Title: Countergradient variation in reptiles: thermal sensitivity of developmental and metabolic rates across locally adapted populations

Contribution to: “Coping with Environmental Fluctuations: Ecological and Evolutionary Perspectives”

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Abstract

Environmental temperature is a key driver of variation in physiological developmental rates in reptiles. Cooler temperatures extend development time and increase the amount of energy required to achieve hatching success, and which can pose fitness consequences later in life. Yet, for locally-adapted populations, genetic variation can oppose environmental variation across ecological gradients, known as countergradient variation (CnGV). Not only presence, but absence of phenotypic variation that can reveal insights into the mechanisms underlying local adaptation across environmental gradients. While evidence for genetic variation opposing environmental variation in physiological rates has been summarised in other taxa, the generality of CnGV variation in reptiles is yet unknown. Here I present a summary of studies measuring developmental time and metabolic rates in locally-adapted populations across thermal clines for 15 species of reptiles across 8 families. CnGV in developmental time is found to be common, while no clear pattern emerges for the thermal sensitivity of metabolic rates across locally-adapted populations. CnGV in developmental time may be an adaptive response in order to decrease the costly development in cool climates, however empirical work is needed to disentangle plastic from genetic responses, and to uncover potentially general mechanisms of local thermal adaptation in reptiles.

Introduction

Thermal regimes often vary considerably across spatio-temporal gradients, yet similar developmental phenotypes can be maintained when genetic variation opposes environmentally-induced variation (Levins, 1969; Conover and Schultz, 1995). Biologists have long sought to understand sources of phenotypic variation along thermal gradients, such as genotype-environment co-gradient variation (CoGV) that occur when genotypes non-randomly and positively affect phenotypes across environments (**Box 1**). Yet geographic variation in genotypes can also oppose environmental effects, thereby reducing, or masking observable phenotypic variation across a species thermal range (Taylor et al., 2015). This form of countergradient variation (CnGV) in thermally-sensitive traits such as physiological rates is important because it likely reflects an adaptive response, whereby selection acts to reduce phenotypic variance across environmental gradients in response to local selection regimes. Thus, investigating patterns of phenotypic uniformity in nature, rather than just phenotypic variability, can help us to understand potentially general mechanisms underlying local adaptation.

There are many instances of both co- and counter- gradient variation among populations spanning a range of taxa, where natural selection drives variation across thermal gradients, such as altitudinal and latitudinal clines (Conover et al., 2009). Physiological rates often show countergradient variation: in a review by Conover et al., (2009), 87% of the studies showing evidence for CnGV were for measures of growth and developmental rates, while evidence for CoGV in physiological rates was found to be comparatively rare (Kelly, 2019). It is unclear why CnGV in the thermal sensitivity of physiological traits is so prevalent, however it may be due to relatively lower genetic constraints in physiological (compared with for example, morphological traits which generally show CoGV (Li et al., 2011)). Temperature poses a strong influence on physiological rates underlying energy acquisition and utilisation in ectotherms that often misalign with the direction of selection. For example, an acute decrease in environmental temperature increases developmental time, yet cold climates often select for faster development so that embryos can complete development and commence feeding and growth before the onset of winter (Edge et al., 2017). CnGV can enable populations to compensate for the direct effects of temperature on physiological rates, to ensure persistence of populations under extreme climactic regimes (Angilletta Jr, 2009; Conover et al., 2009).

In egg laying species, temperature experienced during embryonic development can impart significant fitness consequences, either through hatching survival or effects imparted later in life, for example reductions in size at hatching, growth rates and reproductive success (Warner et al., 2010; Andrews and Schwarzkopf, 2012; DuRant et al., 2013; Ospina et al., 2018). Low temperatures can affect key physiological rates during development, including increasing time from fertilisation to hatching (developmental time) and decreasing rates of energy expenditure (metabolic rate). Across a species thermal range, it is reasonable to assume both developmental time and metabolic rate are under stabilising selection since adequate time and energy is needed to successfully complete cell division and differentiation, however increases in either trait could expose embryos to higher mortality risk via predation, desiccation, or depletion of energy reserves (Martin et al., 2007; Burton et al., 2011; Nord and Nilsson, 2011).

Combined, the thermal sensitivities of developmental and metabolic rates determine how energy use scales with temperature (Pettersen et al., 2019). Increasing either developmental time (D), or metabolic rate (MR) will increase the costs of development, and therefore reduce the amount of residual energy at hatching. The recently proposed Development Cost Theory (DCT) posits that the relative temperature sensitivity of D and MR together determine the amount of energy expended at any given temperature (Marshall et al., In press). At cooler developmental temperatures, *D* is often increased more than *MR* decreases, hence cold environments generally increase total energy use, thereby reducing energy available for fitness-enhancing processes such as growth, maintenance and foraging (Booth and Thompson, 1991; Angilletta Jr et al., 2000; DuRant et al., 2011; Pettersen et al., 2019). Based on DCT, the temperature dependence of development rate, has the greatest influence on the relative costs of development, and is therefore expected to evolve more rapidly than the thermal sensitivity of metabolic rate (Marshall et al., In press). DCT can thus provide a useful framework for detecting local adaptation by providing a mechanistic link between population-level reaction norms and fitness across thermal gradients.

