn length (bottom), respectively.

tions have diverged dramatically in the threshold body size that separates alternative male morphologies (Moczek 2002b). We showed previously that threshold divergence is maintained fully in the field and laboratory over generations (Moczek et al. 2002) and is mediated via genetic modifications of the endocrine control mechanisms that specify adult morphology during late larval development (Moczek and Nijhout 2002). Here we contrast patterns of male phenotype expression in native and exotic and ancestral and descendant populations of *O. taurus* and explore the magnitude, direction, and speed of threshold evolution in different populations. We begin by quantifying the scaling relationship between body size and horn length of male *O. taurus* collected in the 1960s from native populations in Spain, Greece, and Turkey that constituted the source material for the introduction of *O. taurus* to Western Australia. We then compare this scaling relationship with the one expressed by descendant present-day Western Australian populations less than 40 years after introduction. We then extend our comparison and include samples from the second exotic distribution of *O. taurus* in the eastern United States. Because in this case the origin of the founding populations is unknown, we include additional scaling relationships from the remainder of the circum-Mediterranean distribution of *O. taurus* into our analysis. Finally, we compare differences in scaling relationships between exotic and native *O. taurus* populations with those between native *O. taurus* populations and its sympatric sister species, *O. illyricus* (Balthasar 1965; Baraud 1992; Lohse and Lucht 1992).

MATERIALS AND METHODS

We used beetles collected in the field and museum specimens for our study. Present-day populations from the eastern United States were sampled in 1996 and 1997 from pastures in Durham and Orange Counties, North Carolina. Populations in Western Australia were sampled in 1997 at Margaret River and in 1998 near Busselton. Beetles were collected using whole dung pad samples. Specimens belonging to the original pool of individuals used to breed *O. taurus* for release into Western Australia were made available by the Australian National Insect Collection, CSIRO, Canberra, Australia. Additional specimens of European and North African origin were made available by the Australian National Insect Collection, Canberra, Australia; the Museo Nacional de Ciencias Naturales, Madrid, Spain; the South Africa National Collection of Insects, Pretoria, South Africa; and the Università di Torino, Italy. Specimens of the sibling species *O. illyricus* were made available by the Università di Torino, Italy, and the Australian National Insect Collection, Canberra, Australia.

Morphometric measurements

All individuals were measured using a standard two-dimensional image analysis system and Image™ software at the Duke University Morphometrics Laboratory (for details see Moczek and Emlen

1999). Thorax width was used as an estimate for body size (for justification see Emlen 1994; Moczek and Emlen 1999).

Statistical analysis

To compare the location of the threshold body size between different populations, we fitted a modified sigmoid equation of the form

$$\text{horn length} = y_0 + \frac{a(\text{body size})^b}{c^b + (\text{body size})^b} \quad (1)$$

to all individuals collected from Spain, Greece, and Turkey (the source populations for the introduction into Western Australia), where a is the range of horn lengths in the sample, b is a slope coefficient, c is the body size at the point of inflection of the sigmoid, and y_0 is the minimum horn length. We used c , or the inflection point of the sigmoid, as an estimate of the average body size threshold at which males switch from the hornless to the horned phenotype. To compare two samples, we first applied the above regression model to both samples combined (= simple model) and determined the parameter values that maximized the likelihood L of our data given this model using the likelihood function:

$$L(\sigma^2 a, b, c, y_0; x) = \frac{1}{(2\pi\sigma^2)^{\frac{n}{2}}} \exp\left\{-\frac{1}{2\sigma^2} \sum_{i=1}^n (y_i - \hat{y}(a, b, c, y_0; x_i))^2\right\} \quad (2)$$

where x_i is the body size of male i , σ^2 is the variance of the data about the fitted values, n is the number of beetles in the combined sample, and

$$\hat{y}_i(a, b, c, y_0; x) = y_0 + \frac{ax^b}{c^b + ax_i^b} \quad (3)$$

We then repeated this analysis for each sample separately (complex model). We obtained a probability value by comparing the test statistic

$$T = 2 \ln(\text{likelihood of the complex model} \div \text{likelihood of the simple model}) \quad (4)$$

to a χ^2 distribution with degrees of freedom equal to the difference in the number of parameters between the two models (Edwards 1972; Weir 1990). If significant differences were indicated, we used repeated Welch's T-tests to examine the degree to which differences in particular regression parameters, such as the inflection point or slope, explained allometric differences between samples (Sachs 1992; Sokal and Rohlf 1995). All significance levels reported below are adjusted for multiple comparisons using sequential Bonferroni corrections where this was necessary (Sachs 1992; Sokal and Rohlf 1995). Unless otherwise noted, all data are presented as means \pm standard error. Results from Welch's T-tests for pair-wise comparisons of parameter estimates are presented as T_{df} = test statistic.

All males collected in Greece and Turkey came from the original pool of individuals used to breed *O. taurus* for release into Western Australia. Males collected from Spain were of two origins: one subsample belonged to the original field sample used to seed

the Australian introduction and one subsample came from other Spanish populations. Using the method outlined above, we tested whether both subsamples of males differed significantly in the average horn length–body size allometry. We found no significant differences between the two subsamples and therefore combined both subsamples for further analyses to increase statistical power.

RESULTS

Western Australian populations and their ancestors

Log-likelihood contrasts and subsequent pair-wise parameter comparisons for individuals collected from Spain, Greece, and Turkey revealed significant differences in allometric parameter values in three instances (Table 1A). *Onthophagus taurus* males collected in Spain exhibited a significantly higher slope coefficient than males collected in Turkey ($T_{191} = 2.059$, $P < 0.05$) or Greece ($T_{35} = 2.51$; $P < 0.05$). In addition, *O. taurus* collected in Greece exhibited a significantly smaller range of horn lengths (parameter y_0) than males collected in Spain ($T_{130} = 2.96$; $P < 0.01$). We found no significant differences in body size thresholds (parameter c) or minimum horn length (parameter a) between any of the three source populations for the Western Australian introduction (Table 1A).

