

Carryover effects and the evolution of polyphenism

SOFIA M. DE LA SERNA BUZON¹, RYAN A. MARTIN², and DAVID W. PFENNIG^{1*}

¹Department of Biology, CB#3280, University of North Carolina, Chapel Hill, NC 27599, USA

²Department of Biology, Case Western Reserve University, Cleveland, OH 44106, USA

Received 18 June 2020; revised 29 July 2020; accepted for publication 3 August 2020

An individual's early-life environment and phenotype often influence its traits and performance as an adult. We investigated whether such 'carryover effects' are associated with alternative, environmentally-induced phenotypes ('polyphenism'), and, if so, whether they influence the evolution of polyphenism. To do so, we studied Mexican spadefoot toads, *Spea multiplicata*, which have evolved a polyphenism consisting of two, dramatically different forms: a carnivore morph and an omnivore morph. We sampled both morphs from a fast-drying and a slow-drying pond and reared them to sexual maturity. Larval environment (pond) strongly influenced survival as well as age and size at metamorphosis and sexual maturity; i.e. environment-dependent carryover effects were present. By contrast, larval phenotype (morph) did not affect life-history traits at sexual maturity; i.e. phenotype-dependent carryover effects were absent. These results are consistent with theory, which suggests that by amplifying selective trade-offs in heterogenous environments, environment-dependent carryover effects might foster the evolution of polyphenism. At the same time, by freeing selection to refine a novel phenotype without altering the existing form, the absence of phenotype-dependent carryover effects might enable polyphenism to evolve in the first place. Generally, carryover effects might play an underappreciated role in the evolution of polyphenism.

ADDITIONAL KEYWORDS: larval polyphenism – life-history – novelty – phenotypic plasticity – pleiotropy.

INTRODUCTION

Ecologists have long known that the environment an organism experiences early in life can impact its traits and performance as an adult, and such phenomena are known as 'carryover effects' (O'Connor *et al.*, 2014; Moore & Martin, 2019). However, not only is early-life environment important, an individual's early-life phenotype can also affect its traits and performance later in life (Kirkpatrick *et al.*, 1990). Remarkably, this holds even for organisms with complex life cycles—the predominant developmental mode among animals (Moran, 1994)—where juvenile and adult life stages are separated by metamorphosis. Although metamorphosis is thought to decouple juvenile and adult stages from one another (Wilbur, 1980; Moran, 1994; Goedert & Calsbeek, 2019), larval traits often influence adult traits [e.g. limb length (Relyea, 2001); life-history traits (Watkins, 2001); brain architecture (Schmidt *et al.*, 2012); behaviour (Müller & Müller, 2015); body size (Tarvin *et al.*, 2015)]; reviewed in Collet & Fellous (2019).

Essentially, carryover effects can assume two forms: 'environment-dependent' carryover effects, in which the environment that an individual experiences early in life influences its adult phenotype and/or fitness, and 'phenotype-dependent' carryover effects, in which the phenotype that an individual expresses early in life influence its adult phenotype and/or fitness (Fig. 1). By influencing an organism's performance in later life stages, both forms of carryover effects can either constrain or intensify natural selection acting on early life stages (Moore & Martin, 2019). However, the evolutionary consequences of carryover effects remain largely unexplored empirically (O'Connor *et al.*, 2014; Moore & Martin, 2019).

An evolutionary context in which carryover effects might be especially important is the evolution of phenotypic plasticity. Although carryover effects are themselves a manifestation of phenotypic plasticity (Moore & Martin, 2019), their role in shaping plasticity's evolution has received scant attention. For example, among the most dramatic examples of plasticity are polyphenisms (*sensu* Michener, 1961)—discrete, environmentally-induced, alternative phenotypes. Polyphenisms have been hypothesized

*Corresponding author. E-mail: dpfennig@unc.edu

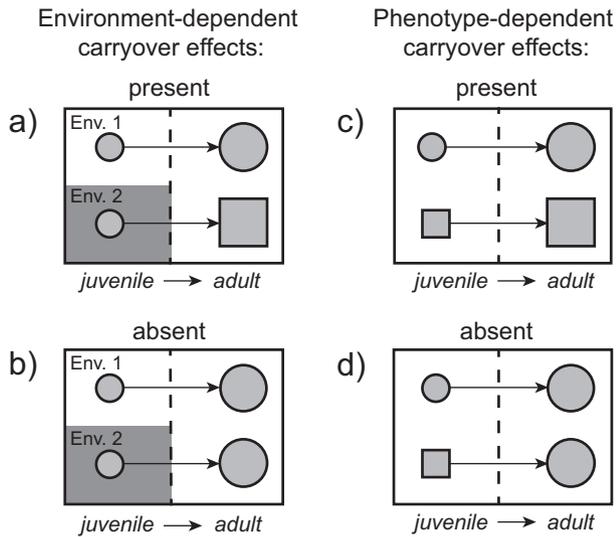


Figure 1. Distinction between environment-dependent and phenotype-dependent carryover effects (here, different shapes signify different phenotypes; the vertical dashed line demarks sexual maturity). (a) Environment-dependent carryover effects are present when the juvenile environment influences the adult phenotype (here, absence/presence of shading signifies different juvenile environments) but (b) absent when the juvenile environment does not influence the adult phenotype. (c) Phenotype-dependent carryover effects are present when the juvenile phenotype influences the adult phenotype but (d) absent when the juvenile phenotype does not influence the adult phenotype.

to represent a key phase in major, lineage-specific innovations (West-Eberhard, 1986, 1989, 2003). According to this idea, if alternative phenotypes are subject to independent selection—such that selection acting on one phenotype does not impact the other—then the evolution of a polyphenism could permit elaboration of a novel phenotype without affecting the established form (Snell-Rood *et al.*, 2010; Van Dyken & Wade, 2010; Levis & Pfennig, 2019b).

Similarly, an uncoupling of selection between juvenile and adult could foster novelty within either life stage (West-Eberhard, 2003). Such developmental uncoupling could enable selection to refine a novel larval form without affecting the existing adult phenotype (or vice versa). However, whether uncoupling occurs between life stages depends on whether carryover effects are present. In the absence of such pleiotropy, selection could refine a novel phenotype at one life stage without altering the existing phenotype at the other life stage. Thus, an absence of phenotype-dependent carryover effects should render polyphenism more likely to evolve.

