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An experimental investigation of how intraspecific competition and phenotypic plasticity can promote the evolution of novel, complex phenotypes

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Running title: Competition and plasticity promote novelty

Abstract

Intraspecific competition has long been considered a key driver of evolutionary diversification, but whether it can also promote evolutionary innovation is less clear. We examined the interplay between competition and phenotypic plasticity in fueling the origins of a novel, complex phenotype—a distinctive carnivore morph found in spadefoot toad tadpoles (genus *Spea*) that specializes on fairy shrimp. We specifically sought to explore the possible origins of this phenotype by providing shrimp to *Scaphiopus holbrookii* tadpoles (the sister genus to *Spea* that does not produce carnivores) while subjecting them to competition for their standard diet of detritus. We found that: 1) some individuals used behavioral and morphological plasticity to expand their diet to include shrimp; 2) there was heritable variation in this plasticity; and 3) individuals received a growth and development benefit by eating shrimp. Thus, novel resource use can arise via plasticity as an adaptive response to intraspecific competition. More generally, our results show how competition and plasticity may interact to pave the way for the evolution of complex, novel phenotypes, such as the distinctive carnivore morph in present-day *Spea*.

Keywords: Competition, diversification, novelty, phenotypic plasticity, plasticity-led evolution

Introduction

Competition's contribution to evolutionary diversification is widely accepted. For example, niche-width expansion, arising as an adaptive response to intraspecific competition, is well supported theoretically (Van Valen 1965; MacArthur and Wilson 1967; MacArthur 1972; Roughgarden 1972) and empirically (e.g., Robinson et al. 1993; Robinson and Wilson 1994; Swanson et al. 2003). In contrast to this widespread acceptance of competition's role in diversification, its role in evolutionary innovation is not generally appreciated. Yet, by depressing the fitness of individuals such that some would do better by seeking alternative resources that are less in demand—or even previously avoided—intraspecific competition might favor novel phenotypes that exploit unique resources (reviewed in Pfennig and Pfennig 2012). Such a process has been demonstrated experimentally in the lab (e.g., Rozen and Lenski 2000; Bolnick 2001; Friesen et al. 2004; Bono et al. 2013; Ferguson et al. 2013) and might explain the evolution of novel resource-use traits in natural populations, including the novel trophic morphology of soapberry bugs, *Jadera haematoloma* (Carroll et al. 1998), the novel cecal valve of Italian wall lizards, *Podarcis sicula* (Herrel et al. 2008), and the novel head shape of tiger snakes, *Notechis scutatus* (Aubret and Shine 2009).

Phenotypic plasticity may play a crucial role in the early stages of such competitively mediated niche-width expansion. For example, the individuals of many species can facultatively adjust their resource-use (and associated phenotypes) depending on the intensity of competition and the availability of underutilized resources (reviewed in Pfennig and Pfennig 2012). Essentially, such plasticity enables individuals to shift their morphology, physiology, and/or behavior *in real time* from resources that are in high demand to those that are less in demand (Svanbäck and Bolnick 2007; Svanbäck et al. 2008).

Niche-width expansion arising via plasticity might even pave the way for the evolution of novel resource-use traits. Indeed, such traits might often evolve from an initial phase in which they arise through plasticity to one in which these traits become genetically fixed in a population. This scenario is thought to play a key role in mediating an adaptive evolutionary response to competition between species via character displacement (Pfennig and Pfennig 2012, pp. 94–102), and it may similarly explain how novel complex traits arise as an adaptive response to competition *within* species via niche-width expansion. Such an evolutionary pathway seems plausible, given that intraspecific competition is more common and frequently stronger than interspecific competition (Gurevitch et al. 1992; Dybzinski and Tilman 2009) and that niche-width expansion is the intraspecific analog of character displacement (West-Eberhard 2003, p. 397; Dayan and Simberloff 2005; Pfennig and Pfennig 2010). Yet, few studies have empirically evaluated whether intraspecific competition can combine with plasticity to promote the evolution of novel features involved in resource use.