In contrast, Western Australian males did not differ significantly in slope coefficients when compared with any of the source populations. Instead, Western Australian males

differed highly significantly in the average body size threshold that separates horned and hornless male phenotypes ($P < 0.0001$ for each comparison, Table 1A). Extant Western Australian populations expressed the hornless morph over a much wider range of body sizes and switched to the horned morph at a much larger body size than any of their Mediterranean ancestors (Fig. 2, A and B). In addition, *O. taurus* collected in Greece exhibited a significantly smaller range of horn lengths (parameter a) than Western Australian males ($T_{103} = 3.09$; $P < 0.01$).

North Carolinian and Mediterranean populations

Onthophagus taurus males collected in North Carolina also differed highly significantly from males collected in Spain, Greece, or Turkey. Significant differences between samples could be attributed entirely (Spain, Turkey) or largely (Greece) to differences in the body size threshold (parameter c , $P < 0.0001$ in each case; Table 1A). In these cases, however, males in the exotic North Carolinian range switched to the horned phenotype at much smaller body sizes and expressed the horned morph over a much wider range of body sizes than their Mediterranean counterparts (Fig. 2, A and B). *Onthophagus taurus* collected in Greece also exhibited a significantly smaller range of horn lengths (parameter a) than North Carolinian males ($T_{54} = 3.19$; $P < 0.05$).

Because the founding populations that gave rise to the North Carolinian introduction could have originated from Mediterranean regions other than Spain, Greece, or Turkey, we extended our analysis to include males collected from

Table 1. Intra- and interspecific allometric variation in onthophagine beetles

| Sample | Regression parameters | | | | <i>n</i> |
|--|---------------------------------|--------------------------------------|---------------------------------|---------------------------------|----------|
| | <i>a</i> | <i>b</i> | <i>c</i> | y_0 | |
| A. Intraspecific allometric parameter values (mean \pm SE) for <i>O. taurus</i> collected from native and exotic ranges. | | | | | |
| <i>Native</i> | | | | | |
| Spain | 4.09 \pm 0.169 ^a | 38.55 \pm 4.358 ^{a,f} | 5.12 \pm 0.016 ^a | 0.42 \pm 0.074 ^a | 159 |
| Greece | 3.49 \pm 0.114 ^b | 61.26 \pm 7.912 ^c | 5.14 \pm 0.015 ^a | 0.48 \pm 0.056 ^b | 22 |
| Turkey | 3.72 \pm 0.125 ^{a,b} | 53.20 \pm 5.623 ^{b,c} | 5.15 \pm 0.012 ^a | 0.54 \pm 0.029 ^{a,b} | 91 |
| France | 3.83 \pm 0.225 ^{a,b} | 58.09 \pm 9.037 ^{c,d,e,f} | 5.10 \pm 0.029 ^a | 0.48 \pm 0.072 ^{a,b} | 21 |
| Italy | 4.21 \pm 1.063 ^{a,b} | 29.36 \pm 12.99 ^{a,b,c} | 5.10 \pm 0.084 ^{a,b} | 0.25 \pm 0.789 ^{a,b} | 33 |
| Iran | 4.07 \pm 0.199 ^{a,b} | 34.35 \pm 4.422 ^{a,b,d} | 5.16 \pm 0.024 ^a | 0.45 \pm 0.082 ^{a,b} | 117 |
| Morocco and Tunisia | 4.13 \pm 0.312 ^{a,b} | 35.38 \pm 5.986 ^{a,e} | 5.19 \pm 0.031 ^a | 0.42 \pm 0.095 ^{a,b} | 64 |
| <i>Exotic</i> | | | | | |
| North Carolina | 3.985 \pm 0.090 ^a | 38.50 \pm 2.037 ^{a,b} | 4.98 \pm 0.007 ^b | 0.42 \pm 0.056 ^a | 1016 |
| Western Australia | 4.02 \pm 0.129 ^a | 45.97 \pm 3.059 ^{c,d,e} | 5.33 \pm 0.010 ^c | 0.50 \pm 0.036 ^a | 644 |
| B. Interspecific allometric parameter values (mean \pm SE) for native sympatric populations of <i>O. taurus</i> and <i>O. illyricus</i>. | | | | | |
| Native <i>O. taurus</i> | 3.99 \pm 0.091 ¹ | 38.19 \pm 2.331 ¹ | 5.14 \pm 0.010 ¹ | 0.45 \pm 0.036 ¹ | 507 |
| Native <i>O. illyricus</i> | 4.00 \pm 0.262 ¹ | 135.2 \pm 43.43 ² | 5.42 \pm 0.013 ² | 0.50 \pm 0.142 ¹ | 30 |

a specifies the range of horn lengths within a sample (amplitude), *b* specifies a slope coefficient, *c* represents the body size at the point of inflection of the sigmoid, and y_0 specifies minimum horn length. Different exponents indicate significant differences between samples ($P < 0.05$; multiple Welch's T-tests including sequential Bonferroni correction for multiple comparisons).

other Mediterranean populations, including France, Italy, Iran, Tunisia, and Morocco. In each comparison, differences between males collected in North Carolina and the Mediterranean were significant ($P < 0.01$ for each comparison; Table 1A). With one exception (Italy, see below), differences between males collected in North Carolina and in other Mediterranean samples were attributable entirely (Morocco, Tunisia, Iran) or largely (France) to differences in the body size threshold (parameter c , $P < 0.001$ in each case). As before, North Carolinian males switched to the horned phenotype at much smaller body sizes and expressed the horned morph over a much wider range of body sizes than their Mediterranean counterparts (Fig. 2, A and C). French *O. taurus* also exhibited a marginally significantly higher slope coefficient than males collected in North Carolina ($T_{22} = 2.11$; $P = 0.046$). Interestingly, although log-likelihood contrasts between males collected in North Carolina and Italy suggested significant allometric differences between these two populations, none of the four regression parameters was able to account for these differences. A potential explanation for this observation may lie in the unusually wide spread of data points within the Italian sample (see Fig. 2C) and a relatively modest sample size ($n = 33$).