By contrast, environment-dependent carryover effects should facilitate the evolution of polyphenism. Theory predicts that polyphenism should evolve in

heterogeneous environments, where one environment selects for one phenotype and another environment selects for an alternative phenotype (Levene, 1953; Levins, 1968; West-Eberhard, 1989; Wilson, 1989; Moran, 1992; Roff, 1996; Sultan & Spencer, 2002). Environment-dependent carryover effects should amplify such environment-specific fitness trade-offs. Essentially, by causing the environmental conditions an organism experiences early in life to impact its fitness as an adult, the presence of environment-dependent carryover effects should facilitate the evolution of polyphenism.

Thus, polyphenisms should be characterized by an absence of phenotype-dependent carryover effects and a presence of environment-dependent carryover effects. However, to our knowledge, these predictions have not been tested empirically. Consequently, little is known about whether and how carryover effects impact the evolution of polyphenism specifically or plasticity more generally (but see Lackey *et al.*, 2019).

Here, we tested these predictions by evaluating whether carryover effects are associated with a larval polyphenism in Mexican spadefoot toads, *Spea multiplicata*. This species occurs in deserts of northern Mexico and the southwestern USA, where their tadpoles develop in ephemeral ponds (Pfennig, 1990, 1992). *S. multiplicata* tadpoles are born as an ‘omnivore’ morph that eats detritus, algae and small crustaceans. However, if *S. multiplicata* tadpoles eat fairy shrimp or tadpoles (of the same or other species) (Pfennig, 1990; Levis *et al.*, 2015), some individuals facultatively develop as an alternative ‘carnivore’ morph (Fig. 2a). Carnivores differ from omnivores morphologically [carnivores have larger jaw muscles, more serrated mouthparts, fewer denticle rows and a shorter gut (Pfennig, 1990, 1992; Pfennig & Murphy, 2002)], developmentally [carnivores are younger at metamorphosis (Pfennig, 1992); see also *Results*], and ecologically [unlike the generalist omnivores, carnivores are meat specialists (Paull *et al.*, 2012)]. Because carnivores develop faster than omnivores, they are favoured in ephemeral ponds (Pfennig, 1992). By contrast, because omnivores can consume a wider range of food items, they benefit from reduced intra-morph competition (Paull *et al.*, 2012) and are therefore favoured in more slowly drying ponds (Pfennig, 1992).

Using *S. multiplicata* as our model system, we tested for both phenotype-dependent and environment-dependent carryover effects. Based on theoretical expectations (Fig. 1), we expected to find environment-dependent carryover effects but not phenotype-dependent carryover effects. As described below, our data largely support these predictions.

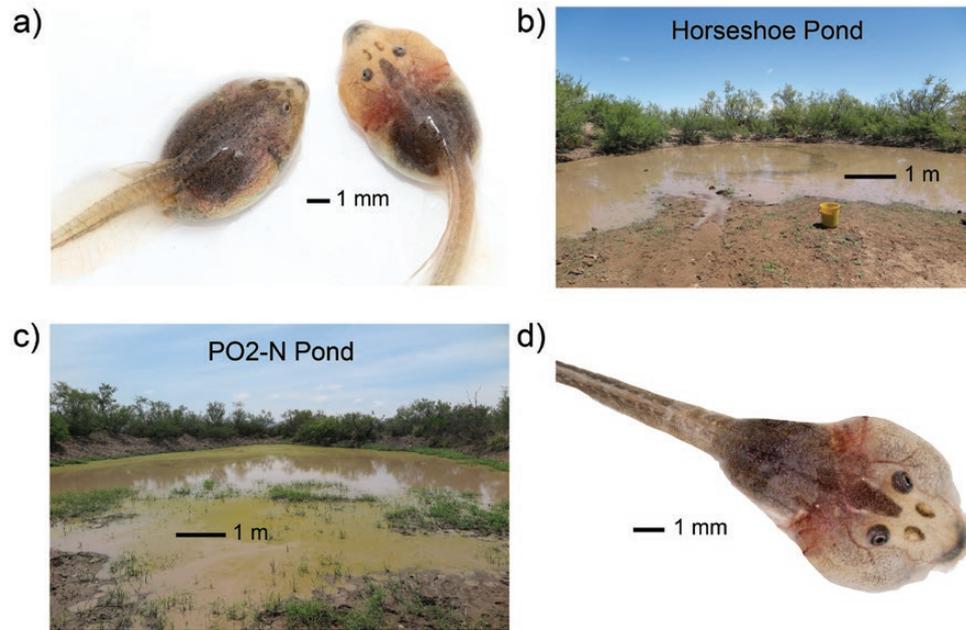


Figure 2. Study system. (a) Tadpoles of *S. multiplicata* occur as alternative, environmentally-induced morphs: an omnivore (left) or a carnivore (right). We sampled both morphs from: (b) Horseshoe Pond, which filled once and was therefore a smaller, shorter-duration pond (photo shows Horseshoe shortly after filling), and (c) PO2-N Pond, which filled twice and was therefore a larger, longer-duration pond (photo shows PO2-N shortly after second filling). (d) An emaciated-looking carnivore from PO2-N Pond after the second filling, when their shrimp and tadpole prey had undergone a dramatic reduction in density.

MATERIAL AND METHODS

SAMPLING PROCEDURES

We sampled late-stage tadpoles from two temporary ponds in the San Simon Valley near Portal, Arizona: ‘Horseshoe’ Pond (lat. 31.9389, long. -109.0864; Fig. 2b) and ‘PO2-N’ Pond (lat. 31.9142, long. -109.0836; Fig. 2c). These ponds are 2.8 km apart and occur in similar desert scrub. Population genetic analyses have revealed little genetic differentiation between the *S. multiplicata* that breed in the two ponds (Levis *et al.*, 2020).

On 2 July 2016, both ponds filled from the same rainstorm and were initially similar in size (~50 m² in area and 15 cm in maximum depth). That same day, about 12 female *S. multiplicata* and several Couch’s spadefoot toads, *Scaphiopus couchii*, bred in each pond. On 4 July, most of the eggs hatched. On 14 July, one of us (D.W.P.) estimated conspecific tadpole, fairy shrimp and *S. couchii* tadpole density by sweeping a net throughout each pond and categorizing densities as ‘high’, ‘moderate-high’, ‘moderate’, ‘moderate-low’ and ‘low’ [these subjective estimates have been used previously on this system (e.g. Levis *et al.*, 2017) and have been corroborated by previously published, intensive, quantitative sampling (Pfennig, 1990; Pfennig *et al.*, 2006)]. Because both ponds initially contained moderate densities of conspecifics, but moderate-high densities of fairy shrimp and *S. couchii* tadpoles, a

substantial fraction (~50%) of *S. multiplicata* tadpoles in each pond developed into carnivores [(Levis *et al.*, 2017); recall from the *Introduction* that carnivores are induced by meat consumption].

