

Allometric plasticity and the evolution of environment-by-environment (ExE) interactions during a rapid range expansion of a dung beetle

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Abstract

Plastic responses to environmental conditions may themselves depend on other environmental conditions, but how such environment-by-environment (ExE) interactions may impact evolution remains unclear. We investigate how temperature shapes the nutritional polyphenism in horn length in a beetle and test whether “allometric plasticity” (a form of ExE) predicts latitudinal differentiation during a rapid range expansion. Rearing populations under common garden conditions demonstrates that increased temperatures reduce the body size threshold separating two male morphs in all populations but also that the magnitude of temperature-dependent changes in allometry diverged across recently established populations. Furthermore, we found a latitudinal increase in the threshold in the species’ exotic range at one of the temperatures, suggesting that allometric plasticity in response to temperature may predict evolved clinal differences. Our findings demonstrate that ExE interactions can be similar in magnitude to G×E interactions and that allometric plasticity and its evolution may impact population’s responses to environmental changes.

Keywords: developmental plasticity, invasion, temperature, multidimensional plasticity, allometry, *Onthophagus*

Introduction

Phenotypic plasticity, i.e., the capacity of a single genotype to underpin different phenotypes depending on the environment, plays a major role in shaping species’ responses to environmental changes (Crispo, 2008; Ghalambor et al., 2007; West-Eberhard, 2003). By decoupling phenotypes from their genotypes, plasticity can both hamper and/or facilitate adaptation to novel environments (Pfennig et al., 2010). In addition, plastic responses often exhibit genetic variation and respond to selection (Via, 1991). Consequently, the role of genotype-by-environment (G×E) interactions in adaptation to changing environments is receiving increased attention (Kelly, 2019). However, plastic responses to an environmental variable do not only vary across genotypes (G×E) but also depend on other environmental variables (ExE) (De Block & Stoks, 2003; Rodrigues et al., 2021; Rohner et al., 2018; Verspagen et al., 2020; Westneat et al., 2019). That is, the magnitude and form of plasticity can itself be plastic. Such ExE interactions may further vary across genotypes or species, leading to environment-by-environment-by-genotype (G×ExE) interactions (Sardi et al., 2018; Verspagen et al., 2020). Because development is intrinsically context dependent, these interactions are probably common and likely important in complex and often fluctuating natural environments. However, their role in shaping evolution is poorly understood (Rodrigues & Beldade, 2020). Here, we study how ExE interactions shape evolution when populations encounter new environments.

One of the most common forms of plasticity are nutritional responses in relative trait size to body size—i.e., allometry (*sensu lato*) (Schmidt-Nielsen, 1984). Allometric variation accounts for much of phenotypic variation and its role in evolution has long been recognized (Gould, 1966; Thompson, 1917). However, nutritional scaling relationships can themselves vary across environments (e.g., Koumoundouros et al., 1999; Okada & Miyatake, 2010; Rhebergen et al. 2022). This type of ExE interaction, coined “allometric plasticity” (Emlen, 1997), has been largely overlooked, but may play a significant role during adaptation if nutritional plasticity is sensitive to environmental variables that change in new habitats. Taking advantage of the recent range expansion of the bull-headed dung beetle *Onthophagus taurus* (Schreber, 1759), we investigate whether and how allometric plasticity shapes phenotypic variation along environmental gradients.

Male *Onthophagus taurus* have very strong morphological responses to larval nutrition. Larvae with access to abundant food develop into large adults yielding a pair of large, curved head horns used as weapons in aggressive combat over mating opportunities. In contrast, male larvae with limited access to larval nutrition emerge at a smaller adult size, develop minute horns, and engage in non-aggressive sneaking behaviors (Moczek & Emlen, 2000). The scaling relationship between horn size and body size is strongly sigmoidal, with a critical threshold size separating small, hornless “minor” males from large, fully horned, “major” males. Previous work shows that the shape of the allometry, and specifically its inflection point (i.e., the threshold body size that separates the two morphs),

is dependent on the quality of larval nutrition and thus shows allometric plasticity (Moczek, 2002). Here, we test whether this polyphenism is also dependent on temperature and whether this impacts local adaptation to new climatic environments.