Pair-wise comparison of all Mediterranean populations

Eighteen pair-wise log-likelihood contrasts of all Mediterranean populations, excluding those between Spain, Greece, and Turkey presented above, yielded additional significant differences in seven cases. Three of these (two of which involved Italy, see above) could not be accounted for by any of the regression parameters. In the three remaining cases, differences were due to significant differences in the slope coefficient (parameter b) between samples (Table 1A). No pair-wise comparison indicated significant differences in parameter c , suggesting that over most of their native geographic range, male *O. taurus* employ a similar threshold body size to switch from hornless to horned morphologies (Fig. 2, B and C).

Intra- versus interspecific differences

When compared with each other, Western Australian and North Carolinian populations differed most dramatically in the threshold body size ($T_{1191} = 28.92$; $P < 0.0001$), causing both populations to express opposite phenotypes over a wide range of body sizes (Table 1A, Fig. 2A). In addition, Western Australian males also expressed a slightly but significantly higher slope coefficient than their North Carolinian counterparts ($T_{1191} = 2.03$; $P < 0.05$).

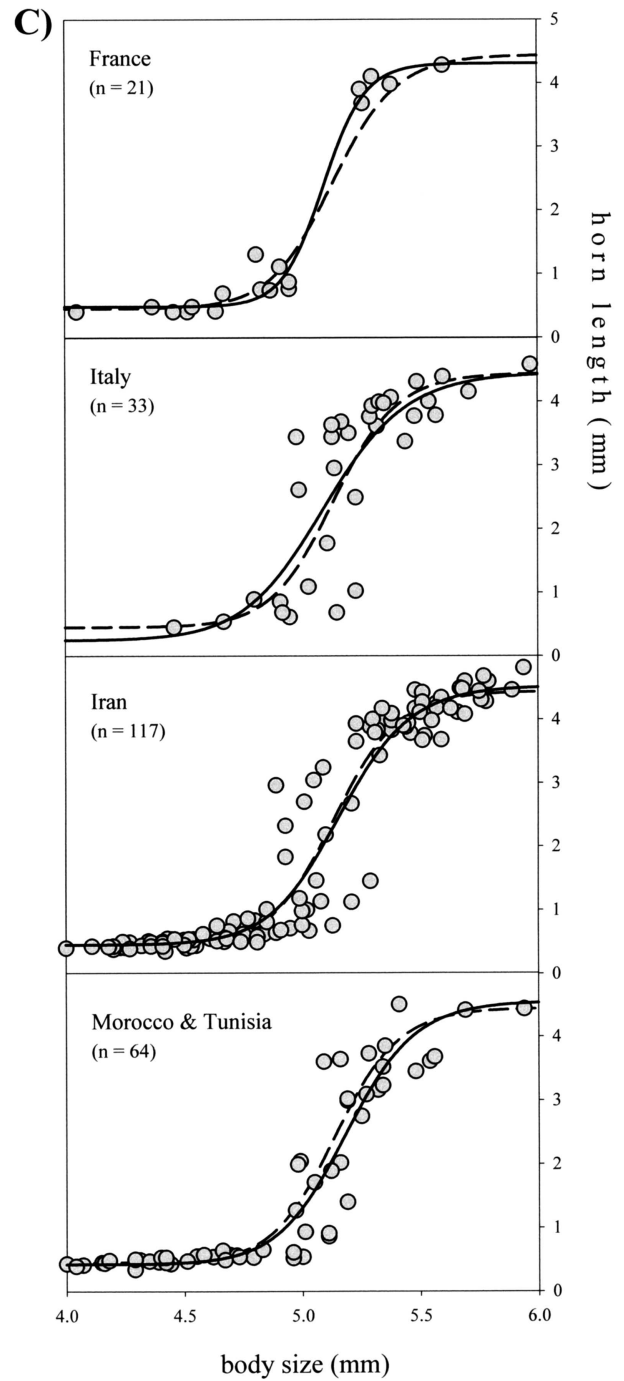
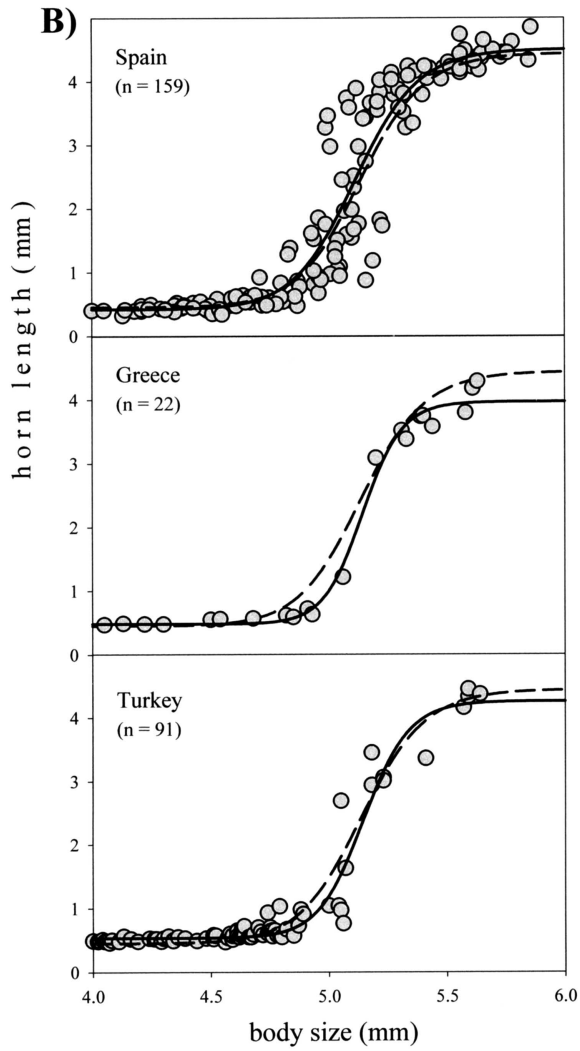
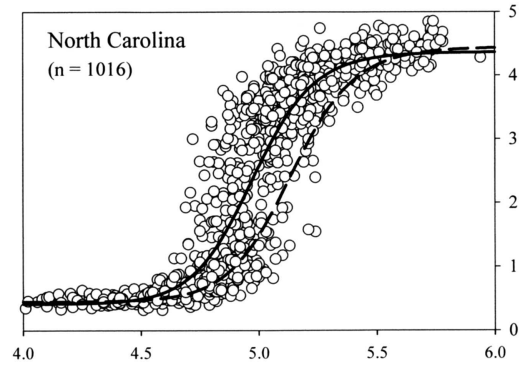
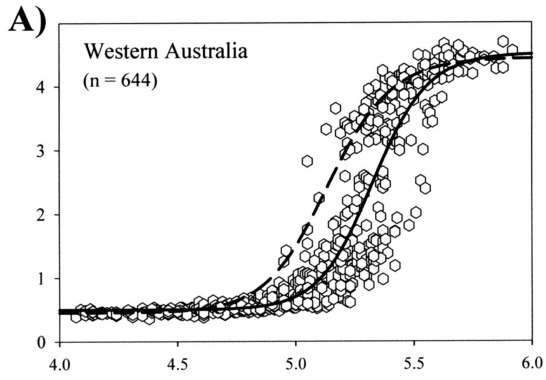
Onthophagus taurus shares much of its native range with *O. illyricus* (Balthasar 1963). *Onthophagus illyricus* also expresses two alternative male horn morphologies very similar to those of *O. taurus* (Balthasar 1963; Baraud 1992; Lohse