We sought to examine the interplay between intraspecific competition and plasticity in evolutionary innovation. Specifically, we asked if: 1) competition and plasticity can combine to promote niche-width expansion, and 2) these two processes might have contributed to an evolutionary sequence leading to evolutionary innovation. To do so, we focused on the possible origins of a novel, complex phenotype found in certain amphibians.

Spadefoot toad tadpoles in the genus *Spea,* like most anurans (Wells 2007), normally develop as an 'omnivore' morph, characterized by small jaw muscles, smooth mouthparts, many denticle rows, and a long gut. This morph is a dietary generalist that eats detritus, algae, and small crustaceans. However, if *Spea* tadpoles consume fairy shrimp or other tadpoles (Pfennig 1990; Levis et al. 2015), some proportion of individuals facultatively develop (during ontogeny in a single animal's lifetime) as an alternative 'carnivore' morph (Figure 1a). Carnivores differ from omnivores in behavior (carnivores are more active); development (carnivores are quicker to undergo metamorphosis; Pfennig 1992); physiology (carnivores have larger livers; de la Serna Buzón 2019); and morphology (carnivores have large jaw muscles, notched mouthparts, few denticle rows, and a short gut; Pfennig 1990; Pfennig 1992; Pfennig and Murphy 2002). The evolution of this carnivore morph facilitated the exploitation of rapidly drying ponds with an abundance of fairy shrimp and other tadpoles (Pfennig 1992; Pfennig et al. 2006).

The carnivore morph is a derived trait restricted to *Spea* (Ledón-Rettig et al. 2008) whose evolutionary origins have recently been the focus of intense scrutiny (reviewed in Levis and Pfennig 2019b). Previous work has supported the hypothesis that this evolutionary novelty arose when pre-existing plasticity was expressed in an ancestral lineage, and later refined by selection into an adaptive phenotype in derived lineages (Ledón-Rettig et al. 2008; Ledón-Rettig et al. 2010; Levis et al. 2018; Levis and Pfennig 2019c). Earlier studies have also demonstrated that some of the constituent morphological and molecular components of the carnivore phenotype exhibit plasticity in response to consumption of a shrimp diet in the genus sister to *Spea* (Ledón-Rettig et al. 2008; Ledón-Rettig et al. 2010; Levis et al. 2018). Specifically, *Scaphiopus couchii* (a species that does not produce the carnivore morph and is a member of the sister genus to *Spea*) developed shorter guts when fed shrimp (a novel diet for this species, but the normal diet of *Spea* carnivores) than when fed its typical detritus diet (a short gut is a component trait of the carnivore morph found in *Spea*). Further evidence from *Sc. couchii* also suggested that cryptic genetic variation in morphological components of the carnivore phenotype was uncovered by shrimp consumption (Ledón-Rettig et al. 2008; Ledón-Rettig et al. 2010). Similarly, a different outgroup species *Sc. holbrookii* (also in the sister genus to *Spea*) exhibited diet-dependent plasticity in several component traits that characterize the carnivore morph in *Spea*, including gut length (Figure 1b), denticle rows, and mouthparts (Levis et al. 2018). Despite some evidence of trait plasticity in *Scaphiopus* tadpoles, this plasticity is not necessarily coordinated (some traits show plasticity and others do not) nor is it fully adaptive (plasticity in some traits is in the maladaptive direction; Levis et al. 2018). Indeed, the induced form found in *Scaphiopus* is a rudimentary version of the carnivore morph found in *Spea* (Figure 1c).

