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REVIEW

Lessons from evolution: developmental plasticity in vertebrates with complex life cycles

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Developmental plasticity is the property of a given genotype to produce different phenotypes in response to the environmental conditions experienced during development. Chordates have two basic modes of development, direct and indirect. Direct development (mode of humans) was derived evolutionarily from indirect development (mode of many amphibians), the major difference being the presence of a larval stage with indirect development; larvae undergo metamorphosis to the juvenile adult. In amphibians, environmental conditions experienced during the larval stage can lead to extreme plasticity in behaviour, morphology and the timing of metamorphosis and can cause variation in adult phenotypic expression (carry-over effects, or developmental programming). Hormones of the neuroendocrine stress axis play pivotal roles in mediating environmental effects on animal development. Stress hormones, produced in response to a deteriorating larval habitat, accelerate amphibian metamorphosis; in mammals, stress hormones hasten the onset of parturition and play an important role in pre-term birth caused by intra-uterine stress. While stress hormones can promote survival in a deteriorating larval or intra-uterine habitat, costs may be incurred, such as reduced growth and size at metamorphosis or birth. Furthermore, exposure to elevated stress hormones during the tadpole or foetal stage can cause permanent neurological changes, leading to altered physiology and behaviour later in life. The actions of stress hormones in animal development are evolutionarily conserved, and therefore amphibians can serve as important model organisms for research on the mechanisms of developmental plasticity.

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Introduction

The developmental origins of disease hypothesis[1] was developed to explain the impact of the environment experienced during early human development on later life health and disease. The long-term effects of the environment experienced by developing organisms on phenotypic expression has long been known in diverse species from plants to vertebrate animals.[2,3] The term phenotypic plasticity is commonly used to describe the property of a given genotype to produce different phenotypes in response to different environmental conditions.[2,4] Developmental plasticity is a form of phenotypic plasticity, in which the environment experienced during development influences the phenotype expressed.[3] Virtually all organisms display some form of developmental plasticity, which is now recognized as an important effector of evolutionary change.[2,5–7]

Developmental plasticity may generate adaptive morphological, physiological or behavioural traits that promote survival during embryonic or early post-embryonic life. For example, the presence of invertebrate or vertebrate prey can induce dramatic changes in cranial and jaw morphology of amphibian larvae, which allow them to capitalize on the protein-rich diet, and may thus enhance fitness (discussed below). Experiences during early life stages that cause, or are independent of, discrete plastic responses during these stages can influence traits expressed in the juvenile or adult stage of the life cycle. Such influences are complex and can be difficult to isolate since the sets of traits may be co-dependent, complementary, co-specialised or compensatory.[5] Furthermore, the consequences of some developmental experiences may not be recognizable until the juvenile or adult stage, perhaps being exposed by the physiological, social or environmental factors experienced during these later life stages; for example, early life exposure to stressors (elevated glucocorticoids) can cause long-term stable changes in physiology and behaviour that are only seen in juvenile/adult animals, and their expression may depend on the nutritional, physiological or social context.[8] Such phenomena are called 'carry-over' effects, or developmental 'programming' – experiences during one life history stage (generally during early development) that affect phenotypic expression in a subsequent life history stage.

There are many examples of developmental plasticity in diverse taxa, far too numerous to describe here. The reader is referred to the book, *Developmental Plasticity*, by Mary Jane West-Eberhard[3] for a comprehensive discussion of this topic. Here we focus primarily on amphibians as case studies for organismal responses to environmental change that lead to variable phenotypic expression at different stages of the life

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cycle. Where appropriate, we draw parallels with mammals, including humans. Most amphibians have complex life cycles (discussed below), with a free-swimming larval stage that undergoes a metamorphosis to the juvenile adult. The amphibian larva, which is a post-embryonic feeding stage, is exposed to diverse environmental conditions that can impact its morphology, behaviour, timing of metamorphosis and subsequently the juvenile/adult phenotype. Hormones play central roles in mediating environmental effects on development; the endocrine system of tetrapod vertebrates is evolutionarily conserved, and recent findings show that hormone action during amphibian development causes similar phenotypic outcomes to those observed in mammals. Therefore, we discuss the endocrine and molecular mechanisms that underlie developmentally plastic responses in amphibians, and describe how amphibian model systems can contribute to the study of developmental plasticity and programming in vertebrates.

Modes of animal development

Animals have two basic modes of development (life history modes), direct and indirect (Fig. 1). The major difference between these developmental modes is that species with direct development do not have a larval phase, which is a growth and dispersal life history stage. Amniotes, which include the reptiles, birds and mammals, all have direct development. Many extant fishes and most extant amphibians have indirect development, although there are also examples of species in these classes that have direct development. Paleontological and phylogenetic evidence supports that the ancestral chordate mode of development was indirect, and that direct development evolved multiple times in different lineages.[9]

Animals with indirect modes of development are said to have complex life cycles, with a larval stage that is commonly a feeding stage of variable duration, and then metamorphosis to the juvenile adult form. Animals with direct development have a simple life cycle. Because larvae often exploit different ecological niches from adults they can avoid competition for resources. The complex life cycle, which generates a 'sequential polymorphism',[10] may have evolved to match the body plan to the physical environment (e.g. water *v*. air, limnetic *v*. benthic) throughout an individual's lifetime, thus allowing species to exploit different habitats at different life history stages. The ability to change form and/or function across life stages allows pre- and post-metamorphic body forms to specialize in growth, dispersive or reproductive roles