On 16 July (12 days after the tadpoles hatched), a second rainstorm flooded PO2-N (but not Horseshoe), doubling PO2-N in size (i.e. to ~100 m² in area; Fig. 2c). The increase in water volume greatly reduced the densities of the carnivore morph’s prey (fairy shrimp and *S. couchii*), and shortly after this second filling, we began to observe emaciated-looking carnivores in PO2-N (Fig. 2d). Thus, PO2-N transformed from a short-duration, high-shrimp-density pond (like Horseshoe) into a long-duration, low-shrimp-density pond. The fact that both morphs were equally abundant in both ponds—even though the ponds ended up diverging in selective environment—thereby allowed us to separate phenotype-dependent carryover effects from environment-dependent carryover effects.

One of us (D.W.P.) sampled and visually inspected dozens of *S. multiplicata* tadpoles in each pond daily. On July 26 (22 days after the tadpoles hatched), a few individuals in Horseshoe Pond were observed approaching metamorphosis [Gosner (1960) stage 42]. We therefore immediately sampled about 100 late-stage carnivores and 100 late-stage omnivores from Horseshoe Pond by sweeping a

dip net throughout the pond (the only tadpoles not kept from this sampling were those relatively early in development). Each tadpole was categorized in the field as a carnivore or an omnivore by visually characterizing the shape its head and mouthparts (see [Levis *et al.*, 2015](#) and references therein); all morph assignments were unambiguous (we collected late-stage tadpoles [[Gosner \(1960\)](#) stages 40–41], rather than metamorphs [[Gosner \(1960\)](#) stage 46], because the two morphs are morphologically similar at metamorphosis). Tadpoles were transported to the nearby Southwestern Research Station (~18 km away), where each morph was placed into a separate outdoor enclosure (plastic wading pools 1.8-m diameter) filled 15 cm deep with well water and provided with live fairy shrimp and detritus from their natal pond *ad libitum*. After 3 days, these tadpoles were placed (in groups of ten same-morph individuals) into 1.6-L plastic bags filled with pond water and shipped overnight to the University of North Carolina (UNC).

PO2-N tadpoles developed more slowly (presumably, because food was less dense after the second filling). One of us (D.W.P.) therefore collected carnivores and omnivores from PO2-N on 30 July (26 days after hatching). Otherwise, PO2-N tadpoles were treated the same as those from Horseshoe.

LABORATORY REARING PROCEDURES

Immediately upon arrival at UNC, tadpoles were housed in groups of twelve and fed both live brine shrimp and crushed fish food *ad libitum*. Brine shrimp simulate fairy shrimp in behaviour and nutrition, whereas crushed fish food simulates naturally occurring detritus in form and nutrition ([Pfennig *et al.*, 1991](#)). Different morphs and populations were reared separately.

When we observed a fore limb protruding from a tadpole [defined as metamorphosis; [Gosner \(1960\)](#) stage 42], that individual was immediately moved to a 5-cm deep container filled 2-cm deep with water with a sandy shore on one end. Once its tail was reabsorbed [[Gosner \(1960\)](#) stage 46], each individual was transferred to a small cage lined with moist paper towels. These individuals were housed in groups of three and fed small, live crickets (dusted with vitamins and calcium powder) *ad libitum* every other day. After 6 weeks, these individuals were transferred (again, in groups of three) to larger terraria (11.9 × 7.8 × 8.1 inches) with moistened sand as substrate. Twice a week, any individuals on the surface were fed crickets *ad libitum*. Every other week, any buried individuals were dug up and fed crickets. Terraria were assigned to randomized locations on shelves and kept in the same room at 23 °C, 40% humidity and with lights

on a reversed cycle from 20:00h to 08:00h. Sand was changed every 2 months.

We held our adults in sand, because adult spadefoots cope with their arid habitat by burrowing underground ([Ruibal *et al.*, 1969](#)) and estivating there for a year or longer ([Mayhew, 1965](#); [McClanahan, 1967](#); [Seymour, 1973](#)). They emerge for only a few weeks following warm rains to breed and feed ([Ruibal *et al.*, 1969](#)).

MEASURING LIFE-HISTORY TRAITS

We measured individuals at two life stages: (1) the larval/juvenile stage (which terminates at metamorphosis); and (2) the adult stage. Specifically, we measured each individual's size and age at metamorphosis and at sexual maturity. The former captured any responses to the larval (aquatic) environment, whereas the latter captured any responses to the adult (terrestrial) environment. By comparing size and age at these two life stages, we tested for carryover effects both across and within larval morphs and environments.

For age at metamorphosis, we recorded the age (starting from hatching; all tadpoles hatched on 4 July; see above) at which an individual's tail was completely reabsorbed [[Gosner \(1960\)](#) stage 46]. For age at sexual maturity, we recorded the age (again, starting from hatching) at which an individual first expressed sexual traits: nuptial pads in males and eggs in females. For size, we recorded the individual's snout-vent length (SVL) at metamorphosis and again at maturity. Finally, we also recorded each individual's survival to 400 days old (the age when most individuals had achieved sexual maturity).

STATISTICAL ANALYSES

We assessed whether body size at metamorphosis (natural-log transformed to improve normality) was influenced by pond, morph or their interaction with a general linear model with the *lm* function from the *stats* library (R Development Core Team, 2018). To determine if size at sexually maturity was affected by pond, morph, sex or their two-way interactions, we again fit a general linear model (body size was not log transformed because it was normally distributed). We then evaluated whether age at metamorphosis was influenced by either of these same two factors or their interaction by using a generalized linear model with a negative binomial distribution to account for overdispersion using the *glm.nb*, function from the *Mass* library ([Venables & Ripley, 2002](#)). Using a general linear model (the response variable was normally distributed) we then evaluated if age at sexual maturity was influenced by pond, morph, sex or their two-way interactions. For our models of both age and body size we also tested, post-hoc, for a difference between

morphs within each pond using the *emmeans* function from the eponymous library (Lenth, 2019). To directly compare the magnitude of differences across ontogeny, where our sample sizes differed due to mortality, we additionally calculated standardized effect sizes (calculated as the pairwise difference divided by the pooled variance) and confidence intervals for these comparisons using the *effsize* function in the *emmeans* library. Finally, we looked at how survival throughout the period observed (400 days) was influenced by pond or morph. We assessed survivorship by a parametric survival analysis following a Weibull distribution with natal pond and larval morph and their interaction as

factors (Therneau, 2015). All analyses were carried out in R v.3.5.2 (R Development Core Team, 2018).