In this study, we further explored diet dependent plasticity in *Sc. holbrookii* to clarify the possible ecological circumstances that may have started *Spea*'s ancestor down the evolutionary trajectory that ultimately led to the novel carnivore morph. Because the absence of the carnivore morph represents the 'ancestral' condition, we used this species to help inform possible features and responses of the last common ancestor of *Scaphiopus* and *Spea*, before the evolution of the carnivore morph (for further justification—and additional examples—of this general approach, see Badyaev and Foresman 2000; Gomez-Mestre and Buchholz 2006; Rajakumar et al. 2012; Schlichting and Wund 2014; Standen et al. 2014; Allf et al. 2016; Levis and Pfennig 2016; Jones et al. 2017; Casasa and Moczek 2018). Moreover, although tadpoles of *Sc. holbrookii* (like tadpoles of most species of anurans) feed mostly on plankton and detritus (Wells 2007), they will opportunistically consume macroinvertebrates (such as fairy shrimp) when conspecific densities are high and competition for their standard resources is severe (McDiarmid and Altig 1999; Levis and Pfennig personal observation). We hypothesized that if we found evidence of selectable variation in plasticity in *Sc. holbrookii*, then the common ancestor of both genera (before the evolution of the carnivore morph) may have possessed such variation as well. Moreover, if we found that the expression of this plasticity increased growth during resource competition, then this might indicate that competitively mediated selection, acting on heritable variation in the expression of plasticity, helped fuel the adaptive refinement of the carnivore phenotype, from a rudimentary version (such as that in *Scaphiopus*; Figure 1b) to the fully functional, complex carnivore morph in *Spea* (Figure 1a).

Methods

General approach

We examined the interplay between competition and phenotypic plasticity in fueling the origins of a novel, complex phenotype—the distinctive carnivore morph found in *Spea* that specializes on fairy shrimp. Because previous studies supported the idea that this evolutionary novelty arose via plasticity (see **Introduction**), we designed two experiments to investigate the initial steps in this scenario by using tadpoles of *Sc. holbrookii*.

To begin, we gauged the amount of phenotypic plasticity and phenotypic variation produced by *Sc. holbrookii* tadpoles when reared on live shrimp (the novel diet of the carnivore morph) versus their standard diet of detritus (the standard diet of most anuran tadpoles). As noted in the **Introduction**, earlier studies found that cryptic genetic variation was uncovered by shrimp consumption in *Sc. couchii* (Ledón-Rettig et al. 2008; Ledón-Rettig et al. 2010), but this species may have secondarily become a detritus specialist and become less informative of the common ancestor with *Spea* (Ledón-Rettig and Pfennig 2012). Therefore, characterization of additional species is needed to improve our understanding of possible attributes of the last common ancestor with *Spea*. Next, we determined if there was a growth and development benefit to consuming shrimp when competition for the standard resource (detritus) was severe. Presumably, because most individuals would be utilizing the detritus resource, those individuals that were able to switch to the underutilized shrimp resource (which is also more nutritious than detritus; Pfennig 2000) would grow more and develop faster. Thus, we expected that individuals exhibiting behavioral plasticity in resource use would gain a fitness advantage.

Based on our hypothesis that competition and plasticity can combine to promote niche-width expansion, and that these two processes can jumpstart the evolutionary sequence leading to evolutionary innovation (in this case, the carnivore morph), we expected to find: 1) the existence of variation in trait plasticity among sibships, 2) greater trait variation on a live shrimp diet (the novel resource) than on a detritus diet (the ancestral resource), and 3) that individuals that consumed more shrimp during competition had greater growth and development. More generally, we predicted that variation in traits whose plasticity is adaptive in *Spea* (the polyphenic lineage), will also have adaptive value on alternative resources and during competition in *Scaphiopus* (the monomorphic lineage).

Diet-dependent variation

We bred 10 pairs of *Sc. holbrookii* that were collected from Hoffman, North Carolina and that had been maintained in a laboratory colony at the University of North Carolina. We injected adults with 0.04 ml luteinizing hormone-releasing hormone (Sigma L-7134) at a concentration of 0.01µg/µl to induce breeding. We then left pairs overnight to breed, removed adults 24 h after injection, and kept eggs in separate nursery tanks until hatching. Three days after hatching, we placed thirty tadpoles from each sibship individually into opaque 88mL cups (56mm diameter x 55mm tall). Half of the tadpoles from each sibship were fed plant-based fish food (Ken's Premium Vegetable Flake) that simulates the organic detritus upon which these tadpoles normally eat in the wild. To accommodate growth over time, their feeding regime was 10mg of detritus on the first day, 10mg on the third day, 20mg on the fifth day, 20mg on the seventh day, 10mg on the eighth day, 20mg on the ninth day, and 20mg on the eleventh day. The other half of the tadpoles were fed live brine shrimp (*Artemia* sp.) that simulate the fairy shrimp (*Thamnocephalus* sp. and *Streptocephalus* sp.) that *Spea* carnivores eat in nature (Pfennig et al. 2006). The feeding regime of this group consisted of 1mL of concentrated brine shrimp nauplii on the first day, 4mL of concentrated brine shrimp nauplii on the second day, 30 small brine shrimp on the third day, 40 small brine shrimp on the fourth, fifth, and sixth days, 60 small brine shrimp on the seventh day, 40 adult brine shrimp on the eighth day, 60 adult brine shrimp on the ninth day, and 40 adult brine shrimp on the tenth and eleventh days. Water in all tanks was changed every other day and any uneaten carcasses of shrimp were removed before each feeding. We ended this experiment on the twelfth day by euthanizing tadpoles in a 0.8% aqueous solution of tricane methanesulfonate (MS-222) and preserving them in 95% ethanol.

