PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

**Cite this article:** Levis NA, McKay DJ, Pfennig DW. 2022 Disentangling the developmental origins of a novel phenotype: enhancement versus reversal of environmentally induced gene expression. *Proc. R. Soc. B* **289**: 20221764.
https://doi.org/10.1098/rspb.2022.1764

Received: 6 September 2022
Accepted: 3 October 2022

**Subject Category:**
Evolution

**Subject Areas:**
evolution, developmental biology, ecology

**Keywords:**
gene expression, phenotypic accommodation, phenotypic plasticity, plasticity-led evolution, spadefoot toad

**Author for correspondence:**
Nicholas A. Levis
e-mail: nicholasalevis@gmail.com

†Present address: Department of Biology, Indiana University, Bloomington, IN 47405, USA.

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.6250553.

THE ROYAL SOCIETY PUBLISHING



# Disentangling the developmental origins of a novel phenotype: enhancement versus reversal of environmentally induced gene expression

Nicholas A. Levis†, Daniel J. McKay and David W. Pfennig

Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA

NAL, 0000-0001-7650-2371; DWP, 0000-0002-1114-534X

Increasing evidence suggests that many novel traits might have originated via plasticity-led evolution (PLE). Yet, little is known of the developmental processes that underpin PLE, especially in its early stages. One such process is 'phenotypic accommodation', which occurs when, in response to a change in the environment, an organism experiences adjustments across variable parts of its phenotype that improve its fitness. Here, we asked if environmentally induced changes in gene expression are enhanced or reversed during phenotypic accommodation of a novel, complex phenotype in spadefoot toad tadpoles (*Spea multiplicata*). More genes than expected were affected by both the environment and phenotypic accommodation in the liver and brain. However, although phenotypic accommodation primarily reversed environmentally induced changes in gene expression in liver tissue, it enhanced these changes in brain tissue. Thus, depending on the tissue, phenotypic accommodation may either minimize functional disruption via reversal of gene expression patterns or promote novelty via enhancement of existing expression patterns. Our study thereby provides insights into the developmental origins of a novel phenotype and the incipient stages of PLE.

## 1. Introduction

A longstanding problem in biology is to explain how novel, complex features come about [1–4]. According to the 'plasticity-led evolution' hypothesis ('PLE'; *sensu* [5,6]), novel phenotypes first appear in a rudimentary form when a change in the environment triggers a shift in phenotype via phenotypic plasticity [4,7]. Because different genotypes typically differ in whether and how they express plasticity [8], revealing such formerly 'cryptic' genetic variation by plasticity can fuel an evolutionary response in which selection moulds this environmentally induced phenotype into a new adaptive form (figure 1). Although PLE is increasingly viewed as a route leading to novelty [10], little is known of the developmental processes that underpin PLE, especially in its early stages [11]. Identifying these processes is crucial for fully elucidating whether and how PLE occurs [11].

One such critical, early process occurs when, in response to a change in the environment (and an initial modification of some aspect of its phenotype through phenotypic plasticity), an organism undergoes adjustments across variable parts of its phenotype that improve its fitness. For example, upon encountering a new environment, many organisms experience a change in gene expression [12–15]. This initial change is often immediately followed—in developmental time—by changes in numerous other phenotypic attributes (the expression of other genes or even changes in physiology, behaviour or morphology) that improve the match between the organism's overall phenotype and its environment and thereby enhance fitness [16,17]. Such a process has been dubbed 'phenotypic accommodation' [4] (N.B.: phenotypic accommodation may lead to, but is distinct from, 'genetic accommodation', in which gene frequencies change due to selection on the regulation or form of a trait [4,18]). One extreme form of genetic

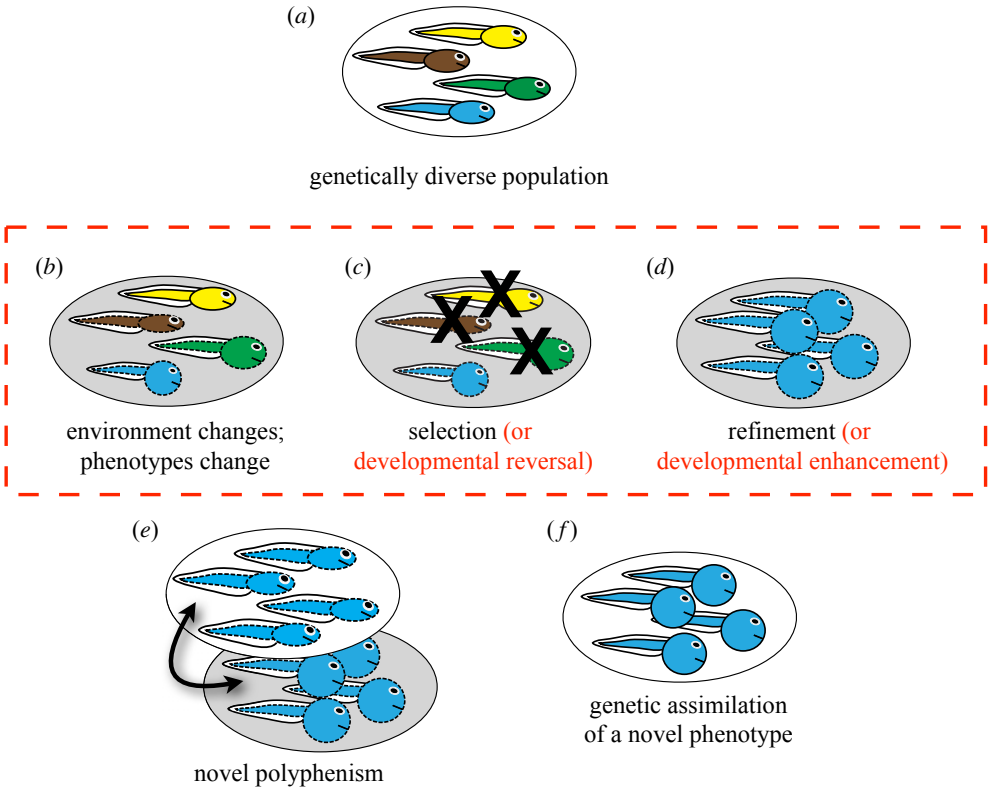


**Figure 1.** How PLE can facilitate the evolution of a novel, complex phenotype (N.B.: the focus of this paper is on the steps outlined by the dashed box). (*a*) A genetically diverse population (different colours: different genotypes) (*b*) experiences a novel environment (shading), which induces novel phenotypes (dashed lines), but genotypes differ in whether and how they respond (different shapes). (*c*) Selection acts on this formerly cryptic genetic variation (revealed by a change in environment) and disfavours genotypes that produce poorly adapted phenotypes (X). Alternatively, this process might occur through development via the 'reversal' of environmentally induced phenotypes that are poorly suited for the new environment. (*d*) This leads to the adaptive refinement of the favoured phenotype (enlargement of the blue tadpole). Again, this process might occur through development via the 'enhancement' of environmentally induced phenotypes that are well suited for the new environment. (*e*) If individuals produce either this novel phenotype or the ancestral phenotype depending on their environment, then the result is a novel polyphenism. (*f*) Alternatively, selection might favour the loss of plasticity (i.e. genetic assimilation), resulting in a novel phenotype that is produced regardless of the environment (indicated by the loss of dashed lines). Modified from [9].