and Lucht 1992). In fact, the two species are very difficult to distinguish on the basis of external morphological characters alone and were originally considered varieties of the same species (Balthasar 1963). Male genital morphology, however, allows for a clear and unambiguous separation of both species and provides a reliable species-diagnostic character (Lohse and Lucht 1992; Krell, personal communication). *Onthophagus taurus* and *O. illyricus* are now considered sister species (Baraud 1992; Lohse and Lucht 1992). Exotic *O. taurus* populations do not contain *O. illyricus* (Krell, personal communication).

Mediterranean *O. taurus* and sympatric male *O. illyricus* differed highly significantly in the average horn length–body size allometry ($L_4 = 159.73$). Differences between the two species were attributable largely to highly significant differences in the infection point c ($T_{72} = 17.21$; $P < 0.0001$), suggesting that apart from genital characters, both species can also be distinguished by the body size threshold between alternate morphs (Fig. 3). In addition, *O. illyricus* exhibited a marginally significantly higher slope coefficient ($T_{29} = 2.23$; $P = 0.0336$) than Mediterranean *O. taurus*. Differences in threshold body sizes and slope coefficients between Mediterranean *O. taurus* and *O. illyricus* were similar in kind and magnitude to the differences between North Carolinian and Western Australian populations of *O. taurus* and between exotic and native *O. taurus* populations (Table 1B). Combined, these data suggest that the current morphological divergences of exotic *O. taurus* populations in North Carolina and Western Australia may be similar to patterns of morphological differentiation associated with the early stages in the formation of new species in the genus *Onthophagus*.

Discussion

Polyphenisms are a common extreme of phenotypically plastic trait expression and rely on threshold responses to produce discrete alternative phenotypes (Nijhout 1994; Roff 1996; Stearns 1989). Threshold responses are widespread and essential components of many physiological and developmental processes yet have only relatively recently regained attention from evolutionary biologists (Schmalhausen 1949; Tauber and Tauber 1970; Hazel and West 1982; West-Eberhard 1989, 1992; Kingsolver 1995; Emlen 1996, 2000; Roff 1996; Zera and Denno 1997; Hazel et al. 1998; Schlichting and Pigliucci 1998; Lively et al. 1999; Nijhout 1999; Tomkins 1999). Here we show that exotic and native populations of the polyphenic beetle *O. taurus* differ most dramatically in the threshold body size that separates alternative male phenotypes. The kind and magnitude of threshold divergences between native and exotic populations do not reflect variation in the native range of *O. taurus* but instead resemble differences normally observed between



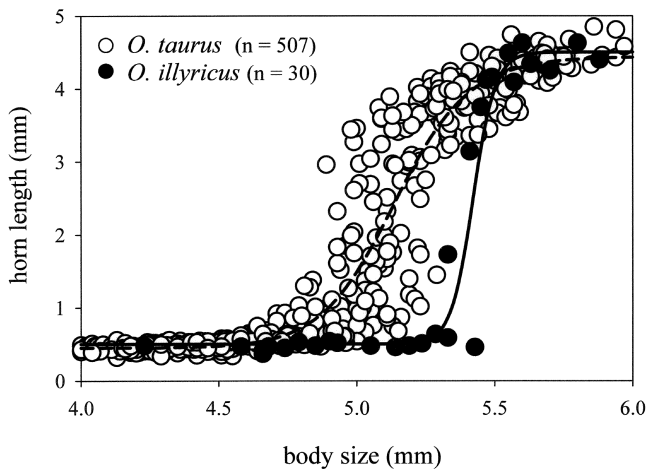


Fig. 3. Scaling relationship between body size and horn length of male *O. taurus* (open circles and dashed line) and *O. illyricus* (black circles and solid line) collected from native Mediterranean populations. Lines represent best-fit nonlinear regressions calculated separately for both samples (see text for details and Table 1 for parameter values).