We measured the following four morphological traits (Table S1; Pfennig and Murphy 2002) that are diagnostic of morphotype in *Spea*: 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 also measured body size (snout-vent length [SVL]; body size serve as a reliable fitness proxy in spadefoots: larval size predicts juvenile survival and adult reproductive success; Pfennig et al. 2007; Martin and Pfennig 2009). Measurements were done blind with respect to diet treatment. We standardized OH for body size (SVL) by regressing log OH on log SVL (Pfennig et al. 2007) and using the residuals from the regression as our measure for OH width. For each sibship, we measured plasticity as the slope of the line between diet treatments (i.e., shrimp – detritus) for each morphological trait. Thus, plasticity was determined at the sibship-level, and these levels of sibship plasticity are included as explanatory variables in the competition experiment below (see *Growth and development benefits of shrimp consumption during competition*).

To evaluate if there was variation among sibships in the plastic responses of their trophic traits (i.e., if there was selectable variation for plasticity *per se*), we performed a full factorial Type III sum of squares ANOVA (with the function 'Anova' in the R package 'car'). Each trophic trait was used as a response variable and sibship and diet were explanatory variables. Finding a significant interaction between sibship and diet would indicate variation in trait plasticity among sibships. Finding that only sibship or diet was significantly explanatory would indicate variation in mean trait values among sibships or trait means across diets (with all sibships showing a similar response), respectively.

To test for the uncovering of cryptic genetic variation, we followed Ledon-Rettig et al. (2010) and calculated the environment-specific broad-sense heritability (H^2) of each trait. For each diet treatment, we calculated among-sibship (genetic) variances of traits using linear mixed effects models including sibship as a random effect. We then calculated H^2 according to Roff (1997):

$$H^2 = \frac{2V_{AS}}{V_T}$$

where V_{AS} is the variance among sibships and V_T is the total variance. We performed 5000 bootstrap replicates to obtain a distribution of H^2 for each diet and then compared these distributions with Welch's two sample t-test. Finding that the H^2 is significantly greater on shrimp than on detritus, would provide evidence of cryptic genetic variation being uncovered on the novel diet (Ledón-Rettig et al. 2010; Ledón-Rettig et al. 2014). This approach cannot rule out the contribution of maternal effects, which can influence carnivore development in some, but not all, *Spea* populations (Pfennig and Martin 2009; Pfennig and Martin 2010). Thus, while our measure of heritability captures additive genetic variation, we cannot say with certainty that all of the variation we see is indeed additive genetic variation. We also compared the variance in each of the morphological traits across diet treatments using Levene's test via the function leveneTest in the 'car' package. All analyses were carried out in R (R core team 2019).

Finally, we were interested in which diets, traits, or trait plasticities best predicted growth (body size; SVL) and development (Gosner developmental stage) under these 'ideal' conditions (i.e., no competition and an abundance of food). To test this, we combined these two variables (SVL and Gosner developmental stage) into a single metric using a principal component analysis (the function 'prcomp') in R (developmental rate, like body size [see above], serves as a reliable fitness proxy: spadefoots experience strong directional selection for fast development in their rapidly drying ponds; Pfennig et al. 2007; Martin and Pfennig 2009). Based on this proxy fitness measure, individuals that were larger and more developmentally advanced were considered to have a selective advantage. We then used a linear mixed effects model and a type II sum of squares ANOVA and Wald chi-square tests (with the function 'Anova' in the R package 'car') to determine the ability of morphological traits, trait plasticities, and diet to predict this proxy for fitness. We included both an individual's trait value and its sibship-level trait plasticity because one measure captures a realized developmental response (trait) and the other captures genetic developmental potential (trait plasticity), and we wanted to see if these had different relationships with fitness. Sibship was included as a random effect, and our fitness proxy was the response variable.