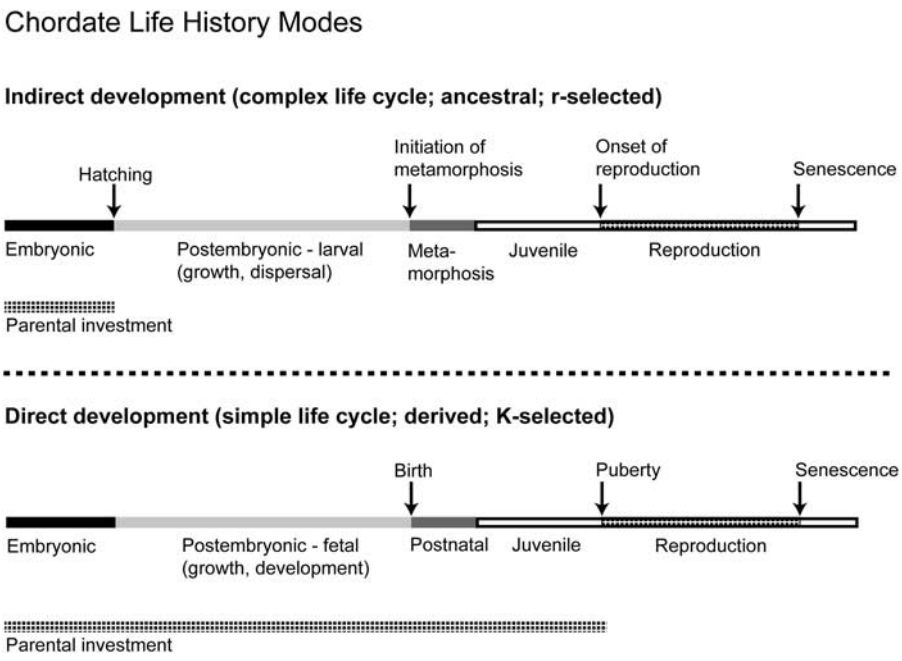

Fig. 1. Comparison of the two general chordate life history modes, indirect and direct development. Species with indirect development have complex life cycles with a free-living larval phase, which is a growth and dispersal life history stage. The larvae then undergo a metamorphosis to the juvenile adult, which is the reproductive life stage. Some species become reproductively mature while retaining larval characteristics (neoteny or paedomorphosis). Indirect development represents the ancestral life history mode of chordates. Species with complex life cycles are generally r-selected, that is, they have high fecundity, offspring are often dispersed widely, parental investment is often limited to the deposition of yolk in the eggs, among other characteristics. Species with direct development are said to have simple life cycles characterized by the lack of a larval stage. This developmental strategy was derived evolutionarily from indirect development. Species with simple life cycles are generally K-selected, that is, they produce fewer offspring that require extensive parental care until they mature, among other characteristics. For both indirect and direct development, the arrows indicate significant life history stage transitions. The indicated durations of each life history stage is not to scale and varies widely among species.

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when those components of fitness are concentrated in a particular life stage.[11]

For organisms in which juvenile and adult phenotypes are not independent, phenotypes exhibited in one stage may reflect the action of selective pressures in the other stage.[12] Extreme differences in habitat and lifestyle in different stages of a life cycle may even result in opposing selective pressures and thus some conflict in phenotype. For example, among Darwin's finches, which have a simple life cycle, juveniles experience selection for small body size while adults are selected for large body size; as body size is correlated through development, adaptive change in one stage may be accompanied by maladaptive responses in another stage.[12,13] As organisms with complex life cycles often experience dramatic niche shifts in subsequent life history stages, individuals are expected to experience increasingly dissimilar selective regimes across the metamorphic boundary, and thus the existence of phenotypic correlations between character states in subsequent stages would have important consequences for both individual fitness and life cycle evolution. Although some phenotypic correlations among stages of a complex life cycle may be the result of pleiotropy or linkage disequilibrium, such carry-over effects may also arise when an environmentally induced phenotype influences the development of other characters. Research on different taxa with complex life cycles has shown that larval history can substantially impact post-metamorphic traits.[14]

Environmentally induced phenotypic correlations between stages of a complex life cycle can arise through direct effects on phenotypic expression, for example, direct influence on the development of cells, tissues and organs, leading to long-term changes in form and function.[15] For example, in wood frogs, predator-induced changes in tadpole body allometry correspond with changes in juvenile traits such as leg length.[16] Exposure to stressful environmental conditions may affect neural circuits or metabolic pathways in the larva (perhaps mediated by stress hormones[17]) that lead to long-term programming of behaviour or metabolism expressed in the juvenile adult. Alternatively, the environment can indirectly affect post-metamorphic phenotype by altering larval life history traits such as developmental timing that can affect size at transformation.[18]

Most amphibians have complex life cycles, and the aquatic larvae of anuran amphibians (frogs and toads; tadpoles) are perhaps the best-studied chordates with indirect developmental modes. Environmental conditions experienced during the larval stage, such as conspecific density, food availability, pond drying and predation risk, affect metamorphic timing, body size and morphology of the tadpole and can also lead to variation in adult phenotypic expression. On reaching a species-dependent minimum body size, tadpoles develop competence to undergo metamorphosis, characterized by dramatic morphological, biochemical and physiological transformation into the terrestrial juvenile adult (Fig. 2). The timing of the initiation of metamorphosis is strongly influenced by the external environment, and is controlled by the production of hormones by the thyroid gland (iodothyronines; thyroxine – T_4; 3,5,3′-triiodothyronine – T_3). Iodothyronines are necessary for the metamorphosis of amphibians,[19] flatfishes[20] and echinoderms[21–23] and they play essential roles in the development of direct-developing vertebrates such as humans, in whom thyroid deficiency during foetal or neonatal life causes profound mental retardation and skeletal malformation (cretinism).[24] On account of their external, free-living post-embryonic stage of development, amphibians are ideal for investigating environmental effects on early development, the roles of hormones in mediating these effects and impacts on future phenotypic expression and fitness.

Fig. 2. (Colour online) Metamorphosis of the South African clawed frog *Xenopus laevis*. Shown are tadpoles in premetamorphosis (top), prometamorphosis (middle) and metamorphic climax (bottom). The tadpole is a growth stage in the anuran amphibian life cycle (premetamorphosis). The first external sign that metamorphosis has begun is the growth and differentiation of the hind limbs (prometamorphosis). Metamorphic climax is a period of rapid and dramatic tissue transformations that involve cell proliferation (e.g. the limbs), cell death (e.g. the tail) and tissue remodelling (e.g. the intestine and brain). Metamorphosis is controlled by thyroid hormone, and thyroid hormone is controlled by the stress neuropeptide corticotropin releasing factor that stimulates the release of pituitary thyroid-stimulating hormone.[31] Photos by David Bay.

