Received: 5 September 2018 | Revised: 1 April 2019 | Accepted: 3 April 2019

DOI: 10.1111/jeb.13475

RESEARCH PAPER

JOURNAL OF Evolutionary Biology eseb WILEY

How stabilizing selection and nongenetic inheritance combine to shape the evolution of phenotypic plasticity

Nicholas A. Levis | David W. Pfennig

Department of Biology, University of North Carolina, Chapel Hill, North Carolina

Correspondence
Nicholas A. Levis, Department of Biology, University of North Carolina, CB#3280, Chapel Hill, NC 27599.
Email: nicholasalevis@gmail.com

Funding information
Division of Environmental Biology, Grant/Award Number: DEB-1019479 and DEB-1643239

Abstract

Relatively little is known about whether and how nongenetic inheritance interacts with selection to impact the evolution of phenotypic plasticity. Here, we empirically evaluated how stabilizing selection and a common form of nongenetic inheritance—maternal environmental effects—jointly influence the evolution of phenotypic plasticity in natural populations of spadefoot toads. We compared populations that previous fieldwork has shown to have evolved conspicuous plasticity in resource-use phenotypes ("resource polyphenism") with those that, owing to stabilizing selection favouring a narrower range of such phenotypes, appear to have lost this plasticity. We show that: (a) this apparent loss of plasticity in nature reflects a condition-dependent maternal effect and not a genetic loss of plasticity, that is "genetic assimilation," and (b) this plasticity is not costly. By shielding noncostly plasticity from selection, nongenetic inheritance generally, and maternal effects specifically, can preclude genetic assimilation from occurring and consequently impede adaptive (genetic) evolution.

KEYWORDS

genetic assimilation, maladaptation, maternal effects, phenotypic plasticity

1 | INTRODUCTION

Phenotypic plasticity is increasingly viewed as playing a key role in adaptive (i.e. genetic) evolution (Badyaev, 2005; Gilbert, Bosch, & Ledon-Rettig, 2015; Laland et al., 2015; Lande, 2009; Moczek et al., 2011; Pfennig et al., 2010; Pigliucci, Murren, & Schlichting, 2006; Price, Qvarnstrom, & Irwin, 2003; Schlichting & Wund, 2014; Susoy, Ragsdale, Kanzaki, & Sommer, 2015; West-Eberhard, 2003; Whitman & Agrawal, 2009). "Plasticity-led" evolution begins when a novel, adaptive trait is first produced in a rudimentary form through plasticity following a change in the environment (Levis & Pfennig, 2016; West-Eberhard, 2003). If underlying genetic variation exists in whether and how individuals respond to this environmental change (Scheiner, 1993, 2002), then selection can act on such variation and cause an evolutionary adjustment in the trait's form as well as its regulation. In some cases, this selection can even favour the complete loss of plasticity through a process known as "genetic assimilation" (sensu Waddington, 1953), the end result of which is a new genetically encoded trait (for possible examples from natural populations, see Aubret & Shine, 2009; Scoville & Pfrender, 2010; Parsons et al., 2016; Badyaev, Potticary, & Morrison, 2017; Kulkarni, Denver, Gomez-Mestre, & Buchholz, 2017; Levis, Serrato-Capuchina, & Pfennig, 2017; Bock, Kantar, Caseys, Matthey-Doret, & Rieseberg, 2018; Levis, Isdaner, & Pfennig, 2018).

A critical factor that can influence the likelihood of genetic assimilation occurring is whether or not the initial plastic response can be transmitted across generations (West-Eberhard, 2003, p. 416; Pfennig & Pfennig, 2012, p. 101; but see Nishikawa & Kinjo, 2018). Indeed,

Data deposited at Dryad: https://doi.org/10.5061/dryad.rm041kb.

environmentally induced phenotypic responses in the *parental* generation (i.e. "within-generation" plasticity) sometimes alter development in the *offspring* generation, even when these offspring do not experience the inducing environmental factor (reviewed in Jablonka & Lamb, 1995; Rossiter, 1996; West-Eberhard, 2003; Bonduriansky & Day, 2009). A common form of such "between-generation" or "transgenerational" plasticity (sensu Galloway & Etterson, 2007) involves environmentally induced phenotypic responses that are transmitted maternally, that is "maternal effects" (Mousseau & Fox, 1998). Maternal effects are, in turn, a form of "nongenetic inheritance" (sensu Bonduriansky & Day, 2009), which occurs when factors other than altered DNA sequence are transmitted from parents to offspring (e.g. structural, cytoplasmic, epigenetic, symbiotic, environmental, and behavioural factors; although we use the terms "maternal effects," "nongenetic inheritance" and "transgenerational plasticity" interchangeably, different subfields might prefer one term over another).

Nongenetic inheritance generally, and maternal effects specifically, have been proposed to both facilitate and impede genetic assimilation. On the one hand, many studies have suggested that maternal effects are often an evolutionary precursor to, and are especially effective at promoting, genetic assimilation (Pfennig & Pfennig, 2012, p. 101; West-Eberhard, 2003, p. 416). On the other hand, because maternal effects can result in offspring phenotypes adaptively matching prevailing environmental conditions in the absence of genetic change (Galloway & Etterson, 2007), they might weaken selection for any genetic changes (Bonduriansky, Crean, & Day, 2012), thereby impeding genetic assimilation. However, despite these opposing predictions, whether and how nongenetic inheritance interacts with stabilizing selection (i.e. selection favouring phenotypes near the population mean) to promote the loss of phenotypic plasticity (and, hence, genetic assimilation) has not been explored empirically in natural populations (for additional insights, see Hoyle & Ezard, 2012; Ezard, Prizak, & Hoyle, 2014; Kuijper & Hoyle, 2015; Walsh, Cooley, Biles, & Munch, 2015; Walsh et al., 2016; Auge, Leverett, Edwards, & Donohue, 2017; Greenspoon & Spencer, 2018; Cavieres, Alruiz, Medina, Bogdanovich, & Bozinovic, 2019).