species. Our results suggest that threshold differences between exotic *O. taurus* populations have evolved in less than 40 years since the introduction to a new habitat and that threshold evolution proceeded in opposite directions in both exotic ranges. Combined, our results support the view that certain components of polyphenic development, such as the response threshold, can evolve rapidly in natural populations and may provide important avenues for phenotypic differentiation and diversification in nature.

Developmental basis of allometric diversification

Threshold response

We used four parameters to characterize the average scaling relationship between horn length and body size in native and exotic *O. taurus* populations. Of those, the threshold body size c , or point of inflection of the sigmoid, is understood best with respect to its underlying developmental mechanisms. Earlier studies showed that horns develop only in male larvae that exceed a critical weight threshold (Emlen and Nijhout 1999). Horn expression can be induced experimentally in smaller male larvae via topical application of juvenile hormone analogs, provided a certain minimum dosage

is applied and application occurs during a particular time window or sensitive period (Moczek and Nijhout 2002). Combined, these findings suggested that juvenile hormone titers may be correlated with larval weight during a sensitive period, where they are then used to differentiate between two alternative developmental pathways. Although juvenile hormone titers have yet to be measured in *O. taurus*, similar regulatory mechanisms have been observed in a variety of other insect polyphenisms (Nijhout 1994, 1999). Apart from providing a first insight into the developmental regulation of horns, these findings also suggested at least two avenues for evolutionary modifications of the body size threshold. For example, changes in sensitivity to juvenile hormone, or changes in the timing of sensitivity to juvenile hormone relative to other developmental events, could modify the range of larval body weights that would fall above or below the threshold required for horn induction (Moczek and Nijhout 2002). Both hypotheses recently received experimental support. Allometrically divergent laboratory strains derived from North Carolinian and Western Australian populations were found to differ in both the degree and timing of sensitivity to the juvenile hormone analog methoprene. To induce horn expression, presumptive hornless male Western Australian larvae required higher methoprene dosages and expressed a delay in their sensitive period by 2–3 days compared with their North Carolinian counterparts (Moczek and Nijhout 2002). These findings therefore suggest that the divergence in body size thresholds between exotic and native *O. taurus* populations may have been mediated by evolutionary modifications in the degree and timing of sensitivity to juvenile hormone.

Range of horn lengths and slope

The present study occasionally detected significant differences between samples in the range of horn lengths (amplitude, a) and, more commonly, in slope (b), though some of these results may have been influenced by small sample sizes. However, in at least two cases, significant differences in slope could not be attributed to insufficient sample sizes. For example, Western Australian males ($n = 644$) expressed significantly higher slope coefficients than their North Carolinian ($n = 1016$) counterparts. Like differences in threshold body sizes, differences in slope coefficients were of a magnitude similar to differences between *O. taurus* and *O. illyricus*. Unlike differences in threshold body sizes, however,

Fig. 2. Scaling relationship between body size and horn length of male *O. taurus* from native (open symbols) and exotic (gray circles) populations. (A) Body size–horn length allometry of males collected from exotic populations in Western Australia (left) and North Carolina (right). (B) Body size–horn length allometry of males preserved from ancestral Mediterranean populations collected in Spain, Greece, and Turkey to found the Australian introduction. (C) Body size–horn length allometry of males collected from the remainder of the native geographic range of *O. taurus*. Solid lines represent best-fit nonlinear regressions calculated separately for each sample. For ease of interpretation the dashed line is provided as a visual reference (identical in all graphs) and represents the best-fit nonlinear regression for all Mediterranean samples combined (see text for details and Table 1 for parameter values).

significant differences in slope coefficients were also detected between populations *within* the native range of *O. taurus*. For instance, males collected in Turkey ($n = 91$) expressed significantly higher slope coefficients than males collected in Spain. This suggests that differences in slope could have been present in the native range of *O. taurus* before introduction to the United States and Australia. Our results nevertheless indicate that significant phenotypic and possibly genetic variation in slope exists in nature, which may provide additional, and previously overlooked, opportunities for evolutionary diversification of horn length–body size allometries.

Unfortunately, relatively little is known about the developmental basis of allometric parameters such as slope or amplitude. Recent studies have shown, however, that horns develop from imaginal disk-like structures that undergo rapid cell divisions during the prepupal stage, much like the imaginal disks that give rise to legs, mouthparts, antennae, and genitalia in holometabolous insects (Emlen and Nijhout 1999; Moczek and Nagy, unpublished data). Therefore, insights into the nature and regulation of imaginal disk development may be of help in understanding the developmental basis of allometric shape parameters and their evolutionary significance. For example, imaginal disks undergo most of their growth in the closed environment of the prepupal and pupal stage at the expense of nutrients accumulated during larval life. Because growth occurs in a closed environment without further input of resources from the outside, there is a potential for trade-offs for resources during growth of imaginal disks, such that an increase in one disk is often associated with the relative diminution of another disk. Increased horn size in *O. taurus*, for instance, occurs at the expense of eye size (Nijhout and Emlen 1998). Such competition among disks has the potential to result in the highly nonlinear (hyperbolic and sigmoid) allometries that are so common in adult insects (Nijhout and Wheeler 1995; Emlen and Nijhout 2000). The exact shape of such allometries is further influenced in part by the sizes of the disks relative to the available nutrient pool and by the relative ability of different disks to compete for resources during growth (Nijhout and Wheeler 1995). Thus, evolutionary changes in insect scaling relationships, including changes in the slope or amplitude of an allometry, may not only proceed via evolutionary changes in endocrine control mechanisms but may also be brought about through evolutionary changes in the growth and competition parameters of imaginal discs.

