Seminars in Cell & Developmental Biology xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians

Nicholas A. Levis, David W. Pfennig*

Department of Biology, CB#3280, University of North Carolina, Chapel Hill, NC, 27599, USA

ARTICLE INFO

Article history:
Received 18 September 2017
Received in revised form 25 January 2018
Accepted 29 January 2018
Available online xxx

Keywords:
Canalization
Genetic assimilation
Hormones
Intraspecific macroevolution
Plasticity-first evolution

ABSTRACT

A growing number of biologists have begun asking whether environmentally induced phenotypic change––'phenotypic plasticity'––precedes and facilitates the origin and canalization of novel, complex phenotypes. However, such 'plasticity-first evolution' (PFE) remains controversial. Here, we summarize the PFE hypothesis and describe how it can be evaluated in natural systems. We then review the evidence for PFE from amphibians (a group in which phenotypic plasticity is especially widespread) and describe how phenotypic plasticity might have facilitated macroevolutionary change. Finally, we discuss what is known about the proximate mechanisms of PFE in amphibians. We close with suggestions for future research. As we describe, amphibians offer some of the best support for plasticity's role in the origin of evolutionary novelties.

Contents

1. Introduction

Among biology's enduring problems is to explain how novel, complex phenotypes originate [1,2]. New phenotypes have often been assumed to arise exclusively from genetic changes [3]. Consistent with this assumption, many features have now been traced to single gene mutations [e.g., 4,5], duplications of large regions of the genome [e.g., 6,7], alterations in regulatory sequence [e.g., 8–10], and/or a variety of other changes in DNA sequence or content.

Yet, in recent years, an increasing number of biologists have begun questioning whether novel features arise solely from genetic changes. These researchers have been asking if environmentally induced phenotypic change––i.e., "phenotypic plasticity" (hereafter just "plasticity" and used synonymously with 'developmental plasticity')––might also play a role in initiating novelty [e.g., 11,12–20]. Indeed, some researchers have noted that plasticity can trigger phenotypic divergence within species as great as that *between* species [21], suggesting that it might even contribute to *large-scale* evolutionary change; i.e., "macroevolution" [22,23].

* Corresponding author.
E-mail address: dpfennig@unc.edu (D.W. Pfennig).

https://doi.org/10.1016/j.semcdb.2018.01.012
1084-9521/

Here, we discuss plasticity's possible contribution toward the origins of complex, canalized (i.e., fixed) phenotypes. We begin by introducing the theory of how plasticity might promote novelty. Next, we discuss some ways by which this theory can be evaluated. We then review case studies that have used these approaches to test the theory. We focus on amphibians because they display abundant and striking plasticity, and they have been subjects of considerable research. Indeed, as we describe, amphibians offer some of the strongest support implicating plasticity in macroevolutionary change. We then review what is known about the proximate (i.e., genetic, developmental, and physiological) mechanisms underlying plasticity-mediated evolution in amphibians. Finally, we close with a brief discussion of potential future research directions.

2. Plasticity-first evolution

Plasticity is commonplace [reviewed in 24], and it has long been thought to play a role in fostering evolutionary innovation [12,25–27]. Although a number of routes by which plasticity might promote novelty have been proposed [12,26], we focus here on a widely accepted pathway dubbed "plasticity-first evolution" [hereafter, 'PFE'; 28,29].

PFE rests on the observation that plasticity often enhances fitness under stressful conditions [14,30,31]. When an individual encounters an environment that induces such a change in its phenotype, the multidimensional nature of developmental and physiological processes can help stabilize the expression of the altered phenotype, such that the individual can persist in the new environment [i.e., "phenotypic accommodation" occurs; sensu 12]. If underlying genetic variation exists in either the tendency or manner in which individuals respond to the environment [i.e., if different genotypes exhibit different "reaction norms"; 32], then selection can act on such variation [or de novo variation induced by the environmental shift; sensu 33,34] and improve this new phenotype's functionality. Moreover, depending on whether or not plasticity is favored [35,36], selection can also respectively promote either *increased* environmental sensitivity––which might ultimately maintain the new phenotype as part of a "polyphenism" [sensu 37]—or *decreased* environmental sensitivity––in which plasticity is lost and the phenotype becomes canalized. The latter outcome, known as "genetic assimilation" [sensu 27] might occur for two reasons. First, when plasticity is costly [38,39], selection can actively eliminate it, leading to the canalization of the favored phenotype. Second, plasticity can be lost through mutational degradation or genetic drift [40], as might occur when non-favored phenotypes are seldom expressed and thereby experience relaxed selection [41–43].

Regardless of whether the outcome is a polyphenism or genetic assimilation, the key point is that selection can act on an initially environmentally induced phenotype and promote an adaptive change in the form and/or regulation of that phenotype [a process known as "genetic accommodation"; sensu 12]. In this way, plasticity could precede––and facilitate––the origin and canalization of a novel, complex phenotype (Fig. 1). Essentially, plasticity might often "jump start" phenotypic change in an adaptive direction [44].

PFE may be a more common route to novelty than is generally recognized [28,29]. Indeed, environmentally triggered novelties could have greater evolutionary potential than genetic mutations, for at least three reasons [12]. First, in contrast to most genetic mutations (which initially affect only one individual and its descendants), changes in the environment often impact *many* individuals simultaneously. This widespread recurrence means that environmentally induced novelties are less likely to be lost through drift and are more likely to be tested in diverse genetic backgrounds, both of which increase the chances of genetic accommodation [12]. Second, with environmentally triggered novelties, the inducing cue(s) is often associated with the environment in which the trait is adaptive [e.g., see 45,46–50]. In contrast, a mutation occurs regardless of whether or not the organism is in an environment in which that mutation is advantageous [but see 33,51,52]. Consequently, environmentally induced traits are more likely to experience consistent selective pressures when expressed, which also facilitates genetic accommodation [12].

Finally, plasticity promotes the storage and release of "cryptic genetic variation"––i.e., variation that is expressed only under new conditions [53–56]. Specifically, genetic variation that is not expressed (as is often the case with alleles associated with environmentally triggered traits) accrues because it is not exposed to, and removed by, selection [43,53,55]. However, when novel phenotypes are triggered by environmental change, the formerly cryptic genetic variation underlying these phenotypes becomes exposed to selection. Indeed, genetic variation influencing the regulation of plastic traits is plentiful [36,57], and the expression of such variation often increases when populations encounter novel environments [58]. The release of such variation fuels and thereby facilitates genetic accommodation [59] (Fig. 1b).

Lab studies have shown that PFE *can* lead to canalization of environmentally-induced phenotypes [e.g., 27,60,61]. Yet, some researchers remain skeptical of PFE, primarily because of a perceived lack of evidence from the wild [62,63]. However, recent years have seen growing support for PFE from numerous natural populations [reviewed in 19,29].

Here, we review some of this support. Specifically, we discuss the evidence for PFE from studies of natural populations of amphibians. Before doing so, however, we discuss some general approaches that can be used to evaluate PFE in natural populations.

3. Empirical approaches for evaluating PFE

The most direct evidence of PFE from natural systems involves comparing plasticity in ancestral and derived (sister) lineages, such as when derived populations have undergone a recent range expansion from progenitor ancestral populations [e.g., 64]. When ancestors and descendants cannot be evaluated directly, however, phylogenetic inference allows indirect tests of PFE [19,65]. Alternatively, a widely-used approach is to compare the reaction norms (i.e., the trait's plasticity as estimated from the slope of the line between trait values in alternative environments) of two different types of lineages: one that possesses the focal, potentially canalized, trait (representing the "derived" condition) and one that is closely related to the former lineage but that lacks the focal trait (representing the "ancestral" condition and that can therefore serve as an "ancestor-proxy" lineage). Reaction norms of these two types of lineages can be compared when both lineages are reared in both the derived environment (i.e., the environment in which the trait is associated) and the ancestral environment [66]. This approach can be effective in evaluating the following critical predictions of PFE [29]:

Prediction 1––The focal trait can be environmentally induced in lineages showing the ancestral state. A fundamental prediction of PFE is that the novel trait should exhibit "ancestral plasticity." In other words, the derived trait (or components thereof) should be environmentally induced in lineages that normally lack the trait, but only when they experience the derived environment [12].

Prediction 2––The focal trait's heritability should increase when lineages with the ancestral state experience the derived environment. As noted above, cryptic genetic variation can accrue when genetic variants are not expressed phenotypically. Such could be the case if lineages still showing the ancestral state remain in the ancestral environment. However, once these lineages experience a

Please cite this article in press as: N.A. Levis, D.W. Pfennig, Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2018.01.012