accommodation is 'genetic assimilation', in which the regulation of plasticity evolves to the point that the plasticity is lost and the trait becomes fixed [19]; another extreme is 'polyphenism', where environmental responsiveness is accentuated and refined into two or more discrete, environmentally induced forms [20]). Phenotypic accommodation is thought to be a widespread adaptive mechanism by which organisms respond to changing environmental circumstances [16,17,21], and it might be crucial in promoting PLE and the evolution of a novel complex phenotype [10].

But how does phenotypic accommodation unfold? One possibility is that phenotypic changes initially induced by the environment are reversed during phenotypic accommodation. In such cases involving gene expression, the magnitude and direction of gene expression differences between individuals developing an adaptive versus non-adaptive phenotype (i.e. differences owing to development) would be negatively associated with the magnitude and direction of gene expression differences between individuals in the old versus new environment that exhibit the same phenotype (i.e. differences owing to the environment). Such reversal might occur, for example, if the new environment is stressful, and individuals who become adapted to it are better at regulating their stress responses such that these responses more closely resemble those of individuals in the old environment. Indeed, the stress caused by exposure to a new environment might manifest as an elevated metabolic rate or increase in oxidative damage (or gene expression changes associated with these). Individuals that are better able to regulate this stress may have fewer changes (e.g. no metabolic elevation or major oxidative damage) and exhibit responses similar to those of individuals who never encountered the stressor. By contrast, individuals who are poor regulators of stress responses could show a departure from the pre-stress environment and thereby be more dissimilar to the pre-stress condition (e.g. elevated metabolic rate or oxidative damage). Consistent with these arguments, theory predicts that phenotypic accommodation should reduce the amount of functional disruption caused by a new environment [4,17]. A signature of such robustness might be a reversal of environmentally induced gene expression during phenotypic accommodation.

An alternative scenario is that rather than being reversed, phenotypic changes initially induced by the environment might be *enhanced* by phenotypic accommodation. Consider that phenotypic accommodation is implicated in producing new developmental variants when a new environment is encountered [4,17]. Such developmental novelty likely involves breaking buffering mechanisms (e.g. [21–23]). A signature of this outcome might be the enhancement of environmentally induced changes such that effects of the environment are further exaggerated in individuals that develop a high-fitness phenotype. In this case, the magnitude and direction of gene expression differences between individuals developing an adaptive versus non-adaptive phenotype

(i.e. differences owing to development) would be positively associated with the magnitude and direction of gene expression differences between individuals in the old versus new environment that exhibit the same phenotype (i.e. differences owing to the environment).

In short, environmentally induced changes in gene expression may either be reversed or enhanced during phenotypic accommodation. While seemingly at odds, these patterns are not mutually exclusive when considering multiple tissues or genes and may depend on the nature of the environments being studied. However, the context-dependence of phenotypic accommodation and the co-occurrence of these two outcomes have not been explicitly tested.

Here, we addressed how changes in gene expression that are associated with phenotypic accommodation unfold during the development of a novel, complex phenotype in Mexican spadefoot toads, *Spea multiplicata* [24]. The tadpoles of this species can adaptively adjust development depending on environmental conditions [24–26]. Like most anurans [27,28], they typically develop as an 'omnivore' morph that primarily consumes detritus on the pond bottom [29,30]. However, when they eat live animal prey (fairy shrimp and other tadpoles), some (but not all) individuals deviate from the default omnivore morph and develop into a behaviourally and morphologically distinctive 'carnivore' morph that specializes on meat and metamorphose earlier [31,32]. Switching to the carnivore morph in the presence of abundant animal prey (such as fairy shrimp) is adaptive because shrimp are most abundant in the most ephemeral ponds [31], where the faster-developing carnivore morph is selectively favoured [26,29]. In less ephemeral ponds (where shrimp are less abundant), by contrast, the generalist omnivore morph is favoured [26,29,33]. Finally, several studies, taken together, suggest that the novel carnivore morph arose when pre-existing plasticity was expressed in an ancestral lineage and later refined by selection via PLE into an adaptive phenotype (reviewed in [9]).

In *Spea* tadpoles, phenotypic accommodation begins when an individual experiences a change in its environment (e.g. when animal prey becomes present) and ends when various aspects of its molecular, behaviour, and morphological phenotype 'accommodate' this environmental change to produce a complex, coordinated phenotype: the distinctive carnivore morph. To disentangle the developmental origins of this novel phenotype, we specifically evaluated whether environmentally induced changes in gene expression were enhanced or reversed during phenotypic accommodation. To do so, we measured the change in gene expression between omnivores reared in (1) an environment where omnivores are favoured (i.e. where tadpoles were reared alone and fed detritus only) versus (2) an environment where carnivores are favoured (i.e. where tadpoles were crowded and fed meat). We then asked if—and in what direction—changes in genome-wide gene expression between these environments were correlated with the corresponding differences between carnivores and omnivores in a carnivore-favouring environment. We defined a pattern of 'reversal' and 'enhancement' as either a negative or positive relationship, respectively, between the gene expression changes of omnivores in different environments and those of carnivores and omnivores in the same environment (i.e. the cue treatment; figure 2). We measured gene expression in liver and brain tissue because our treatments differed in resource environment (affecting the liver) and social environment (affecting the brain). Moreover, previous work suggested that these two tissues are foci of numerous, environmentally induced changes in gene expression [34].