Growth and development benefits of shrimp consumption during competition

Our second experiment evaluated whether individuals received a growth and development benefit altering resource use patterns during intense competition. Specifically, we sought to determine if *Sc. holbrookii* individuals that were experiencing competition for detritus gained an advantage by switching to a diet of shrimp. We generated 30 ‘competition’ tanks by filling a plastic box (18 × 13 × 8.5 cm each) with 1 L of water and placing ten sibling tadpoles in each tank (using tadpoles created in the first experiment; see *Diet-dependent variation*). The resulting density of tadpoles was five to 30 times higher than typical spadefoot tadpole competition experiments (e.g., Pfennig and Murphy 2000; Pfennig and Rice 2007; Levis and Pfennig 2019c), over an order of magnitude higher than densities in other manipulations of tadpole competition (e.g., Woodward 1982; Relyea 2002; Jones et al. 2011), but comparable to (albeit slightly higher than) the most extreme densities seen in natural spadefoot ponds (Newman 1987; Pfennig et al. 1991). Thus, competition for resources was strong.

We created five replicates of these competition tanks for each of six sibships (n = 300 tadpoles). Prior to their introduction in the competition tanks, all tadpoles within a sibship were reared together under identical conditions for fifteen days and fed detritus only *ad libitum*. Thus, within each replicate, tadpoles were all from similar rearing conditions and of similar levels of plasticity (because they were from the same sibship), which allowed us to assess the benefits of eating shrimp when individuals were subjected to intense competition for food (based on the fact that all individuals were presumably similar in competitive ability). Every tank was fed both 160 live adult brine shrimp and 30 mg of detritus daily and water was changed and shrimp carcasses were cleared prior to each feeding.

After seven days we ended the experiment and euthanized tadpoles in MS-222 and preserving them in 95% ethanol. We then measured SVL, Gosner developmental stage, GC, DR, MP, and OH of every tadpole as in the first experiment. To account for possible variation among sibships in SVL or Gosner stage, we first took the residuals of each individual's SVL or Gosner stage from its sibship mean. We then added this residual value to the grand mean among all sibships to standardize sibship-level differences (Levis et al. 2016), and helped ensure that the largest and most developed individuals were not simply a result of having come from sibships that already tended to be the largest. In addition to these morphological traits, we assessed individual behavioral plasticity by determining the amount of shrimp each tadpole consumed. Specifically, we measured the $\delta^{15}N$ content of tail tissue from each individual following a modified version of the protocol of Paull et al. (2012). Briefly, we removed the entire tail from each tadpole, dried it in an oven at 65ºC for 72 h. We then placed a sample of 1.0 mg of dried tissue into a tin capsule (5 × 8 mm) and submitted the samples to the University of California at Davis Stable Isotope Facility for analysis. To gauge what individuals reared in competition were eating, we also measured the $\delta^{15}N$ content of pure detritus, pure brine shrimp, tadpoles fed an exclusive diet of detritus, and tadpoles fed an exclusive diet of shrimp (n = 4 per group) as controls. We interpreted greater consumption of shrimp as greater behavioral plasticity.