RESULTS

When both morphs were considered together, tadpoles from the two ponds differed in size at metamorphosis ($F_{1,482} = 44.48$, $P < 0.001$), with Horseshoe producing larger metamorphs than PO2-N (Fig. 3a; Supporting Information, Fig. S1). Morphs also differed in size at metamorphosis ($F_{1,482} = 5.37$, $P = 0.02$), and because we found a significant interaction between pond and morph

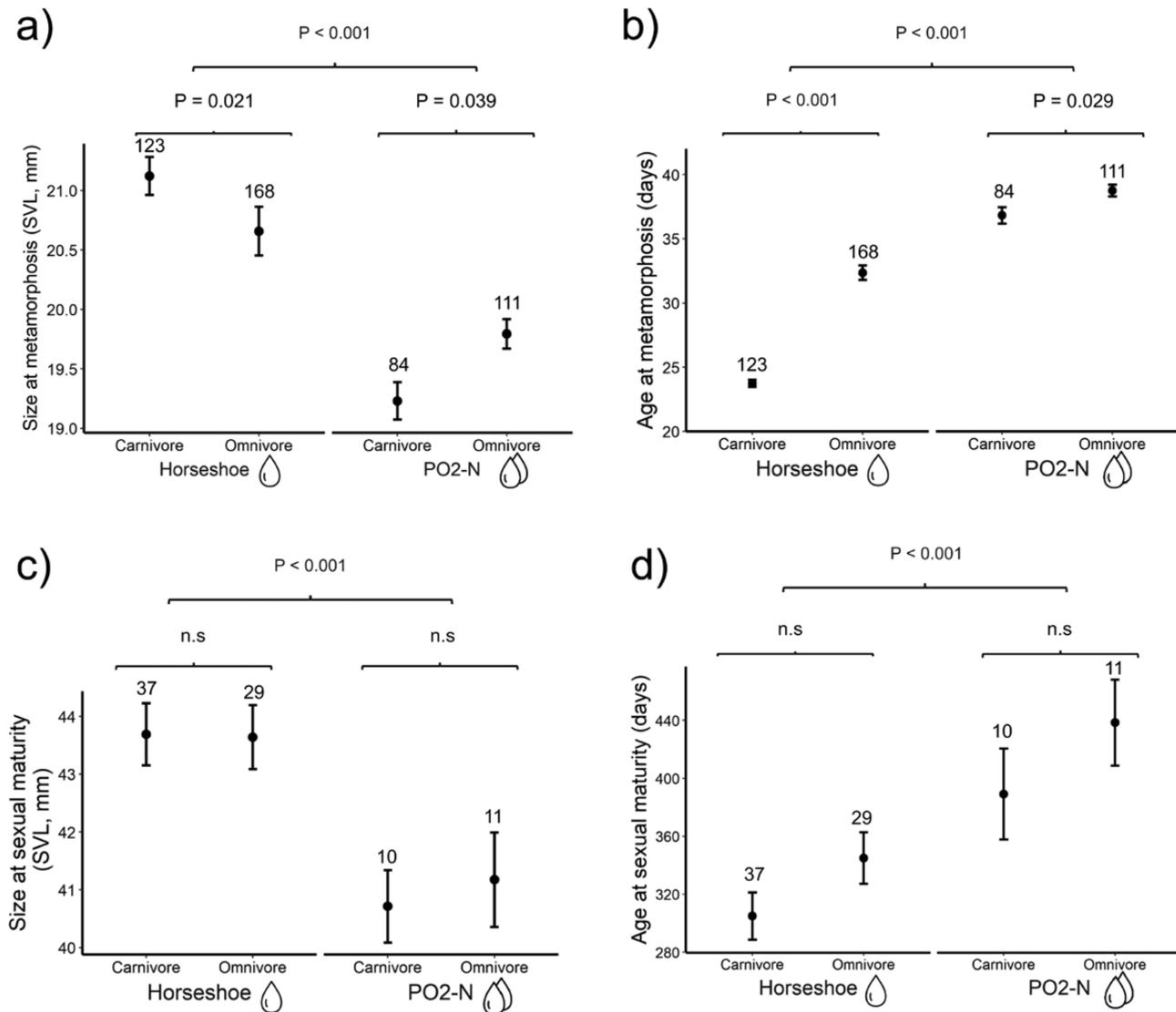


Figure 3. (a) Size and (b) age at metamorphosis and (c) size and (d) age at sexual maturity (mean \pm s.e.m.) for carnivores and omnivores from two different larval environments: Horseshoe Pond (a single-filling, shorter-duration pond) and PO2-N Pond (a double-filling, long-duration pond). Number of individuals shown above each data point (for plots of the raw data, see Supporting Information, Fig. S1).

($F_{1,482} = 9.42, P = 0.002$), we compared body size of carnivores and omnivores within each pond separately. Carnivores were larger than omnivores in Horseshoe (estimated marginal means back-transformed from natural-log scale \pm SE for carnivores: 21.04 ± 0.19 mm, omnivores: 20.48 ± 0.16 mm; carnivore—omnivore t -ratio = 2.31, $P = 0.021$; Fig. 3a), but smaller than omnivores in PO2-N [carnivores: 19.17 ± 0.21 mm, omnivores: 19.75 ± 0.18 ; carnivore—omnivore t -ratio = $-2.074, P = 0.039$ (Fig. 3a; Supporting Information, Fig. S1)].

Age at metamorphosis also differed between ponds [$\chi^2 = 288.64, df = 1, P < 0.001$; (Fig. 3b; Supporting Information, Fig. S1)] and morphs ($\chi^2 = 187.62, df = 1, P < 0.001$). Because we found a significant interaction between pond and morph ($\chi^2 = 61.97, df = 1, P < 0.001$), we compared age at metamorphosis for carnivores and omnivores within each pond separately. Carnivores metamorphosed sooner than omnivores in both ponds, with this difference between morphs being greater in Horseshoe (estimated marginal means back-transformed from log scale \pm SE for carnivores: 23.73 ± 0.44 days, omnivores: 32.36 ± 0.44 days; carnivore—omnivore z -value = $-13.89, P < 0.001$) than in PO2-N [carnivores: 36.82 ± 0.66 days, omnivores: 38.76 ± 0.59 days; z -value = $-2.18, P = 0.029$ (Fig. 3b; Supporting Information, Fig. S1)].

