

Research

Adaptive maternal behavioral plasticity and developmental programming mitigate the transgenerational effects of temperature in dung beetles

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Phenotypic plasticity allows organisms to cope with rapid environmental change. Yet exactly when during ontogeny plastic responses are elicited, whether plastic responses produced in one generation influence phenotypic variation and fitness in subsequent generations, and the role of plasticity in shaping population divergences, remains overall poorly understood. Here, we use the dung beetle *Onthophagus taurus* to assess plastic responses to temperature at several life stages bridging three generations and compare these responses across three recently diverged populations. We find that beetles reared at hotter temperatures grow less than those reared at mild temperatures, and that this attenuated growth has transgenerational consequences by reducing offspring size and survival in subsequent generations. However, we also find evidence that plasticity may mitigate these consequences in two ways: 1) mothers modify the temperature of their offspring's developmental environment via behavioral plasticity and 2) in one population, offspring exhibit accelerated growth when exposed to hot temperatures during very early development ('developmental programming'). Lastly, our study reveals that offspring responses to temperature diverged among populations in fewer than 100 generations, possibly in response to range-specific changes in climatic or social conditions.

Keywords: developmental plasticity, compensatory growth, maternal effects, niche construction

Introduction

Understanding how organisms respond to environmental challenges is critical in order to forecast the impacts of a globally changing climate on populations within their native range, as well as to accurately project the potential outcomes of biological invasions. Both natural selection and phenotypic plasticity can provide adaptive population-level responses to contend with novel environmental conditions: the former by exploiting standing genetic variation and the latter by relying on the ability of individuals to adjust aspects of their development (Snell-Rood et al. 2010, Gilbert et al. 2015).



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While originally considered disparate avenues to maintain high fitness, recent theoretical and empirical work increasingly suggests a close connection between the two mechanisms: while phenotypic plasticity affords individuals some measure of immediate ecological adjustment in the face of a novel challenge, it may at the same time facilitate subsequent longer term evolutionary adaptation via genetic accommodation and assimilation (Wcislo 1989, West-Eberhard 2005, Pfennig et al. 2010, but see Ghilambor et al. 2015).

At the same time, adaptive phenotypic plasticity is itself a highly heterogeneous phenomenon. Not only may plastic responses involve diverse morphological, physiological, or behavioral processes within individuals, they may also act across life stages (Stillwell and Fox 2005) and generations (reviewed by Schlichting and Pigliucci 1998, Nijhout 2003a, West-Eberhard 2005, Uller 2008, Danchin et al. 2011, Bonduriansky et al. 2012, Ledón-Rettig 2013, Gilbert et al. 2015, Donelson et al. 2017). For example, plasticity in parental investment has the potential to adjust the environmental circumstances faced by developing offspring (Moczek 1999), or improve the ability of offspring to deal with novel, challenging conditions (Buzatto et al. 2012) or both (Uller 2008, Burgess and Marshall 2011). Plasticity therefore has great potential to facilitate both short-term ecological adjustments and long-term evolutionary adaptation to novel environments. Yet few study systems exist that allow simultaneous assessment of the role of phenotypic plasticity in short-term adaptive responses, its potential effects on phenotypes and fitness in subsequent generations, and its contribution to population divergences (Pfennig et al. 2010, Donelson et al. 2017, Casasa and Moczek 2018). In this study, we investigated the adaptive significance of both maternal behavioral plasticity and larval developmental plasticity in mitigating the fitness-reducing effects of temperature stress among rapidly diverging populations of dung beetles.

Scarabaeid dung beetles depend on animal excrement to support both their larval and adult development. Constituting one of the most successful radiations of insects, these beetles can be found on all continents save Antarctica, utilizing diverse dung types from ungulate and marsupial herbivores to carnivores and primates (Matthews 1972, Hanski and Cambefort 1991). Dung beetles provide critical ecosystem services in both natural and agricultural habitats (e.g. through nutrient cycling and parasite suppression; Nichols et al. 2008) via three principal reproductive strategies: ‘dwelling’ dung beetles lay eggs within dung pads; ‘rollers’ fragment, roll away and bury dung balls some distance away from the original pad; and ‘tunnelers’ dig tunnels underneath pads and construct breeding chambers with dung provisions that are consumed by their larvae (‘brood balls’, containing a single egg each; Halffter and Edmonds 1982). In this study, we focused on *Onthophagus taurus*, a tunneling species whose offspring size and fitness are critically affected by maternal investment (Moczek and Emlen 1999, Snell-Rood et al. 2016). Native to the Mediterranean, this species expanded its distribution range during the 1970s to several locations either accidentally (e.g. eastern United States; Fincher and Woodruff 1975)

or as part of biocontrol programs (e.g. Western Australia; Tyndale-Biscoe 1996). Intriguingly, in less than 50 years (fewer than 100 generations), non-native *O. taurus* populations have undergone varying degrees of climatic niche evolution (Silva et al. 2016) and have diverged in morphological (Moczek and Nijhout 2002, Pizzo et al. 2008, Macagno et al. 2011, 2016), physiological (Macagno et al. 2015), and behavioral traits (Macagno et al. 2016) to an extent normally only observed among true species.

