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Adaptive responses of the embryos of birds and reptiles to spatial and temporal variations in nest temperatures

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Natural nests of egg-laying birds and reptiles exhibit substantial thermal variation, at a range of spatial and temporal scales. Rates and trajectories of embryonic development are highly sensitive to temperature, favouring an ability of embryos to respond adaptively (i.e. match their developmental biology to local thermal regimes). Spatially, thermal variation can be significant within a single nest (top to bottom), among adjacent nests (as a function of shading, nest depth etc.), across populations that inhabit areas with different weather conditions, and across species that differ in climates occupied and/or nest characteristics. Thermal regimes also vary temporally, in ways that generate differences among nests within a single population (e.g. due to seasonal timing of laying), among populations and across species. Anthropogenic activities (e.g. habitat clearing, climate change) add to this spatial and temporal diversity in thermal regimes. We review published literature on embryonic adaptations to spatio-temporal heterogeneity in nest temperatures. Although relatively few taxa have been studied in detail, and proximate mechanisms remain unclear, our review identifies many cases in which natural selection appears to have fine-tuned embryogenesis to match local thermal regimes. Developmental rates have been reported to differ between uppermost versus lower eggs within a single nest, between eggs laid early versus late in the season, and between populations from cooler versus warmer climates. We identify gaps in our understanding of thermal adaptations of early (embryonic) phases of the life history, and suggest fruitful opportunities for future research.

1. Introduction

The embryo is a critical stage of the life cycle in many organisms, because significant within-population variation among individuals in lifetime reproductive success is driven by mortality prior to hatching, and by the impact of developmental conditions on offspring phenotypes [1]. Unlike viviparous species in which the mother can buffer the impact of external conditions on her embryos, the eggs of oviparous species are exposed to environmental conditions that vary in both abiotic and biotic factors (e.g. temperature, moisture, pollution, pathogens and predation). Temperature is one of the most important environmental influences on embryonic survival, and can induce significant variation in offspring phenotypes [2,3]. In natural nests, embryos experience environmental temperatures that vary both spatially and temporally despite the buffering effect of maternal nest-site selection. For example, mean ambient temperature tends to decrease with increasing latitude, and (within a site) is higher in summer than spring [4,5]. Given the sensitivity of embryogenesis to external temperature [2,3,6], we expect that embryos evolving under different thermal environments will exhibit adaptations that fine-tune developmental biology to match local conditions.

To clarify adaptive responses of embryos to thermal variation, we need to explore the developmental correlates of different thermal environments via inter- and intra-specific comparisons at a range of temporal and spatial scales. In post-embryonic stages, these kinds of intraspecific and interspecific comparisons over temporal and spatial scales have attracted detailed research [7,8]. In contrast, the patterns and underlying mechanisms of embryonic responses to environmental variation have been under-studied both for logistical reasons (embryos inside eggs are less accessible to direct observation) and, more importantly, because embryos traditionally have been regarded as a passive stage unable to respond to environmental changes [9]. However, increasing evidence suggests that embryos possess adaptive strategies that enable them to survive and develop viable phenotypes over a wide range of environmental conditions [9,10].

2. Scope of this review

Thermal regimes experienced by eggs vary over small temporal and spatial scales due to stochastic local weather conditions and physical characteristics (e.g. due to vegetation coverage and soil features). Such stochastic variations may have important consequences for embryonic development, favouring the evolution of developmentally plastic responses that modify developmental trajectories based on short-term thermal cues [9]. Over longer time frames, however, thermal variations associated with temporal shifts (e.g. spring versus summer) or spatial shifts (e.g. high versus low latitudes) are relatively consistent [4,5]. In such cases we expect to see evolutionary responses to longer-term local characteristics, via canalized (hard-wired) variation in maternal nest-site choice and/or embryonic norms of reaction. These latter responses—adaptations of embryos to consistent and thus predictable differences in thermal regimes through time and space—are the main focus of the current review.

Our aim is to summarize recent findings in the emerging research field of thermal adaptation in reptile and bird embryos, and to point out some future challenges to stimulate studies in this emerging research field. Rather than attempting to comprehensively review all publications on the topic, we have chosen studies that represent the taxonomic and geographical breadth of examples of embryonic adaptation. To facilitate discussion, we use a conceptual scheme that recognizes embryonic adaptations to three scales of spatial variation in incubation temperatures (within a single nest; among nests within the same area; and across latitudes and elevations) and two scales of temporal variation (across seasons; and in response to anthropogenic change) (electronic supplementary material, table S1).

3. Spatial variation in egg temperature

The thermal regimes experienced by an embryo can vary predictably over a range of spatial scales—from a few centimetres to many kilometres—as discussed below.

(a) Thermal variation within a nest

In species where a female lays a very large clutch (as in some turtles), the mass of eggs within a nest may be large enough to allow significant variation in thermal regimes experienced by different eggs. For example, diel variation in temperature predictably decreases with depth below the sun-heated ground surface, such that eggs laid deeper in a nest will develop at less variable temperatures than do shallower eggs in some turtles [11,12]. Mean incubation temperatures also may shift; for example eggs at upper and central locations of the nest were consistently warmer than were the base and side of the nest in sea turtles [13–15], as well as in freshwater turtles [11,12] (electronic supplementary material, figure S1a). Temperature clines within a nest depend upon a range of attributes both of the substrate and the nest cavity; for example, as a consequence of thermal variation at various depths beneath the soil surface [16]. Thermal differentials between uppermost and lowest eggs are greater within the relatively small and shallow nests of some freshwater turtles (5–20 cm deep) than within the deeper nests of sea turtles (over 40 cm deep). For example, thermal differentials can reach up to 6°C in nests of the freshwater turtle *Emydura macquarii* [11], but only up to 1.4°C in the nests of sea turtles *Chelonia mydas* and *Caretta caretta* [13]. In the latter nests, however, the large volume of rapidly developing embryonic tissue creates metabolic heat in the second half of the incubation period, which can increase nest temperatures by 1.5–6°C above substrate temperatures [15,17] and generates higher temperatures for eggs in the centre of the nest than those at the periphery [18]. Most birds have clutch sizes small enough to minimize within-nest variation in temperature, but central eggs may be in more intimate connection to heat-providing parental brood patches and hence, kept warmer than are peripheral eggs; that differential can be buffered if the brooding female moves eggs around the nest cup [19]. In addition, eggs of avian brood parasites (e.g. cuckoos) have higher temperature than host eggs during incubation within a nest [20].