Developmental plasticity that promotes survival to metamorphosis

Environmental conditions experienced during the tadpole stage can have profound effects on tadpole behaviour, metamorphic timing, body size and morphology, which can influence survival to metamorphosis.[25–30] Under deteriorating environmental conditions (i.e. limited resources, high predation pressure and habitat desiccation) growth is generally reduced, and if experienced early in development, the

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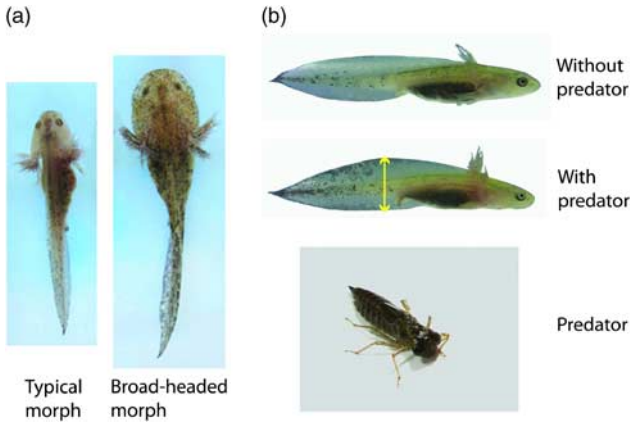

Fig. 3. (Colour online) Phenotypic plasticity caused by the presence of prey (*a*) or predators (*b*) in larvae of the salamander, *Hynobius retardatus*. In panel *a* the typical and broad headed morphs are shown at the same scale. Note the larger body size and head shape of the broad-headed morph.[33,78] In panel *b*, salamander larvae were raised without (top) or with (bottom) caged predators (dragonfly larvae) for 28 days. Typical morphs responded to predation threat by developing deeper tail fins than in the absence of predators (the arrow shows the increased tail height in larvae raised in the presence of predators[109]; photos courtesy of Hirofumi Michimae).

rate of development may be slowed. However, after a minimum body size and developmental stage is reached, when the animals have become competent to upregulate thyroid hormone production, tadpoles may respond to adverse environmental conditions by accelerating metamorphosis.[31]

Amphibian larvae have a remarkable capacity for behavioural and morphological plasticity, which are often adaptive responses to variations in the developmental habitat.[32,33] For example, prey presence and type of prey can have profound effects on larval morphology. In tadpoles of some spadefoot toad species, the presence of fairy shrimp in the larval habitat leads to the generation of a carnivorous morphology characterized by dramatic changes in jaw musculature, and mouth and beak morphology.[34,35] In the larvae of the Hokkaido salamander the presence of prey (tadpoles) induces the broad-headed morphology[36,37] (Fig. 3a). Remarkably, this morphology can be induced in salamander larvae simply by exposing them to the rhythmic water currents generated by the flapping motion of tadpole tails in their environment.[38]

The presence of predators influences tadpole behaviour, morphology and rates of growth and development. A common behavioural response of tadpoles (and many other animals) to the presence of predators is to freeze. While the acute effects of predators are to inhibit swimming, chronic predator presence causes increased tail height (and in some species tail colour) and changes in body shape[39–41] (Fig. 3b). The increased size of the tail may serve to lure predators away from the more vulnerable body, and may improve escape behaviour through enhanced burst locomotion.[42] Chronic predator presence also causes complex and variable effects on the timing of metamorphosis (development rate) and size at transformation.[30,43–45]

The most important environmental variable for an aquatic organism such as an amphibian tadpole is the presence of water (indeed, the mammalian foetus is an aquatic organism, bathed in amniotic fluid until the transition to the terrestrial environment at parturition). Drying of the natal pond accelerates metamorphosis in many amphibian species, a form of developmental plasticity that affects the timing and size at transformation.[27,46] Developmental acceleration in response to pond drying is adaptive for amphibians that live in arid environments since it can increase the probability of survival to reproduction.[27,29] However, there are trade-offs to accelerated metamorphosis such as smaller body size at transformation, which may be associated with future fitness costs. For example, small post-metamorphic frogs may incur costs such as slower growth rates under natural conditions in which resources are limited; although they may have significant potential for catch-up growth when sufficient resources are available.[17] Larger juvenile amphibians have been shown to resist desiccation better, and travel through disturbed, open land with greater success.[47–49] In addition, larger metamorphs may have higher stored lipids,[50] which could allow them to better survive the high-density conditions immediately surrounding a pond. Behavioural measures also suggest that larger metamorphs are not as active on the terrestrial surface, perhaps due to a reduced need to forage, which would decrease their exposure to predators.[51] Small size at metamorphosis in nature may also lead to inferior locomotor ability, greater susceptibility to starvation and higher mortality.[28,50,52–59] The body size disadvantage at metamorphosis may be retained through the age at first reproduction, thus compromising reproductive fitness.[28,50,52,53,58]

Developmental plasticity that leads to variable phenotypic expression in the juvenile/adult stage

From arthropods to humans, it is clear that experiences during early development can carry over to subsequent life history stages affecting phenotypic expression in the juvenile adult.[14,60,61–63] As discussed earlier, there are two ways by which environmentally induced phenotypic correlations between stages of a life cycle can arise: through direct effects on phenotypic expression, or through indirect effects on traits such as body size at life history transitions caused by alterations in the timing of development. As an example of a direct effect, exposure to predation risk during the larval stage has been shown to generate longer limbs in several frog species,[15,16,56] and hind limb morphology is an important determinant of jumping distance in frogs, as longer legs increase leverage and greater muscle mass increases power.[64]

The timing of metamorphosis can indirectly affect traits expressed later in life through effects on body size at metamorphosis. Smaller metamorphic frogs tend to have lower rates of dispersal from the natal pond and survival to first

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reproduction compared with larger animals.[62,65] Frogs show a positive relationship between body size and fecundity such that larger females reach reproductive maturity earlier, and produce larger eggs and larger clutch sizes.[50,66,67] Changes in the age of first reproduction can have important demographic effects, as an earlier reproductive maturity increases the proportion of individuals that will survive to reproduce at least once. Earlier reproductive onset can thus substantially increase the population growth rate[68,69] as well as individual fitness (greater lifetime fecundity) in species that reproduce multiple times.[70]