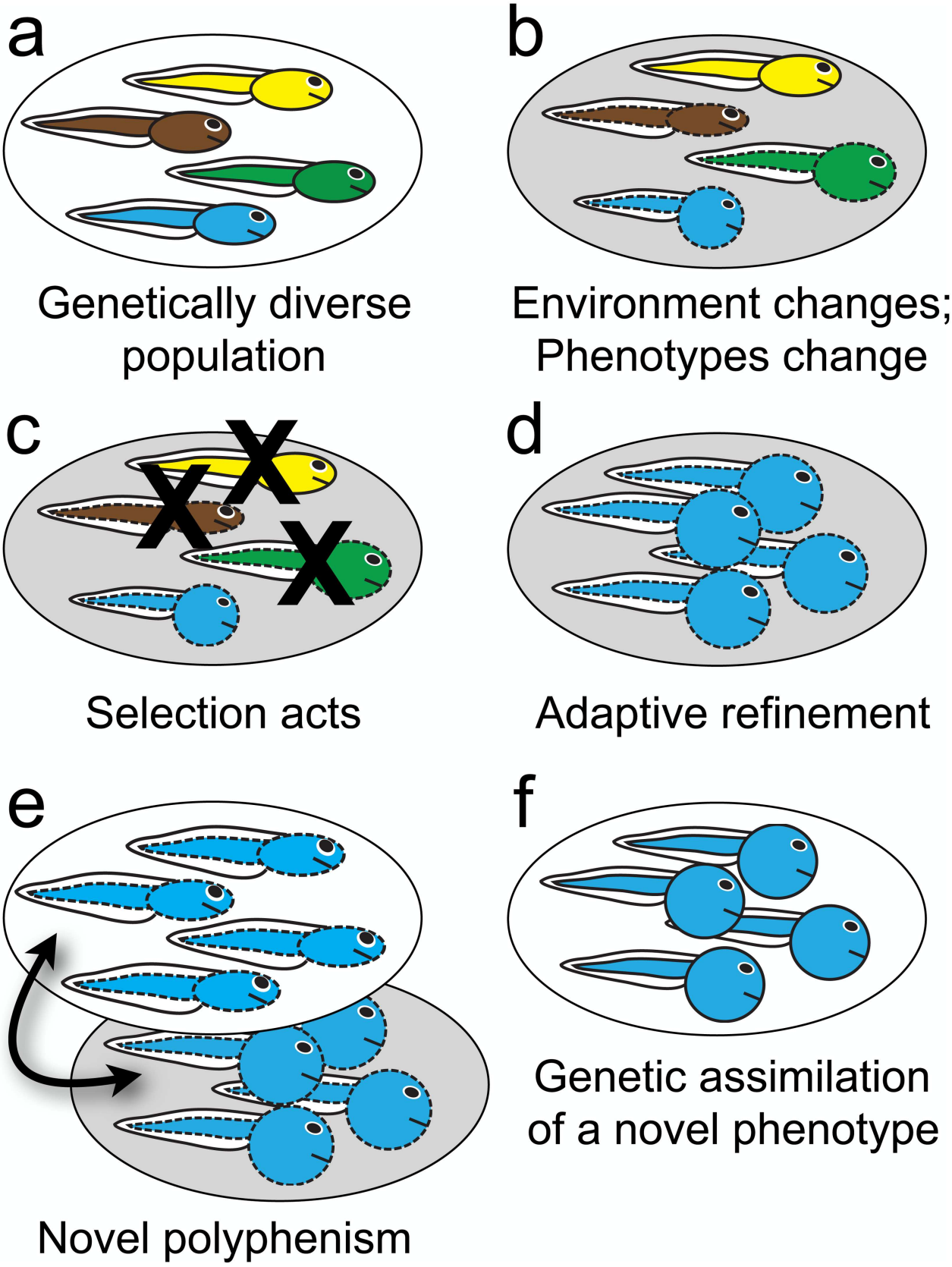

Fig. 1. How plasticity can facilitate the evolution of a novel, complex phenotype. (a) A genetically diverse population (different colors: different genotypes) (b) experiences a novel environment (shading), which induces novel phenotypes (dashed lines), but genotypes differ in whether and how they respond (different shapes). (c) Selection acts on this formerly cryptic genetic variation (revealed by a change in environment) and disfavors genotypes that produce poorly adapted phenotypes ('X'). (d) This leads to the adaptive refinement of the favored phenotype (enlargement of the blue tadpole). (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 favor 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).

Please cite this article in press as: N.A. Levis, D.W. Pfennig, Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2018.01.012

novel environment, this formerly cryptic genetic variation might be expressed. In the case of an environmentally induced, novel trait, this uncovering of cryptic genetic variation should be manifest as an increase in the trait's heritability [67]. Observing such an increase would confirm the presence of cryptic genetic variation upon which selection could act [13,68]. This confirmation is necessary to eliminate the possibility that the novel trait arose solely through lineage-specific mutations [as in 34,69].

Prediction 3––The focal trait should exhibit evidence of having undergone an evolutionary change in its degree of plasticity and/or form in lineages with the derived trait. During genetic accommodation, a trait that is initially environmentally induced undergoes an evolutionary shift in degree of plasticity or form [12]. Such shifts would manifest as changes to the slope, curvature, and/or elevation of the reaction norm [70]. Finding that selection has led to the complete loss of plasticity would imply that the trait has been genetically assimilated [i.e., its expression is now canalized; 27,70].

Prediction 4––The focal trait should exhibit evidence of having undergone adaptive refinement as it is induced and exposed to selection repeatedly. During genetic accommodation, selection improves the functionality of a trait. Thus, as a novel trait is expressed (and exposed to selection) more frequently, it should experience greater and more rapid refinement [12,39].

Note that validation of any one of the above four predictions, by itself, is insufficient to establish that PFE has occurred. For instance, although ancestral plasticity has been documented in many systems [Prediction 1; 19,28], such documentation (by itself) is insufficient to demonstrate PFE. This is because the PFE hypothesis specifically requires that *selection* favored an evolutionary change in the degree of plasticity [12]. Non-selective processes (e.g., drift, mutation) can also promote such shifts and must therefore be ruled out to demonstrate PFE. Thus, studies that verify several (ideally, all four) predictions provide the strongest support for PFE. Below, we review some of these studies.

4. Empirical tests of PFE in amphibians

There is increasing support for PFE. Indeed, many researchers have (intentionally or not) validated one or more of the above predictions in numerous natural populations of plants and animals [reviewed in 12,19,28,29,71]. Here, we discuss the evidence from amphibians, which (as described below) have furnished some of the strongest support to date for plasticity's role evolutionary innovation.

4.1. Development time of spadefoot toads

The rapid development time of North American spadefoot toads (genera *Scaphiopus* and *Spea*; Fig. 2) has potentially arisen via PFE. Unlike their Old World counterparts of the genera *Pelobates* and *Pelodytes*, which breed in permanent or long-lasting ponds and have long larval periods (>180 days in some species), the New World species in the genera *Scaphiopus* and *Spea* occupy ephemeral ponds and are canalized for extremely reduced larval periods [<8 days in some species; 72–74].

Gomez-Mestre and Buchholz [75] reconstructed the reaction norm for development time between short- and long-lasting aquatic environments for the common ancestor of Old World and New World spadefoots [these lineages split 160–170 Mya; 76,77] and compared it to the reaction norms of extant spadefoots. They found that spadefoots from the Old World (where pond duration is generally longer and more variable) more closely resemble the common ancestor in that they have an overall longer development time and exhibit greater plasticity in development time than the relatively canalized development of New World (North American) spadefoots. The authors also showed that these evolved changes in larval period have been accompanied by concomitant changes in morphological development (limb length) in these derived lineages. Two additional studies corroborate these observations. In both, the authors compared the development time (larval period) of two species of North American spadefoot (*Sc. couchii* and *Sp. multiplicata*) that inhabit a desert environment with an Old World ancestor-proxy species (*P. cultripes*) from longer-duration, more variable ponds [78,79]. They found that the desert-adapted species had greater canalization in larval period and that larval period in nature predicted degree of plasticity to simulated pond drying in the laboratory. Furthermore, the desert adapted species were smaller at metamorphosis and had fewer fat bodies, regardless of pond duration. Finally, they found that degree of plasticity in development time corresponded to changes in standard metabolic rate and hormone levels, such that the most plastic species (*P. cultripes* and *Sp. multiplicata*) had the greatest changes in these physiological measures and the least plastic species (*Sc. couchii*) had constitutively high metabolic rate and hormone levels.

Thus, for this system, there is evidence of *ancestral plasticity* (consistent with Prediction 1). There is further evidence that this ancestral plasticity has undergone an *evolutionary* shift in derived (North American) lineages (consistent with Prediction 3) such that plasticity has been reduced (i.e., canalization has been increased). Additionally, there is the suggestion that this ancestral plasticity might have undergone *adaptive refinement* in derived lineages because of concomitant evolved changes in morphology and fat body formation (consistent with Prediction 4). Furthermore, the correspondence between the degree of plasticity in development time and magnitude of physiological changes (i.e., hormone levels and metabolic rate) associated with pond drying suggest adaptive refinement as well.

Despite the compelling evidence presented above, the case for PFE could be strengthened in this system. For instance, there has not been an explicit test of Prediction 2 (uncovering of cryptic genetic variation). In addition, more explicit tests are needed to evaluate whether or not a change in environment (i.e., transition to desert habitat) drove the observed changes in plasticity of North American spadefoots [76]. Nevertheless, this example illustrates nicely how plasticity might have promoted evolutionary change in development, which might have enabled these amphibians to invade novel habitats.

4.2. Alternative ecomorphs in spadefoot toads

In addition to evolved changes in development time, spadefoot toads of the genus *Spea* have also evolved a unique "resource polyphenism", in which their tadpoles produce alternative, environmentally induced morphs [46,80,81]. Most anuran larvae feed on detritus and microorganisms and possess small jaw muscles, smooth keratinized mouthparts, numerous denticle rows, and a long gut [82,83]. Although *Spea* tadpoles develop these features by default (the "omnivore" morph), they can also develop into an alternative "carnivore" morph that is characterized by large jaw muscles, notched and keratinized mouthparts, few denticle rows, and a short gut (Fig. 3). This carnivore morph is specialized for eating fairy shrimp and other tadpoles [84], and it is a derived, novel phenotype restricted to *Spea* [66].

This distinctive carnivore morph can be induced by the consumption of shrimp or tadpoles [46,85]. However, the frequency with which carnivores are produced––and how extreme they are––varies among species, populations, and even sibships [86–88], suggesting underlying genetic variation in propensity to produce and express the carnivore phenotype.