Native to the Mediterranean region and parts of Central Europe, *O. taurus* was accidentally introduced to Florida, United States in the early 1970s (Hoebeke & Beucke, 1997), followed by rapid range expansion toward the North. Within 40 years—corresponding to about 80–100 generations—*O. taurus* was collected at the Canadian border (Rounds & Floate, 2012). Previous studies show that this invasion was associated with population differentiation and local adaptation, including a decrease in body size with increasing latitude (Rohner & Moczek, 2020). This provides us with an ideal system to test whether the polyphenism depends on climatic variables and whether this shapes evolutionary responses when populations colonize new environments.

There are a number of routes through which scaling relationships of secondary sexual traits may evolve during range expansions. For instance, local adaptation may change major life history traits, and in particular, size at maturation. If the selective agents that drive polyphenic development are dependent on relative body size, as has been proposed for dung beetle polyphenisms (status-dependent selection [SDS] model *sensu* Hunt & Simmons [2001]), shifts in size distribution may impact the strength and mode of selection. That is, genetic or plastic shifts in body size distribution are expected to be accompanied by corresponding parallel shifts in the threshold.

We investigated the effect of clinal variation and rearing temperature on male horn polyphenism by rearing four populations obtained from a 1,500 km latitudinal cline of *O. taurus* in the Eastern US as well as one population obtained from the native range in a common garden experiment with two temperature treatments. We predicted that a temperature-mediated change in average population size should alter the distribution of male morphs. Based on the SDS model, we further expected that a plastic or genetic shift in body size should be accompanied by a corresponding shift of the threshold separating the two morphs. That is, we expected threshold functions to track body size shifts to keep morph ratios constant. We show that horn length polyphenism is indeed dependent on temperature and that both threshold position and body size range change with latitude, providing support for a role of E×E interactions in contributing to populations' responses to new environments. However, the observed patterns are unexpected, inconsistent with a simple adaptive scenario of SDS, and instead highlight the complex way by which E×E interactions influence developmental outcomes.

Methods

Common garden rearing

To study population differences among native and exotic populations, we conducted a common garden experiment described in detail in Rohner and Moczek (2020). Adults were collected in the native range (Monte Cucco, Italy, 43.3° latitude) and along a latitudinal cline in the Eastern United States (Santa Fe, Florida (29.9°); Chapel Hill, North Carolina (35.9°); Bloomington, Indiana (39.2°); Lake City, Michigan (44.3°)) and brought into the laboratory to establish colonies. Females were then isolated in individual containers and

allowed to produce so-called “brood balls” for 5 days at 24°C (see [Supplementary Table S1](#) for the number of females producing male offspring). Brood balls are ovoid, compact bodies of cow dung constructed underground by adult females. Each brood ball contains a single egg, which upon hatching completes its development within the brood ball and in complete isolation of other individuals. Because morphological and life history traits depend on larval nutrition and maternal provisioning (Moczek 1998), we then retrieved brood balls produced in the lab, removed the offspring, and reared the latter in standardized, artificial brood balls as described in Shafei et al. (2001). In brief, we placed each larva into a well of a standard 12-well tissue culture plate equipped with approximately 3.2 g of dung obtained from grass-fed cows (cf. Rohner & Moczek, 2021). Larvae then complete their development inside their artificial brood ball without any direct contact to other conspecifics.