Here, we examine how stabilizing selection interacts with a maternal effect to influence the evolution of phenotypic plasticity (including its possible loss). To do so, we studied natural populations of Mexican spadefoot toads, *Spea multiplicata*, that have evolved a resource polyphenism, a widespread form of within-generation plasticity in which alternative, environmentally induced, resource-use phenotypes coexist in the same population (Smith & Skúlason, 1996). We specifically sought to evaluate how this resource polyphenism has evolved when: (a) stabilizing selection disfavours one of the two alternative resource-use phenotypes and (b) a maternal effect mediates the loss of this disfavoured phenotype.

2 | MATERIALS AND METHODS

2.1 | Study system

Spea multiplicata tadpoles develop into either: (a) an omnivore morph, which feeds on detritus, algae and small crustaceans, or (b) a distinctive carnivore morph, which specializes on large animal prey (i.e. fairy shrimp and tadpoles), and is characterized by larger jaw muscles, notched (as opposed to smooth) mouthparts, fewer denticle rows, and a shorter gut (Pfennig, 1992). The carnivore morph is found only in the genus *Spea* (Ledón-Rettig, Pfennig, & Nascone-Yoder, 2008) and is normally induced when a young tadpole eats fairy shrimp or other tadpoles (Levis, de la Serna Buzón, & Pfennig, 2015; Pfennig, 1990).

Omnivore–carnivore polyphenism has evolved as an adaptive response to the extreme variation often present among ponds in drying rate and resource availability (Martin & Pfennig, 2010, 2012; Pfennig, 1990, 1992; Pfennig, Rice, & Martin, 2007). Because carnivores metamorphose at an earlier age, they are favoured in rapidly drying ponds (Pfennig, 1992). In slowly drying ponds, however, intraspecific competition drives negative frequency-dependent disruptive selection (Martin & Pfennig, 2009, 2010, 2012; Pfennig, 1992; Pfennig et al., 2007), which maintains both morphs at an equilibrium frequency that is determined by the relative abundance of two main resource types: small, low-nutritive quality prey (i.e. detritus, algae, and small crustaceans) versus large, high-nutritive quality prey (i.e. fairy shrimp and tadpoles) (Pfennig, 1992).

In some populations, however, selection has favoured the loss of this polyphenism, and most tadpoles become omnivores. In areas of the San Simon Valley of south-eastern Arizona and south-western New Mexico, USA (where our study took place) where *S. multiplicata* occur alone (i.e. in allopatry), they typically produce both morphs. However, in nearby areas where *S. multiplicata* co-occur with *S. bombifrons* (i.e. in sympatry), they produce mostly omnivores, even when fed shrimp (Pfennig & Murphy, 2000, 2002). This shift from producing both morphs in allopatry, to producing mostly omnivores in sympatry, is favoured by competitively mediated, stabilizing selection imposed by *S. bombifrons*, which is the superior competitor for the carnivore's diet of fairy shrimp and tadpoles; that is, this shift reflects character displacement (Pfennig & Murphy, 2000, 2002; Pfennig et al., 2007; Rice, Leichty, & Pfennig, 2009). Indeed, manipulative experiments, in which *S. multiplicata* tadpoles were reared in the presence of varying proportions of *S. bombifrons*, have established that the presence of *S. bombifrons per se*, and not other environmental factors, has favoured this loss of resource polyphenism (Pfennig & Murphy, 2000, 2002; Pfennig & Pfennig, 2005).

Importantly, maternal condition appears to mediate this loss of within-generation plasticity (Pfennig & Martin, 2009). Because *S. multiplicata* produce both morphs in allopatry, but only omnivores in sympatry, females from sympatry mature smaller and in poorer body condition than females from allopatry (presumably because omnivores metamorphose at a smaller body size than carnivores; Pfennig & Pfennig, 2005). Consequently, sympatric and allopatric populations have diverged in maternal investment: sympatric females produce smaller eggs, whereas allopatric females produce larger eggs. Larger eggs hatch into larger tadpoles, which have a greater likelihood of becoming carnivores (Martin & Pfennig, 2010). Thus, plasticity in egg size and tadpole size, and (ultimately) morph production are apparently transmitted across generations via a

TABLE 1 Alternative hypotheses and specific predictions we evaluated and whether or not our data support a given prediction. See main text for more detail on each hypothesis and prediction

Hypothesis	Predictions	Support?
H1. Canalization hypothesis	P1. Plasticity is lost or reduced in sympatry compared to allopatry	No
	P2. Erosion mechanism: There is no evidence of selection acting on plasticity in sympatry	Yes*
	P3. Genetic assimilation mechanism: Plasticity is costly	No
H2. Best-of-both hypothesis	P1. Plasticity is maintained in sympatry	Yes
	P2. Greater plasticity confers greater fitness (as estimated by growth and/or development)	Yes*
	P3. Environmental cues from a mother are more reliable than cues experienced by an individual itself	Yes

Note. Asterisks (*) indicate that different analyses show contrasting patterns of support for that prediction.

condition-dependent maternal effect. Indeed, when the body condition of sympatric and allopatric females was experimentally equilibrated in the laboratory (by supplemental feeding), their tadpoles are equally successful at consuming shrimp (a common metric of propensity to ultimately become a carnivore; Pfennig & Martin, 2009). In other words, following this manipulation, sympatric and allopatric tadpoles no longer differed in morph production.

Thus, although within-generation plasticity and transgenerational plasticity are potentially acting on different traits (resource acquisition traits in the former; possibly egg size in the latter), both ultimately impact the probability of an individual becoming a carnivore morph. Therefore, allopatric and sympatric populations have diverged in the likelihood of expressing alternative resource-use morphs (Pfennig & Murphy, 2000, 2002), and the fitness consequences of expressing a particular morph depend on whether an individual is in allopatry or sympatry (Pfennig et al., 2007).

2.2 | Study aims

Using natural populations of *S. multiplicata*, we evaluated two alternative hypotheses for how nongenetic inheritance might interact with stabilizing selection to affect the evolution of phenotypic plasticity.

H1: Canalization hypothesis.