Disruptive selection favors both omnivores and carnivores in natural populations [89–92]. Such selection arises because tadpoles

Please cite this article in press as: N.A. Levis, D.W. Pfennig, Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2018.01.012

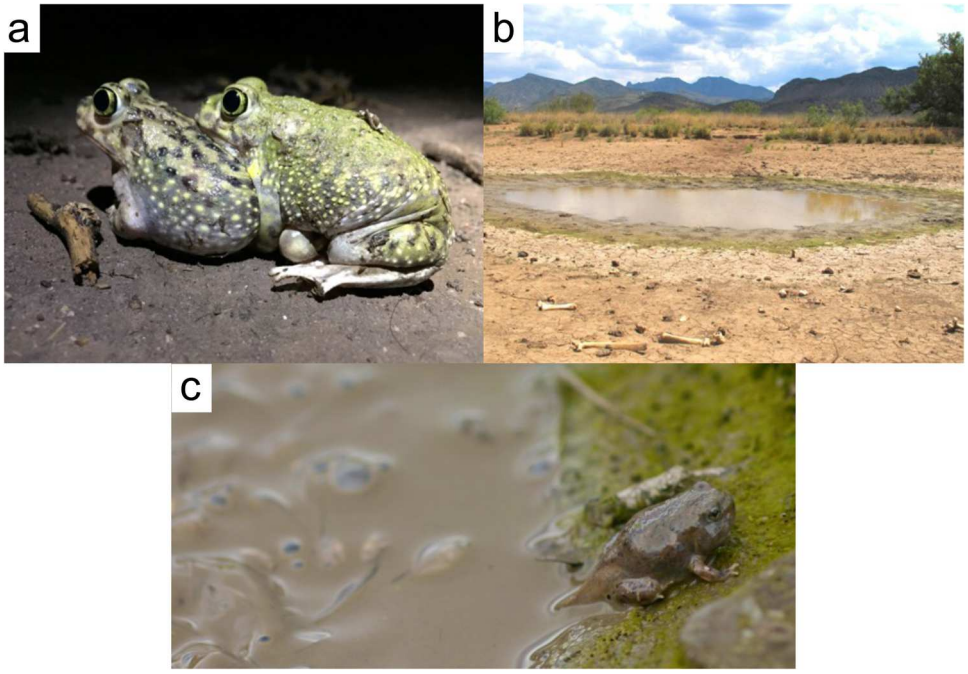

Fig. 2. A model amphibian for studying PFE. (a) Spadefoots from the southwestern U.S. (such as this Couch's spadefoot toad, *Scaphiopus couchii*) typically breed in (b) temporary rain-filled ponds. (c) This harsh environment has favored rapid, but environmentally sensitive, development (here, a metamorph of a Mexican spadefoot toad, *Spea multiplicata*, emerges from a drying pond).

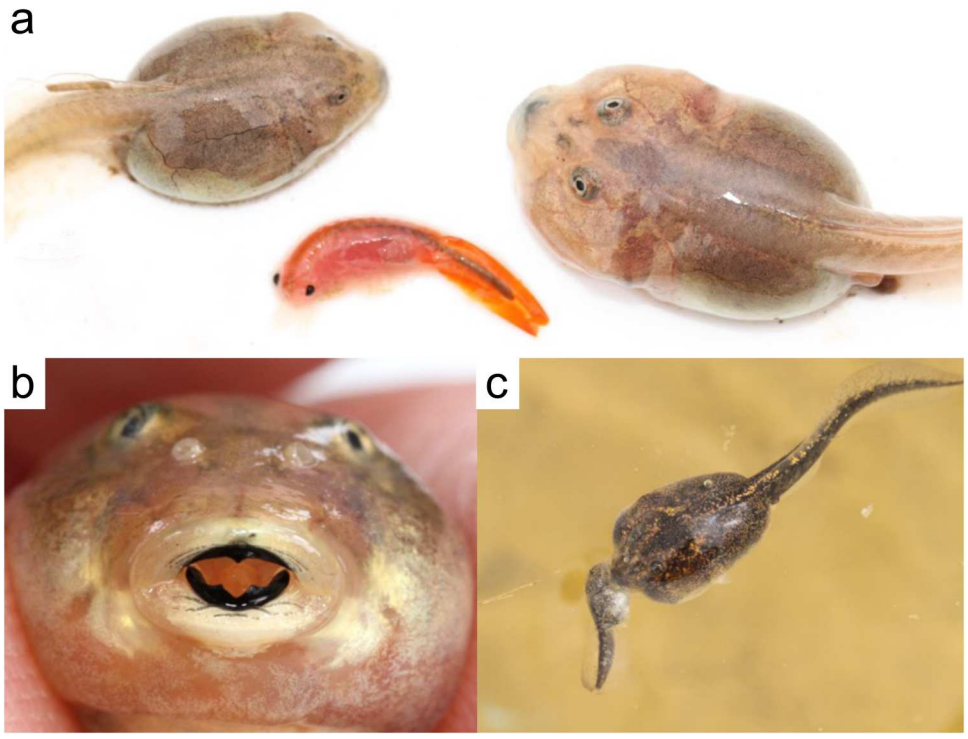

Fig. 3. North American spadefoot toads of the genus *Spea* have evolved a unique resource polyphenism. (a) Like most anuran tadpoles, spadefoots normally develop into a typical 'omnivore' morph by default (pictured on the left). However, if a young tadpole ingests large animal prey, such as Anostracan fairy shrimp (center), it might develop into a novel carnivore morph (right). (b) Among other novel features, carnivores develop a keratinized beak, which they use to grasp large prey, such as (c) other tadpoles.

with intermediate phenotypes are outcompeted by carnivores for shrimp/tadpoles and by omnivores for detritus [89]. Additionally, carnivores are favored in shrimp-rich, short-duration ponds (because they develop faster) and omnivores are favored in shrimp-poor, longer-duration ponds [because they can consume a wider range of resources; 46,80,81,84].

Several studies, taken together, suggest that the carnivore morph might have arisen via PFE. First, this phenotype appears to have started out when selection acted on continuous, diet-induced

Please cite this article in press as: N.A. Levis, D.W. Pfennig, Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2018.01.012

plasticity in a non-polyphenic ancestor. Using *Scaphiopus couchii* as a proxy for *Spea*'s non-plastic ancestors (*Sc. couchii* produce only omnivores), Ledón-Rettig et al. [66,67] found that *Sc. couchii* tadpoles developed shorter guts when fed shrimp than when fed detritus, suggesting that diet-induced plasticity is present in this representative of the ancestral state (consistent with Prediction 1). This species also exhibited greater heritability in gut length when fed shrimp vs. detritus, suggesting that the derived stimulus (shrimp) uncovered cryptic genetic variation [67], which is consistent with Prediction 2. Finally, although *Sp. bombifrons* tadpoles showed an increase in gut cell proliferation when fed shrimp vs. detritus, no such increase was detected in *Sc. couchii* [gut cell proliferation is a measure of gut functionality; 66]. Moreover, *Sp. bombifrons* grew and survived better when fed shrimp vs. detritus; again, the opposite pattern was observed in *Sc. couchii* [93]. This is evidence that shrimp consumption has been adaptively refined, consistent with Prediction 4 of PFE.

The novel carnivore morph appears to have undergone subsequent canalization in certain populations of *Sp. bombifrons* as a result of character displacement with its congener *Sp. multiplicata* [87,92,94]. In ponds containing only a single species (i.e., allopatry), both species produce similar, intermediate frequencies of both morphs. By contrast, in ponds where both species occur (i.e., sympatry), each species becomes nearly monomorphic, with *Sp. multiplicata* producing mostly omnivores, and *Sp. bombifrons* producing mostly carnivores as a result of interspecific competition. These differences between species in morph production in sympatric populations persist even when tadpoles are reared under laboratory conditions, suggesting that the differences are fixed [87].

This evolution of reduced plasticity in sympatry appears to reflect genetic assimilation (i.e., Prediction 3). Experiments have shown that plasticity-mediated shifts in allopatric (ancestral) populations mirror the more highly canalized trait differences observed in sympatric (derived) populations that have undergone character displacement [because these two species have come into secondary contact following a range expansion, allopatry represents the ancestral condition and sympatry the derived condition; 95–97]. In these experiments, allopatric *Sp. multiplicata* that were reared with *Sp. bombifrons* facultatively produced mostly omnivores, which mirrors morph production among *Sp. multiplicata* in natural sympatric populations. Conversely, allopatric *Sp. bombifrons* that were experimentally exposed to *Sp. multiplicata* facultatively produced mostly carnivores, which mirrors morph production among *Sp. bombifrons* in natural sympatric populations. *Spea bombifrons* produce more carnivores in the presence of *Sp. multiplicata*, because they are more effective at capturing and consuming shrimp [87], a diet that induces carnivores. Thus, in sympatric populations, the environmentally induced (i.e., plastic) morphs found in allopatry are more highly canalized, suggesting that PFE may have occurred. Thus, all four predictions suggesting that the novel carnivore morph in *Spea* arose via PFE are satisfied.

4.3. Salamander life history strategies

Differences in life history strategy among eastern newt (*Notophthalmus viridescens*) subspecies and among tiger salamander (*Ambystoma* spp.) species might have evolved through PFE. Four subspecies of eastern newt differ in their color pattern, body size, and life history strategy [reviewed in 98]. The 'typical' life history strategy of eastern newts consists of a larval stage followed by a terrestrial eft stage for up to seven years before returning to an aquatic life as adults [reviewed in 98]. Notably, in the wild, these subspecies differ in the frequency with which their development proceeds through one of three possible trajectories: (1) metamorphosis to an aquatic lunged adult after passing through a terrestrial juvenile (eft) stage, (2) metamorphosis directly to a lunged aquatic adult, or (3) maturation to a gilled aquatic adult and skipping metamorphosis (i.e., paedomorphosis).

Takahashi and Parris [98] performed a common garden experiment in which they subjected larvae from each of these subspecies to various hydroperiods and determined what developmental trajectory they followed. They found that one of the subspecies (*N. viridescens viridescens*) obligately metamorphosed into an eft stage across all hydroperiods, but two others (*N. v. dorsalis* and *N. v. louisianensis*) adjusted their strategies to match pond duration—a short hydroperiod only produced efts, but long and constant hydroperiods produced paedomorphs and/or aquatic adults without an eft stage. Thus, they demonstrated that some populations have become genetically canalized for production of only a single developmental trajectory (i.e., *N. v. viridescens*) while others have maintained their plasticity (consistent with Prediction 3). Furthermore, these differences in plasticity appear to improve fitness of these different lineages in the form of greater growth efficiency [99,100] and earlier sexual maturity [98; possibly consistent with Prediction 4]. Despite the convincing evidence in support of Prediction 3, further work is needed to elucidate: 1) which developmental trajectory(ies) and environmental conditions are the ancestral state [despite the ancestor likely possessing the developmental mechanisms that selection could refine; 101]; 2) if a change in hydroperiod uncovered cryptic genetic variation for these alternative trajectories; and 3) the extent to which selection has adaptively refined these alternatives in different lineages.