In this study, we investigate the roles of behavioral and developmental plasticity in adaptation and population-level divergence among populations of *O. taurus* in response to a common ecological stressor, temperature. We exposed *O. taurus* parents to experimentally-controlled mild and hot breeding conditions to determine: 1) whether mothers exhibited behavioral plasticity in response to thermal variation by altering the depth at which they buried their brood balls, and therefore the thermal environment of their offspring; 2) whether offspring themselves exhibited developmental plasticity in response to the thermal environment to which they were exposed during early development (i.e. ‘developmental programming’); 3) whether either type of plasticity was adaptive; 4) whether any developmental responses occurring in one generation affected fitness-related traits in subsequent generations; and 5) whether populations have diverged in the degree and nature of their plastic responses to thermal conditions. We discuss the implications of our results in light of the evolvability of and constraints on plastic responses to novel temperature regimes.

Material and methods

Animal collection and rearing

Onthophagus taurus beetles were collected from cow pastures at similar latitudes (37.2°, 36.0° and -33.7°; Supplementary material Appendix 1) in the two hemispheres: a) Seville, Spain (SP), a population within the native range for the species (Balthasar 1963); b) Chapel Hill, North Carolina, USA (NC), a non-native population resulting from an accidental introduction to the eastern US ~50 years ago (Fincher and Woodruff 1975); and c) Busselton, in the southwestern region of Australia (WA), a non-native population resulting from an intentional introduction ~50 years ago (Tyndale-Biscoe 1996). All beetles were brought to Bloomington, IN, and reared at 24°C in separate colonies, as in (Macagno et al. 2016). NC and SP colonies contained beetles collected in the spring of 2016 and one generation of their lab-generated offspring. The WA colony included only lab-generated individuals derived from beetles collected in the field in December 2014 and 2015; using lab-generated animals allowed us to compare this Southern Hemisphere population to Northern Hemisphere populations during a time when they would otherwise be experiencing diapause (i.e. during the Southern Hemisphere winter). Experimental breeding started in August 2016 to allow sufficient time for acclimation (Macagno et al. 2016).

Experimental design

Phase 1. Effects of ovipositing and rearing temperature on maternal behavior and offspring fitness

We randomly selected 18 males and 18 females from each population, measured their thoracic width as a proxy for body size using calipers, haphazardly paired females and males, and placed each couple in a cylindrical, light-impermeable ovipositing container filled to a height of 21 cm with sterilized soil (Beckers et al. 2015, Macagno et al. 2015). After adding 200 g of homogenized cow dung, we covered these containers with window screen and perforated black plastic foil (Beckers et al. 2015, Macagno et al. 2015).

For each population, nine ovipositing containers were incubated at 24°C ('Mild' treatment; top $23.7 \pm 0.4^\circ\text{C}$, bottom $24.1 \pm 0.5^\circ\text{C}$), while nine were placed under two 250 W heating lamps ('Hot' treatment), thereby generating a temperature gradient from the top ($32.4 \pm 2.6^\circ\text{C}$) to the bottom ($27.3 \pm 1.2^\circ\text{C}$) of the containers (Fig. 1). The 'Mild' and 'Hot' surface temperatures of containers mirror the mean (NC: 25°C; SP: 24°C; WA: 22°C) and maximum (NC: 32°C; SP: 29°C; WA: 27°C) temperatures experienced by *O. taurus* in nature (Supplementary material Appendix 1; Fick and Hijmans 2017). Temperatures were measured every 30 mins with iButton loggers placed at the top and bottom of two containers per treatment per population. Containers in the hot treatment were shuffled once a day to ensure even exposure to heat. After two days, we replaced the dung with fresh dung. After five days, we separated the soil in the ovipositing containers into three 7-cm layers (Top, Middle and Bottom), and retrieved and weighed all brood balls; thus, early development for these offspring spanned their time as unfertilized eggs within the mother during oviposition to their time as an embryo or very young larva outside the mother up to five days. Due to constraints on incubator space, phase 1 was carried out in two rounds spaced one week apart. Round 1.1 included nine replicates per treatment from SP and WA; round 1.2 included nine replicates per treatment from NC and WA. The WA brood balls harvested in round 1.2 were counted and weighed, but not incubated as described below.

In our design, the mild and hot laying environments were not orthogonally manipulated across temperature and layer depth. However, we demonstrated the absence of a gradient effect by performing an additional experiment using Western Australian beetles only, orthogonally crossing temperature and gradient treatments (i.e. we included a Hot gradient, Mild gradient, Hot constant, and Mild constant treatment), showing that gradient per se did not drive behavioral differences. The experimental conditions were otherwise identical to those described for our original experiment, and the results are described in detail in Supplementary material Appendix 2.

Brood balls from all populations and ovipositing temperatures were placed individually in 1-oz polystyrene cups (3 cm in height) filled with sterile soil and covered with a lid.