We then turned to adult life-history traits, starting with body size at sexual maturity. Again, ponds differed in body size at maturity ($F_{1,83} = 13.55, P < 0.001$), with Horseshoe individuals larger than PO2-N individuals (Fig. 3c; Supporting Information, Fig. S1). However, neither morph [$F_{1,83} = 0.018, P = 0.89$ (Fig. 3c; Supporting Information, Fig. S1)], sex ($F_{1,83} = 0.005, P = 0.95$) or any pairwise interactions were significant (all interactions were $P > 0.05$ and were removed from the final model).

Age at maturity also differed between ponds [$F_{1,83} = 13.52, P < 0.001$ (Fig. 3d; Supporting Information, Fig. S1)], with Horseshoe toads maturing an average of 92 days earlier than PO2-N toads. However, neither morph [$F_{3,83} = 3.10, P = 0.082$ (Fig. 3d; Supporting Information, Fig. S1)], sex ($F_{1,83} = 1.65, P = 0.20$) or any pairwise interactions were significant (all interactions were $P > 0.50$ and were removed from the final model).

Because our sample sizes—and therefore our statistical power—differed across ontogeny due to mortality, we additionally calculated standardized effect sizes and confidence intervals to compare the magnitude of the difference between morphs within each pond in the age and size at metamorphosis, and in the age and size at sexual maturity. The effect size calculations confirm that the morphs differed in age and size at metamorphosis within each pond; however, these differences did not carry over to sexual maturity (Table 1).

Table 1. Standardized effect sizes and confidence intervals (CI), calculated using the *effsize* function in the *emmeans* library, to evaluate the difference between morphs (carnivore—omnivore) within each pond in size and age at metamorphosis and size and age at sexual maturity

Response	Pond	Effect size	Lower CI	Upper CI
Size at metamorphosis	Horseshoe	0.275	0.041	0.509
	PO2-N	-0.299	-0.584	-0.015
Size at sexual maturity	Horseshoe	0.017	-0.477	0.51
	PO2-N	-0.154	-1.02	0.715
Age at metamorphosis	Horseshoe	-8.85	-10.22	-7.49
	PO2-N	-1.99	-3.78	-0.197
Age at sexual maturity	Horseshoe	-0.409	-0.907	0.088
	PO2-N	-0.502	-1.375	0.370

To further investigate the degree of decoupling of traits across life stages, we also calculated correlations between body size at metamorphosis and at sexual maturity, and between age at metamorphosis and at sexual maturity. Body size was not significantly correlated across life stages ($r = 0.15, t = 1.40, df = 85, P = 0.17$). There was, however, a weak correlation between age at metamorphosis and at sexual maturity ($r = 0.26, t = 2.48, df = 85, P = 0.02$).

Finally, we examined whether the pond or morph affected survival. Survival differed between populations ($\chi^2 = 25.52, df = 1, P < 0.001$), with Horseshoe individuals having higher survival rates (Fig. 4a). Overall, morphs did not differ in survival rates ($\chi^2 = 0.82, df = 1, P = 0.367$). However, because there was a significant pond by morph interaction ($\chi^2 = 7.87, df = 1, P = 0.005$), we compared survival rates for carnivores and omnivores within each pond separately. In Horseshoe, carnivores had higher survival rates than omnivores (t -ratio = 2.55, $P = 0.011$; Fig. 4b), whereas in PO2-N, morphs did not differ in survival rates (t -ratio = $-1.38, P = 0.167$; Fig. 4c).

DISCUSSION

Polyphenisms may play an underappreciated role in the origins of novel phenotypes. With polyphenism, a new phenotype evolves alongside an existing phenotype (West-Eberhard, 1986, 1989, 2003). However, polyphenism should be more likely to evolve if the phenotypic alternatives experience independent

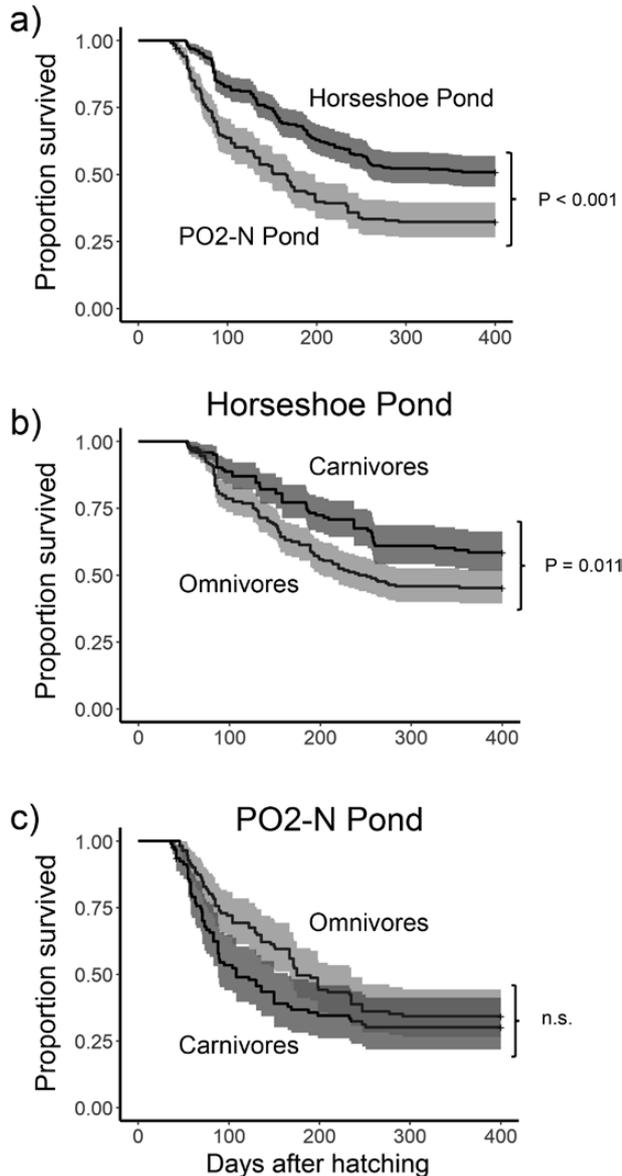


Figure 4. Survival curves (black lines; grey shading: 90% CI) for (a) tadpoles of both morphs derived from two different ponds (Horseshoe Pond and PO2-N Pond), and (b, c) for carnivores and omnivores separately from each pond, respectively.

selection, such that selection favours elaboration of the novel phenotype without impacting the established form (West-Eberhard, 1986, 1989, 2003; see also Levis & Pfennig, 2019b).