(b) Geographical variation in nest temperatures

(i) Variations among nest-sites within a single region

Many natural habitats comprise a complex mosaic of thermal environments, even over very small spatial scales within a single area. Thus, nest-site choice by females can expose their embryos to different thermal conditions. For example, females lay their eggs in two types of nests in grass snakes *Natrix natrix* (compost piles or manure heaps) and water pythons *Liasis fuscus* (the burrows of varanid lizards or cavities beneath tree-roots) [21,22]. In both cases, the two types of nest sites provide different thermal environments for developing embryos, generating differences in phenotypic traits of the hatchling snakes [22,23]. In sociable weavers (*Philetairus socius*), older females construct nests in the centre of the communal nesting area and their eggs experience more stable temperatures [24].

Nest temperature may also vary among subpopulations within a region. For example, nest temperatures differ significantly between two beaches just a few kilometres apart in a single, island-breeding population of the green turtle *Chelonia mydas*, due to the different physical characteristics of sand (biogenic pale sand versus volcanic dark sand) [25] (electronic supplementary material, figure S1b). In nests of the snapping turtle *Chelydra serpentina*, nests in sandy patches are warmer than those in grassy patches, and nests at natural sites are warmer than those at disturbed sites with a higher percentage of overstory vegetation cover [26].

(ii) Variations among nest-sites due to latitude and elevation

Developmental temperatures for embryos may differ strongly between geographically separated populations of oviparous vertebrates, reflecting local abiotic conditions. As a general rule, nests are expected to be warmer and less variable at lower latitudes, consistent with field data (e.g. [4,27]) (electronic supplementary material, figure S1c). Nonetheless, variation in maternal behaviours (e.g. nest selection and nesting phenology) may compensate for those geographical differences in nest temperatures to some degree [4]. Such behavioural shifts may actually reverse latitudinal patterns in nest temperature, as in high-latitude populations of some freshwater turtles, where females select more open (sunlight-exposed) nesting sites than do low-latitude females [28,29].

The impacts of elevation are similar to those of latitude, with montane nests of lizards typically cooler and more variable in temperature than low-elevation nests of the same taxa [30] (electronic supplementary material, figure S1d). Again, however, nests may be shallower at high elevations than at low elevations, creating thermally variable (and effectively, hotter) conditions in a direction opposite to overall mean air temperatures [31]. Nest temperatures typically show similar latitudinal and elevational patterns in birds [32], although parental nest construction and brooding behaviour partially buffer the impact of ambient temperature (e.g. females construct better-insulated nests and spend more time incubating as ambient temperatures decrease [33,34]). For example, the median temperature during incubation was between 33°C and 35°C in seven species of Arctic-nesting species of birds [35], lower than the nest temperature (*ca* 38°C) of most birds from other regions [36].

4. Temporal variations in egg temperature

(a) Seasonal variation in nest temperatures

Reproductive timing determines the developmental temperature of embryos; nest temperatures show a strong seasonal change. Unlike many reptiles from tropical areas that experience high and relatively constant ambient temperatures, reptiles that inhabit temperate areas experience seasonal variation in nest temperatures during the reproductive season. First, nest temperatures in spring are generally lower than those in summer. Second, the eggs laid by early-breeding females in spring experience rising temperatures during incubation, whereas the eggs laid by late-breeding females in summer experience falling temperatures [5,37,38] (electronic supplementary material, figure S1e). By contrast, seasonal variation in nest temperatures of birds and facultatively endothermic reptiles are buffered by parental incubation behaviour [36]. For example, female blue tits *Cyanistes caeruleus* use less cup-lining material in response to increasing ambient temperatures [39]. Reptiles may also achieve relatively high incubation temperatures year-round by laying their eggs inside or beside active termite mounds [40,41], or on the slopes of active volcanoes [42].

(b) Nest warming in the context of global change

The ongoing trend for global warming inevitably will increase nest temperatures of many oviparous species. For example, nest temperatures of the three-lined skink *Bassiana duperreyi* overall increased from 1997 to 2006, even though the impact of ambient changes was buffered by the lizards adjusting both nest depth and seasonal timing of oviposition over that period [43] (electronic supplementary material, figure S1f). Under climate change, the nests of tropical sea turtle populations are predicted to experience extreme high temperatures and therefore a severe decline in hatching success, whereas temperate populations will experience rising temperatures that may enhance or have no impact on hatching success [44]. Increasing evidence has demonstrated that environmental warming results in female-biased populations in sea turtles [45,46]. Moreover, anthropogenic activities have extensively modified many natural habitats of wildlife, affecting the thermal environments of developing embryos in some species. For example, compared to adjacent natural habitats, cities may offer warmer nest conditions in terms of mean and extreme temperatures due to the urban heat island effect [47]. Similarly, nests in anthropogenically disturbed areas are warmer than those in natural areas in the snapping turtle, because anthropogenic nest sites have less ground-level vegetation and paler soil [48].

5. The response of embryos to variations in nest temperature

In response to predictable patterns of thermal variation across time and space, embryos of oviparous species may demonstrate adaptive responses at levels from the whole-organism to the molecular. Below, we discuss adaptive responses to the types of predictable variation outlined in the preceding section.

(a) Embryonic adaptation to thermal variation within a nest

The first eggs laid by a female reptile (i.e. those in the posterior part of her oviduct) will probably end up at the bottom of the nest, and hence be exposed to more stable (and perhaps, cooler) thermal conditions than will the last eggs laid within that same clutch. That predictability suggests that females might be able to match embryonic norms of reaction to an egg's location within the oviduct; but as yet information to test that hypothesis remains limited. The general prediction is that eggs taken from the uppermost layer of a nest would develop most successfully at high and/or variable temperatures, whereas those from the lower levels of the same nest would benefit from low- and/or constant-temperature incubation. Consistent with prediction, embryos of the freshwater turtle *Emydura macquarii* from the upper levels of a nest developed faster than embryos of lower-level eggs when incubated at fluctuating temperatures, whereas embryos of lower-level eggs developed faster when kept at more stable temperatures [49]. In addition, embryos inside the upper and lower eggs use physiological mechanisms to adjust developmental rate, and thereby minimize impacts of the within-nest temperature difference on total incubation period, leading to synchronous emergence of hatchlings in some turtles despite significant within-nest thermal variation [16]. These findings suggest adaptive responses of embryos to within-nest different in thermal developmental environments. Analogously, embryos from eggs laid late in the nesting season have higher metabolic rates and therefore develop faster than those in earlier-laid eggs within a clutch in some avian species, but the underlying driving forces for this phenomenon have yet to be elucidated [50,51].