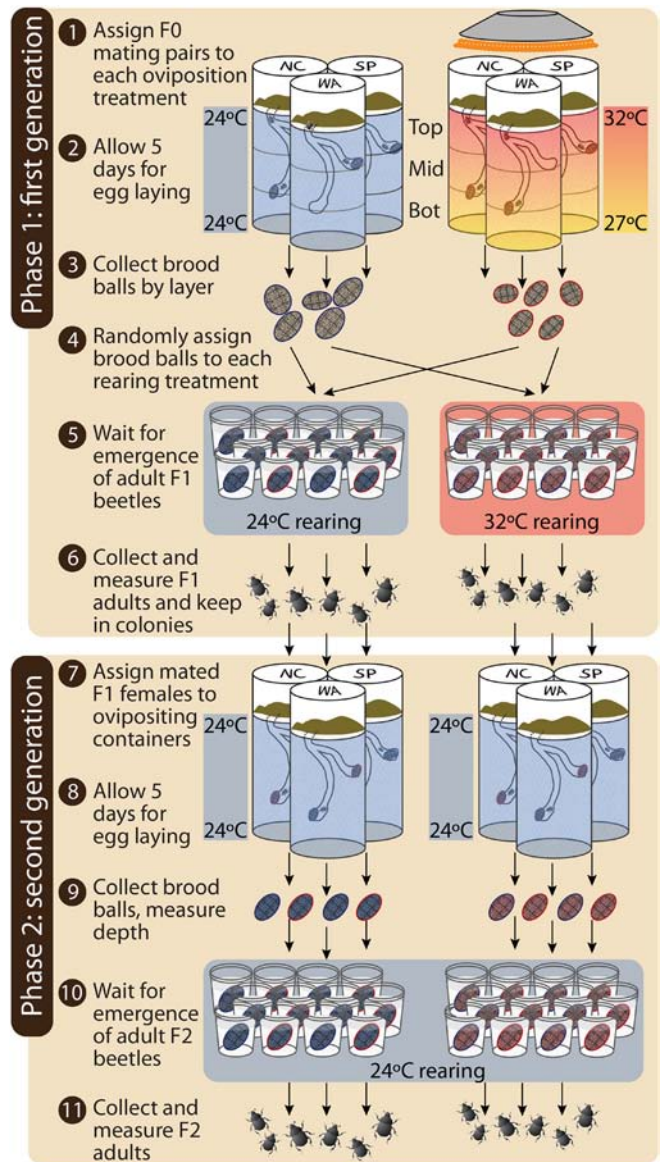


Figure 1. Experimental design. Phase 1 assessed maternal behavioral plasticity and offspring responses to variation in temperature. Phase 2 assessed transgenerational effects of variation in maternal temperature.

Brood balls from each container were evenly assigned to each of two incubation temperatures (24°C or 32°C), and checked every day for adult emergence (Fig. 1). Newly-emerged F₁ adult beetles were promptly removed, measured (thoracic width), and transferred to new colonies divided by population and rearing temperature; thus, late development for these offspring spanned their time as young larvae until eclosion. Beetles were maintained in these colonies at 24°C with a light:dark cycle of 16:8 h, and fed ad libitum for at least three weeks before being used for phase 2. Any brood balls not yielding an adult beetle after seven weeks were opened and inspected; in all cases the developing individual had perished.

Phase 2. Transgenerational effects of parental rearing temperature

F₁ females and males from phase 1 were kept in maturation colonies with individuals of the same rearing treatment (e.g. one maturation colony contained Western Australian offspring reared at 32°C) and were allowed to mate freely. Female *Onthophagus* are capable of sperm storage, therefore it was not necessary to include males in the phase 2 oviposition containers. Mature, phase 1 F₁ females from all populations and rearing temperatures were transferred to ovipositing containers as described above, except that all were kept at 24°C. After five days, we disassembled the containers, weighed all brood balls, and measured brood ball burial depth with a ruler. We then incubated brood balls individually at 24°C, and measured thoracic widths of emerging F₂ adults. Data were collected during two rounds spaced one week apart. Round 2.1 included five replicates from SP, WA and NC; round 2.2 included five replicates from SP and WA, and three from NC.

Statistical analyses

Statistical analyses were performed using R (< www.r-project.org >). A more detailed description of the statistical tests used is reported in Supplementary material Appendix 3 and final models as well as likelihood ratio tests are reported in Supplementary material Appendix 4. Generalized linear models (GLMs) were used to model variables where the response variable included measurements from all offspring laid within an ovipositing container (proportion data). Where multiple measures from a container were considered, mixed models were used (GLMMs) to incorporate a random effect associated with replicate containers (package 'lme4'; Bates et al. 2015). By including replicate container – and by extension the mother within – as a random effect, these multiple measures did not result in pseudoreplication (Bolker et al. 2009). The numbers of brood balls used across populations and treatments are provided in Supplementary material Appendix 5 Table A1. Models were simplified by removing non-significant explanatory variables as determined by χ^2 -tests or by F-tests when data were overdispersed (Bolker et al. 2009). Significant differences between specific contrasts in the final mixed models were determined using post hoc Tukey's honest significant difference tests (package 'lsmeans'; Lenth 2016).