For organisms that undergo metamorphosis, a further uncoupling of selection between larval and adult stages would permit selection to refine a novel larval form without affecting the adult phenotype (or vice versa). However, this argument assumes that the early-life phenotype does not influence the adult phenotype and/or fitness (Fig. 1d). Thus, the absence

of such phenotype-dependent carryover effects should render polyphenism more likely to evolve. In contrast, environment-dependent carryover effects should amplify environment-specific fitness trade-offs. Essentially, by causing the environmental conditions an organism experiences early in life to impact its fitness as an adult, the presence of environment-dependent carryover effects should facilitate the evolution of polyphenism. Although few tests of carryover effects in species with larval polyphenisms have been conducted (Lackey *et al.*, 2019), these tests are vital for evaluating whether larval polyphenisms are indeed a crucible for evolutionary innovation and diversification (e.g. see Wray, 1992; Raff, 1996; Hall & Wake, 1999).

We conducted such a test by assessing whether carryover effects are present in a well-characterized larval polyphenism in *S. multiplicata* (Fig. 2a). Specifically, we asked if either environment-dependent or phenotype-dependent carryover effects are associated with the alternative phenotypes that comprise this polyphenism. We also asked if these different forms of carryover effects conformed to the theoretical expectations outlined in the previous paragraph.

We found that larval environment (natal pond) dramatically influenced survival as well as age and size at metamorphosis and at maturity; i.e. environment-dependent carryover effects were present. In contrast, although morphs did differ in age and size at metamorphosis (as expected; see Pfennig, 1992), they did not differ in life-history traits at maturity, suggesting that phenotype-dependent carryover effects were absent at sexual maturity. Thus, the two types of carryover effects did indeed conform to our theoretical expectations.

The lack of phenotype-dependent carryover effects was surprising, given the pronounced differences between carnivores and omnivores in morphology (Fig. 1a), behaviour (Pfennig, 1999), patterns of gene expression (Leichty *et al.*, 2012), and (crucially) size and age at metamorphosis (Figs 3a, b; see also Pfennig, 1992). Moreover, phenotype-dependent carryover effects have been reported in another amphibian polyphenism: tiger salamanders, *Ambystoma tigrinum*, whose larvae develop into either an aquatic paedomorph that retains larval traits and stays in its natal pond or a terrestrial metamorph that undergoes metamorphosis (Lackey *et al.*, 2019). Our finding of no phenotype (morph)-dependent carryover effects suggests that *S. multiplicata* has evolved growth-rate plasticity in the adult stage, in which initial size differences disappear over time. Such plasticity in species with indeterminate growth would allow individuals to adjust their growth over the course of their ontogeny and enhance fitness. Thus, poor

growth early in life can be compensated for later in life, as has been documented in diverse taxa (reviewed in Hector & Nakagawa, 2012; Schmidt *et al.*, 2012; Hyeun-Ji & Johansson, 2016). Such growth-rate plasticity would be especially important in anurans, where size at reproduction predicts mating success for males and fecundity for females (Duellman & Trueb, 1986; Wells, 2007).

In addition, an individual's age at metamorphosis was only weakly correlated with its age at maturity ($r = 0.26$, $P = 0.02$); size was not correlated across life stages ($r = 0.15$, $P = 0.17$). Generally, in amphibians, an individual's age and size at metamorphosis correlate with its age and size at sexual maturity (e.g. Semlitsch *et al.*, 1988; Morey & Reznick, 2001; Altwegg & Reyer, 2003; but see Earl & Whiteman, 2015). Our finding that these characters are either only weakly correlated or not correlated at all further suggests a decoupling of selection between life stages in our system.

Our findings come with two caveats. First, we compared life-history traits for individuals from two ponds only. Although this lack of replication of each pond type was not a problem for our study—we were not interested in the effects of a particular pond type on life-history traits; we were merely interested in establishing whether or not carryover effects were present—future studies using additional populations are needed to establish the generality of our findings. A second caveat is that our focal subjects were reared in a favourable laboratory environment which likely enhanced growth and survival by mitigating the hazards that these animals would have experienced in the wild. At the same time, many animals did die, possibly of malnutrition or disease [similar mortality rates have been observed previously in the laboratory (Pfennig, 1992)]. Regardless, our common-or-garden approach was informative in that it suggests an absence of *intrinsic* phenotype-dependent carryover effects associated with omnivores and carnivores.

In contrast to the absence of phenotype-dependent carryover effects, there were consistent—and pronounced—environment-dependent carryover effects at metamorphosis and sexual maturity. Compared to tadpoles derived from Horseshoe (Fig. 2b), those derived from PO2-N (Fig. 2c) were smaller at both metamorphosis (Fig. 3a) and at sexual maturity (Fig. 3c), took longer to reach both metamorphosis (Fig. 3b) and sexual maturity (Fig. 3d), and were less likely to survive to sexual maturity (Fig. 4a). These differences are consistent with the difference between ponds in size and duration: Horseshoe was a smaller, shorter duration pond whereas PO2-N was a much larger, longer duration pond. That individuals from the longer-duration pond (PO2-N) had lower fitness than individuals from the shorter-duration

pond (Horseshoe) is, at first glance, counterintuitive. However, this poor performance likely reflected the reduced levels of shrimp and tadpoles [the highest nutritive value resources (Pfennig, 2000)] in the longer-duration pond (PO2-N). More generally, these results suggest that environmental effects experienced early in life cannot be easily decoupled from performance later in life.