While developmental time and the costs of development are generally increased at low environmental temperatures, countergradient variation can compensate for these effects. Countergradient variation can reduce the costs of development associated with cool temperatures via variation in developmental and metabolic rates that oppose the acute effects of environment on phenotype – for example, higher physiological rates can be maintained despite decreases in environment temperature. In order to identify whether the genetic component for the change in mean *D* or *MR* is statistically correlated with thermal gradient, three criteria must be met: 1) measures of the pattern of change in *D* and *MR* across a spatially or temporally varying environmental gradient; 2) the norm of reaction for *D* and *MR* in response to temperature; 3) a measure of the magnitude of thermal variation across the gradient. It is often difficult to unequivocally demonstrate that inter-populational divergence in thermal sensitivity of a trait is a result of adaptive genetic divergence, and not due to a plastic response. Obtaining evidence for CoGV and CnGV requires collecting data from common garden or reciprocal transplant studies, conducted across a range of temperatures in order to distinguish between V_{GxE} and Cov(*G,E*) (Yamahira and Conover, 2002; Yamahira et al., 2007). Norms of reaction that are parallel, and those that lie above or below one another in trait value provide evidence for CnGV and CoGV, respectively. Whereas, both V_{GxE} and Cov(G,E) are acting simultaneously when norms of reaction are not parallel and do not cross (see Box 2 in Conover and Schultz, 1995). While the prevalence of CoGV and CnGV in physiological traits has been summarised for fish (Conover et al., 2006), amphibians (Morrison and Hero, 2003), marine invertebrates (Sanford and Kelly, 2011) and insects (Sinclair et al., 2012), examples in reptiles are less well documented. This is surprising, given that reptiles represent one of the largest study groups in vertebrate thermal physiology.

It is important to develop a clear understanding of patterns of countergradient variation in nature, before designing experiments to evaluate causal mechanisms (Conover et al., 2009). This paper therefore compiles data from common garden (CG) and reciprocal transplant (RT) studies testing for temperature-by-population interactions on variations in developmental time (*D*) and metabolic rate (*MR*) across cold- and warm-adapted populations of reptiles (Li et al., 2018). Effect sizes for each study, weighted by sample size can then be calculated in order to test whether selection has modified reaction norms of *D* and *MR* across climactic regimes (Table S1). It is anticipated that despite a decrease in environmental temperatures, cold-adapted populations maintain similar *D* and (or) *MR* across a species' thermal gradient, relative to warm-adapted populations. Reptiles provide a useful model system to study local adaptation because developmental trajectories in reptiles are highly sensitive to environmental temperatures (Angilletta Jr, 2009), and many reptile species have limited dispersal ability between populations (Uller and While, 2015). This review aims to elucidate broad-scale mechanisms underlying local adaptation in reptiles by evaluating the generality of phenotypic plasticity (Cov(G,E) = 0; Figure 1a,d), cogradient variation (Cov(G,E) >1; Figure 1b, e), and countergradient variation (Cov(G,E) <1; Figure 1c, f) in developmental physiological rates across populations experiencing different thermal regimes. If populations maintain similarity in *D* and (or) *MR* under thermal change then evolution is likely the result of CnGV, whereas rapid trait divergence in *D* or *MR* in the direction of thermal change is due to the evolution of CoGV. Due to the paucity of data on thermal sensitivity of *D* and *MR*, it is not yet feasible to present a formal, comprehensive meta-analysis on the topic here. Rather, this review serves as a summary of existing data on thermal reaction norms across locally adapted populations, and points towards future avenues of research that require further work in order to continue developing our understanding of adaptation along thermal gradients.

Discussion

Countergradient variation of thermal sensitivity in reptiles is prevalent in developmental but not metabolic rates