As we describe below, we found that, depending on the tissue, phenotypic accommodation may either minimize functional disruption via reversal of gene expression patterns or promote novelty via enhancement of existing expression patterns. Our study thereby provides a window into the developmental origins of a novel phenotype and the early stages of PLE. This study also complements recent work on this system [34–36] regarding how gene expression changes undergird phenotypic plasticity and the evolution of the distinctive carnivore phenotype.

## 2. Material and methods

### (a) Sample collection and experimental design

On 10 July 2018, we collected six pairs of Mexican spadefoot toads (*Spea multiplicata*) in amplexus from a newly formed, temporary pond near Portal Arizona ('PO2-N Pond'; lat. 31.9142, long. −109.0836). We transported pairs to the nearby Southwestern Research Station and allowed them to breed. Two days after their eggs hatched, for each sibship, 12 tadpoles were reared individually and fed 10 mg of fish food daily to mimic an omnivore-favouring pond. In this 'control' treatment group, tadpoles were not exposed to cues that elicit carnivore production (i.e. crowding and live meat consumption). The remaining tadpoles per sibship were divided into five boxes of 80 tadpoles each and fed fish food (as before) as well as live fairy shrimp and live *Scaphiopus couchii* tadpoles. This was the 'cue' treatment group because competition, shrimp consumption, and tadpole consumption contribute to the development of carnivores (N.B.: not all individuals that experience these cues develop into a carnivore; some remain omnivores even though they experienced the carnivore-favouring environment [31]). Mortality was low, and when the tadpoles were 10 days old, we randomly sampled five control omnivores, five cue omnivores and five cue carnivores per sibship (the cue omnivores and cue carnivores were sampled from the same boxes). We euthanized these tadpoles with a 0.8% aqueous tricaine methanesulfonate (MS-222) solution and placed them in a microcentrifuge tube filled with RNAlater. These samples remained at room temperature for 24 h to allow RNAlater penetration and then were frozen at −20°C until being shipped to the University of North Carolina overnight on dry ice. The remaining tadpoles per sibship were euthanized and preserved in 95% ethanol for morphological measurements.

### (b) Sample processing

We obtained standard trophic morphology measurements [30] for our gene expression samples while submerged in RNAlater immediately before tissue extraction. The brain and liver were removed from three randomly selected tadpoles (out of five) per sibship among the three experimental conditions ($n = 9$ tadpoles for each of the six sibships). We extracted RNA and obtained measures of gene expression for a total of approximately 14 000 genes using 3′ RNA-seq methods described in detail elsewhere [34,37,38], but additional details are provided in the electronic supplementary material.

### (c) Data analysis

First, we ensured that our cue treatment favoured carnivore-like tadpoles by fitting cubic splines [39] for the trophic morphology for each sibship while controlling for microcosm identity. In each case (electronic supplementary material, figure S1), the most

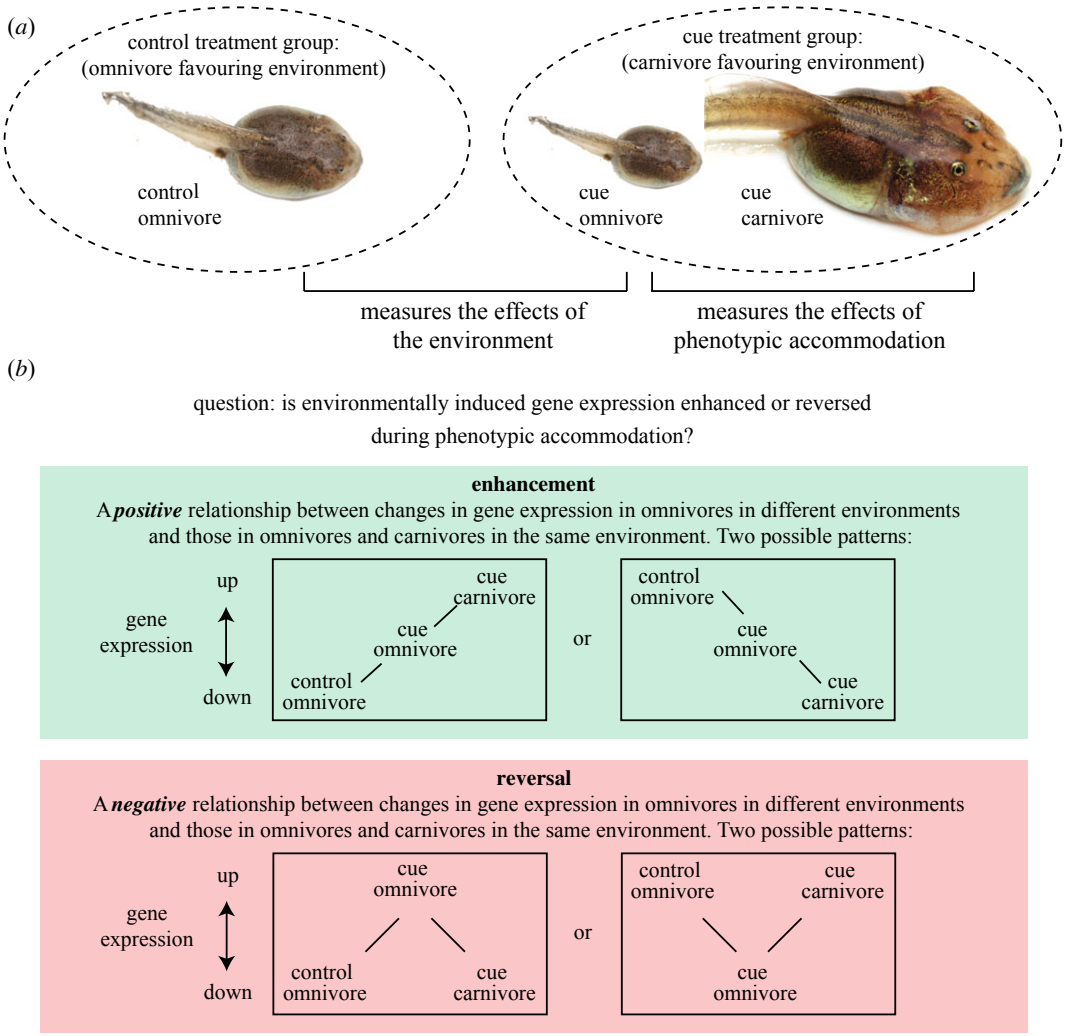


**Figure 2.** Diagrammatic representation of our (*a*) experimental design and (*b*) predictions. See text for details. Tadpole images are not to scale and are only for visualization purposes. (Online version in colour.)

carnivore-like individuals had the highest relative fitness as measured by body size; i.e. the log(snout–vent length)/mean(-log[snout–vent length]) [40]. Body size is a reliable proxy for fitness in this system because larger tadpoles are more likely to (1) survive to metamorphosis [41], (2) survive to sexual maturity, and (3) mature as larger adults [42]. Adult body size is also associated with mating success in males [43] and fecundity in females [41].