Such environment-dependent carryover effects likely favoured the evolution of carnivore-omnivore polyphenism. As noted in the *Introduction*, theory predicts that environmental heterogeneity favours polyphenism (Levene, 1953; Levins, 1968; West-Eberhard, 1989; Wilson, 1989; Moran, 1992; Roff, 1996; Sultan & Spencer, 2002). Essentially, selection should favour polyphenism if one environment selects for one phenotype and another environment selects for another phenotype. By causing the environmental conditions an organism experiences early in life to also affect its fitness as an adult, environment-dependent carryover effects should amplify such fitness trade-offs. For example, ponds in which *S. multiplicata* breed vary in duration, from one location to another (Pfennig, 1990) and from one year to another (Pfennig, 2007; Levis & Pfennig, 2019a). In shorter-duration ponds, the faster-developing carnivore morph is favoured, whereas in longer-duration ponds, the slower-developing (but dietary generalist) omnivore morph is favoured (Pfennig, 1992; Paull *et al.*, 2012). As expected, we found that carnivores did indeed metamorphose at a larger size than omnivores in the shorter-duration pond (Horseshoe; Fig. 3a), whereas the opposite was true in the longer-duration pond (PO2-N; Fig. 3a). Larger size at metamorphosis is, in turn, associated with higher survival in the wild: larger metamorphs are less likely to starve, desiccate and be eaten (Pfennig; unpubl. data; see also Cabrera-Guzman *et al.*, 2013). Thus, these results support theoretical expectations that environmental heterogeneity—coupled with environment-dependent carryover effects, which amplify environment-specific fitness trade-offs associated with alternative phenotypes—favours the evolution of polyphenism.

Additional studies are needed, however, to evaluate these ideas more completely. One approach would be to study populations that vary in expression of carnivore-omnivore polyphenism; specifically, in degree of bimodality and divergence between trophic phenotypes (Martin & Pfennig, 2010). Finding that more pronounced polyphenism (i.e. greater bimodality and divergence) is associated with more pronounced environment-dependent carryover effects would support the hypothesis that environment-dependent carryover effects promote the evolution of polyphenism.

ACKNOWLEDGEMENTS

We thank K. Pfennig for help in the field, and K. Pfennig, N. Levis, E. Harmon, A. Isdaner, P. Kelly and two anonymous referees for comments on the manuscript. This research was funded by grants from the U.S. National Science Foundation (DEB-1643239 to K. Pfennig and D.W.P. and DEB-1753865 to D.W.P.). All procedures were carried out in compliance with the Institutional Animal Care and Use Committee at the University of North Carolina. Field collections were conducted under Arizona Scientific Collecting Permit SP SP745794.

REFERENCES

- Altwegg R, Reyer HU. 2003.** Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* **57**: 872–882.
- Cabrera-Guzman E, Crossland MR, Brown GP, Shine R. 2013.** Larger body size at metamorphosis enhances survival, growth and performance of young cane toads (*Rhinella marina*). *PLoS One* **8**: 10.
- Collet J, Fellous S. 2019.** Do traits separated by metamorphosis evolve independently? *Proceedings of the Royal Society B* **286**: 20190445.
- Duellman WE, Trueb L. 1986.** *Biology of amphibians*. New York: MacGraw Hill.
- Earl JE, Whiteman HH. 2015.** Are commonly used fitness predictors accurate? A meta-analysis of amphibian size and age at metamorphosis. *Copeia* **103**: 297–309.
- Goedert D, Calsbeek R. 2019.** Experimental evidence that metamorphosis alleviates genomic conflict. *The American Naturalist* **194**: 356–366.
- Gosner KL. 1960.** A simplified table for staging anuran embryos with notes on identification. *Herpetologica* **16**: 183–190.
- Hall BK, Wake MH. 1999.** Introduction: larval development, evolution, and ecology. In: Hall BK, Wake MH, eds. *The origin and evolution of larval forms*. San Diego: Academic Press, 1–19.
- Hector KL, Nakagawa S. 2012.** Quantitative analysis of compensatory and catch-up growth in diverse taxa. *Journal of Animal Ecology* **81**: 583–593.
- Hyeun-Ji L, Johansson F. 2016.** Compensating for a bad start: compensatory growth across life stages in an organism with a complex life cycle. *Canadian Journal of Zoology* **94**: 41–47.
- Kirkpatrick M, Lofsvold D, Bulmer M. 1990.** Analysis of the inheritance, selection and evolution of growth trajectories. *Genetics* **124**: 979–993.
- Lackey ACR, Moore MP, Doyle J, Gerlanc N, Hagan A, Geile M, Eden C, Whiteman HH. 2019.** Lifetime fitness, sex-specific life history, and the maintenance of a polyphenism. *American Naturalist* **194**: 230–245.
- Leichty AR, Pfennig DW, Jones CD, Pfennig KS. 2012.** Relaxed genetic constraint is ancestral to the evolution of phenotypic plasticity. *Integrative and Comparative Biology* **52**: 16–30.
- Lenth R. 2019.** *Estimated marginal means, aka least-squares means*. Available at: <https://cran.r-project.org/web/packages/emmeans/emmeans.pdf>
- Levene H. 1953.** Genetic equilibrium when more than one ecological niche is available. *American Naturalist* **87**: 331–333.
- Levins R. 1968.** *Evolution in changing environments*. Princeton: Princeton University Press.
- Levis NA, Pfennig DW. 2019a.** How stabilizing selection and nongenetic inheritance combine to shape the evolution of phenotypic plasticity. *Journal of Evolutionary Biology* **32**: 706–716.
- Levis NA, Pfennig DW. 2019b.** Plasticity-led evolution: evaluating the key prediction of frequency-dependent adaptation. *Proceedings of the Royal Society B* **286**: 20182754.
- Levis NA, de la Serna Buzon S, Pfennig DW. 2015.** An inducible offense: carnivore morph tadpoles induced by tadpole carnivory. *Ecology and Evolution* **5**: 1405–1411.
- Levis NA, Martin RA, O'Donnell KA, Pfennig DW. 2017.** Intraspecific adaptive radiation: competition, ecological opportunity, and phenotypic diversification within species. *Evolution* **71**: 2496–2509.
- Levis NA, Reed EMX, Pfennig DW, Buford Reiskind MO. 2020.** Identification of candidate loci for adaptive phenotypic plasticity in natural populations of spadefoot toads. *Ecology and Evolution* in press.
- Martin RA, Pfennig DW. 2010.** Field and experimental evidence that competition and ecological opportunity promote resource polymorphism. *Biological Journal of the Linnean Society* **100**: 73–88.
- Mayhew WW. 1965.** Adaptations of the amphibian, *Scaphiopus couchii*, to desert conditions. *American Midland Naturalist* **74**: 95–109.
- McClanahan LJ. 1967.** Adaptations of the spadefoot toad *Scaphiopus couchii*, to desert environments. *Comparative Biochemistry and Physiology* **20**: 73–99.
- Michener CD. 1961.** Social polymorphism in Hymenoptera. *Symposium of the Royal Entomological Society of London* **1**: 43–56.
- Moore MP, Martin RA. 2019.** On the evolution of carry-over effects. *Journal of Animal Ecology* **88**: 1832–1844.
- Moran NA. 1992.** The evolutionary maintenance of alternative phenotypes. *American Naturalist* **139**: 971–989.
- Moran NA. 1994.** Adaptation and constraint in the complex life-cycles of animals. *Annual Review of Ecology and Systematics* **25**: 573–600.
- Morey S, Reznick D. 2001.** Effects of larval density on postmetamorphic spadefoot toads (*Spea hammondi*). *Ecology* **82**: 510–522.
- Müller T, Müller C. 2015.** Behavioural phenotypes over the lifetime of a holometabolous insect. *Frontiers in Zoology* **12**: S8.
- O'Connor CM, Norris DR, Crossin GT, Cooke SJ. 2014.** Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere* **5**: 1–11.
- Paull JS, Martin RA, Pfennig DW. 2012.** Increased competition as a cost of specialization during the evolution of resource polymorphism. *Biological Journal of the Linnean Society* **107**: 845–853.