The matching of developmental rates to thermal regimes in eggs from high and low in a nest may be attributable to the embryo's capacity to increase its heart rate in the last few weeks of incubation [49]. The mechanisms that drive within-clutch differences in thermal sensitivities of reptile embryonic development remain unclear. An additional uncertainty relates to whether any within-clutch divergence in developmental responses is due to processes acting prior to laying (such as maternal allocation of steroids) as opposed to developmentally plastic responses of embryos to the conditions that they encounter post-oviposition [9]. To tease apart those possibilities, it would be interesting to test first-laid versus last-laid eggs within a single clutch, rather than waiting until the eggs had been deposited at different levels within a nest and hence, may have triggered facultative responses to the thermal conditions that they encountered post-oviposition.

(b) Local adaptation to geographical variation

Spatial heterogeneity in the consequences of specific phenotypic trait values for organismal fitness causes corresponding spatial variation in optimal values for those traits [52]. As a result, individuals within a population are expected to exhibit traits that maximize lifetime reproductive success (fitness) under the conditions applying at that site. Reptile and bird embryos vary in thermal physiology across their geographical ranges. Much of that variation probably results from adaptation in response to ambient thermal heterogeneity at a range of spatial scales.

(i) Thermal variation among nests

Adjacent nests may provide very different thermal regimes, based on characteristics such as shading, depth and substrate type (see above). Are there maternally derived mechanisms by which embryonic reaction norms are matched to the abiotic characteristics of their nest-sites? For example, a female nesting in a cool (shaded) site could produce embryos that develop most effectively under this kind of thermal regime. Diamondback terrapins *Malaclemys terrapin* have been reported to lay clutches of small eggs in cool nests, and clutches of large eggs in hotter nests, thereby reducing overall variance in the seasonal time of hatching (because at standard temperatures, incubation period increases with egg size) [53]. However, a field experiment with reciprocal transplantation of eggs among natural nests found no evidence that female *Bassiana duperreyi* match incubation optima of their embryos to thermal characteristics of the nest-sites in which those eggs are deposited [54].

At a slightly larger spatial scale, thermal physiology of embryos may differ among populations. In the example of island-nesting green turtles noted above, where nest temperatures are higher on beaches with dark sand, embryos from the 'hot beach' population tolerate higher thermal maxima than do those from the 'cool beach' population, and that difference has a genetic basis [25]. Adaptive matches between local nest temperatures and embryonic developmental biology are likely to be widespread in geographically wide-ranging species [55].

(ii) Latitudinal variation

Correlations between ambient thermal regimes and embryonic thermal tolerances or optimal developmental temperatures provide strong support for the idea of adaptive matching of norms of reaction to local incubation conditions [29]. Thermal tolerance is a critical physiological trait that has been extensively investigated in ectotherms; such tolerances may well set geographical limits for species distributions and determine their vulnerability to climate change [56]. Here we focus on latitudinal patterns in thermal tolerance of embryos among species and populations. The lower thermal limit for embryonic development tends to increase as latitude decreases in reptiles [57]. Relationships between embryonic heat tolerance and latitude vary among lineages: for example, the maximum temperature that permits embryonic development has not diverged among populations along a latitudinal cline in *Sceloporus* lizards of North America [58], but shows a decrease at lower latitudes in *Eumeces* skinks of China (probably as an adaptation to the highly variable nest temperatures in high-latitude regions) [59].

Incubation periods determine the duration of time for which embryos are exposed to external risks such as predation and abiotic extremes, imposing strong selection on any trait that influences the total duration of the egg stage [60]. As a compensatory response to low temperatures that retard embryonic development, eggs of high-latitude populations of some reptile species have a shorter incubation period (when incubated at controlled temperatures) than do those from low-latitude populations [61–64].

In reptiles, shorter incubation periods in high-latitude populations could be attributed to advanced embryogenesis completed prior to oviposition, higher rates of embryonic development during incubation, thermal acclimation and compensation of embryonic developmental rate at low temperatures, or an earlier-hatching but less-developed offspring (electronic supplementary material, table S2). The countergradient variation in incubation period is related to faster development during incubation in the fence lizard *Sceloporus undulatus* and the Mongolian racerunner *Eremias argus*, but to the degree of embryonic development prior to oviposition in the grass lizard *Takydromus wolteri* [62,63]. Moreover, the underlying physiological mechanisms that generate faster developmental rate in cool-climate *S. undulatus* differ between populations, with enlarged hearts in one population and increased heartbeat rates in another [62]. However, high-latitude embryos do not show an enhanced ability to acclimate thermally, or to hatch earlier with less-developed offspring [65]. The situation is complex, with latitudinal differences in incubation period sometimes reversing over different ranges of incubation temperature. For example, the incubation period of Asian yellow pond turtles (*Mauremys mutica*) is shorter in high-latitude populations than in low-latitude populations at low temperatures, but *vice versa* at high temperatures [66].

(iii) Elevational adaptation

Nest temperature decreases at higher elevations, so we expect local adaptation of embryonic development to produce a match between embryogenesis and microhabitat temperatures along an elevational gradient. In response to low ambient temperature, female *Bassiana duperreyi* facultatively retain embryos *in utero* for longer, and therefore produce eggs with more advanced embryonic stages at oviposition in high-elevation populations than in lower-elevation populations [67]. The same pattern is seen in many other reptiles, likely due to adaptation rather than plasticity. For example, some lizards from high-elevation populations have shorter incubation periods than do low-elevation conspecifics, due to retention of eggs *in utero* in high-elevation populations [68,69]. In addition, in the blood pheasant *Ithaginis cruentus* at high-elevation forests (3000 m), embryos can tolerate low temperatures (below 10°C for 3.5 h per day) when the mother leaves the nest [70].