We then determined the relationship between gene expression changes induced by the environment and gene expression resulting from phenotypic accommodation. Specifically, we used DESeq2 [44] in R (version 4.0.1; [45]) to calculate the $\log_2$ fold change (LFC) in expression (with sibship as a covariate) for each gene between cue omnivores and control omnivores and LFC in expression between carnivores and cue omnivores. (N.B.: There were no control carnivores.) We then calculated the Spearman correlation between LFC environment (cue omnivores/control omnivores) and LFC development (cue carnivores/cue omnivores) separately for the liver and brain. We used all genes in this analysis, but confirmed that our observed patterns were not heavily influenced by lowly expressed genes (electronic supplementary material, table S1). To determine if the observed correlations were more or less positive or negative than expected by chance, we randomized transcriptomes among samples, recalculated LFC values and recalculated the correlation coefficient [46,47]. This randomization was performed 1000 times for each tissue. As with previous studies, the null distribution of these analyses is centred around a negative correlation [46–48]; we compared our observed relationship to this distribution. We identified biological process gene ontology (GO) terms that were enriched in the subsets of genes differentially expressed between environments and morphs and that show different patterns of 'enhancement' (i.e. similar sign in both comparisons) or 'reversal' (i.e. opposite signs between comparisons) using the g: Profiler online tool [49].

## 3. Results

Changes in expression induced by the environment tended to be either reversed or enhanced during phenotypic accommodation depending on the tissue. In the liver, we found a significant negative relationship (table 1), suggesting that phenotypic accommodation tended to reverse the environmentally induced change. Indeed, the majority of genes (54%; $\chi^2$ goodness of fit against 50% expectation = 111.834; $p < 0.00001$) showed a pattern of reversal (figure 3*d*). While 54% is not an overwhelming majority of genes, this pattern held even when we removed genes that were not differentially expressed in either contrast or that had low overall expression (electronic supplementary material, table S1). However, the magnitude of the relationship between environmentally and developmentally induced gene expression was weaker than that of the random permutations ($p = 0.002$; figure 3*a*). This was primarily driven by a significantly higher proportion of genes showing a pattern of enhancement than expected by chance ($p = 0.005$; table 1 and figure 3*b*).

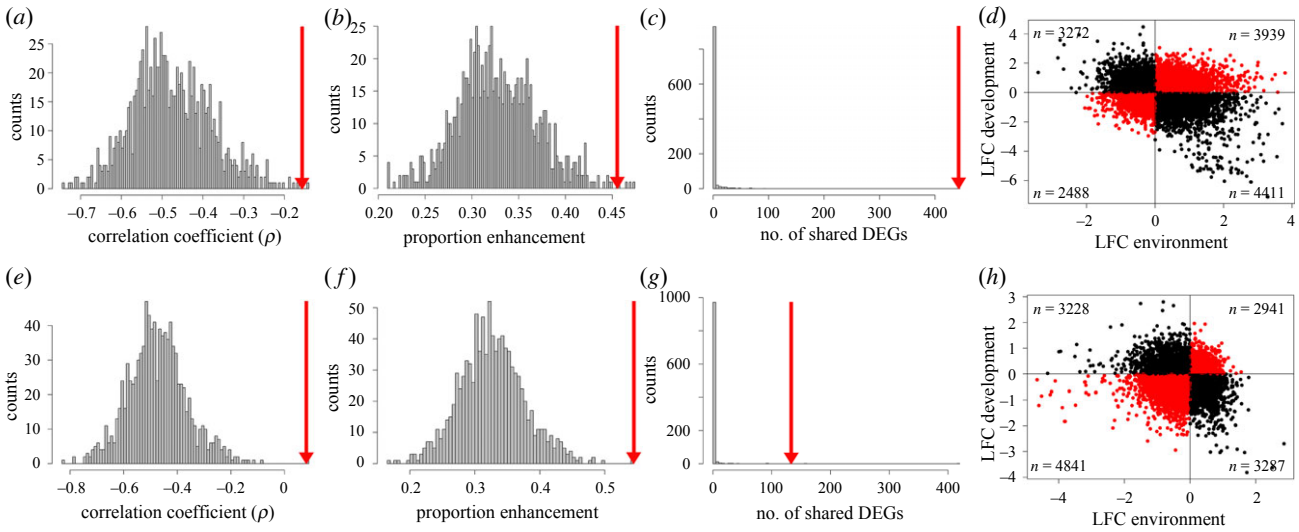


**Figure 3.** The relationship between changes in gene expression caused by the environment and those caused by phenotypic accommodation for genes from two tissues: liver (top) and brain (bottom). Whereas the liver showed a significantly weaker negative relationship between environmentally induced gene expression and gene expression of phenotypic accommodation (*a*), the brain showed a significantly more positive one (*e*). Both tissues had significantly more genes exhibiting a pattern of enhancement than random permutations (*b*,*f*) and had significantly more genes showing differential expression in both environmental and phenotypic accommodation contrasts (*c*,*g*). In the liver, most genes showed a pattern of reversal (*d*, black dots) during phenotypic accommodation, but in the brain, most genes showed a pattern of enhancement (*h*, red dots). Histograms are based on 1000 random permutations that varied transcriptome assignments among samples and red arrows denote the observed values in panels *a*–*c* and *e*–*g*. The number of genes in each quadrant for *d* and *h* is indicated. DEGs, differentially expressed genes; LFC, $\log_2$ fold change.