- Pfennig DW. 1990.** The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. *Oecologia* **85**: 101–107.
- Pfennig DW. 1992.** Polyphenism in spadefoot toad tadpoles as a locally adjusted evolutionarily stable strategy. *Evolution* **46**: 1408–1420.
- Pfennig DW. 1999.** Cannibalistic tadpoles that pose the greatest threat to kin are most likely to discriminate kin. *Proceedings of the Royal Society of London, Series B* **266**: 57–81.
- Pfennig DW. 2000.** Effect of predator-prey phylogenetic similarity on the fitness consequences of predation: a trade-off between nutrition and disease? *American Naturalist* **155**: 335–345.
- Pfennig KS. 2007.** Facultative mate choice drives adaptive hybridization. *Science* **318**: 965–967.
- Pfennig DW, Mabry A, Orange D. 1991.** Environmental causes of correlations between age and size at metamorphosis in *Scaphiopus multiplicatus*. *Ecology* **72**: 2240–2248.
- Pfennig DW, Murphy PJ. 2002.** How fluctuating competition and phenotypic plasticity mediate species divergence. *Evolution* **56**: 1217–1228.
- Pfennig DW, Rice AM, Martin RA. 2006.** Ecological opportunity and phenotypic plasticity interact to promote character displacement and species coexistence. *Ecology* **87**: 769–779.
- Pfennig D, de la Serna Buzón S, Martin R. 2020.** Data from: Carryover effects and the evolution of polyphenism, v2, Dryad, Dataset. <https://doi.org/10.5061/dryad.gflvhmmmf>
- R Development Core Team. 2018.** *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: <http://www.r-project.org>
- Raff RA. 1996.** *The shape of life: genes, development, and the evolution of animal form*. Chicago: University of Chicago Press.
- Relyea RA. 2001.** The lasting effects of adaptive plasticity: predator-induced tadpoles become long-legged frogs. *Ecology* **82**: 1947–1955.
- Roff DA. 1996.** The evolution of threshold traits in animals. *Quarterly Review of Biology* **71**: 3–35.
- Ruibal R, Tevis L, Roig V. 1969.** The terrestrial ecology of the spadefoot toad *Scaphiopus hammondi*. *Copeia* **1969**: 571–584.
- Schmidt BR, Hodl W, Schaub M. 2012.** From metamorphosis to maturity in complex life cycles: equal performance of different juvenile life history pathways. *Ecology* **93**: 657–667.
- Semlitsch RD, Scott DE, Pechmann JH. 1988.** Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology* **69**: 184–192.
- Seymour RS. 1973.** Energy metabolism of dormant spadefoot toads (*Scaphiopus*). *Copeia* **1973**: 435–445.
- Snell-Rood EC, Van Dyken JD, Cruickshank T, Wade MJ, Moczek AP. 2010.** Toward a population genetic framework of developmental evolution: the costs, limits, and consequences of phenotypic plasticity. *Bioessays* **32**: 71–81.
- Sultan SE, Spencer HG. 2002.** Metapopulation structure favors plasticity over local adaptation. *American Naturalist* **160**: 271–283.
- Tarvin RD, Bermudez CS, Briggs VS, Warkentin KM. 2015.** Carry-over effects of size at metamorphosis in red-eyed treefrogs: higher survival but slower growth of larger metamorphs. *Biotropica* **47**: 218–226.
- Therneau T. 2015.** *A package for survival analysis in S. Version 2.38*. Available at: <https://CRAN.R-project.org/package=survival>
- Van Dyken JD, Wade MJ. 2010.** The genetic signature of conditional expression. *Genetics* **184**: 557–570.
- Venables WN, Ripley BD. 2002.** *Modern applied statistics with S*. New York: Springer.
- Watkins TB. 2001.** A quantitative genetic test of adaptive decoupling across metamorphosis for locomotor and life-history traits in the Pacific tree frog, *Hyla regilla*. *Evolution* **55**: 1668–1677.
- Wells KD. 2007.** *The ecology and behavior of amphibians*. Chicago: University of Chicago Press.
- West-Eberhard MJ. 1986.** Alternative adaptations, speciation, and phylogeny. *Proceedings of the National Academy of Sciences* **83**: 1388–1392.
- West-Eberhard MJ. 1989.** Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* **20**: 249–278.
- West-Eberhard MJ. 2003.** *Developmental plasticity and evolution*. New York: Oxford University Press.
- Wilbur HM. 1980.** Complex life cycles. *Annual Review of Ecology and Systematics* **20**: 249–278.
- Wilson DS. 1989.** The diversification of single gene pools by density- and frequency-dependent selection. In: Otte D, Endler JA, eds. *Speciation and its consequences*. Sunderland: Sinauer, 366–383.
- Wray GA. 1992.** The evolution of larval morphology during the post-Paleozoic radiation of echinoids. *Paleobiology* **18**: 258–287.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Box and whisker plots of size and age at metamorphosis and sexual maturity for carnivores and omnivores from two different larval environments.

SHARED DATA

Data available from the Dryad Digital Repository (Pfennig et al., 2020; doi:10.5061/dryad.gflvhmmmf).