Most published studies show evidence for CnGV between developmental time and environmental temperature (Figure 2), supporting the generality of countergradient variation in reptile development. For 17 out of 22 studies, intrinsic (genetic) factors were shown to counter thermal influences on developmental rate. Rather than an outcome of genetic drift, these findings suggest an adaptive countergradient response - selection opposes reaction norms of developmental time (*D*) across climactic regimes. Development under cool conditions necessitates a countergradient adaptive response for faster development and earlier hatching time, enabling embryos to hatch before winter while resources are still available (Du et al., 2012). On the contrary, there is little support to suggest that CnGV is common for metabolic rate (*MR*) – overall, reptile embryos from locally-adapted cooler climates did not maintain higher metabolic rates compared with populations from warmer climates (Figure 2). Despite an apparent lack of evolved response in MR to buffer against reduced energy turnover under cold temperatures, a countergradient response in *D* can itself reduce energy expenditure during development under cold conditions. Across a species natural temperature range, the thermal sensitivity of *D* is often greater than the thermal sensitivity of *MR* and is therefore a more important determinant of how the costs of development scale with temperature (Pettersen et al., 2019, Marshall et al., In press). It may be that embryos counteract increased energy costs associated with development under cold temperatures, by reducing *D* without a concomitant increase in *MR* , and is supported by evidence for CnGV in yolk assimilation in the eastern fence lizard (Storm and Angilletta, 2007). The ability to evolve increases in one physiological rate independently of another has been shown previously for metabolic and growth rates (Williams et al., 2016). It may be that for developmental physiological rates, selection acts to reduce the costs of development, via CnGV in the thermal sensitivity of *D*, resulting in a better alignment of embryo thermal optima to that of local thermal regimes.

Proximal drivers of countergradient adaptation in developmental rates

Various mechanistic explanations have been proposed to explain the prevalence of countergradient variation in developmental rates, and its compensating effects on the costs of development at cooler temperatures, including the role of maternal effects. It is a common view that faster development in cold-, versus warm-adapted populations reared under common garden conditions are a consequence of later stage of embryogenesis at laying, later stage of development at hatching, or larger egg size. Yet, even after accounting for these population-level differences, studies find faster developmental rates in cold-adapted populations (Storm and Angilletta, 2007; Du et al., 2010). Increased heart mass in cold-adapted populations may allow for faster cell division and differentiation associated with development (Du et al., 2010), however how these effects are isolated from effects on the thermal sensitivity of heart rates is difficult to explain. Variation in yolk hormone content and composition (Ruuskanen et al., 2016) or enzymatic activity (Rungruangsak-Torrissen et al., 1998) may also play a role in facilitating faster development rates in cold-, relative to warm-adapted populations. Indeed, there are likely to be multiple mechanisms responsible for countergradient variation in DT, rather than any single factor.

Consequences of countergradient adaptation: when and why is thermal countergradient adaptation absent?

CnGV in developmental time was also found to be absent across native-non-native ranges for species adapting to hot temperatures. For example, for *Anolis cristatellus* and *A. sagrei* across forested (cool) versus urban (hot) populations, co-gradient variation was observed where hot-adapted populations showed lower developmental times relative to warm-adapted populations under common garden conditions (Tiatragul et al., 2017; Hall and Warner, 2018). These findings are congruent with CDT – beyond a species usual temperature range development is more costly because metabolic rate increases more than development time decreases (Marshall et al., In Press). Decreasing *D* at hot temperatures results in reduced costs of development, and therefore likely fitness advantages. Further measures of the relative temperature dependencies of *D* and *MR* in other species are needed to elucidate the temperature-dependent costs of development as a potentially general mechanism for local thermal adaptation to extreme high temperatures.

Trade-offs with other fitness-related traits can also help to explain an absence of CnGV in *D* – reducing developmental time may come at the cost of embryos hatching at smaller sizes such as reduced juvenile growth rate (Angilletta Jr et al., 2003; Buckley et al., 2010). However for reptiles, evidence for trade-offs amongst life-history traits are mixed (Niewiarowski and Angilletta Jr, 2008; Fetters and McGlothlin, 2017). Alternatively, it may be that selection on other traits can compensate for a lack of CnGV in developmental physiological rates. In squamates, behavioural thermoregulation, such as shifts in female body temperature while gravid, may be a more labile, and therefore more important mechanism for adaptation to cold and variable climactic regimes than perhaps more conserved, physiological responses (Navas, 2002).

Other climactic factors that vary across thermal gradients, such as temperature variation, seasonal time constraints, and food availability may confound effects of temperature on developmental rates. One study included in this dataset (Li et al., 2018) used fluctuating temperature manipulations to mimic nest temperatures, rather than constant-temperature incubations, and found evidence for CoGV in *Mauremys mutica* (Figure 2; Table S1). Studies using incubation temperature manipulations that reflect natural thermal conditions are likely to capture more complex, realistic responses in population-level responses in development time, however more studies are needed to reveal any consistent patterns across thermal gradients (Du et al., 2010; Denny, 2017; Li et al., 2018).

Finally, it may be that interactions between genotype and environment are inflating obervations of CnGV across thermal regimes, and that CnGV in developmental time is less common than currently assumed. There may be genotype-dependent effects of environment on developmental time, where for example, a single genotype is superior in all environments, even though the slopes of reaction norms differ (see Conover and Schultz 1995, Box 2). All studies included in this review only observed a single generation – it is unlikely that all sources of V_E are controlled for over this timescale (plastic responses may still play a role), which is a limitation of studying species with relatively long generation times, such as reptiles and other vertebrates (Laugen et al., 2003).