(c) Temporal adaptation to seasonal variation

Temporal variation in environmental conditions causes an ecotype to be well-adapted to functioning at a given time (e.g. seasonally), with the result that it outperforms other ecotypes that evolve to function at other time periods. Seasonal shifts in nest temperatures provide predictable cues to which embryos can respond in adaptive ways, but our understanding of the responses of embryos to seasonal changes in incubation temperatures remains limited.

In many species of reptiles and birds, eggs from early-breeding females are of higher quality, and produce offspring with better performance and higher survival rate than those from late-breeding females [71–73]. In addition, early breeding may provide a better environment (e.g. benign temperature) for embryonic development than late breeding [37,74]. Seasonal shifts in thermal regimes also may be important *per se*. Compared to rising nest temperatures, falling temperatures can have negative impacts on offspring traits of lizards (e.g. a higher incidence of deformities, and reduced locomotor performance) [37]. More surprisingly, embryos from early and late-breeding females respond differently to seasonal change (rising versus falling temperatures). In the toad-headed agamid *Phrynocephalus przewalskii* and the brown anole *Anolis sagrei*, offspring from eggs laid by late-breeding females exhibit higher growth rates or survival rates when incubated at the nest regimes available at that time of year (falling temperatures) compared to mismatched early-season regimes (rising temperature) [5,38] (electronic supplementary material, table S3).

Analogously, late-season chicks may benefit from earlier hatching, especially in temperate-zone species that have to prepare for an autumn migration after hatching [75]. In addition, turtles with environmental sex determination may shift offspring sex ratio seasonally by flexible allocation of yolk oestradiol (E2) at specific times of year [76].

(d) Embryonic responses to anthropogenically-derived warming of the nest

Evolutionary theory predicts that both phenotypic plasticity and evolutionary adaptation may help embryos to cope with anthropogenically derived temperature changes [57,77]. Compared to nests in forest habitats, warmer city temperatures increase developmental rates, but a brief thermal spike was reported to reduce embryonic survival in the Puerto Rican crested anole lizard (*Anolis cristatellus*) [47]. A comparison of nest fates among sympatric species of birds on an urban-to-rural gradient in the western United States indicated that nest survival increased with slight thermal increases during the nestling stage, suggesting moderate warming in spring temperatures may be beneficial [78]. Global warming is likely to affect reproductive success of ground-nesting birds because they lay eggs that often are left unattended for days or even weeks before parental incubation [79]. Nonetheless, the evidence for adaptive responses of embryos to anthropogenically induced nest warming is still rare because of the formidable logistics required to run a long-term experiment to measure changes in embryonic thermal physiology associated with climate change.

6. Future perspectives

(a) Beyond the thermal environment

Our review summarizes information on the adaptive strategies adopted by reptile and bird embryos in response to their nest temperature over a range of spatial and temporal scales, but a number of knowledge gaps remain. First, our understanding of how reptile and bird embryos respond to small-scale spatial variations in nest temperatures is rather limited (e.g. between individuals within a population). Given the significant heritability of nesting behaviour in female turtles and perhaps in other reptiles [80], identifying embryonic adaptations to thermal environments of nests selected by their mother can help us to understand between-individual and between-population variations in thermal adaptation and their ecological consequences [25]. Second, we have few long-term data on nest temperature and embryonic development of any species or population. Only a small number of long-term (greater than 10-year) studies on embryonic response to nest temperatures have been performed in reptiles and birds (e.g. the three-lined skink *Bassiana duperreyi*; the painted turtle *Chrysemys picta*) [43,81]. Such data are invaluable for answering questions about embryonic adaptation, and more general questions in ecology and evolutionary biology. For example, how will embryos respond to climate change phenotypically and genetically? What is the role of developmental plasticity and genetic adaption in determining the distribution of species? Lastly, abiotic and biotic factors other than temperature (e.g. moisture, oxygen, predation) can also affect embryonic fitness, and may impose selection for adaptive modifications of developmental norms of reaction. However, the response of embryos to environmental factors other than temperature has not been extensively studied. Additionally, interactions between factors may be important, reflecting the complexity that occurs in nature. For example, the ability of reptile embryos to tolerate high temperatures is reduced by hypoxia [82]. Interactions among these abiotic and biotic factors provide abundant opportunities for future studies to explore the adaptive responses of embryos to ambient conditions in the nest.

(b) Ecological consequence of thermal adaptive responses in embryos

Knowing that vertebrate embryos have evolved a variety of strategies in response to thermal variation in the nest is important, but it is only the first step. The ecological consequences of these adaptive responses have not been explicitly elucidated, because determining the fitness consequence of embryonic responses to environmental variations is a challenging task. We need well-designed long-term experiments to evaluate the fitness of offspring under ecologically relevant conditions, and such manipulative field experiments are notoriously difficult to conduct. It is clear that the adaptive responses of embryos to their environment have induced variations in fitness-related phenotypic traits of the offspring (e.g. body size and locomotor performance) [83], but the fitness consequences of these adaptive responses are rarely addressed (but see [84,85]). We need long-term fieldwork with factorial experimental designs that follow individuals to maturity and quantify fitness (lifetime reproductive success) in nature [5].

(c) Biochemical and genetic bases underlying embryonic adaptation

Another gap in our understanding involves the proximate mechanisms at molecular, biochemical and physiological levels by which embryos adapt to environmental variation. Recent developments in physiological and molecular technology provide multiple ways to approach the proximate mechanisms underlying embryonic adaptation. Hormones play a critical role in physiological regulation in response to environmental stress during both embryonic development and post-embryonic stages, so that understanding the hormonal regulation of specific physiological processes is an important step towards identifying proximate mechanisms [86]. For example, modifications of thyroid hormone levels could adjust the density and function of mitochondria [87], and developmental rate of the embryo [88]. Furthermore, multi-omics profiling offers a powerful tool to reveal genomic response to thermal variation. For example, *de novo* transcriptomic profiling has enabled ecologists to identify a number of genes associated with heat tolerance, or detrimental effects on the embryo in embryonic sea turtles *Caretta caretta*, subject to biologically realistic thermal stresses [89]. The decreasing costs of next-generation sequencing (NGS) technologies will make these approaches increasingly accessible to evolutionary biologists working on non-model organisms. Moreover, identifying genes underpinning those adaptive responses is critical for unravelling the molecular mechanisms of thermal adaptation. Transgenic technology (e.g. gene knockout) has been widely used to study gene function in model organisms, but is challenging in non-model animals [90]. Nonetheless, RNA interference and vector-mediated gene transfer techniques provide an alternative way to manipulate gene expression [91]. The proximate mechanisms underlying embryonic adaptation are an exciting area for future research.