Each female's brood was evenly allocated to two temperature treatments that mimic local soil temperatures in the breeding season of the most southern (Florida; 27°C) and the most northern (Michigan; 19°C) population. We did not manipulate nutritional quality of the brood balls using discrete treatments but instead took advantage of natural variation in dung quality (see e.g., Kijimoto & Moczek, 2016; Schwab et al., 2017) resulting in enough variation in offspring body size around the inflection point (see below) to help resolve possible population differences therein. We used F1 offspring as opposed to later generations because European populations do not produce a second filial generation under laboratory conditions (Casasa & Moczek, 2018). To keep the number of generations spent under laboratory conditions constant across all populations we therefore only used offspring of wild-caught individuals. Upon eclosion, we measured pronotum width as a standard measure of size in onthophagids (c.f. Rohner, 2021). Because horns are sex-specific in *O. taurus*, we restricted our analysis here to males only. Horn length was estimated as described in Rohner et al. (2020).

Statistical analysis

To test whether populations and temperature treatments explained variation in horn length independent of body size, we used ANOVAs with population and temperature as fixed effects. We repeated this analysis by using morph as a binary variable as a function of population and temperature in a generalized linear model with binomial error distribution (*glm* function as implemented the R-package “stats” (RCoreTeam, 2020)). To test for clinal variation in horn length in the exotic range, we used linear mixed models to fit horn length as a function of latitude (as a continuous variable) and temperature using population as random effect. We used generalized linear mixed models with binomial error structure (*glmer* as implemented in “lme4” (Bates et al., 2015)) to test for latitude and population effects on morph frequency using population as random effect. As estimates of effect size, we computed partial eta square for linear models. For mixed models, we computed partial R^2 values (following Stoffel et al., 2021). Partial R^2 for main effects were estimated using models excluding interactions. The variance explained by interactions was estimated in separate models (see Stoffel et al., 2021).

Plastic and genetic variation in the sigmoidal horn length allometry was assessed using four-parameter log-logistic models as implemented in the functions *drm* and *nls* (as implemented in the R-packages “drc” (Ritz et al., 2015) and “stats”

(RCoreTeam, 2020), respectively). These models contain separate parameters for the curvature (b), a lower (c) and an upper (d) asymptote, as well as an inflection point (e). To test whether there are any population or temperature differences

in the shape of the horn length allometry, we compared seven different models (see [Supplementary Table S2](#)). The first model only included one single sigmoidal curve (i.e., a common allometry across populations and temperatures), whereas the