Future directions for understanding local adaptation via evolution of developmental rates in reptiles

Adaptation of developmental physiological rates is an important, yet underutilised avenue of research for understanding population persistence under changing and novel environments.

Countergradient variation for traits expressed later in the life history have been well documented in reptiles, including growth (Sears and Angilletta, 2003; Uller and Olsson, 2003; Li et al., 2011; Snover et al., 2015; Ortega et al., 2017), body size (Oufiero et al., 2011; Iraeta et al., 2013), scale size (Oufiero et al., 2011), preferred body temperature (Hodgson and Schwanz, 2019), nest date (Knapp et al., 2006; Edge et al., 2017), reproductive output (Knapp et al., 2006; Li et al., 2011; Fetters and McGlothlin, 2017), critical thermal limits and water loss (Kolbe et al., 2014) and locomotor performance (Niewiarowski, 2001; McElroy, 2014). It is possible that CnGV for traits observed later in life are also a consequence of developmental environment, such as food availability, temperature and stress (DuRant et al., 2013; Noble et al., 2018).

Developmental and metabolic rates under selection may in turn affect selection on genetically correlated traits later in life (Artacho et al., 2015; Pettersen et al., 2016, 2018; Ricklefs et al., 2017). Resolving the interplay between plastic and genetic responses to local selective forces throughout the life history and environmental gradients is the next fundamental challenge (Buckley et al., 2010). Isolating the role of maternal effects from environmental effects can be challenging without multi-generational studies, nevertheless, investigating the effect of maternal environment on offspring phenotype can provide insight into mechanisms underlying rapid adaptation to novel environments. Variation in maternal investment along environmental gradients is common in reptiles, even in egg-laying species that buffer their offspring from external temperatures via behavioural (Mathies and Andrews, 1996; Du et al., 2010), physiological (Harlow and Grigg, 1984) or endocinological (Uller et al., 2007) mechanisms which may complement or even drive countergradient variation to facilitate acclimation and adaptation to local thermal regimes.

Conclusions

Across latitudinal and altitudinal clines, cold-adapted populations have genetic capacity for faster development, relative to warm-adapted populations. While these differences in thermal sensitivity to local temperatures did not extend to warm- versus hot-adapted populations such as forested versus city populations, there is overall support for common CnGV in developmental time and metabolic rate in reptiles, which mirrors findings observed in other taxa (Conover et al., 2009). Given the highly sensitive nature of developmental trajectories to acute changes in temperature, maintenance of stable physiological rates in species covering wide distributions offers a fascinating avenue for understanding local adaptation (Du et al., 2010). In particular, evolutionary change in the thermal sensitivity of developmental and metabolic rates is likely to be a crucial component of adaptive responses to environmental change (Kelly, 2019). Identifying the nature of genotype-environment covariances across ecological gradients is key to understanding variation in physiological rates and for predicting population persistence under environmental change (Conover and Schultz, 1995).

Box 1: Genotype-environment covariances: co- and counter-gradient variation

Genotype-environment covariances (Cov(*G,E*)) can be either positive or negative, depending on whether they reinforce or oppose each other. There are three potential ways in which genotype-environment covariances can play out across populations. First, genotypes (*G*) and the environment (*E*) shift trait expression in the same direction, known as cogradient variation where the Cov(*G,E*) term is positive. Second, trait shifts due to genotypes do not align with trait shifts due to the environment (Cov(*G,E*) is negative), referred to as countergradient adaptation. Alternatively, phenotypes that arise from genotypes distributed randomly in a population that change only in response to the environment, are the result of phenotypic plasticity.