**Table 1.** Relationship between environmentally induced changes in gene expression and those based on phenotypic accommodation. The overall correlation (Spearman's $\rho$) among the $\log_2$ fold change (LFC) values in both comparisons (environment and development) for liver and brain tissue. There was a negative relationship between environmental and developmental responses in the liver, but in the brain, the relationship was positive. $P_{cor}$ corresponds to the *p*-value from the Spearman correlation test. $P_{enhancement}$ corresponds to the probability of finding as great or greater a number of genes with a pattern of enhancement as our observed data in 1000 random permutations.

| tissue | correlation ($\rho$) | $P_{cor}$ | proportion enhancement | proportion reversal | $P_{enhancement}$ |
|---|---|---|---|---|---|
| liver | −0.16 | <0.001 | 0.46 | 0.54 | 0.005 |
| brain | +0.08 | <0.001 | 0.54 | 0.46 | 0.001 |

In the brain, by contrast, we found a significant positive relationship (table 1) and a relationship that was substantially more positive than expected by chance ($p = 0.001$; figure 3*e*). Like the liver, more genes showed a pattern of enhancement than expected by chance ($p = 0.001$; table 1 and figure 3*f*). Unlike the liver, where the majority of genes showed a pattern of reversal, the majority of genes in the brain showed enhancement (54%; $\chi^2$ goodness of fit against 50% expectation = 112.313; $p < 0.00001$; figure 3*h*). Again, while 54% is not an overwhelming majority of genes, this pattern held even when we removed genes that were not differentially expressed in either contrast or that had low overall expression (electronic supplementary material, table S1). In both tissues, more genes were shared between the set of genes differentially expressed between environments and the set of genes differentially expressed during phenotypic accommodation than expected by chance (figure 3*c*,*g*). In sum, although the liver tended to show an overall pattern of reversal and the brain an overall pattern of enhancement, both tissues showed more enhancement than expected, and both tissues had more shared differentially expressed genes from both environmental and phenotypic accommodation contrasts.

Finally, we evaluated if the genes occupying the different quadrants of the LFC environment and LFC development relationship (i.e. genes that showed different patterns of enhancement or reversal) and that were significantly differentially expressed between environments and as a result of phenotypic accommodation were enriched for any biological process GO terms. The top 10 terms for each category and tissue (liver or brain) are presented in electronic supplementary material, table S2. In the liver, enhanced genes were primarily involved in cholesterol and steroid metabolism and synthesis. Reversed genes in the liver were more variable, but had associations with response to nutrients (e.g. glucagon and oleic acid), response to external stimulus and nitrogen metabolism. In the brain, aerobic respiration, immune response and stem cell differentiation were the major categories for enhanced genes. No biological processes were enriched in genes showing a reversal in the brain.

## 4. Discussion

To illuminate the early stages of PLE, we assessed the relationship between changes in gene expression stemming from the environment and changes stemming from phenotypic accommodation. In general, we observed that many genes influenced by a change in the environment also played a role in the development of an adaptive form (figure 3*c*,*g*). This suggests that phenotypic accommodation uses many of the

same genes that respond to environmental change. Moreover, we found that the relationship between changes in gene expression based on the environment and changes based on phenotypic accommodation were more positive than expected by chance in both liver and brain (figure 3*a*,*e*). This pattern was driven by a significantly higher proportion of genes showing enhancement in our observed data than in the permuted samples (figure 3*b*,*f*). Despite this overall trend, many genes still showed a negative relationship, especially in the liver, where most genes showed a pattern of reversal (table 1). Indeed, whereas the brain had a significantly positive relationship between environmental and developmental changes in gene expression, the liver still had a significantly negative relationship (table 1).

Although the proportion of genes that showed a pattern of reversal in the liver and enhancement in the brain (54% for each tissue type) was highly statistically significantly different from random expectation (50%; $p < 0.00001$), a near majority of genes in each tissue type (46%) did not follow these trends. Why such a large proportion of genes did not follow the pattern exhibited by the majority of genes in each tissue is not clear. We speculate that it may result from regulatory interactions such that increased expression of some genes causes decreased expression of others (or vice versa), but future studies are needed to address this issue.

The negative relationship observed in the liver between changes in gene expression induced by the environment and those induced by phenotypic accommodation recalls the pattern often seen during evolutionary adaptation. Recent work has shown that adaptation to a new environment often reverses or 'neutralizes' the gene expression responses a population first exhibits in that environment (in fish brain: [46], in lizard skeletal muscle: [47], in fish muscle: [50,51], in bacteria, yeast and fish brains: [52], in whole beetle carcass: [53], in fruit fly: [54]). That such a reversal seen during adaptation is also elicited by phenotypic accommodation supports the longstanding claim that phenotypic accommodation represents an initial stage of adaptive evolution [4,5,10,55].

An important finding to emerge from our study is that the relationship between changes in gene expression induced by the environment and those induced by phenotypic accommodation *differed for different tissues*. In particular, although genes in the liver showed a significantly negative relationship, genes in the brain showed a significantly positive relationship. In other words, although phenotypic accommodation tended to *reverse* environmentally induced changes in gene expression in *liver* tissue, it tended to *enhance* these changes in *brain* tissue (i.e. expression differences between carnivores and omnivores in the cue treatment exaggerated differences between omnivores in the control and cue treatments; figure 3*e*,*f*). Why might we have observed differences between tissues?

Generally, changes in the brain are associated with a dynamic (enriched) environment and can be important for learning, memory and behaviour [56–58]. Thus, a shift to the dynamic cue environment may have caused changes in the brain to cope with social interactions in that environment [59]. These changes were then further enhanced in those individuals who were best suited for that dynamic social environment—carnivores (electronic supplementary material, figure S1).

By contrast, the negative relationship observed in the liver might reflect resource use and metabolic demands and/or overall growth and fitness. Since the liver is among the first tissues to respond and adapt to nutritional changes [60,61], access to diverse resources (detritus, shrimp, other tadpoles) might induce many metabolic changes. Once individuals begin to specialize on one resource type—meat for carnivores in our cue treatment group, detritus for omnivores in our control group—their metabolic systems might stabilize and become more similar. In other words, we might be seeing a signature of resource specialization and/or metabolic robustness in the liver. The pattern of reversal seen in the liver may also be associated with a general increase in size and/or fitness: carnivores were favoured in the cue treatment and omnivores in the control. Thus, we have shown that both predicted patterns of phenotypic accommodation—stabilization (in this case, reversal) and exaggeration (in this case, enhancement)—can co-occur following a change in environment. Furthermore, differences in tissue function and the nature of the new environment might be critical in determining the relationship between environmental change and adaptive development.

The observed patterns of GO term enrichment further highlight the importance of these differences between tissues. Only 10 genes were shared between the genes that were differentially expressed between environments and between morphs in each tissue. This means that 92% and 98% of these genes were unique to the brain and liver, respectively. In the brain, genes showing enhancement tended to have functions related to oxidative phosphorylation, aerobic respiration, and the innate immune system (electronic supplementary material, table S2). Notably, oxidative phosphorylation is the primary source of energy in the brain during presynaptic and postsynaptic information processing [62], and altered levels of oxidative phosphorylation can affect cognitive function (e.g. [63,64]). Moreover, recent studies have shown that plasticity in spadefoots may often entail regulating oxidative stress [34,65]. Thus, phenotypic accommodation to a new environment in the brain may involve energetic changes that can affect cognition. These findings support the expectation that environmental responses can be exaggerated during phenotypic accommodation.