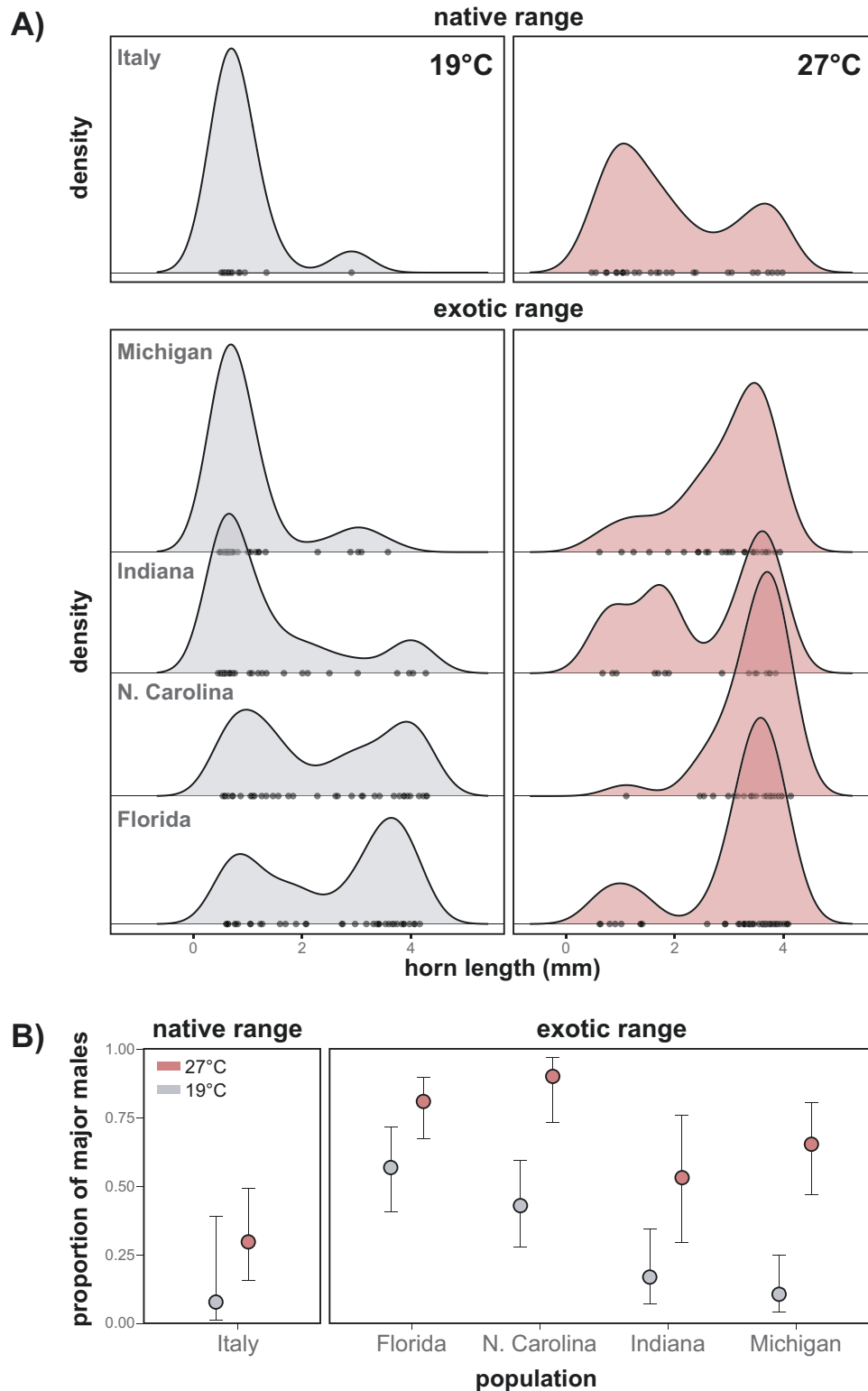


Figure 1. Horn length shows plastic and genetic differences in its distribution. Panel A shows shifts in the bimodal distribution across populations and temperatures. Panel B shows the proportion of major males as a function of population and temperature treatments. Populations in the exotic range are ordered by latitude. Binomial 95% confidence limits for the proportion of major males was calculated based on the logistic parameterization of the observed proportion using the R-package *binom* (Dorai-Raj 2014).

second and third model included separate curves for each population (G×E interaction) or temperature (E×E interaction), respectively. The fourth model allowed temperature effects on the allometric relationship to vary across populations (G×E×E interaction). Because most differences were related to the inflection point of the sigmoid (i.e., the e parameter), we fitted three additional models where we only allowed e to vary. Models 5 and 6 allowed inflection points to vary across populations (G×E) or temperatures (E×E), respectively. The last model allowed the effect of temperature on the inflection point to vary across populations (G×E×E interaction for the inflection point only). Models were compared using Akaike's Information Criterion (AIC).

To test how inflection points vary with latitude in the exotic range, we fitted a four-parameter log-logistic model that allowed inflection points to vary across latitude and temperatures, using population as a random effect as implemented in *nlme* (Pinheiro et al., 2022). We used latitude as an “ordered factor” and used linear contrasts to test whether the inflection point e increases systematically from the southern to the northern populations. To test whether thermal plasticity differed across populations, we compared the above model to one that included additive effects of latitude and temperature (i.e., a separate G×E and E×E for e) using AIC.