Phase 1. Maternal behavioral plasticity and offspring responses

To assess maternal burial behaviors, we determined the proportion of brood balls buried within layers of the oviposition containers using GLMs for each layer (quasibinomial error, logit link). Fixed variables were oviposition temperature, population and their interactions, plus maternal size as a covariate. We used GLMMs to assess offspring days to emergence (Poisson error, log link) and thoracic width (normal error, identity link), with oviposition temperature, rearing temperature, population, brood ball weight and their interactions, plus the offspring individual's sex as fixed variables

and the replicate breeding container as a random effect. For offspring survival, we used a GLM (binomial error, logit link) with explanatory variables oviposition temperature, rearing temperature, population, maternal size and their interactions.

To characterize the effects of temperature and gradient treatment on maternal behavior in our follow-up experiment (Supplementary material Appendix 2), we used a GLMM (normal error, identity link) to assess brood ball burial depth, where container was modeled as a random effect, and explanatory variables included treatment, maternal size and experimental round.

Phase 2. Maternal behaviors and offspring responses

To characterize transgenerational effects of temperature on second-generation maternal behavior, we used GLMMs (normal error, identity link) to assess brood ball burial depth and weight; explanatory variables were maternal size, maternal rearing temperature, population and their interactions, plus experimental round as fixed effects and the replicate breeding container as a random effect. To determine whether the emergence time and growth of offspring from second-generation beetles were affected by the size of their mothers, we used GLMMs (normal error, identity link) with explanatory variables maternal rearing temperature, maternal size, brood ball weight, population and all their interactions, plus experimental round and offspring sex as fixed effects and the replicate breeding container as a random effect. Because there was a significant three-way interaction between maternal size, maternal rearing temperature, and population for offspring emergence time ($\chi^2 = 10.36$, $df = 2$, $p = 0.01$) (Supplementary material Appendix 4 Table A7) we separated the data by population to identify in what population the interaction between maternal size and maternal rearing temperature was significant for this variable. Finally, we determined offspring survival using a GLM (binomial error, logit link) with parental rearing temperature, maternal size, population and their interactions, plus experimental round as explanatory variables.

Data deposition

Data available from the Dryad Digital Repository: < <http://dx.doi.org/10.5061/dryad.g6m34mf> > (Macagno et al. 2018).

Results

Phase 1. Maternal behavioral plasticity

Higher oviposition temperature both significantly increased ($F = 12.28$, $df = 1$, $p < 0.001$) the proportion of brood balls buried in the bottom layer and significantly decreased ($F = 8.34$, $df = 1$, $p = 0.01$) the proportion of brood balls buried in the top layer across populations. Thus, our results suggest that adults bury brood balls at greater depths when surface temperatures are hotter (Fig. 2). This behavior is not the result of a temperature gradient, per se, because in

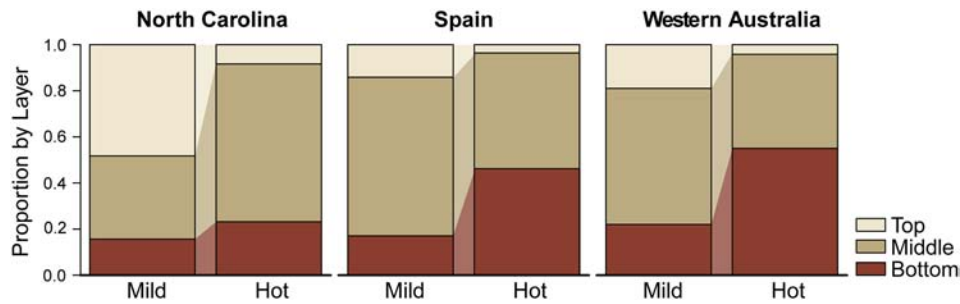


Figure 2. Maternal behavioral plasticity in brood ball burial depth. The effects of surface temperatures (mild = 24°C; hot = 32°C) on brood ball burial depth were assessed across three beetle populations. Across populations, beetles responded to warmer surface temperatures by burying their brood balls deeper.

our follow-up experiment there was a significant interaction between gradient and overall temperature ($\chi^2 = 10.14$, $df = 1$, $p < 0.001$); beetles only buried their brood balls deeper when exposed to both a gradient and hot surface temperatures (Supplementary material Appendix 2 Fig. A1). Further, these patterns are the result of behavioral plasticity as opposed to differential selection between treatments (Supplementary material Appendix 5 Fig. A6–A9); neither mortality nor brood ball production differed substantially between treatments.

Phase 1. Effects of ovipositing and rearing temperature on maternal behavior and offspring fitness

Across populations, higher rearing temperatures had a significant negative effect on days to emergence ($\chi^2 = 219.05$, $df = 1$, $p < 0.001$; Fig. 3) and offspring thoracic width ($\chi^2 = 121.12$, $df = 1$, $p < 0.001$; Fig. 3); i.e. warmer rearing temperatures resulted in faster development but smaller sizes at emergence. Unexpectedly, a post hoc test revealed that hot temperatures experienced by offspring during very early development (oviposition) also had a significant ‘positive’ effect on offspring size in WA beetles ($p < 0.001$), but not on offspring size in beetles from NC or SP.