In the newt example above, plasticity has generated life history differences *within* a species. We now turn to an example in which plasticity appears to have contributed to life history differences *across* species. In tiger salamanders (*Ambystoma* spp.), developmental trajectory varies among species. In these species, there are two, alternative life-history strategies: metamorphosis into a terrestrial adult or paedomorphosis into an aquatic adult [102]. In lineages where paedomorphosis is facultative (i.e., plastic; e.g., *Ambystoma mavortium*), pond quality (often related to larval density) and larvae size are important determinants of which trajectory an individual will follow [103]. Other lineages are canalized (or nearly so) for one alternative (i.e., metamorphosis in *A. tigrinum*) or the other (i.e., paedomorphosis in *A. mexicanum*) [104]. Thus, looking broadly, one can conceive of an evolutionary sequence in which an ancestrally metamorphic lineage (like *A. tigrinum*) develops an alternative paedomorphic strategy (like *A. mavortium*) that ultimately becomes fixed in some lineages (like *A. mexicanum*). While this hypothesis may be provocative, there have been few (if any) direct tests of it. Furthermore, the underlying cause of metamorphic failure in axolotls (*A. mexicanum*) seems to vary among populations [105–107], which casts doubt on whether selection on a single ancestral lineage refined this phenotype. However, the mere presence of parallel loss of metamorphosis suggests an important role for selection, and variation in how the derived phenotype is produced does not necessarily preclude the possibility of PFE. This is because genetic variation in the ancestral lineage may have allowed individuals to accommodate the new phenotype via different underlying mechanisms that, because they produced equivalent phenotypes, are still maintained today [12,108].

4.4. Tadpole insecticide tolerance

Finally, Hua et al. [109,110] evaluated PFE to insecticide exposure using various populations of wood frogs (*Lithobates sylvaticus*). They used multiple populations located at a variety of distances from agriculture: their ancestor-proxy populations were those located farthest (600–800 m) from agricultural areas because these populations were exposed to the insecticide carbaryl least frequently (or not at all) and their most 'derived' populations were those located adjacent to agricultural land (<100 m). They reared

Please cite this article in press as: N.A. Levis, D.W. Pfennig, Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2018.01.012

embryos from each environment (near and far) in the laboratory and outdoor mesocosms to 1) assess the overall tolerance of these populations to the insecticide and 2) evaluate the extent of inducible tolerance (i.e., plasticity) of these populations. Here, the overall tolerance refers to the ability to survive (i.e., time to death) when exposed to a lethal dose of carbaryl, and inducible tolerance refers to the ability to better survive a *lethal* dose of carbaryl after prior exposure to a *sublethal* dose compared to controls that were not previously exposed to the sublethal dose. Consistent with PFE, they observed that ponds located far from agriculture exhibited plasticity in insecticide tolerance (consistent with Prediction 1), and that insecticide tolerance in ponds located near to agriculture had been become canalized (consistent with Prediction 3). Consistent with Prediction 4, a previous study demonstrated that populations located closer to agriculture have overall higher tolerance than those further away [consistent with Prediction 4; 111]. In addition, the extent to which acetylcholine esterase (the enzyme targeted by carbaryl in this species) concentrations in the body increased following sublethal exposure varied by population type, which may also be indicative of adaptive refinement during canalization. What remains to be seen is evidence of Prediction 2 being fulfilled and/or additional physiological and fitness-based tests of Prediction 4.

4.5. Other potential systems

Other amphibian systems and phenotypes that might be useful for testing PFE include: the 'bulgy' morph tadpoles induced by predatory salamanders [e.g., 50]; predatory salamanders that develop a larger gape in response to these bulgy tadpoles [112] and crowding [113]; numerous examples of morphological [i.e., tail and body shape; e.g., 49,114] and behavioral changes [e.g., 49,115] that tadpoles undergo when exposed to chemical cues from predators; plasticity in hatching time in response to predator cues [e.g., 47,116]; cannibalistic tiger salamanders [117,118]; the transition between metamorphosis and paedomorphosis among populations of tiger salamanders [102]; morphological changes in response to herbicide exposure that are reminiscent of predator-induced changes [119–121]; and the ability to facultatively change skin texture from rough to smooth [122].

5. Plasticity and macroevolution

PFE is similar to the "flexible stem hypothesis" [12] in which the phenotypic plasticity of an ancestor generates phenotypically [123] and genetically [71] divergent derived lineages via genetic assimilation of alternative morphs or phenotypes [124]. Indeed, as noted in the previous section, plasticity appears to have facilitated the evolution of a wide array of complex phenotypes in amphibians, from a new mode of reproduction in newts and salamanders (paedomorphosis) to a novel resource-use morph in spadefoot toads (the carnivore morph). These observations therefore lend credence to the claim that plasticity instigates novelty. However, there is another reason for suggesting that plasticity fosters evolutionary innovation: it can generate alternative phenotypes within species that are as divergent from each other––in morphology, behavior, and/or physiology––as are different species or even higher taxonomic categories.

Consider, for instance, the novel carnivore morph in spadefoot toads (Fig. 3). This environmentally induced phenotype is as morphologically distinct from the default omnivore morph––produced by the *same* species––as the omnivore morph is to tadpoles from an entirely different *genus* [Fig. 4; for other examples, see 21,125,126]. In fact, for decades, herpetologists thought that these two morphs belonged to separate species [73].

For these reasons, such extreme plasticity (specifically, polyphenism) has long intrigued evolutionary biologists, at least as far back as Weismann [25], Goldschmidt [127], and Waddington [128]. They theorized that environmentally triggered morphs could be informative about the evolution of novelties and differences between species. Indeed, polyphenism has been dubbed 'intraspecific macroevolution' [12,21]. Plasticity might ultimately accelerate macroevolutionary change because it enables alternative phenotypes to evolve semi-independently toward major new adaptive peaks without the negative fitness consequences that usually accompany such large-scale evolutionary shifts [reviewed in 12,39,71].

Moreover, because phenotypic alternatives can undergo canalization via genetic assimilation (see above), they might eventually become reproductively isolated from each other [129]. In such a situation, plasticity could facilitate speciation and adaptive radiation [reviewed in 16,65,124]. Thus, plasticity might play an underappreciated role in promoting both evolutionary innovation *and* diversification [12,13,16,26,71,123].

6. Proximate mechanisms of PFE in amphibians

We now review the proximate mechanisms by which PFE might unfold in amphibians. First, recall that PFE proceeds through two general phases: 1) phenotypic accommodation, in which a novel phenotype is induced and refined through developmental processes acting within generations; and 2) genetic accommodation, in which selection promotes the adaptive refinement of such an induced phenotype through genetic processes acting across generations. For phenotypic accommodation to occur, numerous developmental and physiological processes must come into play [12]. Furthermore, these processes must subsequently undergo adaptive (evolutionary) refinement through genetic changes. Therefore, it is worth exploring what types of processes have been implicated in PFE in amphibians.

Hormonal changes are a primary mechanism by which plastic phenotypes arise and evolve [24,130]. In amphibians, three hormones are crucial [131]: thyroid hormone (TH), corticotropin-releasing hormone (CRH), and corticosterone (CORT). TH in particular induces a complex suite of morphological and biochemical changes in each of a developing tadpole's tissues (especially the central nervous system) during metamorphosis [132]. Secretion of TH and corticosteroids (e.g., CORT) is controlled by CRH [133] which is responsive to desiccating (e.g., desert-like) conditions [134]. By working synergistically, TH and corticosteroids from interrenal glands accelerate metamorphosis [132]. Thus, desiccating conditions lead to an increase in CRH, which in turn, increases CORT and TH levels and increases development rate [134]. Importantly this pattern is conserved evolutionarily in diverse species, from spadefoot toads to tiger salamanders [134–137].

Evolutionary changes in these hormones have facilitated some of the dramatic changes described above in **Section IV**. First, Hollar et al. [138] demonstrated that *Scaphiopus couchii* (a canalized rapidly-developing spadefoot species) have higher expression of the TH receptor (TRα) throughout development than *Pelobates cultripes* (a slow, variably developing spadefoot species). Moreover, the ability of *P. cultripes* to shorten its larval period in response to reduced water levels is achieved by increased TH, CORT, and TRβ (another TH receptor) [139]. Furthermore, whereas *P. cultripes* increases TH and CORT levels in response to pond drying, *Sc. couchii* exhibits constitutively high levels of both hormones across pond drying regimes [79]. Similarly, whereas *Sc. couchii* has high levels of CORT throughout development, *P. cultripes* (like most amphibians) experiences a spike in CORT at metamorphosis [79]. This suggests that the canalized, rapid development of *Sc. couchii* is achieved, at

Please cite this article in press as: N.A. Levis, D.W. Pfennig, Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2018.01.012