7. Conclusion

(1) Embryos of oviparous species are directly exposed to the external (nest) environment and thus experience temperatures that change at a variety of temporal and spatial scales. Some of these changes in nest temperature are predictable, and may induce adaptive responses by embryos.

(2) The predictable changes in nest temperatures not only occur at large scales (e.g. associated with season or latitude), but also at local scales and even within a single nest. Nest temperatures increase from spring to summer, decrease from summer to autumn and increase with global warming. Nest temperatures decrease with latitude and elevation as well as depth within a nest.

(3) The physiology (e.g. thermal tolerance, developmental rate) of embryos may evolve to match the thermal environment experienced by them (e.g. early versus late eggs laid in the season; cool- versus warm-climate eggs). Nonetheless, identifying the adaptive physiological strategies of embryos in response to those complex patterns of spatial and temporal variation in nest temperatures remains a major challenge.

(4) The proximate mechanisms underlying embryonic adaptation provide ample exciting opportunities for future research, especially given the rapid developments in molecular technology (e.g. multi-omics, gene manipulation techniques).

(5) A better understanding of this topic will clarify not only the ways in which abiotic pressures impose selection on biological attributes in an understudied life-history stage, but also can assist in planning conservation and management by identifying high-priority species and populations, and predicting a species's vulnerability to global change.

Data accessibility. This article has no additional data.

Competing interests. We declare we have no competing interests.

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References

1. Deeming DC, Ferguson MWJ. 1991 *Egg incubation: its effect on embryonic development in birds and reptiles*. Cambridge, UK: Cambridge University Press.
2. Deeming DC. 2004 *Reptilian incubation: environment, evolution and behaviour*. Nottingham, UK: Nottingham University Press.
3. DuRant SE, Hopkins WA, Hepp GR, Walters JR. 2013 Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biol. Rev.* **88**, 499–509. (doi:10.1111/brv.12015)
4. Doody JS, Guarino E, Georges A, Corey B, Murray G, Ewert M. 2006 Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. *Evol. Ecol.* **20**, 307–330. (doi:10.1007/s10682-006-0003-2)
5. Sun BJ, Ma L, Li SR, Williams CM, Wang Y, Hao X, Du WG. 2018 Phenology and the physiological niche are co-adapted in a desert-dwelling lizard. *Funct. Ecol.* **32**, 2520–2530. (doi:10.1111/1365-2435.13201)
6. Noble DWA, Stenhouse V, Schwanz LE. 2018 Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biol. Rev.* **93**, 72–97. (doi:10.1111/brv.12333)
7. Bozinovic F, Calosi P, Spicer JI. 2011 Physiological correlates of geographic range in animals. *Annu. Rev. Ecol. Evol. Syst.* **42**, 155–179. (doi:10.1146/annurev-ecolsys-102710-145055)
8. Williams CM *et al.* 2017 Understanding evolutionary impacts of seasonality: an introduction to the symposium. *Integr. Comp. Biol.* **57**, 921–933. (doi:10.1093/icb/icx122)
9. Du WG, Shine R. 2015 The behavioural and physiological strategies of bird and reptile embryos in response to unpredictable variation in nest temperature. *Biol. Rev.* **90**, 19–30. (doi:10.1111/brv.12089)
10. Warkentin KM, Caldwell MS. 2009 Assessing risk: embryos, information, and escape hatching. In *Cognitive ecology II* (eds R Dukas, JM Ratcliffe),

pp. 177–200. Chicago, IL: University of Chicago Press.