The evolutionary loss of plasticity could occur for at least two, non-mutually exclusive, reasons. First, the buffering capacity of transgenerational plasticity could facilitate the loss—via stochastic processes—of within-generation plasticity. More precisely, if transgenerational plasticity buffers selection from acting on within-generation plasticity (i.e. because transgenerational plasticity prevents within-generation plasticity from being expressed), then this within-generation plasticity might be lost (Latta et al., 2015; Masel, King, & Maughan, 2007; West-Eberhard, 1989). Such a loss may occur because, in the absence of selection favouring plasticity, stochastic processes (e.g. mutation or genetic drift) could eventually degrade the plasticity or the phenotypes that it produces. Thus, if this mechanism of canalization is operating, we should find: (a) the loss of within-generation plasticity in sympatry (i.e. no difference in trait values between treatments) and (b) no evidence of selection on such plasticity in sympatry (i.e. no effect of magnitude of plasticity on growth and development).

Canalization could also occur because plasticity *per se* is disfavoured (through genetic assimilation). For genetic assimilation to occur (and to distinguish this mechanism of canalization from the one above), selection must *disfavour* plasticity, and theory suggests that this can only happen if plasticity is costly (Gomez-Mestre & Jovani, 2013; Scheiner, Barfield, & Holt, 2017). Thus, this hypothesis predicts that: (a) within-generation plasticity has been lost in sympatry (or at least evolved away from that seen in allopatry) and (b) that there is a cost to being plastic (i.e. there is selection *against* within-generation plasticity). To test the latter prediction, we determined whether sibships (genotypes) that exhibit greater levels of within-generation plasticity incur a cost (in terms of their growth and developmental rate) compared to sibships (genotypes) that exhibit lower levels of similar within-generation plasticity.

H2: Best-of-both hypothesis.

Under this hypothesis, within-generation plasticity and transgenerational plasticity could both be maintained in a population by separate agents of selection. Theory and empirical work suggest that within-generation plasticity should be favoured in heterogeneous and/or unpredictable (i.e. unpredictable in regard to how reliably the parental environment matches that of its offspring) environments (Ezard et al., 2014; Gomez-Mestre & Jovani, 2013; Scheiner, 1993). Such work also suggests that transgenerational plasticity should be favoured when the parental environment is a good predictor of the offspring environment (i.e. the environment is temporally stable across generations; Uller, 2008; Ezard et al., 2014; Kuijper & Hoyle, 2015; Walsh et al., 2015, 2016).

In *S. multiplicata*, within-generation plasticity (the resource polyphenism) is an adaptation to pond ephemerality and variation in resource availability, whereas transgenerational plasticity (the condition-dependent maternal effect) is an adaptation to competition with *S. bombifrons* (see Section 2.1). Thus, both forms of plasticity might be favoured if these separate agents of selection differ in stability across generations (Walsh et al., 2016). This hypothesis therefore predicts that: (a) within-generation plasticity has been maintained in sympatry (i.e. the magnitude of trait difference is the same as in allopatry), (b) sibships with greater within-generation plasticity have an advantage (i.e. achieve higher growth and/or development) over those with less plasticity when shrimp are available (i.e. there is selection *for* within-generation plasticity) and (c) competitive environment is more stable across generations than pond duration or resource availability.

We evaluated the above two hypotheses by testing the key predictions of each hypothesis (Table 1). To do so, we used experiments and field measures to answer the following three questions:

Q1. Has within-generation plasticity been reduced (or lost) in sympatry relative to allopatry?
Q2. Is within-generation plasticity costly?
Q3. Do within-generation plasticity and transgenerational plasticity respond to cues that differ in temporal stability?

Below, we describe the laboratory experiment and field observations used to answer these questions.

2.3 | Laboratory experiment

Because morph production can be affected by a condition-dependent maternal effect (Pfennig & Martin, 2009), we first ensured that our females derived from sympatric populations were in equivalent condition to those derived from allopatric populations. We did so by taking the residuals of their mass regressed on snout–vent length (SVL) and comparing these values with a linear mixed-effects model with “selective environment” (allopatry or sympatry) as a fixed effect and “population” (the specific population within each environment from which each female was derived) as a random effect. These residuals described the variability of mass around a given size and thus reflected individual condition: good condition was associated with positive residuals (heavy individuals for a given size), whereas poor condition was associated with negative residuals (light individuals for a given size). These residuals were also the formulation originally used to describe the condition-dependent maternal effect in this system (Pfennig & Martin, 2009). Residual measurements of mass can result in biased parameter estimates in later analyses (e.g. regressions with other variables; Freckleton, 2002). However, because we equilibrated condition in the laboratory, none of the female body size metrics were associated with the trophic traits we measured and were not included in downstream analyses.

We bred 17 pairs of *S. multiplicata* from four populations in allopatry (“Crater,” “Observatory,” “Portal” and “Upper Portal Road”) and 15 pairs of *S. multiplicata* from four populations in sympatry (“410,” “Sky Ranch,” “Rodeo” and “Sulfur Draw”). All populations occur in the San Simon Valley of south-eastern Arizona and south-western New Mexico, USA within 40 km of each other (see map of study sites in Pfennig, Rice, & Martin, 2006), and they experience ongoing gene flow (Pfennig & Rice, 2014; Pierce, Gutierrez, Rice, & Pfennig, 2017). Our breeding stock had been collected from the wild as adults and maintained for one to three years at the University of North Carolina, Chapel Hill.