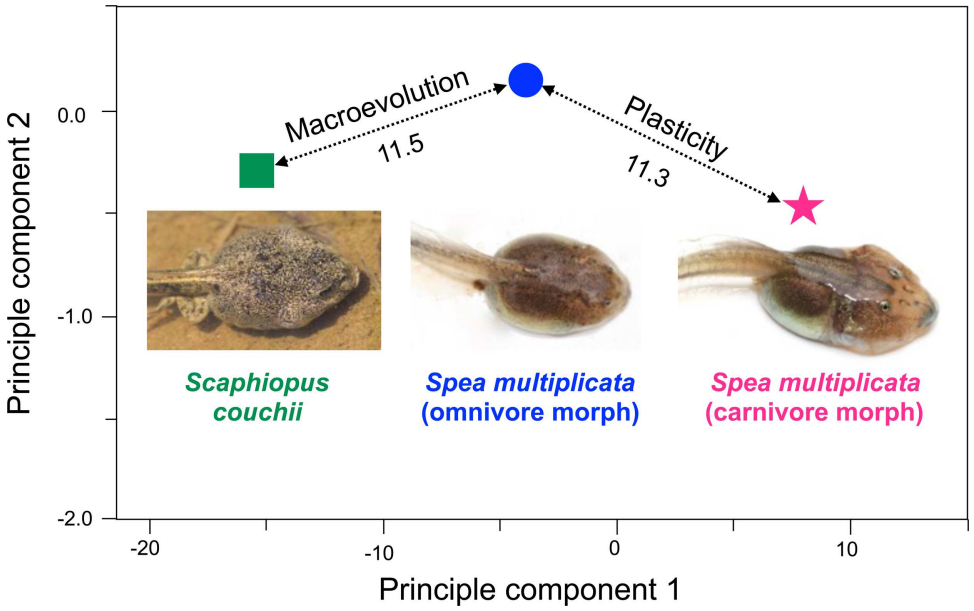

Fig. 4. Plasticity can generate phenotypic divergence within species as great as that between species. Depending on their diet, spadefoot toad tadpoles in the genus *Spea* develop into either an omnivore morph or a carnivore morph (see Fig. 3). An analysis of body shape reveals that these two morphs (in this case, within *Sp. multiplicata*) are as divergent as are the tadpoles of different *genera* of spadefoot toads (numbers denote least squares mean differences between morphs/species in principle component space).

least in part, by this species maintaining its hormonal state near those required for metamorphosis which thereby facilitates rapid exit from a pond.

The evolution of the novel carnivore ecomorph in *Spea* was likely also mediated by changes in hormone expression and/or processing. Pfennig [81] demonstrated that treatment with exogenous TH induced carnivore-like tadpoles even if they were fed a detritus diet [but see 140]. In addition to a role for TH, CORT appears to be important in the evolutionary history of *Spea*. Specifically, unlike *Spea*, the ancestor-proxy of *Spea* (*Sc. couchii*) experienced increased CORT levels when fed a shrimp diet, which suggests that this transition was initially stressful for the *Spea* ancestor, but has since been accommodated in extant *Spea* [93]. Furthermore, Ledón-Rettig et al. [67] demonstrated the uncovering of cryptic genetic variation when *Sc. couchii* tadpoles were exposed to exogenous CORT.

Evolved changes in life history strategy among ambystomid salamanders (and potentially newts) similarly appears to have arisen because of differences in the thyroid hormone cascade [104–107]. Interestingly, even the mudpuppy, *Necturus maculosus* (an obligate paedomorphic species outside of the family Ambystomatidae) has fully functioning TH receptors (TRα and TRβ) that lead to metabolic changes when exposed to TH [141]. Thus, in the *N. maculosus* lineage, loss of metamorphosis may be due to loss of TH-dependent control of genes required for tissue transformation, rather than the ability of TH receptors to form, recognize, and bind TH.

Changes in hormone regulation are ultimately only important because of the impact they have on gene expression. For instance, TH brings about metamorphosis by activating transcription factors (that are likely to be required for the expression of downstream genes); cellular enzymes (which carry out hormone conversions, energy transformations and may possibly mediate extranuclear effects of TH on neural cells); cytoskeletal elements required for axonal development and; secreted signaling molecules that control the production of TH [136]. The synergistic effects of TH and CORT on amphibian development arise because of their influence on gene expression [142], and the differences in development rate and plasticity between *Sc. couchii* and *P. cultripes* occurs through thyroid hormone receptor mediated changes in gene expression [138]. Similarly, the canalization of the carnivore morph in some populations of *Spea bombifrons* has been accompanied by changes in gene expression [143]. Even the origin of the carnivore morph was potentially facilitated by variation in gene expression in response to consuming a shrimp diet (Levis et al. in review).

Evolved changes in the epigenetic mechanisms that moderate gene expression likely play an important role in the maintenance and evolution of plasticity [144]. For example, studies on natural populations of species other than amphibians have demonstrated an excess of DNA methylation relative to genetic variation, and that DNA methylation patterns vary with population, habitat, and/or species [e.g., 145–147]. In addition to the context-specificity of epigenetic regulation, there is increasing evidence of transgenerational epigenetic inheritance in animals [reviewed in 148,149]. Such transgenerational epigenetic inheritance––even if it lasts for only a few generations––could 'buy time' for genetic accommodation to occur [150].

Maternal effects—where a female's phenotype influences its offspring's phenotype, independent of the direct effects of the female's coding sequence on its offspring's phenotype—are one form of epigenetic inheritance that might be particularly important for amphibians undergoing PFE. In many species of amphibians, mothers differentially provision their eggs depending on their own environment and/or phenotype [e.g., 151–153]. For example, larger females often produce larger eggs [152,154], which develop into larger, faster developing tadpoles [152]. Furthermore, differential investment can mediate plasticity in the expression of offspring traits [155,156]. Thus, condition-dependent maternal effects might be a mechanism by which amphibian populations are buffered from extinction while genetic changes accumulate and produce divergent traits in the absence of the maternal effect [156,157]. Consistent with this scenario, a condition-dependent maternal effect maintains the omnivore morph in populations of *Sp. multiplicata* in sympatry with *Sp. bombifrons* [156], but maintenance of the carnivore morph of *Sp. bombifrons* in these same populations appears to have a genetic basis [157]. Importantly, these sympatric populations appear to be at the edge of range expansion by *Sp. bombifrons* [97]. These data imply that co-existence with *Sp. bombifrons* where these studies took place is relatively recent for *Sp.*

Please cite this article in press as: N.A. Levis, D.W. Pfennig, Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2018.01.012

multiplicata in contrast to *Sp. bombifrons*, which has had a longer time to adapt to *Sp. multiplicata* [156,157]. Thus, maternal effects might be important in determining the direction and rate of evolution.

7. Conclusions

Despite growing evidence that PFE might be a common route to evolutionary innovation, several research avenues could advance this field. First and foremost, additional tests of PFE must be performed. The examples mentioned above in **Section IV** (under *Other potential systems*) could be prime candidates for such tests. Additionally, instances in which plasticity helps amphibians adapt to rapid environmental changes [e.g., pathogens, toxins, habitat destruction, climate change; 158] could provide excellent opportunities to observe PFE in the wild. Finally, future studies should seek to identify the molecular signatures of PFE and the proximate mechanisms of plasticity, genetic accommodation, and genetic assimilation [13,159–163]. As noted above, some likely candidates include hormonal regulation, gene expression, and epigenetic modifications. All of these processes should be explored in the context of gene regulatory developmental networks [164] to elucidate the developmental changes that facilitate the transition from environmental induction to canalization [165]. In sum, amphibians adapting to an ever-changing world are ideal for exploring the roles of plasticity and canalization in evolution.

Acknowledgments

We thank Vincent Debat and Arnaud Le Rouzic for inviting us to participate in this special issue and Alice Levis, Vincent Debat, Arnaud Le Rouzic, and two anonymous referees for helpful comments on earlier drafts of the manuscript.