11. Thompson MB. 1988 Nest temperatures in the Pleurodiran turtle, *Emydura macquarii*. *Copeia* **1988**, 996–1000. (doi:10.2307/1445723)
12. Packard GC, Paukstis GL, Boardman TJ, Gutzke WH.N. 1985 Daily and seasonal variation in hydric conditions and temperature inside nests of common snapping turtles (*Chelydra serpentina*). *Can. J. Zool.* **63**, 2422–2429. (doi:10.1139/z85-358)
13. Kaska Y, Downie R, Tippett R, Furness RW. 1998 Natural temperature regimes for loggerhead and green turtle nests in the eastern Mediterranean. *Can. J. Zool.* **76**, 723–729. (doi:10.1139/cjz-76-4-723)
14. Murray CM, Easter M, Padilla S, Marin MS, Guyer C. 2016 Regional warming and the thermal regimes of American crocodile nests in the Tempisque Basin, Costa Rica. *J. Therm. Biol* **60**, 49–59. (doi:10.1016/j.jtherbio.2016.06.004)
15. Booth DT, Astill K. 2001 Temperature variation within and between nests of the green sea turtle, *Chelonia mydas* (Chelonia: Cheloniidae) on Heron Island, Great Barrier Reef. *Aust. J. Zool.* **49**, 71–84. (doi:10.1071/ZO00059)
16. Spencer RJ, Janzen FJ. 2011 Hatching behavior in turtles. *Integr. Comp. Biol.* **51**, 100–110. (doi:10.1093/icb/icr045)
17. Zbinden JA, Margaritoulis D, Arlettaz R. 2006 Metabolic heating in Mediterranean loggerhead sea turtle clutches. *J. Exp. Mar. Biol. Ecol.* **334**, 151–157. (doi:10.1016/j.jembe.2006.01.021)
18. Wallace BP, Sotherland PR, Spotila JR, Reina RD, Franks BF, Paladino FV. 2004 Biotic and abiotic factors affect the nest environment of embryonic leatherback turtles, *Dermochelys coriacea*. *Physiol. Biochem. Zool.* **77**, 423–432. (doi:10.1086/420951)
19. Boulton RL, Cassey P. 2012 How avian incubation behaviour influences egg surface temperatures: relationships with egg position, development and clutch size. *J. Avian Biol.* **43**, 289–296. (doi:10.1111/j.1600-048X.2012.05657.x)
20. Yang C, Huang Q, Wang L, Du W-G, Liang W, Moller AP. 2018 Keeping eggs warm: thermal and developmental advantages for parasitic cuckoos of laying unusually thick-shelled eggs. *Sci. Nat.* **105**, 10. (doi:10.1007/s00114-017-1532-y)
21. Löwenborg K, Shine R, Karvemo S, Hagman M. 2010 Grass snakes exploit anthropogenic heat sources to overcome distributional limits imposed by oviparity. *Funct. Ecol.* **24**, 1095–1102. (doi:10.1111/j.1365-2435.2010.01730.x)
22. Shine R, Madsen TRL, Elphick MJ, Harlow PS. 1997 The influence of nest temperatures and maternal brooding on hatchling phenotypes in water pythons. *Ecology* **78**, 1713–1721. (doi:10.1890/0012-9658(1997)078[1713:TIONTA]2.0.CO;2)
23. Löwenborg K, Gotthard K, Hagman M. 2012 How a thermal dichotomy in nesting environments influences offspring of the world's most northerly oviparous snake, *Natrix natrix* (Colubridae). *Biol. J. Linn. Soc.* **107**, 833–844. (doi:10.1111/j.1095-8312.2012.01972.x)
24. van Dijk RE, Kaden JC, Argueelles-Tico A, Beltran LM, Paquet M, Covas R, Doutrelant C, Hatchwell BJ. 2013 The thermoregulatory benefits of the communal nest of sociable weavers *Philetairus socius* are spatially structured within nests. *J. Avian Biol.* **44**, 102–110. (doi:10.1111/j.1600-048X.2012.05797.x)
25. Weber SB, Broderick AC, Groothuis TGG, Ellick J, Godley BJ, Blount JD. 2012 Fine-scale thermal adaptation in a green turtle nesting population. *Proc. R. Soc. B* **279**, 1077–1084. (doi:10.1098/rspb.2011.1238)
26. Kolbe JJ, Janzen FJ. 2002 Impact of nest-site selection on nest success and nest temperature in natural and disturbed habitats. *Ecology* **83**, 269–281. (doi:10.2307/2680137)
27. Li T, Cao P, Bei YJ, Du WG. 2018 Latitudinal and temperature dependent variation in embryonic development rate and offspring performance in a freshwater turtle. *Physiol. Biochem. Zool.* **91**, 673–681. (doi:10.1086/694856)
28. Refsnider JM, Milne-Zelman C, Warner DA, Janzen FJ. 2014 Population sex ratios under differing local climates in a reptile with environmental sex determination. *Evol. Ecol.* **28**, 977–989. (doi:10.1007/s10682-014-9710-2)
29. Ewert MA, Lang JW, Nelson CE. 2005 Geographic variation in the pattern of temperature-dependent sex determination in the American snapping turtle (*Chelydra serpentina*). *J. Zool.* **265**, 81–95. (doi:10.1017/S0952836904006120)