It is now well established that experiences during early development can affect phenotype, and thus individual fitness in amphibians. However, the effect on fitness may be either negative or positive, depending on the context and informational content of the early experiences. In some cases, carry-over effects may be beneficial to the animal, such as when early-life cues convey information about the conditions likely to be encountered later. In such instances, it is useful for the animal to respond to those cues in such a way that modifies its behavioural repertoire, morphological characteristics or decision-making processes in preparation for the anticipated environment. However, in many cases the adaptive value of carry-over effects may be more complicated. For example, Benard and Fordyce[71] showed that the outcomes of larval experiences may be context-dependent. In western toads, juveniles that developed under larval predation risk produced a higher concentration of toxins found in the skin of adult toads that repel predators.[71] Such a carry-over effect in predation response is intriguing, given that the aquatic predation rate has no predictive value for subsequent terrestrial predation risk. In addition, these animals showed reduced survival with a toxin-resistant predator,[71] suggesting that there is a cost to producing the toxins. Thus, larval predator induction produces juvenile toads with greater protection against toxin-sensitive predators, but these animals had reduced capacity to defend themselves against predators that do not respond to toxins, meaning that the fitness outcome will depend on the type of predator encountered.

Hormones mediate genotype–environment interactions

Variation in organismal form, function and life history traits leads to variation in Darwinian fitness. Hormones have widespread and diverse actions in coordinating the expression of suites of phenotypic traits, and thus play a key role in determining fitness. In the words of Mary Jane West-Eberhard,[72] hormones '…link environmental, genetic and phenotypic variation to selection and evolution through their mediation of gene expression' (see also Gilbert and Epel[73]). The central roles of hormones, especially stress hormones, in amphibian developmental plasticity was recently reviewed.[8] Here we summarize some of the key concepts and their broader implications.

The neuroendocrine stress axis (the hypothalamo–pituitary–adrenal or HPA axis; in amphibians the hypothalamo–pituitary–interrenal axis) plays a central role in mediating physiological and behavioural responses to environmental change.[74] The major hormones produced within the HPA axis, which are evolutionarily conserved among vertebrates, are corticotropin-releasing factor (CRF) and related peptides produced in the hypothalamus, pituitary adrenocorticotropic hormone (ACTH; also known as corticotropin) and glucocorticoids produced by adrenal cortical cells (e.g. cortisol or corticosterone). The end effectors of the HPA axis, the glucocorticoids, function by binding to nuclear receptors (NRs) that regulate gene expression. The NRs cause epigenetic changes in the chromatin structure (e.g. histone methylation, acetylation, phorphorylation and ubiquitination) and possibly DNA methylation,[73] but do not affect the DNA sequence (i.e. the definition of epigenetic). The epigenetic changes alter gene expression, which drive phenotypic expression, and the changes may be stable and persist through later stages of the life cycle,[75,76] or even be passed on to subsequent generations (i.e. transgenerational effects).[77–80]

Hormones control tadpole metamorphosis

Thyroid hormone controls tadpole metamorphosis and stress hormones (glucocorticoids) synergise with thyroid hormone to promote tissue morphogenesis.[8,81] The functioning of the tadpole thyroid axis is regulated at multiple levels, and the rate of thyroid secretion and potency of thyroid hormone action on target tissues determines when larvae enter metamorphosis and the rate at which metamorphosis progresses. Thyroid secretion is controlled by neurohormones produced in the hypothalamus that control the secretion of thyroid-stimulating hormone (TSH) by the anterior pituitary gland. In mammals, the tripeptide amide thyrotropin-releasing hormone (TRH) controls TSH release. Although tadpoles synthesize TRH in their brains, TRH has no effect on TSH secretion from the tadpole pituitary. Instead, the 41 amino-acid peptide, CRF, is the primary TSH-releasing factor in tadpoles, and also functions in controlling the release of ACTH, which controls glucocorticoid production by adrenocortical cells. Glucocorticoids synergise with thyroid hormone, and thus promote thyroid hormone action on target tissues. Thus, tadpole metamorphosis is controlled centrally by CRF, which has a dual role in controlling TSH and ACTH, and peripherally by thyroid hormone and glucocorticoids, which synergise at target tissues to control organogenesis and tissue remodelling.

As CRF neurons show sensitive and robust responses to environmental change, and CRF controls both thyroid and adrenocortical secretion in tadpoles, the neurohormone is ideally positioned to mediate environmental effects on the timing of metamorphosis. The developmental response to pond drying depends on the upregulation of the hypothalamo–pituitary–thyroid axis, and the function of CRF in mediating stressor-induced early metamorphosis has been shown in tadpoles of the western spadefoot toad, which respond to habitat desiccation by accelerating metamorphosis.[29,82–84] This is not a response to osmotic stress, as the animals do not desiccate (i.e. they accelerate metamorphosis before the water disappears – see Denver *et al.*[29]), but is likely caused by

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restricted locomotion that reduces foraging.[82] Corticotropin-releasing factor may be a phylogenetically ancient developmental cue that vertebrates use to assess changes in their habitat and to mount an appropriate developmental/physiological response.[8] The broader significance of this finding is that in mammals, CRF of foetal and/or placental origin controls the timing of the length of gestation and may shorten the gestational period under conditions of foetal stress.[85,86] Thus, a neuroendocrine stress pathway regulated by environmental input controls the timing and character of animal development, that is, developmental plasticity.