References

[1] G.P. Wagner, V.J. Lynch, Evolutionary novelties, Curr. Biol. 20 (2010) R48–R52.
[2] A.P. Moczek, On the origins of novelty in development and evolution, Bioessays 30 (2008) 432–447.
[3] S.B. Carroll, Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution, Cell 134 (2008) 25–36.
[4] P.F. Colosimo, C.L. Peichel, K. Nereng, B.K. Blackman, M.D. Shapiro, D. Schluter, D.M. Kingsley, The genetic architecture of parallel armor plate reduction in threespine sticklebacks, PLoS Biol. 2 (2004) e109.
[5] H.E. Hoekstra, R.J. Hirschmann, R.A. Bundley, P.A. Insel, J.P. Crossland, A single amino acid mutation contributes to adaptive beach mouse color pattern, Science 313 (2006) 101–104.
[6] G.C. Conant, K.H. Wolfe, Turning a hobby into a job: how duplicated genes find new functions, Nat. Rev. Genet. 9 (2008) 938–950.
[7] L.E. Flagel, J.F. Wendel, Gene duplication and evolutionary novelty in plants, N. Phytol. 183 (2009) 557–564.
[8] Y.F. Chan, M.E. Marks, F.C. Jones, G. Villareal Jr., M.D. Shapiro, S.D. Brady, A.M. Southwick, D.M. Absher, J. Grimwood, J. Schmultz, R.M. Myers, D. Petrov, B. Jónsson, D. Schluter, M.A. Bell, D.M. Kingsley, Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of *Pitx1* enhancer, Science 327 (2010) 302–305.
[9] P.A. Cleves, N.A. Ellis, M.T. Jimenez, S.M. Nunez, D. Schluter, D.M. Kingsley, C.T. Miller, Evolved tooth gain in sticklebacks is associated with a cis-regulatory allele of *Bmp6*, Proc. Natl. Acad. Sci. U. S. A. 111 (38) (2014) 13912–13917.
[10] N.M. O'Brown, B.R. Summers, F.C. Jones, S.D. Brady, D.M. Kingsley, A recurrent regulatory change underlying altered expression and *Wnt* response of the stickleback armor plates gene EDA, Elife 4 (2015).
[11] T.D. Price, A. Qvarnstrom, D.E. Irwin, The role of phenotypic plasticity in driving genetic evolution, Proc. R. Soc. Lond. Ser. B 270 (2003) 1433–1440.
[12] M.J. West-Eberhard, Developmental Plasticity and Evolution, Oxford University Press, New York, 2003.
[13] A.P. Moczek, S.E. Sultan, S. Foster, C. Ledon-Rettig, I. Dworkin, H.F. Nijhout, E. Abouheif, D.W. Pfennig, The role of developmental plasticity in evolutionary innovation, Proc. R. Soc. B: Biol. Sci. 278 (2011) 2705–2713.
[14] A.V. Badyaev, Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation, Proc. R. Soc. B: Biol. Sci. 272 (2005) 877–886.
[15] M. Pigliucci, C.J. Murren, C.D. Schlichting, Phenotypic plasticity and evolution by genetic assimilation, J. Exp. Biol. 209 (2006) 2362–2367.
[16] D.W. Pfennig, M.A. Wund, E.C. Snell-Rood, T. Cruickshank, C.D. Schlichting, A.P. Moczek, Phenotypic plasticity's impacts on diversification and speciation, Trends Ecol. Evol. 25 (2010) 459–467.
[17] K.N. Laland, T. Uller, M.W. Feldman, K. Sterelny, G.B. Müller, A.P. Moczek, E. Jablonka, F.J. Odling-Smee, The extended evolutionary synthesis: its structure, assumptions and predictions, Proc. R. Soc. B 282 (2015) 20151019.
[18] V. Susoy, E.J. Ragsdale, N. Kanzaki, R.J. Sommer, Rapid diversification associated with a macroevolutionary pulse of developmental plasticity, eLife 4 (2015) e05463.
[19] C.D. Schlichting, M.A. Wund, Phenotypic plasticity and epigenetic marking: an assessment of evidence for genetic accommodation, Evolution 68 (3) (2014) 656–672.
[20] R. Lande, Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation, J. Evol. Biol. 22 (7) (2009) 1435–1446.
[21] K.F. Liem, L.S. Kaufman, Intraspecific macroevolution: functional biology of the polymorphic cichlid species *Cichlasoma minckleyi*, in: A.A. Echelle, I. Kornfield (Eds.), Evolution of Fish Species Flocks, University of Maine Press, Orono, ME, 1984, pp. 203–215.
[22] M. Pigliucci, C.J. Murren, Genetic assimilation and a possible evolutionary paradox: can macroevolution sometimes be so fast as to pass us by? Evolution 57 (2003) 1455–1464.
[23] D. Jablonski, Approaches to macroevolution: 1. General concepts and origin of variation, Evol. Biol. 44 (2017) 427–450, http://dx.doi.org/10.1007/s11692-017-9420.
[24] S.F. Gilbert, D. Epel, Ecological Developmental Biology: Integrating Epigenetics, Medicine, and Evolution, 2nd ed., Sinauer, Sunderland, MA, 2015.
[25] A. Weismann, in: Raphael Meldola (Ed.), Studies in the Theory of Descent. With Notes and Additions by the author. Translated and Edited, With Notes, Sampson, Low, Marston, Searle, Rivington, London, 1882.
[26] M.J. West-Eberhard, Phenotypic plasticity and the origins of diversity, Annu. Rev. Ecol. Syst. 20 (1989) 249–278.
[27] C.H. Waddington, Genetic assimilation of an acquired character, Evolution 7 (1953) 118–126.
[28] T. Schwander, O. Leimar, Genes as leaders and followers in evolution, Trends Ecol. Evol. 26 (3) (2011) 143–151.
[29] N.A. Levis, D.W. Pfennig, Evaluating 'plasticity-first' evolution in nature: key criteria and empirical approaches, Trends Ecol. Evol. 31 (7) (2016) 563–574.
[30] C.K. Ghalambor, K.L. Hoke, E.W. Ruell, E.K. Fischer, D.N. Reznick, K.A. Hughes, Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature, Nature 525 (7569) (2015) 372–375.
[31] I.I. Schmalhausen, Factors of Evolution: The Theory of Stabilizing Selection, University of Chicago Press, Chicago, 1949 [1986].
[32] C.D. Schlichting, M. Pigliucci, Phenotypic Evolution: A Reaction Norm Perspective, Sinauer, Sunderland, MA, 1998.
[33] L. Piacentini, L. Fanti, V. Specchia, M.P. Bozzetti, M. Berloco, G. Palumbo, S. Pimpinelli, Transposons, environmental changes, and heritable induced phenotypic variability, Chromosoma 123 (2014) 345–354.
[34] L. Fanti, L. Piacentini, U. Cappucci, A.M. Casale, S. Pimpinelli, Canalization by selection of de novo induced mutations, Genetics 206 (2017) 1995–2006.
[35] D. Berrigan, S.M. Scheiner, Modeling the evolution of phenotypic plasticity, in: T.J. DeWitt, S.M. Scheiner (Eds.), Phenotypic Plasticity: Functlonal and Conceptual Approaches, Oxford University Press, New York, 2004, pp. 82–97.
[36] S.M. Scheiner, Genetics and evolution of phenotypic plasticity, Annu. Rev. Ecol. Syst. 24 (1993) 35–68.
[37] C.D. Michener, Social polymorphism in Hymenoptera, Symposium of the Royal Entomological Society of London 1 (1961) 43–56.
[38] C.J. Murren, J.R. Auld, H. Callahan, C.K. Ghalambor, C.A. Handelsman, M.A. Heskel, J.G. Kingsolver, H.J. Maclean, J. Masel, H. Maughan, D.W. Pfennig, R.A. Relyea, S. Seiter, E. Snell-Rood, U.K. Steiner, C.D. Schlichting, Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity, Heredity 115 (2015) 293–301.
[39] E.C. Snell-Rood, J.D. Van Dyken, T. Cruickshank, M.J. Wade, A.P. Moczek, Toward a population genetic framework of developmental evolution: the costs, limits, and consequences of phenotypic plasticity, Bioessays 32 (2010) 71–81.
[40] J. Masel, O.D. King, H. Maughan, The loss of adaptive plasticity during long periods of environmental stasis, Am. Nat. 169 (1) (2007) 38–46.
[41] T.J. Kawecki, Accumulation of deleterious mutations and the evolutionary cost of being a generalist, Am. Nat. 144 (1994) 833–838.
[42] M.C. Whitlock, The red queen beats the jack-of-all-trades: the limitations on the evolution of phenotypic plasticity and niche breadth, Am. Nat. 148 (1996) S65–S77.
[43] J.D. Van Dyken, M.J. Wade, The genetic signature of conditional expression, Genetics 184 (2010) 557–570.
[44] D.W. Pfennig, K.S. Pfennig, Evolution's Wedge: Competition and the Origins of Diversity, University of California Press, Berkeley, CA, 2012.
[45] R.A. Newman, Adaptive plasticity in development of *Scaphiopus couchii* tadpoles in desert ponds, Evolution 42 (4) (1988) 774–783.
[46] D.W. Pfennig, The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole, Oecologia 85 (1990) 101–107.
[47] K.M. Warkentin, Adaptive plasticity in hatching age: a response to predation risk trade-offs, Proc. Natl. Acad. Sci. U. S. A. 92 (8) (1995) 3507–3510.

Please cite this article in press as: N.A. Levis, D.W. Pfennig, Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2018.01.012