30. Du WG, Elphick M, Shine R. 2010 Thermal regimes during incubation do not affect mean selected temperatures of hatchling lizards (*Bassiana duperreyi*, Scincidae). *J. Therm. Biol* **35**, 47–51. (doi:10.1016/j.jtherbio.2009.10.007)
31. Shine R, Elphick MJ, Barrott EG. 2003 Sunny side up: lethally high, not low, nest temperatures may prevent oviparous reptiles from reproducing at high elevations. *Biol. J. Linn. Soc.* **78**, 325–334. (doi:10.1046/j.1095-8312.2003.00140.x)
32. Webb DR. 1987 Thermal tolerance of avian embryos: a review. *Condor* **89**, 874–898. (doi:10.2307/1368537)
33. Heenan CB, Goodman BA, White CR. 2015 The influence of climate on avian nest construction across large geographical gradients. *Glob. Ecol. Biogeogr.* **24**, 1203–1211. (doi:10.1111/geb.12378)
34. Matysiokova B, Remes V. 2018 Evolution of parental activity at the nest is shaped by the risk of nest predation and ambient temperature across bird species. *Evolution* **72**, 2214–2224. (doi:10.1111/evo.13580)
35. Irving L, Krog J. 1956 Temperature during the development of birds in arctic nests. *Physiol. Zool.* **29**, 195–205. (doi:10.1086/physzool.29.3.30155337)
36. Deeming DC. 2002 *Avian incubation: behaviour, environment and evolution*. Oxford, UK: Oxford University Press.
37. Shine R. 2004 Seasonal shifts in nest temperature can modify the phenotypes of hatchling lizards, regardless of overall mean incubation temperature. *Funct. Ecol.* **18**, 43–49. (doi:10.1046/j.0269-8463.2004.00806.x)
38. Pearson PR, Warner DA. 2018 Early hatching enhances survival despite beneficial phenotypic effects of late-season developmental environments. *Proc. R. Soc. B* **285**, 20180256. (doi:10.1098/rspb.2018.0256)
39. Mainwaring MC, Hartley IR. 2008 Seasonal adjustments in nest cup lining in blue tits *Cyanistes caeruleus*. *Ardea* **96**, 278–282. (doi:10.5253/078.096.0213)
40. Ehmann H, Swan G, Smith B. 1991 Nesting, egg incubation and hatching by the heath monitor *Varanus rosenbergi* in a termite mound. *Herpetofauna* **21**, 17–24.
41. Magnusson WE, Lima AP, Sampaio RM. 1985 Source of heat for nests of *Paleosuchus trigonatus* and a review of crocodilian nest temperatures. *J. Herpetol.* **19**, 199–207. (doi:10.2307/1564173)
42. Werner DI. 1983 Reproduction in the iguana *Conolophus subcristatus* on Fernandina Island, Galapagos: clutch size and migration costs. *Am. Nat.* **121**, 757–775. (doi:10.1086/284103)
43. Telemeco RS, Elphick MJ, Shine R. 2009 Nesting lizards (*Bassiana duperreyi*) compensate partly, but not completely, for climate change. *Ecology* **90**, 17–22. (doi:10.1890/08-1452.1)
44. Pike DA. 2014 Forecasting the viability of sea turtle eggs in a warming world. *Glob. Chang. Biol.* **20**, 7–15. (doi:10.1111/gcb.12397)
45. Jensen MP, Allen CD, Eguchi T, Bell IP, LaCasella EL, Hilton WA, Hof CAM., Dutton PH. 2018 Environmental warming and feminization of one of the largest sea turtle populations in the world. *Curr. Biol.* **28**, 154–159. (doi:10.1016/j.cub.2017.11.057)
46. Laloe J-O, Esteban N, Berkel J, Hays GC. 2016 Sand temperatures for nesting sea turtles in the Caribbean: implications for hatchling sex ratios in the face of climate change. *J. Exp. Mar. Biol. Ecol.* **474**, 92–99. (doi:10.1016/j.jembe.2015.09.015)
47. Hall JM, Warner DA. 2018 Thermal spikes from the urban heat island increase mortality and alter physiology of lizard embryos. *J. Exp. Biol.* **22**, jeb181552. (doi:10.1242/jeb.181552)
48. Francis EA, Moldowan PD, Greischar MA, Rollinson N. 2019 Anthropogenic nest sites provide warmer incubation environments than natural nest sites in a population of oviparous reptiles near their northern range limit. *Oecologia* **190**, 511–522. (doi:10.1007/s00442-019-04383-3)
49. Loudon FK. 2014 Influence of temperature on the life history of turtles: an exploration of the embryonic and maternal adaptions to incubation temperature. PhD thesis, University of Western Sydney, Australia.
50. Huo J, Su T, Niu N, Yang C, Liang W. 2018 Last but not the least: effects of laying sequence on egg color variation and embryonic development of Russet Sparrow (*Passer cinnamomeus*). *Avian Res.* **9**, 21. (doi:10.1186/s40657-018-0113-x)
51. Boonstra TA, Clark ME, Reed WL. 2010 Position in the sequence of laying, embryonic metabolic rate, and consequences for hatching synchrony and