Figures

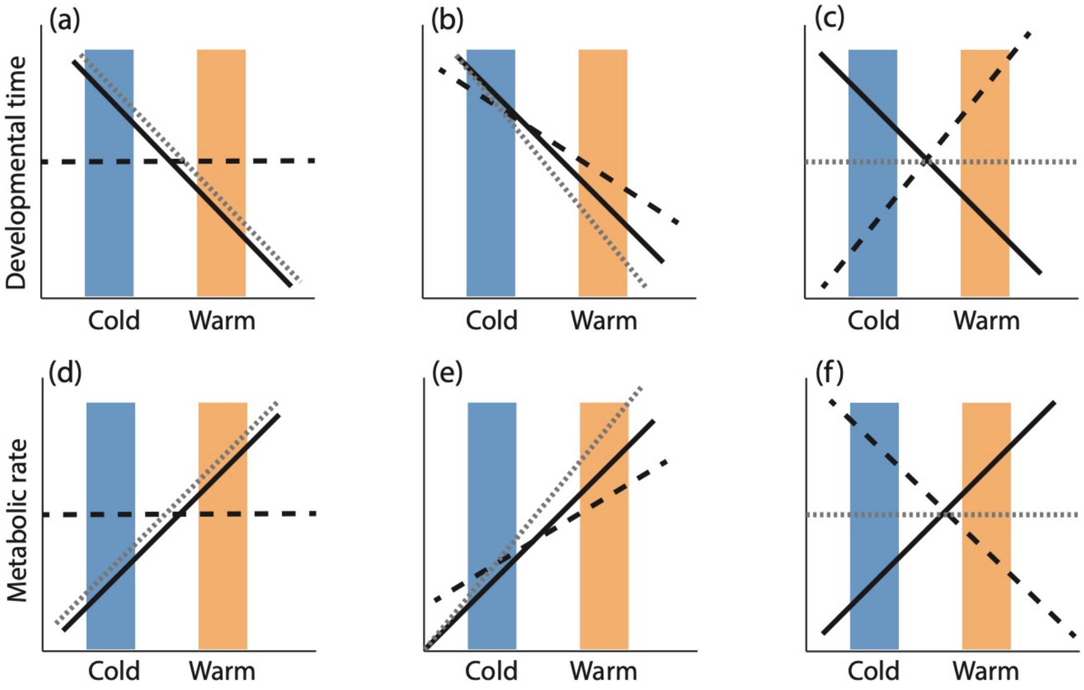

Figure 1: Hypothetical phenotypic variation (grey dashed lines) across locally-adapted cold (i.e., non-native conditions) and warm (i.e., native conditions) populations as a result of environmental temperature (black solid lines) and genetic (black dashed lines) effects. Shifts in phenotypic values of developmental time (a-c) and metabolic rate (d-f) in response to environmental temperature, can be entirely determined by environment (phenotypic plasticity; a,d). Alternatively, genotypic differences can be in the same direction as environmental influences, amplifying environmental effects on phenotypic (co-gradient variation; b,e), or they can oppose environmental temperature effects, resulting in little or no phenotypic change across cold and warm environments (c,f). Note, reaction norms may differ among genotypes, posing environmentally determined effects on phenotype value (V_{GXE}; discussed in Box 2, not shown here).

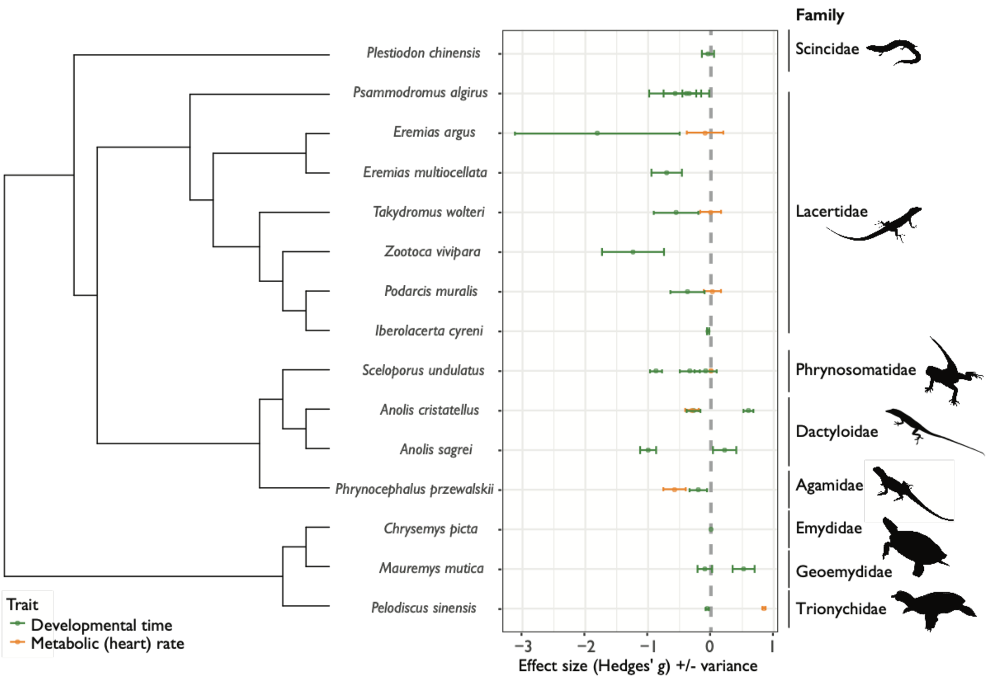

Figure 2: Effect sizes (Hedges' *g*) for differences in the thermal sensitivity of developmental time (time from oviposition until hatching) and metabolic (heart) rate across cold and warm-adapted populations for 15 species of reptiles across 8 families (+/- variance). Positive Hedges' *g* values indicate cold-adapted populations have longer developmental times (*D*) or higher metabolic rates (*MR*) relative to warm-adapted populations (and vice versa). Negative values of *D*, and positive values of *MR*, indicate countergradient variation, where genotypic differences oppose environmental temperature effects.

References

Andrews, R. M., and Schwarzkopf, L. (2012). Thermal performance of squamate embryos with respect to climate, adult life history, and phylogeny. *Biological Journal of the Linnean Society* 106, 851–864.