[48] J. Schmitt, S.A. Dudley, M. Pigliucci, Manipulative approaches to testing adaptive plasticity: phytochrome-mediated shade-avoidance responses in plants, Am. Nat. 154 (1999) S43–S54.
[49] R.A. Relyea, Morphological and behavioral plasticity of larval anurans in response to different predators, Ecology 82 (2) (2001) 523–540.
[50] O. Kishida, K. Nishimura, Bulgy tadpoles: inducible defense morph, Oecologia 140 (3) (2004) 414–421.
[51] P.D. Sniegowski, R.E. Lenski, Mutation and adaptation: the directed mutation controversy in evolutionary perspective, Annu. Rev. Ecol. Syst. 26 (1995) 553–578.
[52] L. Fanti, L. Piacentini, U. Cappucci, A.M. Casale, S. Pimpinelli, Canalization by selection of *de novo* induced mutations, Genetics 206 (4) (2017) 1995–2006.
[53] G. Gibson, I. Dworkin, Uncovering cryptic genetic variation, Nat. Rev. Genet. 5 (2004) 1199–1212.
[54] C.C. Ledon-Rettig, D.W. Pfennig, A.J. Chunco, I. Dworkin, Cryptic genetic variation in natural populations: a predictive framework, Integr. Comp. Biol. 54 (5) (2014) 783–793.
[55] C.D. Schlichting, Hidden reaction norms, cryptic genetic variation, and evolvability, Ann. N. Y. Acad. Sci. 1133 (2008) 187–203.
[56] A.B. Paaby, M.V. Rockman, Cyptic genetic variation: evolution's hidden substrate, Nat. Rev. Genet. 15 (2014) 247–258.
[57] S.M. Scheiner, Selection experiments and the study of phenotypic plasticity, J. Evol. Biol. 15 (2002) 889–898.
[58] M.A. Lardies, F. Bozinovic, Genetic variation for plasticity in physiological and life-history traits among populations of an invasive species, the terrestrial isopod *Porcellio laevis*, Evol. Ecol. Res. 10 (2008) 747–762.
[59] A.P. Moczek, Developmental capacitance, genetic accommodation, and adaptive evolution, Evol. Dev. 9 (3) (2007) 299–305.
[60] V. Sollars, X. Lu, L. Xiao, X. Wang, M.D. Garfinkel, D.M. Ruden, Evidence for an epigenetic mechanism by which Hsp90 acts as a capacitor for morphological evolution, Nat. Genet. 33 (1) (2003) 70–74.
[61] Y. Suzuki, H.F. Nijhout, Evolution of a polyphenism by genetic accommodation, Science 311 (2006) 650–652.
[62] D.J. Futuyma, Can modern evolutionary theory explain macroevolution? in: E. Serrelli, N. Gontier (Eds.), Macroevolution: Explanation, Interpretation and Evidence, Springer, New York, 2015, pp. 29–85.
[63] G.A. Wray, H.E. Hoekstra, D.J. Futuyma, R.E. Lenski, T.F.C. Mackay, D. Schluter, J.E. Strassmann, Does evolutionary theory need a rethink? No, all is well Nature 514 (2014) 161–164.
[64] A.G. Scoville, M.E. Pfrender, Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators, Proc. Natl. Acad. Sci. U. S. A. 107 (9) (2010) 4260–4263.
[65] M.A. Wund, Assessing the impacts of phenotypic plasticity on evolution, Integr. Comp. Biol. 52 (1) (2012) 5–15.
[66] C.C. Ledón-Rettig, D.W. Pfennig, N. Nascone-Yoder, Ancestral variation and the potential for genetic accommodation in larval amphibians: implications for the evolution of novel feeding strategies, Evol. Dev. 10 (3) (2008) 316–325.
[67] C.C. Ledón-Rettig, D.W. Pfennig, E.J. Crespi, Diet and hormone manipulations reveal cryptic genetic variation: implications for the evolution of novel feeding strategies, Proc. R. Soc. B: Biol. Sci. 277 (2010) 3569–3578.
[68] A.P. Moczek, Developmental capacitance, genetic accomodation, and adaptive evolution, Evol. Dev. 9 (2007) 299–305.
[69] M.E. Santos, A. Le Bouquin, A.J.J. Crumière, A. Khila, Taxon-restricted genes at the origin of a novel trait allowing access to a new environment, Science 358 (2017) 386–390.
[70] E. Crispo, The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity, Evolution 61 (2007) 2469–2479.
[71] J.-M. Gibert, The flexible stem hypothesis: evidence from genetic data, Dev. Genes Evol. 227 (2017) 297–307.
[72] R.A. Newman, Effects of density and predation on *Scaphiopus couchii* tadpoles in desert ponds, Oecologia 71 (1987) 301–307.
[73] A.N. Bragg, Gnomes of the Night: The Spadefoot Toads, University of Pennsylvania Press, Philadelphia, PA, 1965.
[74] D.R. Buchholz, T.B. Hayes, Larval period comparison for the spadefoot toads *Scaphiopus couchii* and *Spea multiplicata* (Pelobatidae: Anura), Herpetologica 56 (4) (2000) 455–468.
[75] I. Gomez-Mestre, D.R. Buchholz, Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 19021–19026.
[76] C. Zeng, I. Gomez-Mestre, J.J. Wiens, Evolution of rapid development in spadefoot toads is unrelated to arid environments, PLoS One 9 (5) (2014) e96637.
[77] K. Roelants, D.J. Gower, M. Wilkinson, S.P. Loader, S.D. Biju, K. Guillaume, L. Moriau, F. Bossuyt, Global patterns of diversification in the history of modern amphibians, Proc. Natl. Acad. Sci. U. S. A. 104 (3) (2007) 887–892.
[78] S.S. Kulkarni, I. Gomez-Mestre, C.L. Moskalik, B.L. Storz, D.R. Buchholz, Evolutionary reduction of developmental plasticity in desert spadefoot toads, J. Evol. Biol. 24 (11) (2011) 2445–2455.
[79] S.S. Kulkarni, R.J. Denver, I. Gomez-Mestre, D.R. Buchholz, Genetic accommodation via modified endocrine signalling explains phenotypic divergence among spadefoot toad species, Nat. Commun. 8 (1) (2017).
[80] D.W. Pfennig, Polyphenism in spadefoot toads as a locally adjusted evolutionarily stable strategy, Evolution 46 (1992) 1408–1420.
[81] D.W. Pfennig, Proximate and functional causes of polyphenism in an anuran tadpole, Funct. Ecol. 6 (1992) 167–174.
[82] W.E. Duellman, L. Trueb, Biology of Amphibians, MacGraw Hill, New York, 1986.
[83] K.D. Wells, The Ecology and Behavior of Amphibians, University of Chicago Press, Chicago, IL, 2007.
[84] J.S. Paull, R.A. Martin, D.W. Pfennig, Increased competition as a cost of specialization during the evolution of resource polymorphism, Biol. J. Linn. Soc. 107 (2012) 845–853.
[85] N.A. Levis, S. de la Serna Buzon, D.W. Pfennig, An inducible offense: carnivore morph tadpoles induced by tadpole carnivory, Ecol. Evol. 5 (7) (2015) 1405–1411.
[86] D.W. Pfennig, Cannibalistic tadpoles that pose the greatest threat to kin are most likely to discriminate kin, Proc. R. Soc. Lond. Ser. B 266 (1999) 57–81.
[87] D.W. Pfennig, P.J. Murphy, Character displacement in polyphenic tadpoles, Evolution 54 (2000) 1738–1749.
[88] R.A. Martin, D.W. Pfennig, Evaluating the targets of selection during character displacement, Evolution 65 (2011) 2946–2958.
[89] R.A. Martin, D.W. Pfennig, Disruptive selection in natural populations: the roles of ecological specialization and resource competition, Am. Nat. 174 (2009) 268–281.
[90] R.A. Martin, D.W. Pfennig, Field and experimental evidence that competition and ecological opportunity promote resource polymorphism, Biol. J. Linn. Soc. 100 (2010) 73–88.
[91] R.A. Martin, D.W. Pfennig, Widespread disruptive selection in the wild is associated with intense resource competition, BMC Evol. Biol. 12 (2012) 136.
[92] D.W. Pfennig, A.M. Rice, R.A. Martin, Field and experimental evidence for competition's role in phenotypic divergence, Evolution 61 (2007) 257–271.
[93] C. Ledón-Rettig, D.W. Pfennig, E.J. Crespi, Stress hormones and the fitness consequences associated with the transition to a novel diet in larval amphibians, J. Exp. Biol. 212 (2009) 3743–3750.
[94] D.W. Pfennig, P.J. Murphy, How fluctuating competition and phenotypic plasticity mediate species divergence, Evolution 56 (6) (2002) 1217–1228.
[95] A.M. Rice, D.W. Pfennig, Analysis of range expansion in two species undergoing character displacement: why might invaders generally ẅinduring character displacement? J. Evol. Biol. 21 (2008) 696–704.
[96] A.M. Rice, A.R. Leichty, D.W. Pfennig, Parallel evolution and ecological selection: replicated character displacement in spadefoot toads, Proc. R. Soc. B: Biol. Sci. 276 (2009) 4189–4196.
[97] A.A. Pierce, R. Gutierrez, A.M. Rice, K.S. Pfennig, Genetic variation during range expansion: effects of habitat novelty and hybridization, Proc. R. Soc. B: Biol. Sci. 284 (1852) (2017).
[98] M.K. Takahashi, M.J. Parris, Life cycle polyphenism as a factor affecting ecological divergence within *Notophthalmus viridescens*, Oecologia 158 (1) (2008) 23–34.
[99] W.R. Healy, Life-history variation and growth of juvenile *Notophthalmus viridescens* from Massachusetts, Copeia 1973 (4) (1973) 641–647.
[100] W.R. Healy, Population consequences of alternative life histories in *Notophthalmus v. viridescens*, Copeia 1974 (1) (1974) 221–229.
[101] H.M. Wilbur, J.P. Collins, Ecological aspects of amphibian metamorphosis, Science 182 (4119) (1973) 1305–1314.
[102] H.H. Whiteman, Evolution of paedomorphosis in salamanders, Q. Rev. Biol. 69 (1994) 205–221.
[103] H.H. Whiteman, S.A. Wissinger, M. Denoel, C.J. Mecklin, N.M. Gerlanc, J.J. Gutrich, Larval growth in polyphenic salamanders: making the best of a bad lot, Oecologia 168 (1) (2012) 109–118.
[104] H.B. Shaffer, S.R. Voss, Phylogenetic and mechanistic analysis of a developmentally integrated character complex: alternate life history modes in ambystomatid salamanders, Am. Zool. 36 (1) (1996) 24–35.
[105] S.R. Voss, D.K. Kump, J.A. Walker, H.B. Shaffer, G.J. Voss, Thyroid hormone responsive QTL and the evolution of paedomorphic salamanders, Heredity 109 (5) (2012) 293–298.
[106] S.R. Voss, H.B. Shaffer, Adaptive evolution via a major gene effect: paedomorphosis in the Mexican axolotl, Proc. Natl. Acad. Sci. U. S. A. 94 (25) (1997) 14185–14189.
[107] S.R. Voss, H.B. Shaffer, Evolutionary genetics of metamorphic failure using wild-caught vs. laboratory axolotls (*Ambystoma mexicanum*), Mol. Ecol. 9 (9) (2000) 1401–1407.
[108] M.J. West-Eberhard, Developmental plasticity and the origin of species differences, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 6543–6549.
[109] J. Hua, N.I. Morehouse, R. Relyea, Pesticide tolerance in amphibians: induced tolerance in susceptible populations, constitutive tolerance in tolerant populations, Evol. Appl. 6 (7) (2013) 1028–1040.
[110] J. Hua, D.K. Jones, B.M. Mattes, R.D. Cothran, R.A. Relyea, J.T. Hoverman, The contribution of phenotypic plasticity to the evolution of insecticide tolerance in amphibian populations, Evol. Appl. 8 (2015) 586–596.
[111] R.D. Cothran, J.M. Brown, R.A. Relyea, Proximity to agriculture is correlated with pesticide tolerance: evidence for the evolution of amphibian resistance to modern pesticides, Evol. Appl. 6 (5) (2013) 832–841.
[112] M. Wakahara, Cannibalism and the resulting dimorphism in larvae of a salamander *Hynobius retardatus*, inhabited in Hokkaido, Japan, Zool. Sci. 12 (4) (1995) 467–473.
[113] H. Michimae, M. Wakahara, Factors which affect the occurrence of cannibalism and the broad-headed c̈annibalm̈orph in larvae of the salamander *Hynobius retardatus*, Behav. Ecol. Sociobiol. 50 (4) (2001) 339–345.