In the liver, our finding that steroid and cholesterol biosynthesis were associated with genes showing a pattern of enhancement corroborates previous findings regarding spadefoot plasticity. Specifically, cholesterol is a necessary precursor for producing steroid hormones, two of which have been implicated in spadefoot tadpole morphological development (thyroxine, [29]; corticosterone, [66]). Moreover, carnivore–omnivore plasticity is related to changes in developmental rates [29], and recent work has found that cholesterol and steroid biosynthesis are not only important for trophic plasticity [34], but also plasticity in developmental rate in response to pond drying in spadefoots [67]. Together with the present results, this suggests that the cue environment may trigger the process of upregulating the synthesis of cholesterol and presumably hormones derived from it (likely through a dietary shift), and that those individuals exceeding some threshold level develop into carnivores. Such a threshold model of adaptive plastic development may be common (e.g. [68–70]) and warrants further investigation in this system. Indeed, the ability to develop into carnivore tadpoles may have arisen, at least in part, through the co-option of responses induced by pond drying.

In general, phenotypic accommodation and adaptation can be linked through the process of PLE. Over developmental time, if the new environment is stressful (as is often the case; [71]), adaptive development in, or phenotypic accommodation

to, the new environment usually requires overcoming the initial stress response by altering other aspects of development [17]. This *developmental* process reduces the amount of functional disruption imposed by environmentally induced change (as in our observed patterns of reversal in the liver) and can even generate novel adaptive developmental variants (as in our observed patterns of enhancement in the brain; [4,5,17]). Through subsequent evolution, the population of organisms overcomes any responses that would otherwise be deleterious. During this *evolutionary* process, variation among individuals in how effectively they undergo phenotypic accommodation provides the substrate for selection to drive quantitative genetic changes that enhance or reverse developmental responses to produce evolutionary adaptations [4,5,16]. In this way, environmentally induced change may promote both phenotypic accommodation and evolutionary adaptation.

Ethics. All procedures complied with all relevant ethical regulations, and our study protocol was approved by the University of North Carolina Institutional Animal Care and Use Committee (IACUC IDs 14-297.0 and 17-055.0). Field collections were conducted under scientific collection permit SP745794 provided by the Arizona Game and Fish Department.

Data accessibility. Raw sequencing reads have been deposited in the National Center for Biotechnology Information Sequence Read Archive under Bioproject PRJNA675144. Morphological data, expression data and R code are available as electronic supplementary material.

The data are provided in the electronic supplementary material [72].

Authors' contributions. N.A.L.: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, writing—original draft, writing—review and editing; D.J.M.: funding acquisition, supervision, writing—review and editing; D.W.P.: funding acquisition, methodology, project administration, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. Funding was provided by a seed grant from the UNC Biology Department (to D.J.M., D.W.P. and N.A.L.) and the United States National Science Foundation (DEB 1753865 to D.W.P.).

Acknowledgements. We thank E. Harmon and P. Kelly for help with tadpole husbandry, E. Harmon for morphological measurements, and K. Pfennig, E. Harmon, A. Isdaner, P. Kelly, and two anonymous referees for helpful comments on the manuscript.

## References

1. Darwin C. 1868 *The variation of animals and plants under domestication*. London, UK: John Murray.
2. Goldschmidt R. 1940 *The material basis of evolution*. New Haven, CT: Yale University Press.
3. Mayr E. 1959 The emergence of evolutionary novelties. In *Evolution after Darwin* (ed. S Tax), pp. 349–380. Chicago, IL: University of Chicago Press.
4. West-Eberhard MJ. 2003 *Developmental plasticity and evolution*. New York, NY: Oxford University Press.
5. Levis NA, Pfennig DW. 2016 Evaluating 'plasticity-first' evolution in nature: key criteria and empirical approaches. *Trends Ecol. Evol.* **31**, 563–574. (doi:10.1016/j.tree.2016.03.012)
6. Levis NA, Pfennig DW. 2019 Plasticity-led evolution: evaluating the key prediction of frequency-dependent adaptation. *Proc. R. Soc. B* **286**, 20182754. (doi:10.1098/rspb.2018.2754)
7. West-Eberhard MJ. 1989 Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* **20**, 249–278. (doi:10.1146/annurev.es.20.110189.001341)
8. Scheiner SM. 1993 Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol. Syst.* **24**, 35–68. (doi:10.1146/annurev.es.24.110193.000343)
9. Levis NA, Pfennig DW. 2019 Phenotypic plasticity, canalization, and the origins of novelty: evidence and mechanisms from amphibians. *Semin. Cell Dev. Biol.* **88**, 80–90. (doi:10.1016/j.semcdb.2018.01.012)
10. Levis NA, Pfennig DW. 2021 Innovation and diversification via plasticity-led evolution. In *Phenotypic plasticity and evolution: causes, consequences, controversies* (ed. DW Pfennig), pp. 211–240. Boca Raton, FL: CRC Press.
11. Levis NA, Pfennig DW. 2020 Plasticity-led evolution: a survey of developmental mechanisms and empirical tests. *Evol. Dev.* **22**, 71–87. (doi:10.1111/ede.12309)
12. Projecto-Garcia J, Biddle JF, Ragsdale EJ. 2017 Decoding the architecture and origins of mechanisms for developmental polyphenism. *Curr. Opin Genet. Dev.* **47**, 1–8. (doi:10.1016/j.gde.2017.07.015)
13. Aubin-Horth N, Renn SCP. 2009 Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Mol. Ecol.* **18**, 3763–3780. (doi:10.1111/j.1365-294X.2009.04313.x)
14. Corona M, Libbrecht R, Wheeler DE. 2016 Molecular mechanisms of phenotypic plasticity in social insects. *Curr. Opin. Insect Sci.* **13**, 55–60. (doi:10.1016/j.cois.2015.12.003)
15. Hodgins-Davis A, Townsend JP. 2009 Evolving gene expression: from G to E to GxE. *Trends Ecol. Evol.* **24**, 649–658. (doi:10.1016/j.tree.2009.06.011)
16. Badyaev AV. 2009 Evolutionary significance of phenotypic accommodation in novel environments: an empirical test of the Baldwin effect. *Proc. R. Soc. B* **364**, 1125–1141.
17. West-Eberhard MJ. 2005 Phenotypic accommodation: adaptive innovation due to developmental plasticity. *J. Exp. Zool. B* **304**, 610–618. (doi:10.1002/jez.b.21071)
18. West-Eberhard MJ. 2021 Foreword: a perspective on 'plasticity'. In *Phenotypic plasticity and evolution: causes, consequences, controversies* (eds DW Pfennig), pp. ix–xxi. Boca Raton, FL: CRC Press.
19. Waddington CH. 1953 Experiments in acquired characteristics. *Sci. Am.* **189**, 92–99. (doi:10.1038/scientificamerican1253-92)
20. Michener CD. 1961 Social polymorphism in Hymenoptera. *Symp. R. Entomol. Soc. Lond.* **1**, 43–56.
21. Moczek AP, Sultan SE, Foster S, Ledon-Rettig C, Dworkin I, Nijhout HF, Abouheif E, Pfennig DW. 2011 The role of developmental plasticity in evolutionary innovation. *Proc. R. Soc. B* **278**, 2705–2713. (doi:10.1098/rspb.2011.0971)
22. Rutherford SL, Lindquist S. 1998 Hsp90 as a capacitor for morphological evolution. *Nature* **396**, 336–342. (doi:10.1038/24550)
23. Moczek AP. 2007 Developmental capacitance, genetic accomodation, and adaptive evolution. *Evol. Dev.* **9**, 299–305. (doi:10.1111/j.1525-142X.2007.00162.x)
24. Ledón-Rettig CC, Pfennig DW. 2011 Emerging model systems in eco-evo-devo: the environmentally responsive spadefoot toad. *Evol. Dev.* **13**, 391–400. (doi:10.1111/j.1525-142X.2011.00494.x)
25. Martin RA, Pfennig DW. 2010 Field and experimental evidence that competition and ecological opportunity promote resource polymorphism. *Biol. J. Linnean Soc.* **100**, 73–88. (doi:10.1111/j.1095-8312.2010.01380.x)
26. Pfennig DW. 1992 Polyphenism in spadefoot toad tadpoles as a locally adjusted evolutionarily stable strategy. *Evolution* **46**, 1408–1420.
27. McDiarmid RW, Altig R. 1999 *Tadpoles: the biology of anuran larvae*. Chicago, IL: Chicago University Press.
28. Ledón-Rettig CC, Pfennig DW, Nascone-Yoder N. 2008 Ancestral variation and the potential for genetic accommodation in larval amphibians: implications for the evolution of novel feeding strategies. *Evol. Dev.* **10**, 316–325. (doi:10.1111/j.1525-142X.2008.00240.x)
29. Pfennig DW. 1992 Proximate and functional causes of polyphenism in an anuran tadpole. *Funct. Ecol.* **6**, 167–174. (doi:10.2307/2389751)
30. Pfennig DW, Rice AM, Martin RA. 2006 Ecological opportunity and phenotypic plasticity interact to