Behavioral ecology of threshold evolution

Our results indicate that modifications of the threshold body size that separates hornless and horned morphs can evolve extraordinarily rapidly in geographically isolated populations and may precede, rather than follow, the evolution of reproductive isolation. An important implication of our findings is

therefore that allometric diversification may be relatively easy to achieve, provided appropriate driving forces are in place.

A likely evolutionary mechanism underlying allometric divergence of exotic populations from their ancestors is random genetic drift, in particular if exotic populations are founded by an accidental introduction of a small population, which is likely to have been the case for the introduction of *O. taurus* to North Carolina. Even though the introduction of *O. taurus* to Western Australia was deliberate, with 36 documented releases between 1969 and 1983 with 500–1800 individuals per release (Tyndale-Biscoe 1996), drift could nonetheless have played an important role in the evolution of new body size thresholds. For example, knowledge of the exact habitat requirements of *O. taurus* at the time of introduction was limited, and several releases took place in regions or at times during the season where it would have been difficult for this species to establish itself (Tyndale-Biscoe 1996; Feehan and Weir, personal communication). Genetic drift due to local extinctions could therefore have contributed to threshold divergences in Western Australian populations. If this is correct, however, then present-day allometries in North Carolinian and Western Australian populations should overlap with preexisting allometric variation in the native range of this species. Our present study, however, indicates that this is not the case. Average Western Australian and North Carolinian body size thresholds do not reflect opposite extremes present in the native range of *O. taurus* but instead are both clearly outside the range of body size thresholds documented so far for native *O. taurus* populations. This therefore points toward an alternative explanation for the evolutionary mechanism behind this divergence and suggests that rather than a consequence of random genetic drift, divergent thresholds in exotic *O. taurus* populations may reflect a response to divergent selective environments within which alternative male morphs function.

Male horn polyphenism in *O. taurus* plays an important role in male reproductive behavior, and both male phenotypes adopt strikingly different coadapted behaviors to gain mating opportunities (Emlen 1997, 2000; Moczek 1999; Moczek and Emlen 2000). Large horned males rely exclusively on aggressive behaviors involving fights and the use of horns as weapons to gain and defend access to breeding tunnels that contain females (Moczek and Emlen 2000), and larger males almost always win fights against smaller males (Emlen 1997). Small hornless males instead engage in nonaggressive sneaking behaviors to circumvent large horned males (Moczek and Emlen 2000).

The possession of long horns confers an advantage to males that engage in fights but is detrimental to the performance of males that engage in sneaking behaviors (Moczek and Emlen 2000). Therefore, only males that are sufficiently large for fighting to provide the tactic that yields the highest fitness gain should develop horns. Small males, given their low competitive status, may gain higher fitness by engaging

in sneaking rather than fighting and should therefore remain hornless. Such a selection environment would favor genotypes that match the developmental switch from no to complete horn expression to a body size that corresponds to the point of equal fitness between fighting and sneaking tactics (Moczek et al. 2002; for a general derivation of this model, see Hazel et al. 1990; Gross 1996). However, the optimal body size at which to switch from sneaking to fighting behaviors may vary as a function of external conditions (Gross 1996). For example, because fights are energetically costly, an increase in the local density of competing males, with a corresponding increase in the frequency of male–male encounters, may allow only but the very largest and strongest males to benefit from engaging in fights. Under high density conditions, sneaking behavior may thus become profitable over a wider range of body sizes, which in turn would favor a corresponding shift of the threshold for horn production to larger body sizes (Moczek 2002b, Moczek 2003). Fitness estimates of both male morphs under a range of environmental conditions are clearly needed to test such hypotheses. We are currently exploring whether allometrically divergent populations indeed exhibit consistent differences in demographic factors and the extent to which such factors have the power to result in selection on body size thresholds in onthophagine beetles.

Acknowledgments

We are indebted to T. Weir for his much appreciated support and advice throughout the course of this study and to J. Hunt for collecting *O. taurus* in Western Australia. Special thanks go to D. Higdon and the Duke University Statistical Consulting Center for developing the likelihood analysis and for expert advice on the statistical analysis of allometries. F. T. Krell confirmed species identity for all our samples and ensured that North Carolinian and Western Australian *O. taurus* populations were free of *O. illyricus*. T. Weir, J. Ridsdill-Smith, R. Staals, P. E. Skelley, and E. Barbero helped to make museum material available to us. We thank the following institutions for providing specimen loans: the Australian National Insect Collection, CSIRO, Canberra, Australia; the Museo Nacional de Ciencias Naturales, Madrid, Spain; the South Africa National Collection of Insects, Pretoria, South Africa; and the Università di Torino, Italy. We thank J. Hunt, J. Feehan, J. Ridsdill-Smith, S. Shattuck, L. W. Simmons, and J. Tomkins for logistic support and J. Mercer and the Duke Morphometrics Laboratory for access to equipment. We thank S. Richards for his excellent drawings of *O. taurus*, and C. Patel for her assistance in locating taxonomic literature on *O. illyricus*. A. P. M. was funded by the Department of Zoology and Biology, Duke University, a National Science Foundation Dissertation Improvement Grant IBN 9972567, a Sally Hughes-Schrader International Fellowship, a Duke University Grant for International Studies, a Robert R. Bryden/North Carolina Academy of Science Fellowship, a Sigma Xi Grant-in-Aid of Research, and a Kathryn Stern Fellowship. H. F. N. was funded in part by NSF grant IBN 9728727.