Please cite this article in press as: N.A. Levis, D.W. Pfennig, Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2018.01.012

[114] S.A. McCollum, J.D. Leimberger, Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color, Oecologia 109 (4) (1997) 615–621.
[115] J. Van Buskirk, S.A. McCollum, Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation, J. Evol. Biol. 13 (2) (2000) 336–347.
[116] D.P. Chivers, J.M. Kiesecker, A. Marco, J. DeVito, M.T. Anderson, A.R. Blaustein, Predator-induced life history changes in amphibians: egg predation induces hatching, Oikos 92 (1) (2001) 135–142.
[117] E.A. Hoffman, D.W. Pfennig, Proximate causes of cannibalistic polyphenism in larval tiger salamanders, Ecology 80 (3) (1999) 1076–1080.
[118] J.P. Collins, J.E. Cheek, Effect of food and density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum*, Am. Zool. 23 (1983) 77–84.
[119] N.A. Levis, M.L. Schooler, J.R. Johnson, M.L. Collyer, Non-adaptive phenotypic plasticity: the effects of terrestrial and aquatic herbicides on larval salamander morphology and swim speed, Biol. J. Linn. Soc. 118 (3) (2016) 569–581.
[120] N.A. Levis, J.R. Johnson, Level of UV-B radiation influences the effects of glyphosate-based herbicide on the spotted salamander, Ecotoxicology 24 (5) (2015) 1073–1086.
[121] R.A. Relyea, New effects of Roundup on amphibians: predators reduce herbicide mortality; herbicides induce antipredator morphology, Ecol. Appl. 22 (2) (2012) 634–647.
[122] J.M. Guayasamin, T. Krynak, K. Krynak, J. Culebras, C.R. Hutter, Phenotypic plasticity raises questions for taxonomically important traits: a remarkable new Andean rainfrog (*Pristimantis*) with the ability to change skin texture, Zool. J. Linn. Soc. 173 (4) (2015) 913–928.
[123] M.A. Wund, J.A. Baker, B. Clancy, J.L. Golub, S.A. Foster, A test of the Flexible stem model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation, Am. Nat. 172 (2008) 449–462.
[124] R.F. Schneider, A. Meyer, How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations, Mol. Ecol. 26 (1) (2017) 330–350.
[125] R. Calsbeek, T.B. Smith, C. Bardeleben, Intraspecific variation in *Anolis sagrei* mirrors the adaptive radiation of Greater Antillean anoles, Biol. J. Linn. Soc. 90 (2007) 189–199.
[126] V. Susoy, M. Herrmann, N. Kanzaki, M. Kruger, C.N. Nguyen, C. Rödelsperger, W. Röseler, C. Weiler, R.M. Giblin-Davis, E.J. Ragsdale, R.J. Sommer, Large-scale diversification without genetic isolation in nematode symbionts of figs, Sci. Adv. 2 (1) (2016).
[127] R. Goldschmidt, The Material Basis of Evolution, Yale University Press, New Haven, CT, 1940.
[128] C.H. Waddington, The Strategy of the Genes, George Allen & Unwin, London, U.K, 1957.
[129] D.W. Pfennig, M. McGee, Resource polyphenism increases species richness: a test of the hypothesis, Philos. Trans. R. Soc. Lond. Ser. B 365 (2010) 577–591.
[130] Y. Suzuki, H.F. Nijhout, Evolution of a polyphenism by genetic accommodation, Science (New York, N.Y.) 311 (2006) 650–652.
[131] D.D. Brown, L. Cai, Amphibian metamorphosis, Dev. Biol. 306 (1) (2007) 20–33.
[132] S. Kikuyama, K. Kawamura, S. Tanaka, K. Yamamoto, Aspects of amphibian metamorphosis: hormonal control, Int. Rev. Cytol. 145 (1993) 105–148.
[133] D.R. Denver, Neuroendocrine control of amphibian metamorphosis, in: L.I. Gilbert, J.R. Tata, B.G. Atkinson (Eds.), Metamorphosis: Postembryonic Reprogramming of Gene Expression in Amphibian and Insect Cells, Academic Press, San Diego, CA, 1996, pp. 434–464.
[134] R.J. Denver, Environmental stress as a developmental cue: corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis, Horm. Behav. 31 (2) (1997) 169–179.
[135] G.C. Boorse, R.J. Denver, Endocrine mechanisms underlying plasticity in metamorphic timing in spadefoot toads, Integr. Comp. Biol. 43 (2003) 646–657.
[136] R.J. Denver, N. Mirhadi, M. Phillips, Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation, Ecology 79 (6) (1998) 1859–1872.
[137] G.C. Boorse, R.J. Denver, Acceleration of *Ambystoma tigrinum* metamorphosis by corticotropin-releasing hormone, J. Exp. Zool. 293 (1) (2002) 94–98.
[138] A.R. Hollar, J. Choi, A.T. Grimm, D.R. Buchholz, Higher thyroid hormone receptor expression correlates with short larval periods in spadefoot toads and increases metamorphic rate, Gen. Comp. Endocrinol. 173 (1) (2011) 190–198.
[139] I. Gomez-Mestre, S. Kulkarni, D.R. Buchholz, Mechanisms and consequences of developmental acceleration in tadpoles responding to pond drying, PLoS One 8 (12) (2013) 12.
[140] B.L. Storz, Reassessment of the environmental mechanisms controlling developmental polyphenism in spadefoot toad tadpoles, Oecologia 141 (2004) 402–410.
[141] R. Safi, V. Vlaeminck-Guillem, M. Duffraisse, I. Seugnet, M. Plateroti, A. Margotat, M. Duterque-Coquillaud, E.J. Crespi, R.J. Denver, B. Demeneix, V. Laudet, Pedomorphosis revisited: thyroid hormone receptors are functional in *Necturus maculosus*, Evol. Dev. 8 (3) (2006) 284–292.
[142] S.S. Kulkarni, D.R. Buchholz, Beyond synergy: corticosterone and thyroid hormone have numerous interaction effects on gene regulation in *Xenopus tropicalis* tadpoles, Endocrinology 153 (11) (2012) 5309–5324.
[143] N.A. Levis, A. Serrato-Capuchina, D.W. Pfennig, Genetic accommodation in the wild: evolution of gene expression plasticity during character displacement, J. Evol. Biol. 30 (9) (2017) 1712–1723.
[144] J. Hu, R.D.H. Barrett, Epigenetics in natural animal populations, J. Evol. Biol. 30 (9) (2017) 1612–1632.
[145] R. Massicotte, E. Whitelaw, B. Angers, DNA methylation: a source of random variation in natural populations, Epigenetics 6 (2011) 421–427.
[146] A.L. Liebl, A.W. Schrey, C.L. Richards, L.B. Martin, Patterns of DNA methylation throughout a range expansion of an introduced songbird, Integr. Comp. Biol. 53 (2013) 351–358.
[147] A.J. Lea, J. Altmann, S.C. Alberts, J. Tung, Resource base influences genome-wide DNA methylation levels in wild baboons (*Papio cynocephalus*), Mol. Ecol. 25 (8) (2016) 1681–1696.
[148] E. Jablonka, G. Raz, Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution, Q. Rev. Biol. 84 (2009) 131–176.
[149] E. Heard, R.A. Martienssen, Transgenerational epigenetic inheritance: myths and mechanisms, Cell 157 (2014) 95–109.
[150] D.W. Pfennig, M.R. Servedio, The role of transgenerational epigenetic inheritance in diversification and speciation, Non-Genet. Inherit. 1 (2013) 17–26.
[151] J. Bernardo, The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations, Am. Zool. 36 (1996) 216–236.
[152] R.H. Kaplan, Ovum size plasticity and maternal effects on the early development of the frog, *Bombina orientalis* Boulenger, in a field population in Korea, Funct. Ecol. 3 (5) (1989) 597–604.
[153] R.A. Martin, D.W. Pfennig, Maternal investment influences expression of resource polymorphism in amphibians: implications for the evolution of novel resource-use phenotypes, PLoS One 5 (2010) e9117.
[154] R.H. Kaplan, Developmental plasticity and maternal effects of reproductive characteristics in the frog, *Bombina orientalis*, Oecologia 71 (2) (1987) 273–279.
[155] R.H. Kaplan, P.C. Phillips, Ecological and developmental context of natural selection: maternal effects and thermally induced plasticity in the frog *Bombina orientalis*, Evolution 60 (1) (2006) 142–156.
[156] D.W. Pfennig, R.A. Martin, A maternal effect mediates rapid population divergence and character displacement in spadefoot toads, Evolution 63 (2009) 898–909.
[157] D.W. Pfennig, R.A. Martin, Evolution of character displacement in spadefoot toads: different proximate mechanisms in different species, Evolution 64 (2010) 2331–2341.
[158] S.E. Diamond, R.A. Martin, The interplay between plasticity and evolution in response to human-induced environmental change, F1000 Res. 5 (2016) 2835.
[159] A.V. Badyaev, A.L. Potticary, E.S. Morrison, Most colorful example of genetic assimilation? Exploring the evolutionary destiny of recurrent phenotypic accommodation, Am. Nat. 190 (2) (2017) 266–280.
[160] I.M. Ehrenreich, D.W. Pfennig, Genetic assimilation: a review of its potential proximate causes and evolutionary consequences, Ann. Bot. 117 (2016) 769–779.
[161] S.C.P. Renn, M.E. Schumer, Genetic accommodation and behavioural evolution: insights from genomic studies, Anim. Behav. 85 (5) (2013) 1012–1022.
[162] K.L. Sikkink, R.M. Reynolds, C.M. Ituarte, W.A. Cresko, P.C. Phillips, Rapid evolution of phenotypic plasticity and shifting thresholds of genetic assimilation in the nematode *Caenorhabditis remanei*, G3: genes, Genomes Genet. 4 (2014) 1103–1112.
[163] V. Debat, P. David, Mapping phenotypes: canalization, plasticity and developmental stability, Trends Ecol. Evol. 16 (2001) 555–561.
[164] D.W. Pfennig, I.M. Ehrenreich, Toward a gene regulatory network perspective on phenotypic plasticity, genetic assimilation, and genetic accommodation, Mol. Ecol. 23 (2014) 4438–4440.
[165] V. Debat, A. Debelle, I. Dworkin, Plasticity, canalization, and developmental stability of the Drosophila wing: joint effects of mutations and developmental temperature, Evolution 63 (11) (2009) 2864–2876.

Please cite this article in press as: N.A. Levis, D.W. Pfennig, Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2018.01.012