Breeding was induced by injecting adults with 0.07 ml luteinizing hormone-releasing hormone (Sigma L-7134) at a concentration of 0.01 μg/μl and leaving pairs overnight in nursery tanks. The male and female in each pair were from the same pond population. Eggs from each sibship were kept in separate nursery tanks until hatching. Upon hatching, we placed tadpoles individually into containers (18 × 13 × 8.5 cm) filled with 1.2 L of dechlorinated water and assigned them to one of three diet treatments: (a) crushed fish food (hereafter, “detritus”), which simulates in form and nutrition the detritus on which *Spea* omnivores feed in natural ponds (Pfennig, Mabry, & Orange, 1991), (b) live brine shrimp (*Artemia*), which simulate the fairy shrimp (*Thamnocephalus* or *Steptocephalus*) on which *Spea* carnivores feed in natural ponds or (c) a mixture of detritus and shrimp. Detritus-fed tadpoles received 10 mg of detritus every other day. Shrimp-fed tadpoles received 10 ml of concentrated brine shrimp nauplii twice daily for four days and then switched to 20 adult brine shrimp twice daily until the end of the experiment. Mixed diet tadpoles received half the amount of the detritus and shrimp as the pure diets. Nine tadpoles per sibship were reared on each diet ($n = 864$). After 18 days, we ended the experiment by euthanizing tadpoles in a 0.1% aqueous solution of tricaine methanesulfonate (MS-222) and preserving them in 95% ethanol.

We measured each preserved tadpole's mass, snout–vent length (SVL) and Gosner developmental stage (Gosner, 1960). As in previous studies (Martin & Pfennig, 2009, 2010, 2012; Pfennig et al., 2007), we then measured the following four morphological traits that are diagnostic of morphotype: the width of the jaw muscle (orbitohyoideus muscle; OH), the number of denticle rows (DR), the number of gut coils (GC) and the shape of the mouthparts (MP). We standardized OH for body size (SVL) by regressing ln OH on ln SVL. We also calculated a composite index of trophic morphology using previously described methods (Martin & Pfennig, 2009, 2010, 2012; Pfennig et al., 2007). Briefly, we combined OH, DR, GC and MP into a single multivariate shape variable (the “morphological index”; hereafter, “MI”) with a principal component analysis using a cross-correlation matrix (MI is PC1 of this analysis). In *Spea*, larger values of MI correspond to more carnivore-like tadpoles, with large OH, few DR, short guts and highly keratinized MP. Finally, we calculated the magnitude of within-generation plasticity (referred to as “plasticity” in the questions below) for each sibship as its median MI when reared exclusively on a diet of detritus subtracted from its median MI when reared on a diet exclusively containing shrimp. We used this metric because it reduced the effect outliers had on predicting the “typical” response for a given sibship. Note that although mixed diet tadpoles were not used to determine a sibship's level of plasticity, we could still expect differences in degree of plasticity among sibships to predict differences in the ability of tadpoles from different sibships to utilize and grow when multiple alternative resources are available. For this reason, we included tadpoles reared on a mixed diet in our assessment of costs of plasticity (see below).

To determine whether within-generation plasticity has evolved in sympatry relative to allopatry (Q1), we compared the extent of plasticity across the two selective environments. Specifically, we asked whether plasticity has been retained in sympatry. We did so by using a linear mixed-effects model with "selective environment" (i.e. sympatry vs. allopatry) as a fixed effect and as a random effect "population" (i.e. the specific population from which the sibship's mother was derived). The response variable was "plasticity" (as defined above). In addition, we used Levene's test to determine whether variance in plasticity and in MI on a shrimp diet was greater among tadpoles derived from sympatry than those derived from allopatry. Finding differences in either measure—plasticity or variation in trait values—would point to an evolved change in sympatry relative to allopatry.

Next, we sought to determine whether there is a cost to maintaining the resource polyphenism (Q2). We did so in order to distinguish between alternative canalization mechanisms and to determine whether there was a *benefit* for having greater levels of plasticity. We addressed this issue in two ways: (a) by using a general regression that included all levels of plasticity and (b) by using a narrower comparison focused on the extremes (highly plastic sibships and nonplastic sibships). For the first test, we asked whether sibships that differed in the magnitude of their plastic response also (independently) differed in mass, SVL and/or Gosner stage (all three measures serve as reliable fitness proxies: spadefoots experience strong directional selection for rapid development, and larval size is associated with survival and reproductive success; Pfennig et al., 2007; Martin & Pfennig, 2009) on any diet. Specifically, we determined whether each of these fitness proxies is explained by "selective environment," "plasticity" and/or their interaction using a linear mixed-effects model in JMP Pro 14. Prior to interpretation, we used the package "fdrtool" in R to control for multiple testing. As above, "population" was included as a random effect. If "selective environment" is a significant predictor, then sibships derived from allopatry versus sympatry differ in fitness proxies. If "plasticity" is a significant predictor, then the degree of plasticity affects these fitness proxies such that more (or less) plastic sibships achieve higher fitness (growth). Finally, if the interaction term is significant, then the degree of plasticity impacts fitness (growth) differently between allopatry and sympatry.

A plasticity cost ensues when two genotypes produce the same phenotype, but the genotype that expresses greater levels of plasticity in this phenotype (the "plastic" genotype) has reduced fitness compared to the genotype that expresses lower levels of plasticity in this same phenotype (the "nonplastic" genotype; Murren et al., 2015). Therefore, our second analysis of costs of plasticity compared the extreme ends of the plasticity spectrum: plastic and nonplastic genotypes that possess similar phenotypes. To do so, we identified the four most plastic sibships (PP) and four least plastic sibships (NP) from each selective environment based on their plasticity value described above. For the shrimp and detritus diets individually, we then identified the individual tadpole with largest SVL (putatively the most fit individual). Using this individual's phenotype (MI) as an estimate for the optimal phenotype for a given diet, we then selected the ten PP and ten NP individuals whose phenotypes were closest to this value. Specifically, we chose the closest five individuals above and below the MI value of the largest individual on a given diet. We did so to obtain as equivalent phenotypes as possible between the PP and NP groups. Finally, we subtracted the SVL of these individuals from the SVL of the optimal individual (identified above) and compared the magnitude of these differences (ΔSVL) between PP and NP groups using a *t* test assuming unequal variances. This analysis was performed separately for each selective environment. Thus, diet and morphology were held constant to evaluate whether the plastic group suffered a cost (i.e. greater reduction in growth) compared to the nonplastic group in each selective environment.