As above, we combined the sibship standardized SVL and standardized Gosner developmental stage (Gosner 1960) of individuals into a single metric using a principal component analysis (the function 'prcomp') in R (developmental rate, like body size [see above], serves as a reliable fitness proxy: spadefoots experience strong directional selection for fast development in their rapidly drying ponds; Pfennig et al. 2007; Martin and Pfennig 2009). Based on this measure, individuals that were larger and more developmentally advanced were considered to have a selective advantage. We then used linear mixed effects model and a type II sum of squares ANOVA and Wald chi-square tests (with the function 'Anova' in the R package 'car') to determine the ability of morphological traits, trait plasticities, and δ^{15}N to predict this proxy for fitness. Specifically, each trait, sibship-level trait plasticity, and δ^{15}N were fixed effect predictor variables, replicate was a random effect, and our fitness proxy was the response variable. We compared the δ^{15}N among our control samples using a type I sum of squares ANOVA followed by a Tukey HSD post-hoc test in R.

Results

Diet-dependent variation

Our first experiment assessed the extent of plasticity among sibships and whether a novel shrimp diet induced greater variation in morphology or body size (i.e., SVL; a proxy for fitness in spadefoots) than the typical detritus diet. Regarding plasticity, we found that three of the four traits showed variation in diet-induced morphology and/or variation among sibships in trait values (Table 1). Specifically, gut length (GC) and jaw muscle width (OH) varied by diet and sibship and mouthpart morphology (MP) varied by sibship. Jaw muscle width was nearly significant for variation in plasticity (Diet*Sibship; $P = 0.057$). Denticle rows did not vary by diet, sibship, or their interaction.

We found that every trait except for gut length showed evidence of cryptic genetic variation being uncovered on a shrimp diet (Table 2a). However, none of the trophic traits showed a significant difference in overall phenotypic variation between diets (Table 2b) despite significantly greater variation in SVL on a shrimp diet ($\sigma^2 = 0.49$) than on a detritus diet ($\sigma^2 = 0.35$; F ratio = 4.55; $P = 0.034$).

Finally, we found that, under ideal conditions of no competition and abundant food, tadpole growth (SVL) and development (Gosner developmental stage) was significantly influenced by trophic morphology (Table 3). In particular, our growth and development fitness proxy was explained by variation in gut length and mouthpart morphology. However, Diet and jaw muscle width plasticity were nearly significant ($P \approx 0.06$). Finding these results, we then evaluated a simple mixed effects model (using a type II sum of squares ANOVA and Wald chi-square tests) with gut length as the response variable, mouthpart morphology as the explanatory variable, and sibship as a random effect to determine if there were alternative strategies to achieving higher growth and development or if these two traits were positively (or negatively) associated. We found that gut length and mouthpart morphology values were not associated (Estimate = 0.140, $\chi^2 = 0.848$; $P = 0.357$), suggesting that individuals may deploy different morphological strategies to enhance growth and development.

Growth and development benefits of shrimp consumption during competition

Our control samples indicated that shrimp-fed tadpoles have a lower $\delta^{15}N$ than detritus-fed tadpoles (Table S2). Knowing this relationship, we found that when individuals were reared in competition against individuals of similar plasticity and competitive ability (i.e., siblings), the greatest growth and development (i.e., proxies for fitness) was achieved by having: 1) high behavioral plasticity (i.e., consuming more shrimp); 2) long gut lengths (GC); 3) and carnivore-like mouthparts (Table 4; Figure 2).

As above, we used a simple mixed effects model with type II sum of squares ANOVA and Wald chi-square tests to evaluate the relationships among these variables ($\delta^{15}N$ as the response, GC and MP as explanatory variables, and sibship as a random variable) in order to determine if there were different strategies to enhance growth and development during competition. We found that neither gut length nor mouthpart morphology influenced shrimp consumption directly (GC: Estimate = -0.057, χ^2 = 1.827, P = 0.176; MP: Estimate = -0.089, χ^2 = 1.237, *P* = 0.266). In addition, gut length and mouthpart morphology were not associated with each other (Estimate = 0.032, χ^2 = 0.086; *P* = 0.769). Thus, carnivore-like behavior (shrimp consumption) and morphology (mouthparts) were advantageous during competition, but these attributes may be acting independently to improve fitness.