Phase 2. Transgenerational effects of parental rearing temperature

Across populations, the size of second-generation mothers was significantly positively correlated with both the depth at which they buried their brood balls ($\chi^2 = 4.41$, $df = 1$, $p = 0.04$) and the weight of those brood balls ($\chi^2 = 43.99$, $df = 1$, $p < 0.001$; Fig. 4a), suggesting that the size of a mother – which is in part a function her own rearing temperature (Supplementary material Appendix 5 Table A2, Fig. A3) – plays a major role in her subsequent maternal behavior. Further, across populations, a hot maternal rearing temperature negatively influenced the depth at which mothers buried their brood balls ($\chi^2 = 4.29$; $df = 1$, $p = 0.04$), even after maternal size was accounted for (Supplementary material Appendix 4 Table A9).

In WA beetles, emergence times of offspring from second-generation mothers were significantly positively influenced by maternal size ($\chi^2 = 10.36$, $df = 1$, $p < 0.001$; Fig. 4b);

this relationship was not detected in the other populations. Additionally, for emergence time in WA beetles, there was a significant interaction between maternal size and brood ball weight, such that beetles derived from smaller mothers developed even faster on low investment brood balls than those derived from larger mothers ($\chi^2 = 9.41$, $df = 1$, $p < 0.001$; Supplementary material Appendix 5 Fig. A4). Across populations, thoracic widths of offspring from second-generation mothers were significantly positively influenced by maternal size ($\chi^2 = 22.88$, $df = 1$, $p < 0.001$; Fig. 4b), and additionally there was a significant interaction with brood ball weight, such that beetles derived from smaller mothers grew even less on low investment brood balls than those derived from larger mothers (Supplementary material Appendix 5 Fig. A4). Finally, across populations, maternal size had a significant positive effect on the survival of offspring from second-generation mothers ($\chi^2 = 4.86$, $p = 0.03$) (Fig. 4b). Together, these data suggest that the negative effects of hot rearing temperatures on maternal size result in reduced growth and survival in a subsequent generation, regardless of her offspring’s rearing environment.

Discussion

Phenotypic plasticity, including behavioral and developmental plasticity, is now commonly accepted as a mechanism by which individuals have the potential to respond to rapid environmental change (Charmantier et al. 2008). Indeed, a critical and outstanding question in evolutionary biology is whether and how species use phenotypic plasticity to adapt to anthropogenically-mediated environmental change, in particular, climate change (Charmantier and Gienapp 2014, Merilä and Hendry 2014, Diamond and Martin 2016). However, how behavioral and developmental plasticity interact, how they may diverge among populations, and how their effects may influence subsequent generations remains poorly understood. To address these issues, we used the dung beetle *Onthophagus taurus* to assess behavioral and developmental responses to variation in temperature at several junctures bridging three generations of animals. Further, we replicated this approach across three populations that have evolved independently for only a short period of time (fewer than