We also compared MI and SVL of the entire NP and PP groups on each diet within each selective environment using *F* tests in JMP Pro 14. This analysis provided a general picture of how the most and least plastic sibships looked and performed on each diet and how they compared to each other. It is important to note that our data and these tests for costs of plasticity may not be sufficient for detecting subtle costs associated with being more or less plastic. However, they should provide at the very least, coarse insights that, when combined with the other analyses, will illuminate the processes controlling morph production and evolution of plasticity in our system.

2.4 | Field observations

Finally, we used field observations to determine whether within-generation plasticity and transgenerational plasticity respond to cues that differ in temporal stability (Q3). Essentially, we asked: do the cues driving both types of plastic responses differ in how variable they are through time? To answer this question (and thereby evaluate part of the best-of-both hypothesis), we used previously collected genotypic and ecological data from various sympatric ponds. As a proxy for competitive environment, we identified seven ponds for which a random sampling of tadpoles had been genotyped as *S. multiplicata*, *S. bombifrons* or hybrids in at least two different years. From these data, we determined the proportion of hybrids plus pure *S. bombifrons* (both of which are superior competitors for shrimp than pure *S. multiplicata*) in a pond each year and calculated the coefficient of variation for each pond.

Next, we used pond surface area as a proxy for pond duration (Pfennig, 1990). To do so, we identified seven ponds for which we had pond surface area data for at least two years and calculated each pond's coefficient of variation in this measurement. Finally, we used shrimp density as a proxy for variation in resource availability (Martin & Pfennig, 2010). To do so, we identified seven ponds for which we had data on the density of shrimp in at least two years and calculated the coefficient of variation for each pond.

Because our data did not meet parametric assumptions, we used the function “pairwise.wilcox.test” in R (version 3.4.0) to perform a Kruskal–Wallis rank sum test followed by pairwise Wilcoxon rank sum tests with a Benjamini and Hochberg (1995) correction to determine whether these environmental factors significantly differ in degree of variation. Specifically, we used the coefficient of variation values obtained for each pond as our response variables and the type of variation (i.e. competitive environment, pond duration or resource availability) as the explanatory variables. Assessing such inter-annual variation even over a two-year time scale is a reliable proxy for each factor's reliability as an environmental cue because this species breeds at any given pond explosively once per year. Therefore, the magnitude of change (i.e. coefficient of variation) across years indicates the stability (or lack thereof) of each factor through time.

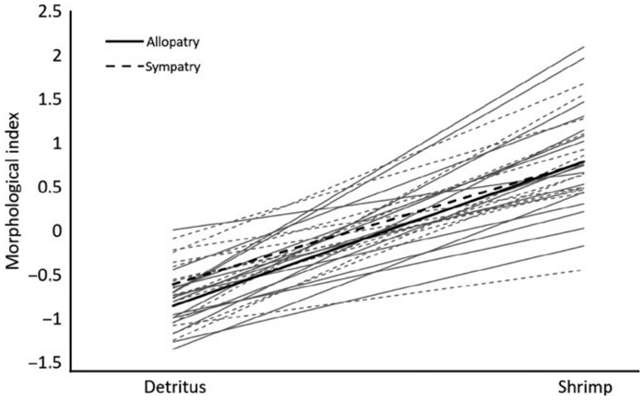

FIGURE 1 Evidence of within-generation plasticity in both allopatry and sympatry. Diet-dependent reaction norms for allopatric (solid) and sympatric (dashed) sibships. Grey lines are for individual sibships, and black lines are the mean reaction norm for each selective environment. The allopatric and sympatric reaction norms (i.e. plasticity) were not significantly different

3 | RESULTS

To remove the influence of the maternal effect (transgenerational plasticity) on the expression of resource polyphenism (within-generation plasticity), we first ensured that the females from the two different selective environments were equivalent in body condition (mass for a given length). Females were indeed similar in body condition ($F_{1,5.5} = 2.71$, $p = 0.1556$), and, if anything, there was a nonsignificant trend towards sympatric females being in better condition than allopatric females [post hoc calculation of the least squares mean of condition (i.e. mean of residuals of mass on SVL) for allopatric females = −0.84 ± 0.82, and for sympatric females = 0.96 ± 0.73].

Having established that the mothers used in our experiments were similar in body condition, we now address each of the three questions (Q1, Q2 and Q3) posed in the Section 2.2.

First, when we compared the degree of diet-induced (within-generation) plasticity expressed by sibships produced by sympatric and allopatric females (Q1), we found that there was no difference in plasticity between these two selective environments ($F_{1,6.1} = 0.34$, $p = 0.5791$; Figure 1). However, tadpoles from both selective environments were more carnivore-like when fed shrimp than when fed detritus (Figure 1). We also found no difference between sympatry and allopatry in variance in plasticity or in the morphological index on a shrimp diet ($F_{1,30} = 0.03$, $p = 0.8589$; $F_{1,30} = 1.13$, $p = 0.2954$, respectively).

Next, when we sought to determine whether there is a cost to maintaining the resource polyphenism (Q2), we found no evidence of any fitness (i.e. growth) costs associated with maintaining this within-generation plasticity. In particular, our broad analysis of costs of plasticity found that: (a) the two selective environments did not differ in the relationship between plasticity and fitness proxies; (b) in general, the degree of plasticity did not affect fitness proxies; and (c) the two selective environments largely did not differ in fitness proxies. Specifically, we found no interaction between degree of plasticity and selective environment for any fitness proxy (SVL, mass and Gosner stage) on any diet (Table 2). However, on a detritus diet, selective environment alone had a significant effect on Gosner stage (Table 2) such that allopatric sibships were more developed than sympatric sibships (Figure S1). All other effects were nonsignificant (Table 2).

A more fine-grained analysis of costs of plasticity (in which we compared individuals from plastic and nonplastic sibships) indicated that, when we held morphology and diet constant, there were no differences in growth (SVL) between individuals from PP sibships and individuals from NP sibships on either shrimp ($t = 1.53$, $p = 0.1469$; Cohen's $d = 0.68$) or detritus ($t = 1.06$, $p = 0.3051$; Cohen's $d = 0.47$) for allopatric individuals (Figure 2a,b). However, when morphology and diet were held constant for sympatric individuals, those from PP sibships experienced a growth cost on a shrimp diet ($t = 2.38$, $p = 0.0316$; Cohen's $d = 1.07$) and a growth benefit of similar magnitude on a detritus diet ($t = 2.38$ $p = 0.0291$; Cohen's $d = 1.06$) compared to phenotypically similar individuals from NP sibships (Figure 2c,d).