offspring survival in Canada geese. *Condor* **112**, 304–313. (doi:10.1525/cond.2010.090043)

52. Zamudio KR, Bell RC, Mason NA. 2016 Phenotypes in phylogeography: species' traits, environmental variation, and vertebrate diversification. *Proc. Natl Acad. Sci. USA* **113**, 8041–8048. (doi:10.1073/pnas.1602237113)
53. Roosenburg WM. 1996 Maternal condition and nest site choice: an alternative for the maintenance of environmental sex determination? *Am. Zool.* **36**, 157–168. (doi:10.1093/icb/36.2.157)
54. Shine R, Elphick MJ, Harlow PS. 1997 The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* **78**, 2559–2568. (doi:10.1890/0012-9658(1997)078[2559:TIONIE]2.0.CO;2)
55. Angilletta MJ, Oufiero CE, Sears MW. 2004 Thermal adaptation of maternal and embryonic phenotypes in a geographically widespread ectotherm. *Int. Congr. Ser.* **1275**, 258–266. (doi:10.1016/j.ics.2004.07.038)
56. Sunday JM, Bates AE, Dulvy NK. 2011 Global analysis of thermal tolerance and latitude in ectotherms. *Proc. R. Soc. B* **278**, 1823–1830. (doi:10.1098/rspb.2010.1295)
57. Andrews RM, Schwarzkopf L. 2012 Thermal performance of squamate embryos with respect to climate, adult life history, and phylogeny. *Biol. J. Linn. Soc.* **106**, 851–864. (doi:10.1111/j.1095-8312.2012.01901.x)
58. Angilletta MJ, Zelic MH, Adrian GJ, Hurliman AM, Smith CD. 2013 Heat tolerance during embryonic development has not diverged among populations of a widespread species (*Sceloporus undulatus*). *Conserv. Physiol.* **1**, cot018. (doi:10.1093/conphys/cot018)
59. Ji X, Huang HY, Hu XZ, Du WG. 2002 Geographic variation in female reproductive characteristics and egg incubation of *Eumeces chinensis*. *Chin. J. Appl. Ecol.* **13**, 680–684.
60. Shine R, Olsson M. 2003 When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizards (Scincidae). *J. Evol. Biol.* **16**, 823–832. (doi:10.1046/j.1420-9101.2003.00600.x)
61. Oufiero CE, Angilletta MJ. 2006 Convergent evolution of embryonic growth and development in the eastern fence lizard (*Sceloporus undulatus*). *Evolution* **60**, 1066–1075. (doi:10.1111/j.0014-3820.2006.tb01183.x)
62. Du WG, Warner DA, Langkilde T, Robbins T, Shine R. 2010 The physiological basis of geographic variation in rates of embryonic development within a widespread lizard species. *Am. Nat.* **176**, 522–528. (doi:10.1086/656270)
63. Sun BJ, Li SR, Xu XF, Zhao WG, Luo LG, Ji X, Du WG. 2013 Different mechanisms lead to convergence of reproductive strategies in two lacertid lizards (*Takydromus wolteri* and *Eremias argus*). *Oecologia* **172**, 645–652. (doi:10.1007/s00442-012-2524-4)
64. Ewert MA. 1985 Embryology of turtles. In *Biology of the Reptilia* (eds C Gans, F Billett, PFA Maderson), pp. 75–268. New York, NY: John Wiley and Sons.
65. Colbert PL, Spencer RJ, Janzen FJ. 2010 Mechanism and cost of synchronous hatching. *Funct. Ecol.* **24**, 112–121. (doi:10.1111/j.1365-2435.2009.01602.x)
66. Zhao B, Chen Y, Lu HL, Zeng ZG, Du WG. 2015 Latitudinal differences in temperature effects on the embryonic development and hatchling phenotypes of the Asian yellow pond turtle, *Mauremys mutica*. *Biol. J. Linn. Soc.* **114**, 35–43. (doi:10.1111/bij.12400)
67. Telemeco RS, Radder RS, Baird TA, Shine R. 2010 Thermal effects on reptile reproduction: adaptation and phenotypic plasticity in a montane lizard. *Biol. J. Linn. Soc.* **100**, 642–655. (doi:10.1111/j.1095-8312.2010.01439.x)
68. Verdu-Ricoy J, Iraeta P, Salvador A, Diaz JA. 2014 Phenotypic responses to incubation conditions in ecologically distinct populations of a lacertid lizard: a tale of two phylogeographic lineages. *J. Zool.* **292**, 184–191. (doi:10.1111/jzo.12091)
69. Rodríguez-Díaz T, Braña F. 2012 Altitudinal variation in egg retention and rates of embryonic development in oviparous *Zootoca vivipara* fits predictions from the cold-climate model on the evolution of viviparity. *J. Evol. Biol.* **25**, 1877–1887. (doi:10.1111/j.1420-9101.2012.02575.x)
70. Jia CX, Sun YH, Swenson JE. 2010 Unusual incubation behavior and embryonic tolerance of hypothermia by the blood pheasant (*Ithaginis cruentus*). *Auk* **127**, 926–931. (doi:10.1525/auk.2010.09254)
71. Warner DA, Shine R. 2007 Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. *Oecologia* **154**, 65–73. (doi:10.1007/s00442-007-0809-9)
72. Olsson M, Shine R. 1997 The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why early clutches are better. *J. Evol. Biol.* **10**, 369–381. (doi:10.1007/s000360050030)
73. Garcia-Navas V, Jose Sanz J. 2011 Seasonal decline in provisioning effort and nestling mass of blue tits *Cyanistes caeruleus*: experimental support for the parent quality hypothesis. *Ibis* **153**, 59–69. (doi:10.1111/j.1474-919X.2010.01077.x)
74. Le Henanff M, Meylan S, Lourdais O. 2013 The sooner the better: reproductive phenology drives ontogenetic trajectories in a temperate squamate (*Podarcis muralis*). *Biol. J. Linn. Soc.* **108**, 384–395. (doi:10.1111/j.1095-8312.2012.02005.x)
75. Reed WL, Clark ME. 2011 Beyond maternal effects in birds: responses of the embryo to the environment. *Integr. Comp. Biol.* **51**, 73–80. (doi:10.1093/icb/icr032)
76. Bowden RM, Ewert MA, Nelson CE. 2000 Environmental sex determination in a reptile varies seasonally and with yolk hormones. *Proc. R. Soc. Lond. B* **267**, 1745–1749. (doi:10.1098/rspb.2000.1205)
77. Kelly M. 2019 Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Phil. Trans. R. Soc. B* **374**, 20180176. (doi:10.1098/rstb.2018.0176)
78. Becker ME, Weisberg PJ. 2014 Synergistic effects of spring temperatures and land cover on nest survival of urban birds. *Condor* **117**, 18–30. (doi:10.1650/CONDOR-14-1.1)
79. Reyna KS, Burggren WW. 2017 Altered embryonic development in northern bobwhite quail (*Colinus virginianus*) induced by pre-incubation oscillatory thermal stresses mimicking global warming predictions. *PLoS ONE* **12**, e0184670. (doi:10.1371/journal.pone.0184670)
80. Janzen FJ, Morjan CL. 2001 Repeatability of microenvironment-specific nesting behaviour in a turtle with environmental sex determination. *Anim. Behav.* **62**, 73–82. (doi:10.1006/anbe.2000.1732)
81. Schwanz LE, Bowden RM, Spencer RJ, Janzen FJ. 2009 Nesting ecology and offspring recruitment in a long-lived turtle. *Ecology* **90**, 1709–1710. (doi:10.1890/08-1676.1)
82. Smith C, Telemeco RS, Angilletta MJ, VandenBrooks JM. 2015 Oxygen supply limits the heat tolerance of lizard embryos. *Biol. Lett.* **11**, 20150113. (doi:10.1098/rsbl.2015.0113)
83. Ma L, Sun BJ, Li SR, Sha W, Du WG. 2014 Maternal thermal environment induces plastic responses in the reproductive life history of oviparous lizards. *Physiol. Biochem. Zool.* **87**, 677–683. (doi:10.1086/678050)
84. Warner DA, Andrews RM. 2002 Laboratory and field experiments identify sources of variation in phenotypes and survival of hatchling lizards. *Biol. J. Linn. Soc.* **76**, 105–124. (doi:10.1111/j.1095-8312.2002.tb01718.x)
85. Dayananda B, Gray S, Pike D, Webb JK. 2016 Communal nesting under climate change: fitness consequences of higher incubation temperatures for a nocturnal lizard. *Glob. Chang. Biol.* **22**, 2405–2414. (doi:10.1111/gcb.13231)
86. Cohen AA, Martin LB, Wingfield JC, McWilliams SR, Dunne JA. 2012 Physiological regulatory networks: ecological roles and evolutionary constraints. *Trends Ecol. Evol.* **27**, 428–435. (doi:10.1016/j.tree.2012.04.008)
87. Lanni A, Moreno M, Goglia F. 2016 Mitochondrial actions of thyroid hormone. *Compr. Physiol.* **6**, 1591–1607. (doi:10.1002/cphy.c150019)
88. McGlashan JK, Thompson MB, Dyke JUV, Spencer R-J. 2017 Thyroid hormones reduce incubation period without developmental or metabolic costs in Murray river short-necked turtles (*Emydura macquarii*). *Physiol. Biochem. Zool.* **90**, 34–46. (doi:10.1086/689744)
89. Bentley BP, Haas BJ, Tedeschi JN, Berry O. 2017 Loggerhead sea turtle embryos (*Caretta caretta*) regulate expression of stress response and developmental genes when exposed to a biologically realistic heat stress. *Mol. Ecol.* **26**, 2978–2992. (doi:10.1111/mec.14087)
90. Sui GC, Soohoo C, Affar E, Gay F, Shi YJ, Forrester WC, Shi Y. 2002 A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. *Proc. Natl Acad. Sci. USA* **99**, 5515–5520. (doi:10.1073/pnas.082117599)
91. Gao J, Zhang W, Dang W, Mou Y, Gao Y, Sun BJ, Du WG. 2014 Heat shock protein expression enhances heat tolerance of reptile embryos. *Proc. R. Soc. B* **281**, 20141135. (doi:10.1098/rspb.2014.1135)