Results

Our common garden experiment revealed that horn length varied across populations ($F_{4,291} = 19.49$, $p < .001$, partial eta squared $[\eta_p^2] = 0.21$) and temperatures ($F_{1,291} = 80.68$, $p < .001$, $\eta_p^2 = 0.22$) and showed a temperature-by-population interaction ($F_{4,291} = 3.27$, $p = .012$, $\eta_p^2 = 0.04$, see Figure 1A). In the exotic range, average horn length decreased toward northern latitudes ($\chi^2_{(1)} = 4.82$, $p = .028$, semi-partial $R^2 = 0.11$) and lower temperatures ($\chi^2_{(1)} = 72.25$, $p < .001$, semi-partial $R^2 = 0.15$, Figure 1B, Supplementary Table S3). The horn length decrease with latitude was stronger at low temperatures (latitude-by-temperature interaction: $\chi^2_{(1)} = 12.50$, $p < .001$, semi-partial $R^2 = .01$, Figure 1B). This resulted in Florida beetles mostly developing into major males when reared at the local temperature (27°C) while beetles from Michigan predominantly developed into minor males when reared at 19°C. These models were largely corroborated when using morph as a binary variable (Figure 1). Taken together, these findings suggest that the proportion of major and minor morphs differs systematically along the invasive range.

To test for differences in the horn length polyphenism between populations and temperatures, we compared several sigmoidal models that differed in the number of parameters. Including separate sigmoid curves for temperatures and population greatly increased model fit compared to a model assuming a common allometry (Figure 2A and B; Supplementary Tables S2 and S4). AICs further decreased when allowing the effect of temperature to vary across populations. Because most of these differences were associated with a shift in the inflection point, we fitted additional models that allowed only e to vary across temperatures and populations while keeping all other parameters fixed. The model that allowed for population differences in the thermal plasticity of the threshold (G×E×E) had the lowest AIC (see Supplementary Table S2), indicating that allometric plasticity evolves across populations (Figure 2C).