Exposure to stressors during larval life causes activation of the neuroendocrine stress axis and elevations of glucocorticoids. Glucocorticoids reduce the growth of tadpoles, which if elevated before metamorphosis is initiated will slow the process of development. This can decrease the likelihood that animals will survive to metamorphosis, and generally leads to reduced body size at metamorphosis. However, the actions of glucocorticoids are complex, being generally inhibitory to growth and development if present before metamorphosis is initiated, but accelerating development once metamorphosis has begun.[31]

Hormones and developmental programming

Stress neurohormones acting to accelerate developmental processes can have a survival value by allowing a tadpole to escape a drying pond, or a mammalian foetus to escape an adverse intra-uterine environment, although with important trade-offs such as immature organ systems or small body size. In these examples, environmentally induced phenotypic correlations between stages of a life cycle may be due to the indirect effects stemming from the effects on body size at metamorphosis or birth. Elevation in stress hormones during the larval or foetal stage can also have direct effects on cells and organ systems, and is implicated as a mechanism for developmental programming that underlies carry-over effects among life stages. Elevated glucocorticoids during early development may programme the phenotype expressed in the juvenile/adult stage of amphibians, birds and mammals.[8] For example, a stressful environment experienced during development can alter later life reactivity to stressful stimuli.[76] The long-term consequences of early-life stressful experience may include changed behaviour, such as increased neophobia and altered social interactions that influence dominance hierarchies and mating success;[87,88] such changes could have a significant impact on lifetime fitness. Some changes could have adaptive value; for example, developing European starling chicks exposed to elevated glucocorticoids during embryogenesis subsequently exhibit enhanced flight performance, which could increase survival in a harsh environment.[89] In humans, exposure to elevated cortisol *in utero* late in gestation may accelerate or enhance neurological development.[90,91]

The effects of early life stress on later life phenotypic expression and susceptibility to disease are well documented in mammals.[76,92–95] Much less is known about such effects in non-mammalian species. It is hypothesized, although not yet directly tested, that glucocorticoids act at critical periods during brain development to cause permanent changes in the functioning of the stress axis, which then alters physiology and behaviour later in life. In mammals, exposure to stressors early in life often leads to a 'hyper-responsive' neuroendocrine stress axis,[96–98] greater anxiety and fearful behaviour[98,99] and increased food intake that can increase chances of becoming obese and developing metabolic syndrome.[100,101]

The hyper-reactivity of the HPA axis may result from reduced glucocorticoid negative feedback, as shown by the simultaneous elevation in basal plasma glucocorticoid concentration and CRF expression in the paraventricular nucleus of the hypothalamus, prolonged elevations in plasma glucocorticoid concentration after a stress response and reduced glucocorticoid receptor (GR) expression in the hippocampus.[98,102,103]

In amphibians, food restriction during the tadpole stage caused increased food intake, catch-up growth and elevated basal corticosterone in juvenile frogs.[17] As food restriction increases corticosterone in tadpoles[104,105] the phenotypic effects of food restriction may have been caused by elevation in stress hormones. Corticosterone stimulates feeding in juvenile frogs;[106] therefore, the elevated corticosterone could be causal for the increased food intake. The treatment of tadpoles with corticosterone for 5 days reduced body weight at metamorphosis (growth inhibition mentioned above), but juvenile frogs showed catch-up growth, reaching similar body size as controls 2 months after metamorphosis.[17] These frogs had higher basal plasma corticosterone concentration, suggesting increased HPA axis activity. In addition, treatment with corticosterone as a tadpole decreased the number of GR-immunoreactive (GR-ir) cells in the brain and pituitary gland, particularly in regions of the brain involved in stress responses.[17] The decreased GR expression may underlie the altered negative feedback reflected in the elevated plasma corticosterone concentration. Therefore, in frogs as in mammals, exposure to elevated glucocorticoids during early development leads to altered neuroendocrine gene expression and elevated HPA axis activity in later life stages.[76] Such changes could have long-term fitness consequences.

The molecular developmental mechanisms by which GR expression is altered by early life exposure to glucocorticoids may involve epigenetic changes at the GR locus. In mammals, it is known that early life experience can influence the degree of DNA methylation at CpG islands located in the promoter region of the GR gene.[75,107,108] The methylation state of the promoter is hypothesized to influence the expression level of the gene, with greater methylation causing lesser gene expression. A similar mechanism may occur in the frog as the frog GR gene has conserved CpG islands that may be modified by DNA methylation (Y. Kyono and R. J. Denver, unpublished data), and may account for the decreased GR-ir observed in juvenile frogs following exposure to corticosterone as a tadpole.[17]

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Relevance to human biology and directions for future research

There are many parallels between the effects of environmental stress on tadpole growth and development and the effects of intra-uterine stress on foetal growth and development in mammals. Maternal malnutrition or exposure to stressors can lead to intra-uterine growth retardation and pre-term birth,[92–95] which are associated with elevated stress hormones in both the mother and the foetus.[60,97,102] Similarly, tadpoles reared in suboptimal nutritive conditions exhibit small body size at transformation, and the resultant frogs have elevated corticosterone. As in frogs, mammals born at a small size often show catch-up growth. Although this is likely an adaptive response that evolved to allow animals to achieve reproductive maturity sooner given favourable growth conditions, it can have negative consequences in many modern human societies in which food may be abundant, and over-consumption leads to obesity and associated health problems. In mammals, exposure to stress *in utero* or neonatally is associated with reproductive dysfunction and increased susceptibility to disease later in life.[97,100] The activation of the stress axis leading to elevations in plasma glucocorticoid concentrations occurring during critical periods of development has been shown to permanently alter the functioning of the stress axis, the expression of behaviours throughout the life of the animal and metabolic pathways that may predispose to metabolic disorders, obesity and type 2 diabetes (although, exposure late in gestation can have positive effects on neurological development – see Davis and Sandman[91]). Similar findings in frogs suggest that the basic developmental mechanisms whereby glucocorticoids 'programme' the phenotype are phylogenetically ancient and evolutionarily conserved.

Due to their complex life cycles, amphibians are ideal for investigating environmental effects on development, the roles of hormones and the impact of early life experience on future phenotypic expression and fitness. Mammalian model systems are hampered by the inaccessibility of the foetus, which makes it very difficult to distinguish specific effects of elevated foetal or neonatal stress hormones from maternal influences. Post-embryonic development of amphibians is external, so that one can directly test for the roles of specific hormone signalling pathways in developmental outcomes. One can study hormone action in development in the absence of confounding maternal effects by manipulating a tadpole's rearing environment leading to elevations in glucocorticoids, or non-invasively increase or block the production or actions of glucocorticoids simply by adding hormones, hormone synthesis inhibitors or hormone antagonists directly to the aquarium water. The structure and function of the amphibian neuroendocrine stress axis are evolutionarily conserved with mammals,[74] and the genome sequence and associated molecular tools are now available, providing for a powerful model system to investigate the mechanisms of the developmental origins of health and disease.