Angilletta Jr, M. J. (2009). *Thermal adaptation: a theoretical and empirical synthesis*. Oxford University Press.

Angilletta Jr, M. J., Winters, R. S., and Dunham, A. E. (2000). Thermal effects on the energetics of lizard embryos: implications for hatchling phenotypes. *Ecology* 81, 2957–2968.

Angilletta Jr, M., Wilson, R. S., Navas, C. A., and James, R. S. (2003). Tradeoffs and the evolution of thermal reaction norms. *Trends in Ecology & Ecolution* 18, 234–240. doi:10.1016/S0169-5347(03)00087-9.

Artacho, P., Saravia, J., Ferrandière, B. D., Perret, S., and Galliard, J.-F. L. (2015). Quantification of correlational selection on thermal physiology, thermoregulatory behavior, and energy metabolism in lizards. *Ecology and Evolution* 5, 3600–3609. doi:10.1002/ece3.1548.

Booth, D. T., and Thompson, M. B. (1991). A comparison of reptilian eggs with those of megapode birds. *Egg incubation: its effects on embryonic development in birds and reptiles. Cambridge University Press, Cambridge*, 325–344.

Buckley, C. R., Irschick, D. J., and Adolph, S. C. (2010). The contributions of evolutionary divergence and phenotypic plasticity to geographic variation in the western fence lizard, Sceloporus occidentalis. *Biological Journal of the Linnean Society* 99, 84–98.

Burton, T., Killen, S. S., Armstrong, J. D., and Metcalfe, N. B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences* 278, 3465–3473.

Conover, D. O., Clarke, L. M., Munch, S. B., and Wagner, G. N. (2006). Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. *Journal of fish biology* 69, 21–47.

Conover, D. O., Duffy, T. A., and Hice, L. A. (2009). “The Covariance between Genetic and Environmental Influences across Ecological Gradients Reassessing the Evolutionary Significance of Countergradient and Cogradient Variation,” in *Year in Evolutionary Biology 2009*, eds. C. D. Schlichting and T. A. Mousseau (Malden: Wiley-Blackwell), 100–129.

Conover, D., and Schultz, E. (1995). Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends Ecol. Evol.* 10, 248–252. doi:10.1016/S0169-5347(00)89081-3.

Denny, M. (2017). The fallacy of the average: on the ubiquity, utility and continuing novelty of Jensen’s inequality. *Journal of Experimental Biology* 220, 139–146. doi:10.1242/jeb.140368.

Du, W., Warner, D. A., Langkilde, T., Robbins, T., Shine, R., and Benkman, N. H. E. C. W. (2010). The Physiological Basis of Geographic Variation in Rates of Embryonic Development within a Widespread Lizard Species. *The American Naturalist* 176, 522–528. doi:10.1086/656270.

Du, W.-G., Warner, D. A., Langkilde, T., Robbins, T. R., and Shine, R. (2012). The roles of pre- and post-hatching growth rates in generating a latitudinal cline of body size in the eastern fence lizard (Sceloporus undulatus). *Biological Journal of the Linnean Society* 106, 202–209. doi:10.1111/j.1095-8312.2011.01846.x.

DuRant, S. E., Hopkins, W. A., and Hepp, G. R. (2011). Embryonic developmental patterns and energy expenditure are affected by incubation temperature in wood ducks (Aix sponsa). *Physiological and Biochemical Zoology* 84, 451–457.

DuRant, S. E., Hopkins, W. A., Hepp, G. R., and Walters, J. R. (2013). Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biological Reviews* 88, 499–509.

Edge, C. B., Rollinson, N., Brooks, R. J., Congdon, J. D., Iverson, J. B., Janzen, F. J., et al. (2017). Phenotypic plasticity of nest timing in a post-glacial landscape: how do reptiles adapt to seasonal time constraints? *Ecology* 98, 512–524. doi:10.1002/ecy.1665.

Fetters, T. L., and McGlothlin, J. W. (2017). Life histories and invasions: accelerated laying rate and incubation time in an invasive lizard, Anolis sagrei. *Biol J Linn Soc* 122, 635–642. doi:10.1093/biolinnean/blx102.

Hall, J. M., and Warner, D. A. (2018). Thermal spikes from the urban heat island increase mortality and alter physiology of lizard embryos. *Journal of Experimental Biology* 221. doi:10.1242/jeb.181552.

Harlow, P., and Grigg, G. (1984). Shivering Thermogenesis in a Brooding Diamond Python, Python spilotes spilotes. *Copeia* 1984, 959–965. doi:10.2307/1445340.

Hodgson, M. J., and Schwanz, L. E. (2019). Drop it like it’s hot: Interpopulation variation in thermal phenotypes shows counter-gradient pattern. *Journal of Thermal Biology* 83, 178–186. doi:10.1016/j.jtherbio.2019.05.016.