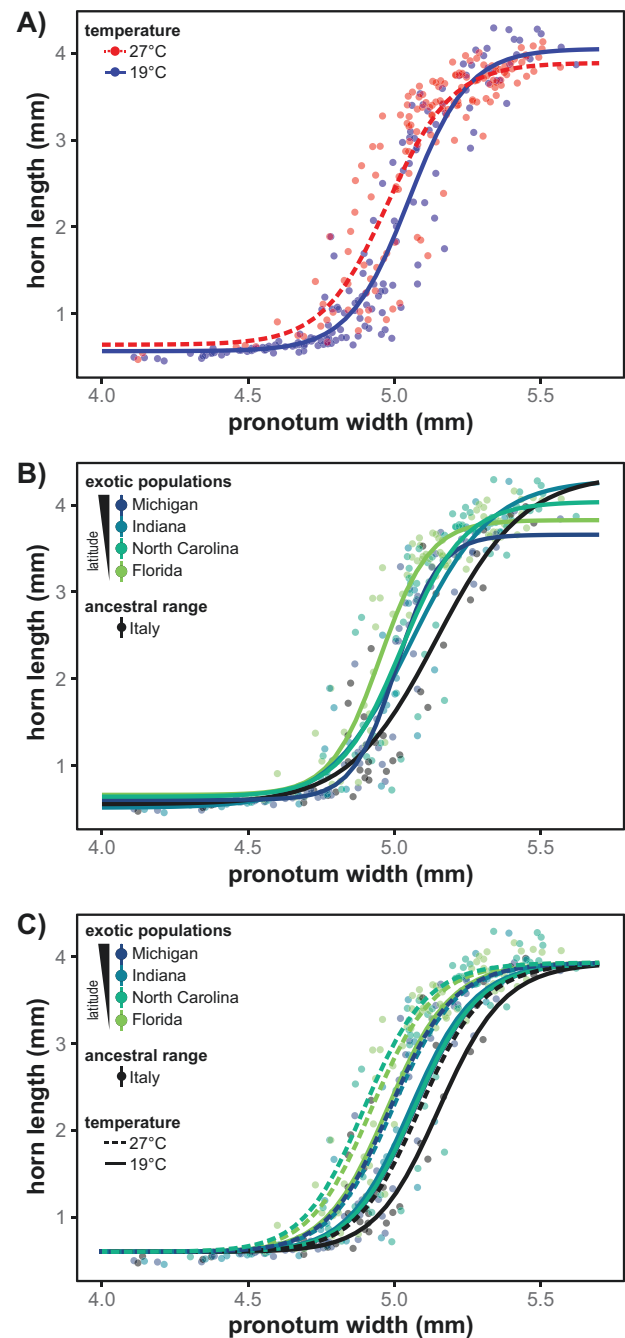


Figure 2. Allometric plasticity and population differentiation in the horn length polyphenism of *O. taurus*. Panel A shows the average allometric plasticity to temperature across populations (AIC = 363.1), while B shows population difference across temperatures (AIC = 339.4). Panel C shows the best fitting four-parameter log-logistic model that allowed the threshold to vary across populations and temperatures simultaneously (G×E×E, AIC = 269.5). AIC = Akaike's Information Criterion.

To test for latitudinal variation in the inflection point, we also fitted models that included latitude and temperature as fixed effects. Allowing thermal plasticity to vary across populations improved the fit of the model ($\Delta\text{AIC} = 12.9$), indicating the evolution of G×E×E interactions. Fitting separate effects for latitude indicated linear increases in inflection point with latitude when reared at 19°C ($t_{1,247} = 3.77$, $p < .001$, Figure 3) but no such trend at 27°C ($t_{1,247} = 0.64$, $p = .524$, Figure 3). Although significant, this latitudinal pattern was heavily influenced by a low threshold in Florida, the most southern population.

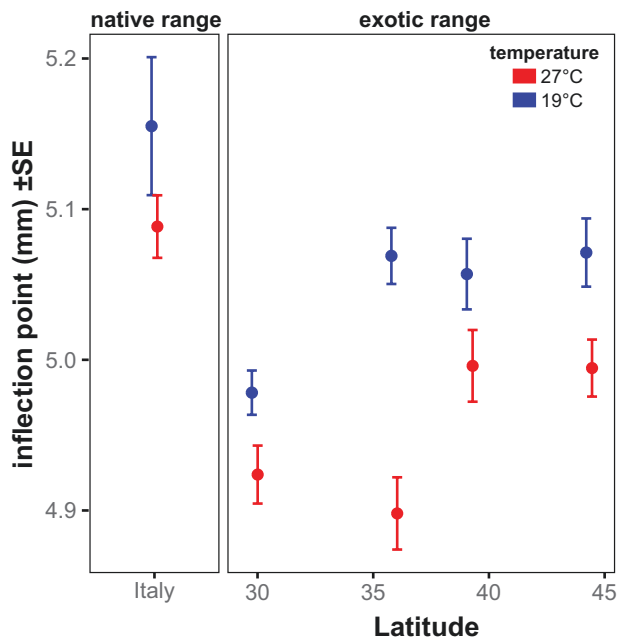


Figure 3. Inflection points (i.e., the body size threshold separating minor from major males, defined as the body size at which a tangent to the sigmoidal curve exhibits maximum slope) and associated standard errors (SE) of the best fit model (four-parameter log-logistic model with separate inflection points for each temperature and population). In the exotic range, inflection points increase with latitude when animals are reared at 19°C.

Discussion

Nutritional scaling relationships are a major contributor to phenotypic variation. Here, we show that the nutritional polyphenism in dung beetle horn length is itself dependent on temperature and that this allometric plasticity differs across populations, indicating G×E×E interactions. The genetic decrease in threshold position in low-latitude populations in the exotic range mirrors the plastic response to high temperatures, indicating that allometric plasticity aligns with threshold evolution. However, the observed patterns were opposite to our expectations under SDS (Hunt & Simmons, 2001, see below), the main model proposed to explain threshold evolution in horn polyphenic beetles. We discuss alternative scenarios driven by selection for dispersal, spatial sorting, and the developmental basis of horn length determination. Lastly, and irrespective of whether allometric plasticity is adaptive or not, our findings highlight the significance of E×E interactions in shaping evolutionary responses on ecological time scales following populations' encounter with novel environments.