REFERENCES

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

- Australian Meat Research Committee (AMRC) Workshop Report. 1982. *The Biological Control of Dung in Australia*. Commonwealth Scientific Research Organization, Canberra.
- 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 scarabaeoidea d'Europe*. Fédération Française des Sociétés des Sciences Naturelles, Paris.
- Davis, L. V. 1958. *The Scarabaeidae of Durham and Orange Counties, North Carolina*. MS thesis, Duke University, North Carolina.
- Denno, R. F., Douglass, L. W., and Jacobs, D. 1986. Effects of crowding and host plant nutrition on a wing-dimorphic planthopper. *Ecology* 67: 116–123.
- Denno, R. F., Roderick, G., Peterson, M. A., Huberty, A. F., Döbel, H. G., Eubanks, M. D., Losey J. E., and Langellotto, G. A. 1996. Habitat persistence underlies intraspecific variation in the dispersal strategies of planthoppers. *Ecol. Monogr.* 66: 389–408.
- Edwards, A. W. F. 1972. *Likelihood*. Cambridge University Press, Cambridge, UK.
- Emlen, D. J. 1994. Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proc. R. Soc. Lond. 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. 1997. Alternative reproductive tactics and male-dimorphism in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Behav. Ecol. Sociobiol.* 41: 335–341.
- Emlen, D. J. 2000. Integrating development with evolution: a case study with beetle horns. *Bioscience* 50: 403–418.
- Emlen, D. J., and Nijhout, H. F. 1999. Hormonal control of male horn length dimorphism in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). *J. Ins. Physiol.* 45: 45–53.
- Emlen, D. J., and Nijhout, H. F. 2000. The development and evolution of exaggerated morphologies in insects. *Annu. Rev. Entomol.* 45: 661–708.
- Fincher, G. T., and Woodruff, R. E. 1975. A European dung beetle, *Onthophagus taurus* Schreber, new to the U.S. (Coleoptera: Scarabaeidae). *Coleopt. Bull.* 29: 349–350.
- Gross, M. R. 1996. Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol. Evol.* 11: 92–98.
- Gross, M. R., and Repka, J. 1998. Stability with inheritance in the conditional strategy. *J. Theor. Biol.* 192: 445–453.
- Harrison, R. G. 1979. Flight polymorphism in the field cricket *Gryllus pennsylvanicus*. *Oecologia* 40: 125–132.
- Hazel, W. N., and Smock, R. 1993. Modeling selection on conditional strategies in stochastic environments. In J. Yoshimura, and C. W. Clark (eds.). *Adaptation in Stochastic Environments*. Springer-Verlag, Berlin, pp. 147–154.
- Hazel, W. N., and West, D. A. 1982. Pupal colour dimorphism in swallowtail butterflies as a threshold trait: selection in *Eurytides marcellus* (Cramer). *Heredity* 49: 295–301.
- Hazel, W. N., Smock, R., and Johnson, M. D. 1990. A polygenic model for the evolution and maintenance of conditional strategies. *Proc. R. Soc. Lond. B* 242: 181–187.
- Hazel, W. N., Ante, S., and Stringfellow B. 1998. The evolution of environmentally-cued pupal colour in swallowtail butterflies: natural selection for pupation site and pupal colour. *Ecol. Entomol.* 23: 41–44.
- Hoebeke, R. E., and Beucke K. 1997. Adventive *Onthophagus* (Coleoptera: Scarabaeidae) in North America: geographic ranges, diagnoses, and new distributional records. *Entomol. News* 108: 345–362.
- Hunt, J., and Simmons, L. W. 1997. Patterns of fluctuating asymmetry in beetle horns: an experimental examination of the honest signalling hypothesis. *Behav. Ecol. Sociobiol.* 41: 109–114.
- Hunt, J., and Simmons, L. W. 1998. Patterns of parental provisioning covary with male morphology in a horned beetle (*Onthophagus taurus*) (Coleoptera: Scarabaeidae). *Behav. Ecol. Sociobiol.* 42: 447–451.
- Kingsolver, J. G. 1995. Fitness consequences of seasonal polyphenism in western white butterflies. *Evolution* 49: 942–954.
- Lively, C. M. 1986a. Predator-induced shell dimorphism in the acorn barnacle *Chtamalus anisopoma*. *Evolution* 40: 232–242.