Iraeta, P., Salvador, A., and Díaz, J. A. (2013). Life-history traits of two Mediterranean lizard populations: a possible example of countergradient covariation. *Oecologia* 172, 167–176.

Kelly, M. (2019). Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philosophical Transactions of the Royal Society B* 374, 20180176.

Knapp, C. R., Iverson, J. B., and Owens, A. K. (2006). Geographic variation in nesting behavior and reproductive biology of an insular iguana (Cyclura cychlura). *Canadian Journal of Zoology* 84, 1566–1575.

Kolbe, J. J., Ehrenberger, J. C., Moniz, H. A., and Angilletta, M. J. (2014). Physiological variation among invasive populations of the brown anole (Anolis sagrei). *Physiol. Biochem. Zool.* 87, 92–104. doi:10.1086/672157.

Laugen, A. T., Laurila, A., Rasanen, K., and Merila, J. (2003). Latitudinal countergradient variation in the common frog (Rana temporaria) development rates - evidence for local adaptation. *J. Evol. Biol.* 16, 996–1005. doi:10.1046/j.1420-9101.2003.00560.x.

Levins, R. (1969). Thermal Acclimation and Heat Resistance in Drosophila Species. *The American Naturalist* 103, 483–499.

Li, H., Qu, Y.-F., Ding, G.-H., and Ji, X. (2011). Life-history variation with respect to experienced thermal environments in the lizard, Eremias multiocellata (Lacertidae). *Zoological science* 28, 332–339.

Li, T., Cao, P., Bei, Y.-J., and Du, W.-G. (2018). Latitudinal and Temperature-Dependent Variation in Embryonic Development Rate and Offspring Performance in a Freshwater Turtle. *Physiological and Biochemical Zoology* 91, 673–681. doi:10.1086/694856.

Marshall, D. J., Pettersen, A. K., Bode, M., and White, C. R. (In Press). Developmental Cost Theory predicts thermal environment and vulnerability to global warming. *Nature Ecology and Evolution*.

Martin, T. E., Auer, S. K., Bassar, R. D., Niklison, A. M., and Lloyd, P. (2007). Geographic variation in avian incubation periods and parental influences on embryonic temperature. *Evolution: International Journal of Organic Evolution* 61, 2558–2569.

Mathies, T., and Andrews, R. M. (1996). Extended Egg Retention and Its Influence on Embryonic Development and Egg Water Balance: Implications for the Evolution of Viviparity. *Physiological Zoology* 69, 1021–1035.

McElroy, M. T. (2014). Countergradient variation in locomotor performance of two sympatric Polynesian skinks (Emoia impar, Emoia cyanura). *Physiol. Biochem. Zool.* 87, 222–230. doi:10.1086/674920.

Morrison, C., and Hero, J.-M. (2003). Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology* 72, 270–279.

Navas, C. A. (2002). Herpetological diversity along Andean elevational gradients: links with physiological ecology and evolutionary physiology. *Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology* 133, 469–485. doi:10.1016/S1095-6433(02)00207-6.

Niewiarowski, P. H. (2001). Energy Budgets, Growth Rates, and Thermal Constraints: Toward an Integrative Approach to the Study of Life-History Variation. *The American Naturalist* 157, 421–433. doi:10.1086/319321.

Niewiarowski, P. H., and Angilletta Jr, M. J. (2008). Countergradient variation in embryonic growth and development: do embryonic and juvenile performances trade off? *Functional Ecology* 22, 895–901. doi:10.1111/j.1365-2435.2008.01441.x.

Noble, D. W. A., Stenhouse, V., and Schwanz, L. E. (2018). Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biological Reviews* 93, 72–97. doi:10.1111/brv.12333.

Nord, A., and Nilsson, J.-Å. (2011). Incubation temperature affects growth and energy metabolism in blue tit nestlings. *The American Naturalist* 178, 639–651.

Ortega, J., López, P., and Martín, J. (2017). Environmental drivers of growth rates in Guadarrama wall lizards: a reciprocal transplant experiment. *Biol J Linn Soc* 122, 340–350. doi:10.1093/biolinnean/blx068.

Ospina, E. A., Merrill, L., and Benson, T. J. (2018). Incubation temperature impacts nestling growth and survival in an open-cup nesting passerine. *Ecology and evolution* 8, 3270–3279.

Oufiero, C. E., Gartner, G. E. A., Adoph, S. C., and Garland Jr, T. (2011). Latitudinal and climactic variation in body size and dorsal scale counts in Sceloporus lizards: a phylogenetic perspective. *Evolution* 65, 3590–3607.

Pettersen, A. K., Marshall, D. J., and White, C. R. (2018). Understanding variation in metabolic rate. *J. Exp. Biol.* 221, jeb166876. doi:10.1242/jeb.166876.

Pettersen, A. K., White, C. R., Bryson-Richardson, R. J., and Marshall, D. J. (2019). Linking life-history theory and metabolic theory explains the offspring size-temperature relationship. *Ecol. Lett.* 22, 518–526. doi:10.1111/ele.13213.