Allometric plasticity and its evolution in novel environments

We show that the nutrition-sensitive threshold separating alternate male beetle morphs is temperature dependent. Low temperatures led to an increase in the threshold, and because low temperatures *also* decrease body size, this synergistically leads to a larger proportion of minor morphs in all populations that we investigated (Figure 1). This shift in the inflection point is comparable in magnitude to evolved differences documented here (see Figure 2B) and in previous studies (c.f., Macagno et al., 2021; Moczek & Nijhout, 2003; Rohner et al., 2020). Allometric plasticity can thus

have large effects and can confound population differences across populations that differ in the local thermal regime. Furthermore, the extent to which allometries respond to temperature may itself differ across populations. Here, this was mostly driven by a much stronger threshold decrease in the North Carolina population compared to all others. We suspect that such G×E×E interactions are also likely common in nature, yet may typically escape conventional experimental designs, despite their possibly profound implications for predicting populations' evolutionary responses, including for instance the likelihood of population persistence following introduction events (Davidson et al., 2011).

Low population density and SDS at range edge

The SDS model predicts that selection should favor genotypes that switch from minor to major morphologies at the body size at which the fitness functions of hornless sneaking and horned fighting tactics intersect (Hunt & Simmons, 2001). By extension, the same model predicts that the resulting threshold body size should track changes in population mean body size. Such tracking could be achieved either via allometric plasticity (as proposed by Emlen (1997) for diet) or genetic differentiation among local populations (Moczek, 2003). Although we found both plastic as well as genetic differences in the threshold, these patterns were in *opposite* directions to what we expected. In fact, when rearing the Florida population under local soil temperatures in the breeding season (27°C), most individuals emerged as major males (proportion of major males: 0.81 [0.67, 0.90] 95% CI). In contrast, almost all Michigan individuals developed into hornless minor males when developing at 19°C (proportion of major males: 0.11 [0.04, 0.25]). Although we lack adequate field sampling across the entire range, our common garden study suggests that the frequency of the two morphs changes drastically, and systematically, with latitude, in a manner that conflicts with the predictions from SDS models.

Likewise, the observed increase in threshold with latitude is also inconsistent with the hypothesis that threshold evolution is driven by population density and intraspecific competition. Moczek (2003) showed that threshold differences between North American and Australian populations can be explained by intraspecific competition. Under such a scenario, we would expect low-density populations that are presumably more common at high latitudes (i.e., the range edge) to evolve reduced thresholds—a prediction inconsistent with our data. Thermal plasticity and latitudinal population differentiation in the position of the threshold thus neither fits the intraspecific competition (density) hypothesis, nor the predictions based on SDS.

Spatial sorting and selection on increased dispersal in the cold

Invasions are expected to be under a strong influence of drift and dispersal. Especially spatial sorting, i.e., the uneven distribution of genotypes with high dispersal capacity along range expansion fronts (Shine et al., 2011) possibly in combination with allele surfing (Klopfstein et al., 2006), may influence latitudinal differentiation (Dudaniec et al., 2022). If increased dispersal capacity trades off with investment into secondary sexual traits, range sorting could indirectly shape the distribution of male morphs in *O. taurus*. However, evidence for a trade-off between horn development and dispersal are mixed. On one hand, males with relatively larger horns

have been shown to also develop larger wings. Such secondary sexual trait compensation suggests that horn development inflicts aerodynamic costs which may be compensated for by adjusting wing size (Hunt et al., 1999; Rohner et al., 2020). However, the aerodynamic costs of horns remain elusive. In the much larger Rhinoceros beetle, elaborate horns only incur marginal costs to flight (McCullough & Tobalske, 2013).