We also found that, in allopatry, PP sibships have a greater MI than NP sibships on a shrimp diet ($F_{1,69} = 20.41$, $p < 0.0001$), but not on a detritus diet ($F_{1,68} = 0.23$, $p = 0.6311$), and that PP sibships and NP sibships do not differ in SVL on either diet (shrimp: $F_{1,69} = 1.30$, $p = 0.2583$; detritus: $F_{1,68} = 3.90$, $p = 0.0524$). For sympatric tadpoles, PP sibships had greater MI on a shrimp diet ($F_{1,65} = 8.07$, $p = 0.0060$) and lower MI on a detritus diet ($F_{1,61} = 4.72$, $p = 0.0337$) than NP sibships. This change in morphology conferred greater growth (SVL) for PP sibships on a detritus diet ($F_{1,61} = 9.48$ $p = 0.0031$), but not on a shrimp diet ($F_{1,65} = 0.45$ $p = 0.5025$). Specifically, when we compared the morphology and growth of all plastic and nonplastic tadpoles in sympatry, we found that, when fed a shrimp diet, plastic tadpoles did not differ in SVL (mean $SVL_{PP} = 13.486$ mm; mean $SVL_{NP} = 13.782$ mm) from nonplastic tadpoles despite the former being more carnivore-like on a shrimp diet (mean $MI_{PP} = 1.0883$; mean $MI_{NP} = 0.2719$). Yet, when fed a detritus diet, plastic tadpoles were more omnivore-like (less carnivore-like;

mean MI_{PP} = −0.950; mean MI_{NP} = −0.495) *and* had greater growth (mean SVL_{PP} = 14.249 mm; mean SVL_{NP} = 13.546 mm) than nonplastic tadpoles when fed a detritus diet. These results further suggest that plasticity is not costly and that it may even be advantageous in sympatry.

Finally, when we investigated whether within-generation plasticity (e.g. resource polyphenism) and transgenerational plasticity (e.g. a maternal effect) respond to cues that differ in temporal stability (Q3), we found that this was indeed the case. Specifically, we found that competitive environment (the selective agent favouring the maternal effect) was significantly more stable (i.e. less variable) than either resource availability or pond duration (the selective agents favouring the resource polyphenism; Table 3; Figure 3). Indeed, competitive environment was, on average, approximately 3.5 and 6.7 times more stable than resource availability and pond duration, respectively.

TABLE 2 Summary statistics for the effects of plasticity, selective environment ("environment") and their interaction on fitness proxies (mass, snout–vent length [SVL] and developmental rate [Gosner stage]) when tadpoles were reared on three alternative diets (see Q3 in Methods). *p* values were corrected for multiple testing using the function "fdrtool" in R. The only significant effect was that of selective environment on Gosner stage when tadpoles were fed a detritus diet (bold)

MASS	Estimate	*F*	*df*	*p*	SVL	Estimate	*F*	*df*	*p*	Gosner Stage	Estimate	*F*	*df*	*p*
Detritus diet														
Plasticity	18.68	1.62	1,28	0.388	Plasticity	0.34	1.80	1,28	0.384	Plasticity	0.21	0.62	1,28	0.410
Environment	6.83	2.80	1,28	0.351	Environment	0.15	3.21	1,28	0.334	**Environment**	0.24	10.52	1,28	0.047
P*E	−0.80	0.01	1,28	0.561	P*E	0.03	0.05	1,28	0.534	P*E	0.06	0.13	1,28	0.498
Shrimp diet														
Plasticity	−14.51	1.15	1,27.2	0.399	Plasticity	−0.29	1.70	1,24.8	0.387	Plasticity	−0.30	0.89	1,19.7	0.405
Environment	9.34	0.78	1,3.8	0.410	Environment	0.17	0.61	1,4.3	0.412	Environment	0.13	0.13	1,2.2	0.507
P*E	−3.69	0.07	1,27.2	0.520	P*E	0.13	0.37	1,24.8	0.439	P*E	0.25	0.63	1,19.7	0.410
Mixed diet														
Plasticity	3.59	0.26	1,26.3	0.458	Plasticity	0.01	0.002	1,25.5	0.571	Plasticity	−0.25	1.02	1,26.1	0.402
Environment	5.00	0.61	1,5.6	0.411	Environment	0.18	1.56	1,4	0.398	Environment	0.21	0.89	1,4.3	0.408
P*E	3.74	0.28	1,26.3	0.454	P*E	0.22	1.94	1,25.5	0.380	P*E	0.33	1.72	1,26.1	0.386

4 | DISCUSSION

We studied natural populations of spadefoot toads to empirically evaluate how stabilizing selection (specifically, selection favouring a narrow range of resource-use phenotypes) combines with non-genetic inheritance (specifically, a condition-dependent maternal effect) to influence the evolution of within-generation plasticity (a resource polyphenism). We did so by testing two alternative hypotheses (Table 1). First, the canalization hypothesis (H1) suggests that within-generation plasticity has been lost and canalization has occurred. According to this hypothesis, a loss of within-generation plasticity occurred either because the maternal effects shielded this plasticity from selection, thereby allowing it to be degraded by stochastic processes, or because costs associated with greater levels of plasticity caused this plasticity to be disfavoured by selection. Second, the best-of-both hypothesis (H2) holds that both within-generation plasticity and transgenerational plasticity are maintained because each represents an adaptive response to different environmental factors that differ in temporal stability.