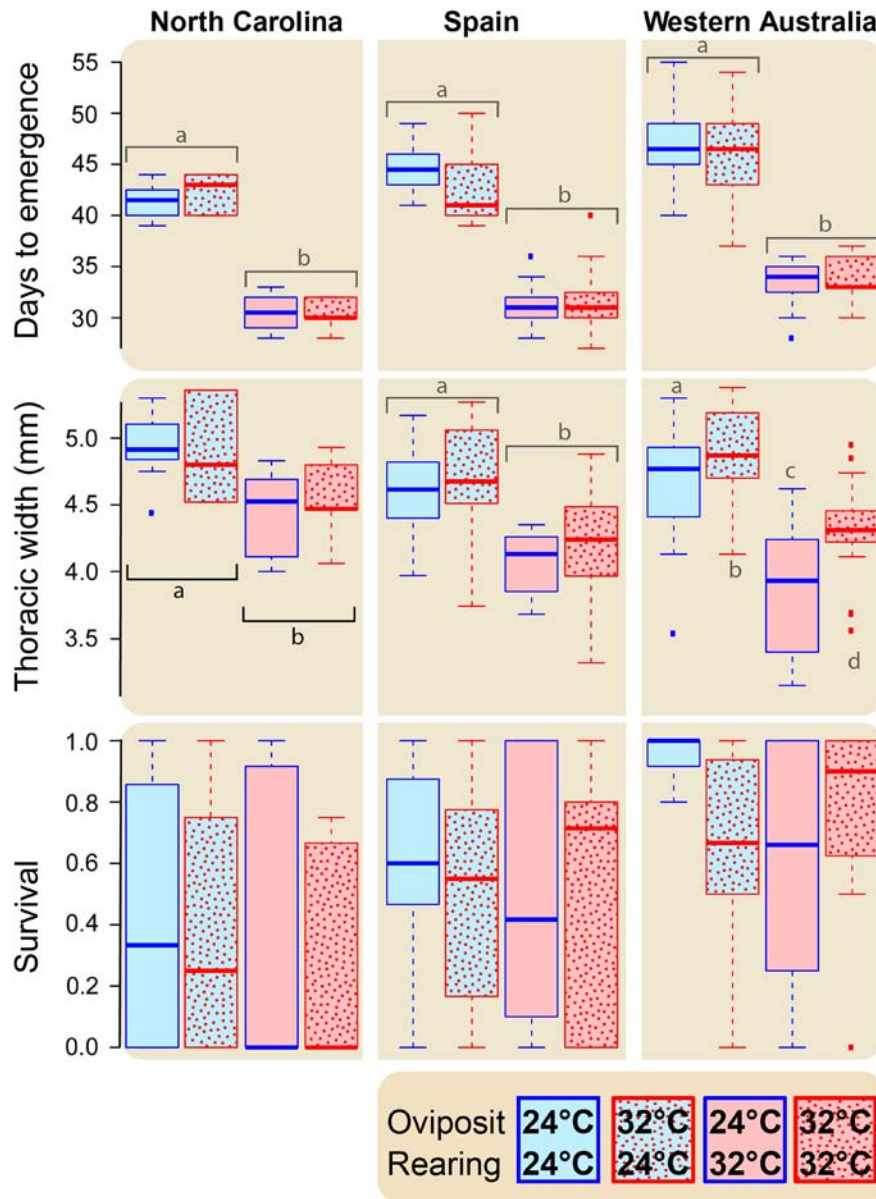


Figure 3. Offspring responses to temperature variation during development. Offspring responses varied by the temperature they experienced during early development (oviposition; blue outline: Mild; red outline and dotted pattern: Hot) and during later development (rearing; light blue background: Mild; pink background: Hot). Across populations, hot rearing conditions significantly accelerated development and reduced growth (top and middle panels; letters and brackets indicate significant differences between sets with different letters – panels with no letters showed no significant differences). Offspring growth in Western Australian beetles also depended on the temperatures experienced during oviposition; experiencing hot oviposition temperatures significantly ‘enhanced’ growth.

100 generations), yet show evidence of rapid divergence in morphological, physiological and behavioral traits (Moczek and Nijhout 2002, Pizzo et al. 2008, Macagno et al. 2011, 2015, 2016, Beckers et al. 2015).

Our study revealed – consistent with several others (Atkinson 1994, Partridge et al. 1994, Nijhout 2003b, Davidowitz and Nijhout 2004) – that variation in temperature during offspring development has a drastic effect on offspring fitness; specifically, across populations, beetles reared at high temperatures during late development grew much less

than those reared at milder temperatures. Importantly, these effects on size manifested as reduced growth in a subsequent generation, even when that generation was reared at cooler temperatures. At the same time our results demonstrated that plastic responses can ameliorate such transgenerational effects of temperature in at least two ways. First, across populations, negative effects of higher rearing temperatures could be mitigated via adaptive plasticity in maternal behavior; when surface temperatures were hot, mothers buried their brood balls deeper, thereby providing a cooler developmental

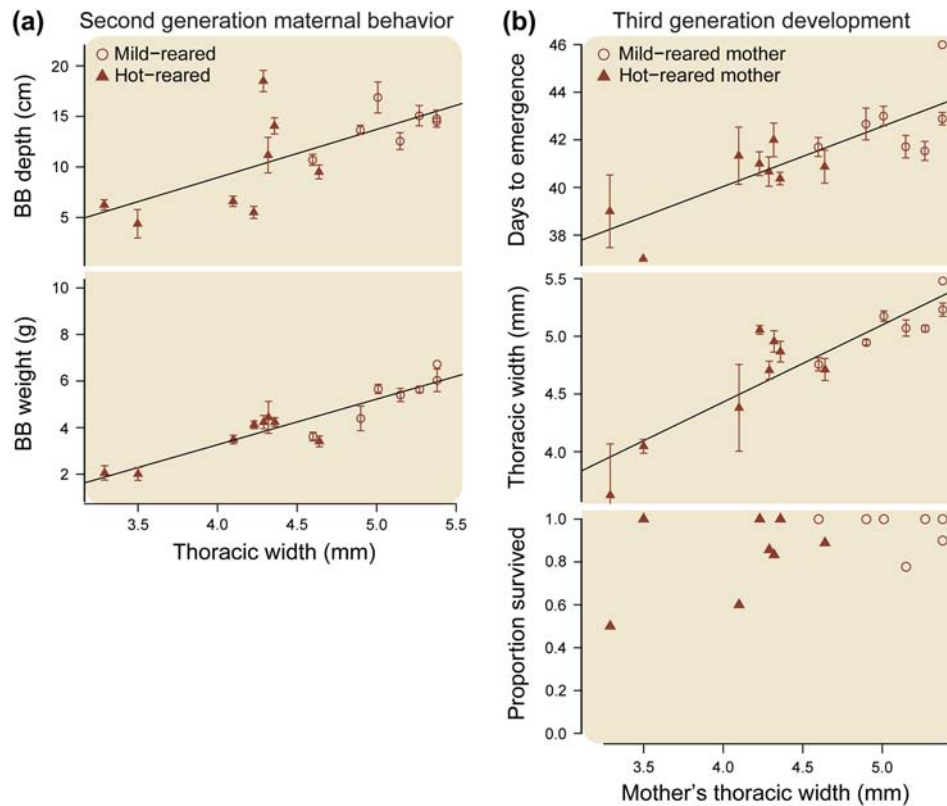


Figure 4. Transgenerational effects of temperature in Western Australian beetles. (a) Brood ball burial depth and weight vary significantly as a function of second-generation maternal size. Maternal size varies as a function of her own rearing temperature; those reared at hot temperatures (closed triangles) were smaller than those reared at mild temperatures (open circles). (b) Offspring from second-generation mothers are, in turn, influenced by the rearing conditions of their mothers; emergence time, growth and survival vary significantly as a function of maternal size. Data plotted are average measures (within oviposition containers) of (a) brood balls or (b) offspring derived from second-generation mothers. Bars represent standard error for measures within a container.