promote character displacement and species coexistence. *Ecology* **87**, 769–779. (doi:10.1890/05-0787)

31. Pfennig DW. 1990 The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. *Oecologia* **85**, 101–107. (doi:10.1007/BF00317349)
32. Levis NA, de la Serna Buzón S, Pfennig DW. 2015 An inducible offense: carnivore morph tadpoles induced by tadpole carnivory. *Ecol. Evol.* **5**, 1405–1411. (doi:10.1002/ece3.1448)
33. Paull JS, Martin RA, Pfennig DW. 2012 Increased competition as a cost of specialization during the evolution of resource polymorphism. *Biol. J. Linnean Soc.* **107**, 845–853. (doi:10.1111/j.1095-8312.2012.01982.x)
34. Levis NA, Kelly PW, Harmon EA, Ehrenreich IM, McKay DJ, Pfennig DW. 2021 Transcriptomic bases of a polyphenism. *J. Exp. Zool. B Mol. Dev. Evol.* **336**, 482–495. (doi:10.1002/jez.b.23066)
35. Levis NA, Isdaner A, Pfennig DW. 2018 Morphological novelty emerges from pre-existing phenotypic plasticity. *Nat. Ecol. Evol.* **2**, 1289–1297. (doi:10.1038/s41559-018-0601-8)
36. Levis NA, Serrato-Capuchina A, Pfennig DW. 2017 Genetic accommodation in the wild: evolution of gene expression plasticity during character displacement. *J. Evol. Biol.* **30**, 1712–1723. (doi:10.1111/jeb.13133)
37. Seidl F, Levis NA, Jones CD, Monroy-Eklund A, Ehrenreich IM, Pfennig KS. 2019 Variation in hybrid gene expression: implications for the evolution of genetic incompatibilities in interbreeding species. *Mol. Ecol.* **20**, 4667–4679. (doi:10.1111/mec.15246)
38. Seidl F, Levis NA, Schell R, Pfennig DW, Pfennig KS, Ehrenreich IM. 2019 Genome of *Spea multiplicata*, a rapidly developing, phenotypically plastic, and desert-adapted spadefoot toad. *G3 (Bethesda)* **9**, 3909–3919. (doi:10.1534/g3.119.400705)
39. Schluter D. 1988 Estimating the form of natural selection on a quantitative trait. *Evolution* **42**, 849–861. (doi:10.1111/j.1558-5646.1988.tb02507.x)
40. Martin RA, Pfennig DW. 2012 Widespread disruptive selection in the wild is associated with intense resource competition. *BMC Evol. Biol.* **12**, 136. (doi:10.1186/1471-2148-12-136)
41. Pfennig KS, Pfennig DW. 2005 Character displacement as the 'best of a bad situation': fitness trade-offs resulting from selection to minimize resource and mate competition. *Evolution* **59**, 2200–2208. (doi:10.1111/j.0014-3820.2005.tb00928.x)
42. Buzón S.M. dlS, Martin RA, Pfennig DW. 2020 Carryover effects and the evolution of polyphenism. *Biol. J. Linnean Soc.* **131**, 622–631. (doi:10.1093/biolinnean/blaa133)
43. Pfennig KS. 2008 Population differences in condition-dependent sexual selection may promote divergence in non-sexual traits. *Evol. Ecol. Res.* **10**, 763–773.
44. Love MI, Huber W, Anders S. 2014 Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550. (doi:10.1186/s13059-014-0550-8)
45. R Core Team. 2020 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See https://www.R-project.org/.
46. Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN, Hughes KA. 2015 Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature* **525**, 372–375. (doi:10.1038/nature15256)
47. Campbell-Staton SC, Velotta JP, Winchell KM. 2021 Selection on adaptive and maladaptive gene expression plasticity during thermal adaptation to urban heat islands. *Nat. Commun.* **12**, 6195. (doi:10.1038/s41467-021-26334-4)
48. Mallard F, Jakšić AM, Schlötterer C. 2018 Contesting the evidence for non-adaptive plasticity. *Nature* **555**, E21–E22. (doi:10.1038/nature25496)
49. Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, Vilo J. 2019 Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res.* **47**, W191–W198. (doi:10.1093/nar/gkz369)
50. Dayan DI, Crawford DL, Oleksiak MF. 2015 Phenotypic plasticity in gene expression contributes to divergence of locally adapted populations of *Fundulus heteroclitus*. *Mol. Ecol.* **24**, 3345–3359. (doi:10.1111/mec.13188)
51. Ho WC, Zhang J. 2019 Genetic gene expression changes during environmental adaptations tend to reverse plastic changes even after the correction for statistical nonindependence. *Mol. Biol. Evol.* **36**, 604–612. (doi:10.1093/molbev/msz002)