There may also be a general difference in dispersal capability among minor and major males. These could be driven by differences in the physiological costs of flight per se, or fitness costs associated with dispersal (e.g., morph differences in opportunity costs; e.g., Bonte et al., 2012). If there is a genetic correlation between the body size threshold separating the two morphs and dispersal capacity, spatial sorting might secondarily lead to a shift in morph frequency. We previously documented an increase in the relative wing size in northern populations, which is expected to provide increased dispersal capacity in cool habitats (Dudley, 2002; Neve & Hall, 2016; Rohner et al., 2018). However, whether this phenotypic correlation is due to a shared genetic basis (or linkage) requires further investigation.

Another alternative explanation could be that exotic North American *O. taurus* populations—due to having been introduced only 80–100 generations ago—are still in the process of adapting to a new location-specific size threshold optimum. North American populations possess consistently lower thresholds compared to populations in the ancestral range (Figure 2; also see: Rohner et al., 2020). This may suggest that selection favors low thresholds throughout North America, with most local populations currently adapting to this new optimum. Because selection may be more effective and have had more time to lead to morphological shifts in the older and putatively larger populations in the South, this could also explain the clinal variation observed. However, the potential selective drivers that could favor lower thresholds in Southern North America remain elusive. Future work, including the estimation of geographic variation in morph-specific fitness functions, will be necessary to test these hypotheses.

Developmental Mechanisms Underpinning Allometric Plasticity

Alternatively, the findings reported here may at least in part also be the result of the nature of organ size determination during insect development. During the larval stages of holometabolous insects, future adult appendages develop increasingly semi-autonomously from the remainder of the body (Klingenberg & Nijhout, 1998). In polyphenic insects, the decision to grow caste- or morph-specific features is frequently made at developmental time points when the body is still adding mass (Nijhout, 1994). Environmental conditions that affect this post-decision mass gain, such as temperature, then have the potential to affect body size—trait scaling (Moczek, 2002). In horned dung beetles, the decision to induce or not to induce horn growth is likely made several days before male larvae cease feeding, and larval mass during the second half of the final instar can be used as an effective predictor of adult morphology (Moczek & Nijhout, 2002, see Supplementary Figure S1). However, colder temperatures extend the duration of the feeding stage substantially (Rohner & Moczek, 2020). While colder temperatures on average cause *O. taurus* to mature to smaller adult body sizes, it is conceivable that in those individuals that manage to just reach the critical size needed for horn

induction this extension of the feeding stage leads to a relatively larger mass gain compared to individuals who have reached the same critical size, but whose feeding period ends earlier due to warm conditions (see Supplementary Figure S1). Assuming that this critical size is largely unaffected by temperature, this would result in individuals reared in colder temperatures to develop relatively large body sizes for the same amount of horn growth, thereby shifting the allometric inflection point to larger body sizes. Future integrative work will be necessary to uncover the developmental mechanisms responsible for allometric plasticity in response to temperature.

Conclusions

Our study highlights that nutritional scaling relationships are themselves environment-sensitive and that the resulting ExE interactions may themselves evolve when species encounter novel environments. Interestingly, the plastic shift in the location of the size threshold separating minor from major males documented here is similar in magnitude to evolved differences among populations. This indicates that ExE interactions can be as strong as microevolutionary divergence or genotype-by-environment interactions. ExE interactions may thus constitute a frequently overlooked but considerable contributor to phenotypic variation in natural environments. Future research will be necessary to investigate the ultimate drivers of allometric plasticity and genetic differentiation therein. More generally, our data highlight that understanding how organisms respond to environmental challenges requires a better understanding of how complex environments shape developmental systems.

Supplementary material

Supplementary material is available online at *Evolution* (<https://academic.oup.com/evolut/article/77/3/682/6965883>)

Data availability

Data and code are accessible on dryad: <https://datadryad.org/stash/share/kKVmkgoLugxm6-1KjBYrz3DfQM23ESYjMK-Bxu87hgT0>

Conflict of interest: The authors declare no conflict of interest.

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