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Statement of Interest

None.

References

1. Ozanne SE, Costancia M. Mechanisms of disease: the developmental origins of disease and the role of the epigenotype. *Nat Clin Pract Endocrinol Metab*. 2007; 3, 539–546.
2. Pigliucci M. *Phenotypic Plasticity. Beyond Nature and Nurture*, 2001. The Johns Hopkins University Press: Baltimore.
3. West-Eberhard MJ. *Developmental Plasticity and Evolution*, 2003. Oxford University Press: New York, NY.
4. Via S, Lande R. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution*. 1985; 39, 505–522.
5. DeWitt TJ, Scheiner SM. *Phenotypic Plasticity. Functional and Conceptual Approaches*, 2004. Oxford University Press: Oxford, UK.
6. West-Eberhard MJ. Developmental plasticity and the origin of species differences. *Proc Natl Acad Sci U S A*. 2005; 102, 6543–6549.
7. Pigliucci M, Murren CJ, Schlichting CD. Phenotypic plasticity and evolution by genetic assimilation. *J Exp Biol*. 2006; 209, 2362–2367.
8. Denver RJ. Stress hormones mediate environment-genotype interactions during amphibian development. *Gen Comp Endocrinol*. 2009; 164, 20–31.
9. Hall BK, Wake MH. Introduction: larval development, evolution and ecology. In *The Origin and Evolution of Larval Forms* (eds. Hall BK, Wake MH), 1999; pp. 1–19. Academic Press: London, UK.
10. Werner EE. Size, scaling, and the evolution of complex life cycles. In *Size-Structured Populations* (eds. Ebenman B, Persson L), 1988; pp. 60–81. Springer-Verlag: Berlin.
11. Moran NA. Adaptation and constraint in the complex life cycles of animals. *Annu Rev Ecol Syst*. 1994; 25, 573–600.
12. Ebenman B. Evolution in organisms that change their niches during the life cycle. *Am Nat*. 1992; 139, 990–1021.
13. Price TD, Grant PR. Life history traits and natural selection for small body size in a population of Darwin's finches. *Evolution*. 1984; 38, 483–494.
14. Pechenik JA, Wendt DE, Jarrett JN. Metamorphosis is not a new beginning. *Bioscience*. 1998; 48, 901–910.
15. Nicieza AG, Alvarez D, Atienza EMS. Delayed effects of larval predation risk and food quality on anuran juvenile performance. *J Evol Biol*. 2006; 19, 1092–1103.
16. Relyea RA. The lasting effects of adaptive plasticity: predator-induced tadpoles become long-legged frogs. *Ecology*. 2001; 82, 1947–1955.
17. Hu F, Crespi EJ, Denver RJ. Programming neuroendocrine stress axis activity by exposure to glucocorticoids during postembryonic development of the frog *Xenopus laevis*. *Endocrinology*. 2008; 149, 5470–5481.

https://doi.org/10.1017/S2040174410000279 Published online by Cambridge University Press

18. Blouin MS, Brown ST. Effects of temperature-induced variation in anuran larval growth rate on head width and leg length at metamorphosis. *Oecologia*. 2000; 125, 358–361.
19. Brown DD, Cai LQ. Amphibian metamorphosis. *Dev Biol*. 2007; 306, 20–33.
20. Dejesus E, Hirano T, Inui Y. Flounder metamorphosis – its regulation by various hormones. *Fish Physiol Biochem*. 1993; 11, 323–328.
21. Chino Y, Saito M, Yamasu K, *et al*. Formation of the adult rudiment of sea-urchins is influenced by thyroid-hormones. *Dev Biol*. 1994; 161, 1–11.
22. Johnson LG, Cartwright CM. Thyroxine-accelerated larval development in the crown-of-thorns starfish, Acanthaster planci. *Biol Bull*. 1996; 190, 299–301.
23. Heyland A, Reitzel AM, Hodin J. Thyroid hormones determine developmental mode in sand dollars (Echinodermata: Echinoidea). *Evol Dev*. 2004; 6, 382–392.
24. Porterfield SP, Hendrich CE. The role of thyroid hormones in prenatal and neonatal neurological development–current perspectives. *Endocr Rev*. 1993; 14, 94–106.
25. Wilbur HM, Collins JP. Ecological aspects of amphibian metamorphosis. *Science*. 1973; 182, 1305–1314.
26. Werner EE. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *Am Nat*. 1986; 128, 319–341.
27. Newman R. Adaptive plasticity in amphibian metamorphosis. *Bioscience*. 1992; 42, 671–678.
28. Goater CP. Growth and survival of postmetamorphic toads: interactions among larval history, density, and parasitism. *Ecology*. 1994; 75, 2264–2274.