environment for their offspring. Second, in one population (Western Australia), offspring themselves responded to the temperature they experienced during early development via developmental programming. Specifically, offspring that experienced a hotter temperature during oviposition exhibited accelerated growth, regardless of what temperature they experienced during later development. Because this offspring response to early-life thermal environments was solely evident in the introduced Western Australian beetles, our study suggests that introduced populations can diverge rapidly in how they respond to thermal variation during early development.

The growth of individuals is determined by early and late thermal environments

In our study, we assessed the effects of temperature on offspring during both early and late development. Here, early development was the period spent in the ovipositing containers, as early as unfertilized eggs within the mother during oviposition and as late as an embryo or very young larva outside the mother for up to five days. Adult attributes that resulted from environmental input during this early developmental period were considered to be a manifestation of

developmental programming. In contrast, late development included the time experienced from late embryo or early larva up until emergence as an adult, thus comprising most if not all of the larval growing period.

As has been observed in other insects (Nijhout et al. 2014), hotter rearing temperatures negatively influence the growth of developing *O. taurus* larvae. Indeed, across populations, growth in beetles was significantly reduced for those reared at hotter temperatures, and this might be intrinsically related to the fact that larvae raised at hotter temperatures develop more quickly (Davidowitz and Nijhout 2004, but see Kingsolver et al. 2007). However, Western Australian beetles that experienced hotter temperatures during very early development actually grew more across both hot and mild rearing conditions. Thus, exposing parents or embryos to hot temperatures for even a short window of time may be enough to activate compensatory mechanisms in anticipation of a warm rearing environment that would, as mentioned, cause a reduction in growth.

Compensatory mechanisms are thought to be ubiquitous in nature; under certain conditions, natural selection should favor the evolution of compensatory mechanisms that allow individuals exposed to unfavorable conditions to grow

as large as possible (Metcalf and Monaghan 2001). Such mechanisms may have evolved in some *O. taurus* populations where larger females generally enjoy higher fecundity (Hunt and Simmons 2002). However, such accelerated growth can also impair other traits that contribute to fitness by interfering with resource allocation to reproduction, immunity, or physiological maintenance. The phenomenon of recovery growth negatively impacting other aspects of fitness is well-documented (Metcalf and Monaghan 2001), with examples from fish (Johnsson and Bohlin 2006, Lee et al. 2013, Ab Ghani and Merilä 2015), birds (Birkhead et al. 1999), rats (Desai and Hales 1997), and correlative studies suggest that these tradeoffs even exist in humans (Forsén et al. 1999). In our study, accelerated growth did not explain differences in survival; when reared under hot conditions, WA *O. taurus* that experienced hot early life conditions survived just as well, if not better than those that experienced mild early life conditions (Fig. 3). Future studies may determine whether this compensatory mechanism in Western Australian beetles results in some trade off with other fitness components.

Late thermal environments have transgenerational effects

Our second generation experiment demonstrates that differences in size – generated by experimental differences in temperature during late development – can be propagated across generations. Specifically, beetles reared at hotter temperatures grew to be smaller individuals, and those smaller sizes led to consequences for both the second-generation mothers and their offspring (i.e. transgenerational effects). Smaller mothers produced smaller brood balls and buried them at shallower depths. Further, across populations, offspring from smaller mothers were, themselves, smaller in size, and were less likely to survive. In all populations, the transgenerational effect of hot conditions was exacerbated under poor conditions, i.e. when mothers allocated less dung to their brood balls (Supplementary material Appendix 5 Fig. A4); after correcting for maternal size, offspring derived from mothers reared under hot conditions grew less under poor conditions (smaller brood balls) than those derived from mothers reared under mild conditions. Together, these results strongly suggest that, in the absence of any maternal intervention via behavioral plasticity, the effects of hot temperatures persist at least one generation after the environmental stressor has been removed.

What are the evolutionary implications of such transgenerational environmental effects? If small mothers are constrained to bury their brood balls at shallower depths and invest less dung into each brood ball, as our data indicate, this means that their offspring will be more susceptible to hot temperatures. In the absence of plasticity, a feedback cycle could ensue resulting in smaller and smaller beetles (Shama and Wegner 2014, Shama et al. 2014). While geographic variation in intraspecific insect sizes are often ascribed to genetic divergence (Mousseau 1997), it is possible that some of this variation is generated (or was initially generated and

later canalized) by the effect of temperature on offspring growth. Indeed, field collected Australian beetles are consistently smaller than beetles from the eastern United States (Moczek 2003), yet these differences at least partly disappear over multiple generations in captivity (Beckers et al. 2015). Although our climatic data did not suggest that Western Australian beetles experience the highest temperatures in nature relative to other populations, our data did suggest that they are most negatively affected by higher temperatures (Supplementary material Appendix 5 Fig. A3). Therefore, warm developmental environments may have contributed to their relatively smaller sizes.