Our data are not consistent with the canalization hypothesis (H1): we did not find the loss of within-generation plasticity in the sympatric environment (Figure 1). However, we did find weak evidence of selection acting on within-generation plasticity in sympatry. Specifically, on a shrimp diet, plastic (PP) sibships had lower growth than nonplastic (NP) sibships when their morphologies were held equivalent (Figure 2d). Hypothetically, this could translate into the selective removal of plastic genotypes from sympatric populations. Yet, our observation that plastic sibships were *favoured* on a detritus diet (Figure 2c) makes this unlikely. If plasticity *per se* is costly, this cost should be detectable even when the plasticity is not elicited (i.e. on a detritus diet; Murren et al., 2015). Clearly, this is not what we observed.

Our data are most consistent with the best-of-both hypothesis (H2). Specifically, we found that: (a) plasticity was maintained in sympatry, (b) plastic sibships performed better in some instances (i.e. on

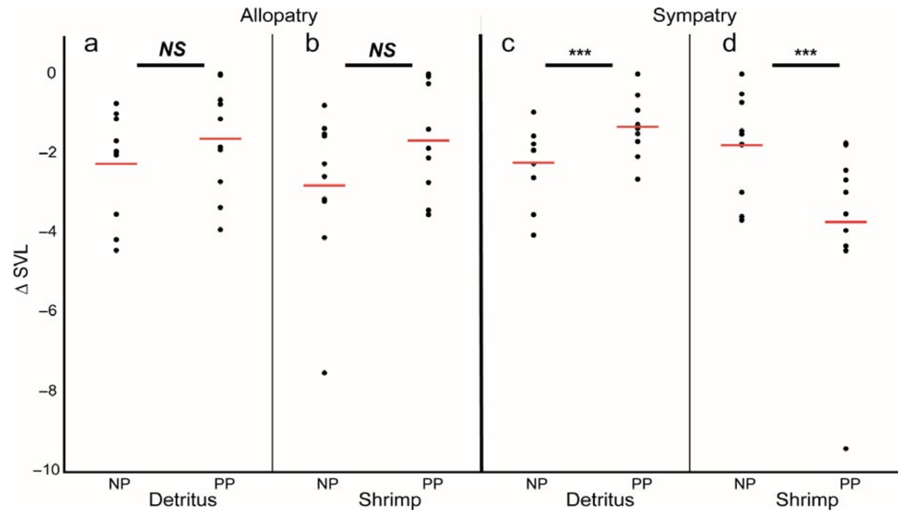

FIGURE 2 Assessment of costs of plasticity in allopatric (a, b) and sympatric (c, d) tadpoles reared on detritus (a, c) or shrimp (b, d). Extent of fitness reduction (ΔSVL) for nonplastic ("NP") and plastic ("PP") sibships when their phenotypes were matched with the largest individual on a given diet in a given selective environment. For allopatric individuals, PP and NP sibships did not differ on either diet. For sympatric individuals, PP sibships had significantly greater growth on detritus (c) and lower growth on shrimp (d). In all panels, the red line indicates the mean for the group. Significant differences are denoted with "***" and nonsignificant differences are denoted with "NS"

TABLE 3 Summary of comparisons between environmental factors in the extent of their variability. Each environmental factor is significantly different from every other

Kruskal–Wallis χ^2	*df*	*p*
11.325	2	0.004
Environmental factor	Resource availability	Pond duration
Competitive environment	0.032	0.012
Resource availability	–	0.040

detritus), and (c) as predicted by theory, within-generation plasticity (e.g. resource polyphenism) and transgenerational plasticity (e.g. a maternal effect) are responses to environmental factors that differ in temporal stability (Figure 3; Table 3). Regarding the second point, the fact that within-generation plasticity is favoured on a detritus diet is important: this is the resource that sympatric *S. multiplicata* primarily consume in natural populations (Pfennig & Murphy, 2000, 2002, 2003). We also found that, in sympatry, plastic tadpoles were more omnivore-like and had greater growth than nonplastic tadpoles when fed a detritus diet. This finding suggests that selection is favouring the maintenance of plasticity in sympatry not because it allows for carnivore production (on a shrimp diet, plastic tadpoles did not differ in SVL from nonplastic tadpoles despite the former being more carnivore-like), but because it facilitates better *omnivore* production. However, there must also be intrinsic differences between plastic and nonplastic sibships: even when morphology was held constant, plastic sibships grew better than nonplastic sibships on a detritus diet (Figure 2c).

An important result to emerge from our study was that within-generation plasticity is apparently not costly. Specifically, sibships (genotypes) that expressed greater levels of plasticity did not grow or

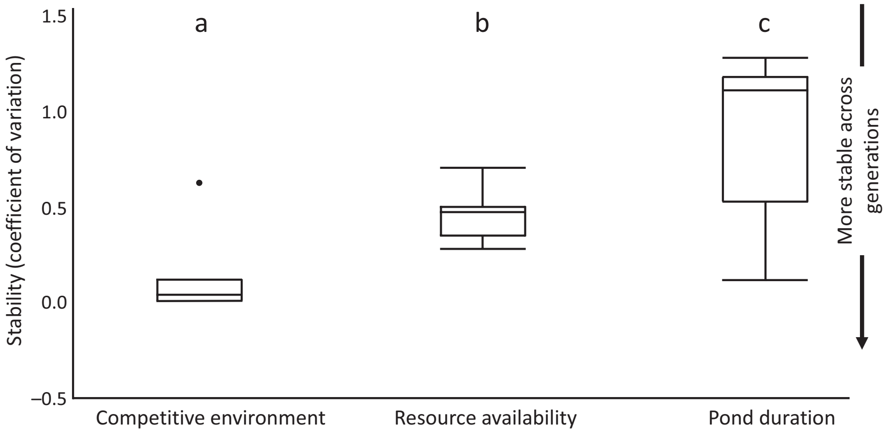

FIGURE 3 Comparisons of the temporal stability of different environmental factors. Box plots of variability of environmental factors in ponds where *Spea multiplicata* occurs in sympatry with *Spea bombifrons*. Competitive environment ($n = 7$) was significantly more stable than resource availability ($n = 7$) and pond duration ($n = 7$), and resource availability was significantly more stable than pond longevity. Different letters denote significant differences between groups

develop more poorly than nonplastic sibships (Table 2; Figure 2). In fact, the most plastic sibships performed better than the least plastic sibships on a detritus diet (greater SVL of PP vs NP sibships). Although we might have detected costs had we reared tadpoles under more stressful conditions, this failure to identify any costs associated with within-generation plasticity makes the selectively driven loss of such plasticity (via genetic assimilation) unlikely to occur among sympatric *S. multiplicata*. This is because, for genetic assimilation to occur, selection must *disfavour* plasticity *per se* (not just a particular phenotype), and theory suggests that this can only happen if plasticity is costly (Gomez-Mestre & Jovani, 2013; Scheiner et al., 2017). Our study adds to the body of literature that has failed to find such costs (Auld, Agrawal, & Relyea, 2010; Murren et al., 2015).