29. Denver RJ, Mirhadi N, Phillips M. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. *Ecology*. 1998; 79, 1859–1872.
30. Relyea RA. Getting out alive: how predators affect the decision to metamorphose. *Oecologia*. 2007; 152, 389–400.
31. Denver RJ. Endocrinology of complex life cycles: amphibians. In *Hormones, Brain and Behavior*, vol. 2 (eds. Pfaff DW, Arnold AP, Etgen AM, Rubin RT, Fahrbach SE), 2009; pp. 707–744. Elsevier: San Diego.
32. Chipman AD. Variation, plasticity and modularity in anuran development. *Zoology*. 2002; 105, 97–104.
33. Newman RA. Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. *Ecology*. 1989; 70, 1775–1787.
34. Pfennig DW. Polyphenism in spadefoot toad tadpoles as a locally adjusted evolutionarily stable strategy. *Evolution*. 1992; 46, 1408–1420.
35. Pfennig D. The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. *Oecologia*. 1990; 85, 101–107.
36. Michimae H, Wakahara M. Factors which affect the occurrence of cannibalism and the broad-headed "cannibal" morph in larvae of the salamander Hynobius retardatus. *Behav Ecol Sociobiol*. 2001; 50, 339–345.
37. Michimae H, Wakahara M. A tadpole-induced polyphenism in the salamander Hynobius retardatus. *Evolution*. 2002; 56, 2029–2038.
38. Michimae H, Nishimura K, Wakahara M. Mechanical vibrations from tadpoles' flapping tails transform salamander's carnivorous morphology. *Biol Lett*. 2005; 1, 75–77.
39. McCollum S, Leimberger J. Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. *Oecologia*. 1997; 109, 615–621.
40. Relyea RA. Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology*. 2001; 82, 523–540.
41. Van Buskirk J, Mccollum SA. Plasticity and selection explain variation in tadpole phenotype between ponds with different predator composition. *Oikos*. 1999; 85, 31–39.
42. Van Buskirk J, McCollum SA. Influence of tail shape on tadpole swimming performance. *J Exp Biol*. 2000; 203, 2149–2158.
43. Benard MF. Predator-induced phenotypic plasticity in organisms with complex life histories. *Ann Rev Ecol Evol Syst*. 2004; 35, 651–673.
44. Fraker ME. The dynamics of predation risk assessment: responses of anuran larvae to chemical cues of predators. *J Anim Ecol*. 2008; 77, 638–645.
45. Fraker ME, Cuddapah V, McCollum SA, *et al*. Characterization of an alarm pheromone secreted by amphibian tadpoles that induces rapid behavioral inhibition and suppression of the neuroendocrine stress axis. *Horm Behav*. 2009; 55, 520–529.
46. Denver RJ. Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. *Am Zool*. 1997; 37, 172–184.
47. Mazerolle MJ, Desrochers A. Landscape resistance to frog movements. *Can J Zool*. 2005; 83, 455–464.
48. Mazerolle MJ. Amphibian activity, movement patterns, and body size in fragmented peat bogs. *J Herpet*. 2001; 35, 13–20.
49. Thorson TB. The relationship of water economy to terrestrialism in amphibians. *Ecology*. 1955; 36, 100–116.
50. Scott D. The effect of larval density on adult demographic traits in *Ambystoma opacum*. *Ecology*. 1994; 75, 1383–1396.
51. Morey S, Reznick D. Effects of larval density on postmetamorphic spadefoot toads (*Spea hammondii*). *Ecology*. 2001; 82, 510–522.
52. Semlitsch RD, Scott DE, Pechmann JHK. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology*. 1988; 69, 184–192.
53. Berven KA. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology*. 1990; 71, 1599–1608.
54. Beck CW, Congdon JD. Effects of individual variation in age and size at metamorphosis on growth and survivorship of southern toad (*Bufo terrestris*) metamorphs. *Can J Zool-Revue Canadienne De Zoologie*. 1999; 77, 944–951.
55. Beck CW, Congdon JD. Effects of age and size at metamorphosis on performance and metabolic rates of Southern Toad, Bufo terrestris, metamorphs. *Funct Ecol*. 2000; 14, 32–38.
56. Van Buskirk J, Saxer G. Delayed costs of an induced defense in tadpoles? Morphology, hopping, and development rate at metamorphosis. *Evolution*. 2001; 55, 821–829.
57. Alvarez D, Nicieza AG. Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. *Oecologia*. 2002; 131, 186–195.
58. Altwegg R, Reyer H-U. Patterns of natural selection on size at metamorphosis in water frogs. *Evolution*. 2003; 57, 872–882.