Pettersen, A. K., White, C. R., and Marshall, D. J. (2016). Metabolic rate covaries with fitness and the pace of the life history in the field. *Proceedings of the Royal Society B: Biological Sciences* 283, 20160323. doi:10.1098/rspb.2016.0323.

Ricklefs, R. E., Austin, S. H., and Robinson, W. D. (2017). The adaptive significance of variation in avian incubation periods. *tauk* 134, 542–550. doi:10.1642/AUK-16-171.1.

Rungruangsak-Torrissen, K., Pringle, G. M., Moss, R., and Houlihan, D. F. (1998). Effects of varying rearing temperatures on expression of different trypsin isozymes, feed conversion efficiency and growth in Atlantic salmon (shape Salmo salar L.). *Fish Physiology and Biochemistry* 19, 247–255. doi:10.1023/A:1007731717021.

Ruuskanen, S., Groothuis, T. G. G., Schaper, S. V., Darras, V. M., de Vries, B., and Visser, M. E. (2016). Temperature-induced variation in yolk androgen and thyroid hormone levels in avian eggs. *Gen. Comp. Endocrinol.* 235, 29–37. doi:10.1016/j.ygcen.2016.05.026.

Sanford, E., and Kelly, M. W. (2011). Local adaptation in marine invertebrates. *Annual Review of Marine Science* 3, 509–535.

Sears, M. W., and Angilletta, M. J. (2003). Life-History Variation in the Sagebrush Lizard: Phenotypic Plasticity or Local Adaptation? *Ecology* 84, 1624–1634. doi:10.1890/0012-9658(2003)084[1624:LVITSL]2.0.CO;2.

Sinclair, B. J., Williams, C. M., and Terblanche, J. S. (2012). Variation in thermal performance among insect populations. *Physiological and Biochemical Zoology* 85, 594–606.

Snover, M. L., Adams, M. J., Ashton, D. T., Bettaso, J. B., and Welsh, H. H. (2015). Evidence of counter-gradient growth in western pond turtles (*Actinemys marmorata*) across thermal gradients. *Freshwater Biology. 60(9): 1944-1963* 60, 1944–1963. doi:10.1111/fwb.12623.

Storm, M. A., and Angilletta, M. J. (2007). Rapid assimilation of yolk enhances growth and development of lizard embryos from a cold environment. *Journal of Experimental Biology* 210, 3415–3421. doi:10.1242/jeb.005652.

Taylor, M. L., Skeats, A., Wilson, A. J., Price, T. A. R., and Wedell, N. (2015). Opposite environmental and genetic influences on body size in North American Drosophila pseudoobscura. *Bmc Evolutionary Biology* 15, 51. doi:10.1186/s12862-015-0323-3.

Tiatragul, S., Kurniawan, A., Kolbe, J. J., and Warner, D. A. (2017). Embryos of non-native anoles are robust to urban thermal environments. *Journal of Thermal Biology* 65, 119–124. doi:10.1016/j.jtherbio.2017.02.021.

Uller, T., Astheimer, L., and Olsson, M. (2007). Consequences of maternal yolk testosterone for offspring development and survival: experimental test in a lizard. *Functional Ecology* 21, 544–551. doi:10.1111/j.1365-2435.2007.01264.x.

Uller, T., and Olsson, M. (2003). Life in the land of the midnight sun: are northern lizards adapted to longer days? *Oikos* 101, 317–322. doi:10.1034/j.1600-0706.2003.12374.x.

Uller, T., and While, G. M. (2015). “The evolutionary ecology of reproductive investment in lizards,” in *Reproductive Biology and Phylogeny of Lizards and Tuatara*.

Warner, D. A., Woo, K. L., Van Dyk, D. A., Evans, C. S., and Shine, R. (2010). Egg incubation temperature affects male reproductive success but not display behaviors in lizards. *Behav Ecol Sociobiol* 64, 803–813. doi:10.1007/s00265-009-0897-0.

Williams, C. M., Szejner-Sigal, A., Morgan, T. J., Edison, A. S., Allison, D. B., and Hahn, D. A. (2016). Adaptation to Low Temperature Exposure Increases Metabolic Rates Independently of Growth Rates. *Integrative and Comparative Biology* 56, 62–72. doi:10.1093/icb/icw009.

Yamahira, K., and Conover, D. O. (2002). Intra-vs. interspecific latitudinal variation in growth: adaptation to temperature or seasonality? *Ecology* 83, 1252–1262.

Yamahira, K., Kawajiri, M., Takeshi, K., and Irie, T. (2007). Inter-and intrapopulation variation in thermal reaction norms for growth rate: evolution of latitudinal compensation in ectotherms with a genetic constraint. *Evolution: International Journal of Organic Evolution* 61, 1577–1589.