- Lively, C. M. 1986b. Competition, comparative life histories, and maintenance of shell dimorphism in a barnacle. *Ecology* 67: 858–864.
- Lively, C. M., Hazel, W. N., Schellenberger, M. J., and Michelson K. S. 1999. Predator-induced defense: variation for inducibility in an intertidal barnacle. *Ecology* 81: 1240–1247.
- Lohse, G. A., and Lucht W. H. 1992. *Die Käfer Mitteleuropas*. Vol. 13. Goecke and Evers, Krefeld, Germany.
- Moczek, A. P. 1996. *Male Dimorphism in the Scarab Beetle Onthophagus taurus Schreber, 1759 (Scarabaeidae: Onthophagini): Evolution and Plasticity in a Variable Environment*. Diplomarbeit, Julius Maximilians University Wuerzburg, 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. *Behav. Ecol.* 9: 636–641.
- Moczek, A. P. 1999. Facultative paternal investment in the polyphenic beetle *Onthophagus taurus*: the role of male morphology and social context. *Behav. Ecol.* 10: 641–647.
- Moczek, A. P. 2002a. Allometric plasticity in a polyphenic beetle. *Ecol. Entomol.* 27: 58–67.
- Moczek, A. P. 2002b. *Evolution of Polyphenic Development in the Beetle Onthophagus taurus (Coleoptera: Scarabaeidae)*. PhD thesis, Duke University, Durham, NC.
- Moczek, A. P. 2003. The behavioral ecology of threshold evolution in a polyphenic beetle. *Behav. Ecol.* (in press).
- Moczek, A. P., and Emlen, D. J. 1999. Proximate determination of male horn dimorphism in the beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). *J. Evol. Biol.* 12: 27–37.
- Moczek, A. P., and Emlen, D. J. 2000. Male horn dimorphism in the scarab beetle *Onthophagus taurus*: do alternative reproductive tactics favor alternative phenotypes? *Anim. Behav.* 59: 459–466.
- Moczek, A. P., and Nijhout H. F. 2002. Developmental mechanisms of threshold evolution in a polyphenic beetle. *Evol. Dev.* 4: 252–264.
- Moczek, A. P., Hunt, J., Emlen, D. J., and Simmons, L. W. 2002. Threshold evolution in exotic populations of a polyphenic beetle. *Evol. Ecol. R.* 4: 587–601.
- Moran, N. 1992. The evolutionary maintenance of alternative phenotypes. *Am. Nat.* 139: 971–989.
- Nijhout, H. F. 1994. *Insect Hormones*. Princeton University Press, Princeton, NJ.
- Nijhout, H. F. 1999. Control mechanisms of polyphenic development in insects. *Bioscience* 49: 181–192.
- Nijhout, H. F., and Emlen D. 1998. Competition among body parts in the development and evolution of insect morphology. *Proc. Natl. Acad. Sci. USA* 95: 3685–3689.
- Nijhout, H. F., and Wheeler, D. E. 1995. Growth models of complex allometries in holometabolous insects. *Am. Nat.* 148: 40–56.
- Paulian, R. 1935. Le polymorphisme des males de coléopteres. In G. Tessier (ed.), *Exposés de Biométrie et Statistique Biologique IV*. Actualités scientifiques et industrielles 255. Hermann and Cie., Paris, pp. 1–33.
- Roff, D. E. 1994. The evolution of dimorphic traits: predicting the genetic correlation between environments. *Genetics* 136: 395–401.
- Roff, D. E. 1996. The evolution of threshold traits in animals. *Q. Rev. Biol.* 71: 3–35.
- Sachs, L. 1992. *Angewandte Statistik*. Springer-Verlag, Berlin.
- Schlichting, C. D., and Pigliucci, M. 1998. *Phenotypic Evolution: A Reaction Norm-Perspective*. Sinauer Associates, Sunderland, MA.
- Schmalhausen, I. I. 1949. *Factors of Evolution*. University of Chicago Press, Chicago.
- Semlitsch, R. D., Harris, R. N., and Wilbur, H. M. 1990. Paedomorphosis in *Ambystoma talpoideum*: maintenance of population variation and alternative life-history pathways. *Evolution* 44: 1604–1613.
- Semlitsch, R. D., and Wilbur, H. M. 1989. Artificial selection for paedomorphosis in the salamander *Ambystoma talpoideum*. *Evolution* 43: 105–112.
- Smith, A. E. 1997. Indian record for *Onthophagus taurus* (Coleoptera: Scarabaeidae): northwestward range extension. *Great Lakes Entomol.* 30: 185–186.
- Sokal, R. R., and Rohlf, F. J. 1995. *Biometry*. W. H. Freeman and Company, New York.
- Stearns, S. C. 1989. The evolutionary significance of phenotypic plasticity. *Bioscience* 39: 436–445.
- Tauber, M. J., and Tauber, C. A. 1970. Photoperiodic induction and termination of diapause in an insect: response to changing day lengths. *Science* 167: 170.
- Tauber, C. A., and Tauber, M. J. 1982. Evolution of seasonal adaptations and life history traits in *Chrysopa*: response to diverse selective pressures. In H. Dingle and J. P. Hegmann (eds.), *Evolution and Genetics of Life Histories*. Springer, New York, pp. 51–72.
- Tauber, C. A., and Tauber, M. J. 1987. Inheritance of seasonal cycles in *Chrysoperla* (Insecta: Neuroptera). *Genet. Res. Camb.* 49: 215–223.
- Tomkins, J. L. 1999. Environmental and genetic determinants of the male forceps length dimorphism in the European earwig *Forficula auricularia* L. *Behav. Ecol. Sociobiol.* 47: 1–8.
- Tyndale-Biscoe, M. 1996. *Australia's Introduced Dung Beetles: Original Releases and Redistributions*. CSIRO, Division of Entomology, Technical Report No. 62, Canberra, Australia.
- Weir, B. S. 1990. *Genetic Data Analysis*. Sinauer, Sunderland, MA.
- West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20: 249–278.
- West-Eberhard, M. J. 1992. Behavior and evolution. In P. R. Grant and H. S. Grant (eds.), *Molds, Molecules and Metazoa: Growing Points in Evolutionary Biology*. Princeton University Press, Princeton, NJ, pp. 57–75.
- Wheeler, D. E. 1986. Developmental and physiological determinants of caste in social hymenoptera: evolutionary implications. *Am. Nat.* 128: 13–34.
- Zera, A. J., and Denno R. F. 1997. Physiology and ecology of dispersal polymorphism in insects. *Annu. Rev. Entomol.* 42: 207–231.
- Zera, A. J., and Tiebel, K. C. 1989. Differences in juvenile hormone esterase activity between presumptive macropterous and brachypterous *Gryllus rubens*: implications for the hormonal control of wing polymorphism. *J. Insect Phys.* 35: 7–17.