https://doi.org/10.1017/S2040174410000279 Published online by Cambridge University Press

59. Relyea RA, Hoverman JT. The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs. *Oecologia*. 2003; 134, 596–604.
60. Matthews SG. Early programming of the hypothalamo-pituitary-adrenal axis. *Trends Endocrinol Metab*. 2002; 13, 373–380.
61. Meaney MJ. Early environmental experience and lifelong changes in behavior and in gene expression. *Biol Psychiatry*. 1999; 45, 143.
62. Chelgren ND, Rosenberg DK, Heppell SS, *et al*. Carryover aquatic effects on survival of metamorphic frogs during pond emigration. *Ecol Appl*. 2006; 16, 250–261.
63. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol Rev*. 2005; 85, 571–633.
64. Emerson SB. Allometry and jumping in frogs: helping the twain to meet. *Evolution*. 1978; 32, 551–564.
65. Smith DC. Adult recruitment in chorus frogs – effects of size and date at metamorphosis. *Ecology*. 1987; 68, 344–350.
66. Prado CPA, Haddad CFB. Size-fecundity relationships and reproductive investment in female frogs in the Pantanal, south-western Brazil. *Herpetol J*. 2005; 15, 181–189.
67. Girish S, Saidapur SK. Interrelationship between food availability, fat body, and ovarian cycles in the frog, *Rana tigrina,* with a discussion on the role of fat body in anuran reproduction. *J Exp Zool*. 2000; 286, 487–493.
68. Birch LC. The intrinsic rate of natural increase of an insect population. *J Anim Ecol*. 1948; 17, 15–26.
69. Cole LC. The population consequences of life history phenomena. *Q Rev Biol*. 1954; 29, 103–137.
70. McGraw JB, Caswell H. Estimation of individual fitness from life-history data. *Am Nat*. 1996; 147, 47–64.
71. Benard MF, Fordyce JA. Are induced defenses costly? Consequences of predator-induced defenses in western toads, Bufo boreas. *Ecology*. 2003; 84, 68–78.
72. West-Eberhard MJ. Phenotypic accommodation: adaptive innovation due to developmental plasticity. *J Exp Zool B Mol Dev Evol*. 2005; 304B, 610–618.
73. Gilbert SF, Epel D. *Ecological Developmental Biology. Integrating Epigenetics, Medicine and Evolution*, 2008. Sinauer Associates, Inc.; Sunderland, Massachusetts.
74. Denver RJ. Structural and functional evolution of vertebrate neuroendocrine stress systems. *Ann N Y Acad Sci*. 2009; 1163, 1–16.
75. Szyf M, Meaney M, Weaver K, *et al*. DNA methylation: an interface between the social environment and our genomes. *Environ Mol Mutagen*. 2007; 48, 531–531.
76. Meaney MJ, Szyf M, Seckl JR. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. *Trends Mol Med*. 2007; 13, 269–277.
77. Anway MD, Cupp AS, Uzumcu M, *et al*. Epigenetic transgenerational actions of endocrine disruptors and mate fertility. *Science*. 2005; 308, 1466–1469.
78. Crews D. Epigenetics and its implications for behavioral neuroendocrinology. *Front Neuroendocrinol*. 2008; 29, 344–357.
79. Gore AC. Developmental programming and endocrine disruptor effects on reproductive neuroendocrine systems. *Front Neuroendocrinol*. 2008; 29, 358–374.
80. Morgan DK, Whitelaw E. The case for transgenerational epigenetic inheritance in humans. *Mamm Genome*. 2008; 19, 394–397.
81. Bonett RM, Hoopfer ED, Denver RJ. Molecular mechanisms of corticosteroid synergy with thyroid hormone in *Xenopus laevis* metamorphosis. *Gen Comp Endocrinol*. 2010; doi:10.1016/j.ygcen.2010.03.014.
82. Denver RJ. Environmental stress as a developmental cue: corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. *Horm Behav*. 1997; 31, 169–179.
83. Denver RJ. Hormonal correlates of environmentally induced metamorphosis in the Western spadefoot toad, *Scaphiopus hammondii*. *Gen Comp Endocrinol*. 1998; 110, 326–336.
84. Boorse GC, Denver RJ. Endocrine mechanisms underlying plasticity in metamorphic timing in spadefoot toads. *Integr Comp Biol*. 2004; 43, 646–657.
85. Smith R, Mesiano S, McGrath S. Hormone trajectories leading to human birth. *Regul Pept*. 2002; 108, 159–164.
86. Challis JRG, Bloomfield FH, Bocking AD, *et al*. Fetal signals and parturition. *J Obstet Gynaecol Res*. 2005; 31, 492–499.
87. Knuth ED, Etgen AM. Long-term behavioral consequences of brief, repeated neonatal isolation. *Brain Res*. 2007; 1128, 139–147.
88. Spencer KA, Wimpenny JH, Buchanan KL, *et al*. Developmental stress affects the attractiveness of male song and female choice in the zebra finch (*Taeniopygia guttata*). *Behav Ecol Sociobiol*. 2005; 58, 423–428.
89. Chin EH, Love OP, Verspoor JJ, *et al*. Juveniles exposed to embryonic corticosterone have enhanced flight performance. *Proc R Soc B-Biol Sci*. 2009; 276, 499–505.
90. Amiel-Tison C, Cabrol D, Denver RJ, *et al*. Fetal adaptation to stress – part I: acceleration of fetal maturation and earlier birth triggered by placental insufficiency in humans. *Early Hum Dev*. 2004; 78, 15–27.
91. Davis EP, Sandman CA. The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. *Child Dev*. 2010; 81, 131–148.
92. Weinstock M, Matlina E, Maor GI, *et al*. Prenatal stress selectively alters the reactivity of the hypothalamic-pituitary adrenal system in the female rat. *Brain Res*. 1992; 595, 195–200.
93. Weinstock M, Poltyrev T, Schorer-Apelbaum D, *et al*. Effect of prenatal stress on plasma corticosterone and catecholamines in response to footshock in rats. *Physiol Behav*. 1998; 64, 439–444.
94. Challis JRG, Sloboda D, Matthews SG, *et al*. The fetal placental hypothalamic-pituitary-adrenal (HPA) axis, parturition and post natal health. *Mol Cell Endocrinol*. 2001; 185, 135–144.
95. Bloomfield FH, Oliver MH, Giannoulias CD, *et al*. Brief undernutrition in late-gestation sheep programs the hypothalamic-pituitary-adrenal axis in adult offspring. *Endocrinology*. 2003; 144, 2933–2940.
96. McCormick CM, Smythe JW, Sharma S, *et al*. Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density in adult rats. *Dev Brain Res*. 1995; 84, 55–61.

https://doi.org/10.1017/S2040174410000279 Published online by Cambridge University Press

97. Weinstock M. Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Prog Neurobiol*. 2001; 65, 427–451.
98. Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annu Rev Neurosci*. 2001; 24, 1161–1192.
99. Smythe JW, McCormick CM, Meaney MJ. Median eminence corticotrophin-releasing hormone content following prenatal stress and neonatal handling. *Brain Res Bull*. 1996; 40, 195–199.
100. Barker DJP, Osmond C, Kajantie E, *et al*. Growth and chronic disease: findings in the Helsinki Birth Cohort. *Ann Hum Biol*. 2009; 36, 445–458.
101. Breier BH, Vickers MH, Ikenasio BA, *et al*. Fetal programming of appetite and obesity. *Mol Cell Endocrinol*. 2001; 185, 73–79.
102. Welberg LAM, Seckl JR. Prenatal stress, glucocorticoids and the programming of the brain. *J Neuroendocrinol*. 2001; 13, 113–128.
103. Weaver ICG, Diorio J, Seckl JR, *et al*. Early environmental regulation of hippocampal glucocorticoid receptor gene expression – Characterization of intracellular mediators and potential genomic target sites. *Ann N Y Acad Sci*. 2004; 1024, 182–212.
104. Glennemeier KA, Denver RJ. Role for corticoids in mediating the response of *Rana pipiens* tadpoles to intraspecific competition. *J Exp Zool*. 2002; 292, 32–40.
105. Crespi EJ, Denver RJ. Roles of stress hormones in food intake regulation in anuran amphibians throughout the life cycle. *Comp Biochem Physiol A Comp Physiol*. 2005; 141, 381–390.
106. Crespi EJ, Denver RJ. Ontogeny of corticotropin-releasing factor effects on locomotion and foraging in the Western spadefoot toad (*Spea hammondii*). *Horm Behav*. 2004; 46, 399–410.
107. Szyf M, Weaver ICG, Champagne FA, *et al*. Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Front Neuroendocrinol*. 2005; 26, 139–162.
108. Szyf M, Weaver I, Meaney M. Maternal care, the epigenome and phenotypic differences in behavior. *Reprod Toxicol*. 2007; 24, 9–19.
109. Michimae H, Hangui J. A trade-off between prey- and predator-induced polyphenisms in larvae of the salamander Hynobius retardatus. *Behav Ecol Sociobiol*. 2008; 62, 699–704.

https://doi.org/10.1017/S2040174410000279 Published online by Cambridge University Press