Behavioral plasticity can mitigate negative transgenerational effects

In spite of the potential negative transgenerational effects of temperature on beetle size, our experiments demonstrate that all three populations have the potential to exhibit adaptive maternal plasticity that can mitigate such effects: all populations shift the distribution of their brood balls deeper when experiencing hot surface conditions. The bottoms of our ‘Hot’ experimental breeding containers (27°C) were cooler than their surface temperatures (32°C), and more similar to temperatures experienced throughout all layers in the ‘Mild’ rearing treatment (24°C, Fig. 1). Importantly, these patterns are the result of behavioral plasticity as opposed to differential selection between treatments (Supplementary material Appendix 5 Fig. A6–A9). Because offspring were larger when reared at mild temperatures, regardless of early developmental conditions, our results suggest that this maternal behavioral plasticity can mitigate the negative effects of a hot rearing temperature on offspring size, and is therefore adaptive.

Possible environmental and ecological drivers of population divergence in offspring responses

The design of our study allowed us to compare the phenotypic responses of both mothers and offspring from three populations: beetles derived from 1) Spain, part of the ancestral range of *O. taurus* (Balthasar 1963), 2) Western Australia, where the species was introduced in the 1970s by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) (Waterhouse 1974, Tyndale-Biscoe 1996) and 3) the United States (NC), where the species has spread since an accidental introduction to Florida in 1971 (Fincher and Woodruff 1975) as far north as New York and Michigan (Hoebeke and Beucke 1997, Rounds and Floate 2012), and as far west as the Mississippi (Fincher et al. 1983). Since these populations were reared and bred under common garden conditions, we can infer that differences in phenotypic responses likely reflect genetic divergence in plasticity. Furthermore, because these beetles are bivoltine, we can infer that divergence between these populations has likely occurred in fewer than 100 generations.

Maternal responses were, in fact, very similar among populations. This is not entirely surprising; while behaviors

are typically thought of as the most labile traits, behavioral responses can be highly stable in the level of their expression (Duckworth 2009) and sometimes slow to evolve (Lahti et al. 2009). In contrast to maternal behavior, we found that *O. taurus* beetles did exhibit population divergence in offspring responses to thermal variation during early development, i.e. divergence in developmental programming of compensatory growth. Aside from non-adaptive founder effects during the introduction of *O. taurus* into its exotic ranges, we present two non-mutually exclusive hypotheses that might explain why the Western Australian population differs from the North Carolinian and Spanish populations in offspring responses to thermal conditions during early development. The first hypothesis regards an abiotic factor, climate. North Carolinian beetles belong to a continuous eastern US population that has expanded its climatic range into wetter and cooler regions (Silva et al. 2016). It is therefore possible that, in the eastern US – because moisture can mitigate thermal stress for developing insects (Birch 1953) – beetle offspring experience thermal stress much less frequently, lessening selection for compensatory mechanisms during early development.

Our second hypothesis considers a biotic factor, competition. Western Australian populations of dung beetles can reach substantially higher densities than those occurring in the eastern United States or Europe (Doube et al. 1991, Moczek 2003). When dung is limited through resource competition, beetles can maximize the number of offspring they produce by minimizing the time they spend building tunnels; thus, it is plausible that competitive conditions favor burying brood balls at a shallower depth. Indeed, Western Australian *O. taurus* beetles position brood balls more superficially than eastern US and Mediterranean beetles when several individuals are forced to breed together (Macagno et al. 2016). If the benefits of burying shallow in a competitive environment outweigh the consequences of burying shallow in a hot environment, this may result in compensatory responses to thermal stress being strongly selected for in Western Australia.

Conclusions

Determining if and how organisms adapt to changes in temperature can critically inform whether biodiversity will be maintained in the face of global climate change for at least two reasons. First, organisms must reckon with increasingly warm temperatures in their native habitats (Parmesan and Yohe 2003, Root et al. 2003) or face extinction (Thomas et al. 2004). Second, the success of introduced species, which can drastically alter biodiversity (Simberloff et al. 2013), to thrive in their non-native ranges may be determined at least in part by their ability to cope with new thermal environments. Our study demonstrates that plasticity in both maternal behavior and offspring development can be adaptive and mitigate the transgenerational effects of stressfully hot temperatures in a dung beetle. At the same time, our comparison of plasticity across three recently diverged beetle populations shows that such adaptive responses can evolve in a remarkably short time

frame, and suggests that the role of plasticity in fostering population health by buffering individuals from thermal stress may depend on each population's unique ecological parameters.

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Supplementary material (available online as Appendix oik-05215 at < www.oikosjournal.org/appendix/oik-05215 >). Appendix 1–5