Theory predicts that transgenerational plasticity should evolve (and be maintained) if the parental environment predicts the offspring environment (Kuijper & Hoyle, 2015; Uller, 2008). In our system, production of the omnivore morph in sympatric populations is largely an adaptive response to resource competition with *S. bombifrons*, which produces the competitively superior carnivore morph (Pfennig & Murphy, 2000, 2002). Since the presence and abundance of *S. bombifrons* are relatively stable through time (Table 3; Figure 3), transgenerational plasticity (i.e. a condition-dependent maternal effect) allows *S. multiplicata* to produce an adaptive phenotype in sympatry in the presence of this heterospecific competitor (Pfennig & Murphy, 2000, 2002). The advantage of being plastic when consuming detritus, the persistent selective pressure of *S. bombifrons* pushing *S. multiplicata* to the detritus resource, and transgenerational plasticity aiding in phenotype–environment matching might thereby work together to enable sympatric *S. multiplicata* to respond adaptively to their highly variable environment. In this way, *S. multiplicata* is potentially receiving the best of both forms of plasticity (within-generation and transgenerational plasticity) in the face of stabilizing selection favouring a limited range of phenotypes. Thus, our study reveals that phenotypic fixation (and the apparent loss of plasticity) need not reflect genetic assimilation, that is *genetically* determined phenotypic fixation. Instead, phenotypic fixation can be underlain solely by nongenetic inheritance, in which apparent phenotypic canalization is mediated by transgenerational plasticity ("*epi*genetic assimilation"; sensu Pfennig & Pfennig, 2012, p. 101).

Our results therefore indicate that the apparent loss of within-generation plasticity (i.e. resource polyphenism) that has long been observed in natural, sympatric populations of *S. multiplicata* does not reflect an actual *genetic* loss of plasticity. Once we controlled for maternal condition, within-generation plasticity was present in sympatric populations to the same degree as in allopatric populations (Figure 1). Instead, the apparent loss of plasticity in nature (i.e. fixation of the omnivore phenotype) reflects a condition-dependent maternal effect. In sympatric populations, *S. multiplicata* tadpoles tend to develop only into omnivores (as a result of competition with *S. bombifrons* for shrimp, a dietary cue for inducing carnivores). Consequently, sympatric *S. multiplicata* metamorphose into smaller juveniles that mature as smaller, poorer condition females that (potentially) lay smaller eggs. This poor body condition, in turn, constrains subsequent generations to produce only omnivore tadpoles (see Section 2.1). In short, a self-reinforcing maternal effect appears to mediate phenotypic fixation in a manner that mirrors a genetic loss of plasticity (i.e. genetic assimilation). In general, our findings are consistent with recent theory (Greenspoon & Spencer, 2018) that suggests transgenerational plasticity can mediate adaptive divergence when costs are not too high (see Section 3) and there is moderate migration between habitats, as has been reported between sympatric and allopatric populations of *S. multiplicata* in the San Simon Valley (Pierce et al., 2017; Rice & Pfennig, 2008).

The precise mechanism of nongenetic inheritance might be crucial in determining how it interacts with within-generation plasticity (Herman, Spencer, Donohue, & Sultan, 2014). Indeed, other mechanisms of nongenetic inheritance might have greater stability and/or interact with genetic factors in different ways than maternal effects. For example, in some organisms, methyl marks can be passed stably for several generations (Cubas, Vincent, & Coen, 1999; Hu & Barrett, 2017; Richards, Bossdorf, Muth, Gurevitch, & Pigliucci, 2006; Schmitz et al., 2011). Unlike the maternal effect in our system, differential methylation might be more directly involved in regulating gene expression (Jaenisch & Bird, 2003; Razin & Cedar, 1991). Therefore, nongenetic inheritance mediated by DNA methylation might be more reliable at transmitting environment–phenotype information—and may therefore be able to evolve in rapidly changing environments—than a maternal effect. However, maternal effects are more directly affected by the environment than methyl marks so they might instead be more reliable at transmitting environment–phenotype information. Thus, the myriad of nongenetic inheritance mechanisms (reviewed in Bonduriansky & Day, 2009) should be explored in conjunction with traditional genetic inheritance to clarify how information is processed to generate functional phenotypes.

Finally, although we found that nongenetic inheritance can shield within-generation plasticity from selection and thereby prevent canalization from occurring, in other cases, nongenetic inheritance might actually facilitate canalization (see Section 1). Further research is needed to clarify the conditions under which each outcome is more likely to occur.

ACKNOWLEDGEMENTS

We thank Emmanuelle Porcher, K. Pfennig, three anonymous referees and the members of the Pfennig laboratory for comments on the manuscript and the Arizona Game and Fish Department for providing scientific collection permits. This research was supported by NSF grants DEB-1019479 to D. Pfennig and DEB-1643239 to D. and K. Pfennig. All procedures were carried out in compliance with the Institutional Animal Care and Use Committee at the University of North Carolina. We declare no conflict of interest.

ORCID

Nicholas A. Levis https://orcid.org/0000-0001-7650-2371

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Levis NA, Pfennig DW. How stabilizing selection and nongenetic inheritance combine to shape the evolution of phenotypic plasticity. *J Evol Biol.* 2019;00:1–11. https://doi.org/10.1111/jeb.13475