52. Ho WC, Zhang J. 2018 Evolutionary adaptations to new environments generally reverse plastic phenotypic changes. *Nat. Commun.* **9**, 350. (doi:10.1038/s41467-017-02724-5)
53. Koch EL, Guillaume F. 2020 Restoring ancestral phenotypes is a general pattern in gene expression evolution during adaptation to new environments in *Tribolium castaneum*. *Mol. Ecol.* **29**, 3938–3953. (doi:10.1111/mec.15607)
54. Huang Y, Lack JB, Hoppel GT, Pool JE. 2022 Gene regulatory evolution in cold-adapted fly populations neutralizes plasticity and may undermine genetic canalization. *Genome Biol. Evol.* **14**, evac050. (doi:10.1093/gbe/evac050)
55. Baldwin JM. 1896 A new factor in evolution. *Am. Nat.* **30**, 441–451. (doi:10.1086/276408)
56. Rampon C, Jiang CH, Dong H, Tang Y-P, Lockhart DJ, Schultz PG, Tsien JZ, Hu Y. 2000 Effects of environmental enrichment on gene expression in the brain. *Proc. Natl Acad. Sci. USA* **97**, 12 880–12 884. (doi:10.1073/pnas.97.23.12880)
57. Chesler EJ, Williams RW. 2004 Brain gene expression: genomics and genetics. In *International review of neurobiology* (eds RJ Bradley, RA Harris, P Jenner), pp. 59–95. Cambridge, MA: Elsevier.
58. Abbey-Lee RN, Uhrig EJ, Zidar J, Favati A, Almberg J, Dahlbom J, Winberg S, Løvlie H. 2018 The influence of rearing on behavior, brain monoamines, and gene expression in three-spined sticklebacks. *Brain Behav. Evol.* **91**, 201–213. (doi:10.1159/000489942)
59. Ledón-Rettig CC. 2021 Novel brain gene-expression patterns are associated with a novel predaceous behaviour in tadpoles. *Proc. R. Soc. B* **288**, 20210079. (doi:10.1098/rspb.2021.0079)
60. Ranalletta M *et al.* 2007 Hepatic response to restoration of GLUT4 in skeletal muscle of GLUT4 null mice. *Am. J. Physiol. Endocrinol. Metab.* **293**, E1178–E1187. (doi:10.1152/ajpendo.00628.2006)
61. Turner N *et al.* 2013 Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. *Diabetologia* **56**, 1638–1648. (doi:10.1007/s00125-013-2913-1)
62. Hall CN, Klein-Flügge MC, Howarth C, Attwell D. 2012 Oxidative phosphorylation, not glycolysis, powers presynaptic and postsynaptic mechanisms underlying brain information processing. *J. Neurosci.* **32**, 8940–8951. (doi:10.1523/JNEUROSCI.0026-12.2012)
63. Rapoport SI, Hatanpää K, Brady DR, Chandrasekaran K. 1996 Brain energy metabolism, cognitive function and down-regulated oxidative phosphorylation in Alzheimer disease. *Neurodegeneration* **5**, 473–476. (doi:10.1006/neur.1996.0065)
64. Manczak M, Park BS, Jung Y, Reddy PH. 2004 Differential expression of oxidative phosphorylation genes in patients with Alzheimer's disease. *Neuromolecular Med.* **5**, 147–162. (doi:10.1385/NMM:5:2:147)
65. Burraco P, Rendón MA, Díaz-Paniagua C, Gomez-Mestre I. 2022 Maintenance of phenotypic plasticity is linked to oxidative stress in spadefoot toad larvae. *Oikos* **2022**, e09078. (doi:10.1111/oik.09078)
66. Ledón-Rettig CC, Pfennig DW, Crespi EJ. 2009 Stress hormones and the fitness consequences associated with the transition to a novel diet in larval amphibians. *J. Exp. Biol.* **212**, 3743–3750. (doi:10.1242/jeb.034066)
67. Liedtke HC, Harney E, Gomez-Mestre I. 2021 Cross-species transcriptomics uncovers genes underlying genetic accommodation of developmental plasticity in spadefoot toads. *Mol. Ecol.* **30**, 2220–2234. (doi:10.1111/mec.15883)
68. Moczek AP, Nijhout HF. 2003 Rapid evolution of a polyphenic threshold. *Evol. Dev.* **5**, 259–268. (doi:10.1046/j.1525-142X.2003.03033.x)
69. Suzuki Y, Nijhout HF. 2006 Evolution of a polyphenism by genetic accommodation. *Science* **311**, 650–652. (doi:10.1126/science.1118888)
70. Bui LT, Ivers NA, Ragsdale EJ. 2018 A sulfotransferase dosage-dependently regulates mouthpart polyphenism in the nematode *Pristionchus pacificus*. *Nat. Commun.* **9**, 1–10. (doi:10.1038/s41467-017-02088-w)
71. Badyaev AV. 2005 Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proc. R. Soc. B* **272**, 877–886. (doi:10.1098/rspb.2004.3045)
72. Levis NA, McKay DJ, Pfennig DW. 2022 Data from: Disentangling the developmental origins of a novel phenotype: enhancement versus reversal of environmentally induced gene expression. Figshare. (doi:10.6084/m9.figshare.c.6250553)