Discussion

We studied the interplay between plasticity and intraspecific resource competition to help evaluate the origins of a novel complex phenotype. We specifically sought to recreate the ecological conditions that may have accompanied the evolution of the distinctive carnivore morph found in spadefoot tadpoles of the genus *Spea.* We did so by studying tadpoles of *Sc. holbrookii*, which are members of the sister genus to *Spea* and do not produce carnivores. When we subjected *Sc*. *holbrookii* to resource competition, we found: 1) heritable variation in morphology; 2) an increase in such heritable variation on the novel shrimp diet; and 3) that the expression of some morphological features and behaviors that characterize the carnivore morph were associated with enhanced growth and development. These data thereby suggest that differences in the expression of plasticity might enable refinement of resource use phenotypes, and might have even contributed to the evolution of a novel carnivore morph (Figure 1a). More generally, our results illustrate how competition and plasticity can interact to promote niche-width expansion and thereby possibly pave the way for evolutionary novelty.

We found that competition for detritus favored individuals that switched to a novel resource (shrimp). Our competition experiment revealed that individuals that expressed alternative foraging behavior through plasticity (i.e., lower δ^{15}N and higher consumption of shrimp) likely had a selective advantage (Figure 2). Presumably, competition for detritus favored those individuals that were able to switch to consuming shrimp because these individuals were thereby released from competition; i.e., they were favored by negative frequency-dependent selection (e.g., see Pfennig 1992; Hori 1993; Benkman 1996; Maret and Collins 1997; Bolnick 2004). As noted in the **Introduction**, such niche-width expansion in the face of intraspecific resource competition has been documented in numerous taxa, in both the lab and in the wild. By favoring increased niche variation among members of a single population, resource competition is thought to act as a key agent of diversifying selection and thereby play a major role in driving evolutionary diversification (Van Valen 1965; MacArthur and Wilson 1967; MacArthur 1972; Roughgarden 1972).

A competitive advantage may have been gained, at least in part, by morphological changes. Specifically, increased mouthpart keratinization and longer gut lengths both enhanced growth and development (and therefore, presumably, fitness) in both non-competitive and competitive conditions. Since *Spea* carnivores exhibit more extreme mouthpart keratinization than omnivores (Table S1), the former observation is consistent with adaptive plasticity. However, *Spea* carnivores typically have *shorter* guts than omnivores. This suggests that our observation of longer guts improving growth and development might represent a trade-off or constraint on morphological development when alternative resources are available. Indeed, individuals with more keratinized mouthparts did not also have longer guts in the trait variation experiment or the competition experiment. In general, these results point to carnivore-like behavior (shrimp consumption) and morphology (more carnivore-like mouthparts) potentially being advantageous. The adaptive refinement and innovation during the evolution of carnivores in *Spea* might have involved breaking developmental constraints among traits (Hallgrimsson et al. 2009; Hallgrimsson et al. 2012) that were limiting simultaneous specialization on alternative resources.

Our finding of heritable and cryptic variation in various traits corroborate findings from a previous study using a different species of *Scaphiopus* (Ledón-Rettig et al. 2010). Finding such among-sibship variation in morphology and plasticity in spadefoots (Levis et al. 2018; Kelly et al. 2019; Levis and Pfennig 2019a) points to the possibility that the common ancestor of *Scaphiopus* and *Spea* (before the evolution of the carnivore morph) may have also harbored heritable (selectable) variation in plasticity of many trophic features. Moreover, the fact that we detected an increase in such variation among tadpoles fed the novel shrimp diet suggests that the efficacy of selection potentially increased during the transition to this novel diet (i.e., cryptic genetic variation was uncovered; Gibson and Dworkin 2004; Paaby and Rockman 2014; Zheng et al. 2019). Thus, the initial transition to the novel shrimp diet (and any morphological changes that accompanied such a transition) may have exposed heritable variation to selection. This exposure then allowed selection to refine the phenotype such that, over evolutionary time, individuals became better at utilizing this resource (e.g., by developing the fully functional carnivore morph). This process is akin to that of ecological character release wherein a species' niche and phenotype expand in the absence of competitors and the presence of greater ecological opportunity (Grant 1972; Bolnick 2001; Svanbäck and Bolnick 2007).

Our results have implications for understanding how novel, complex phenotypes originate (Mayr 1959; West-Eberhard 2003; Wagner and Lynch 2010), and support the idea that an adaptive response to intraspecific competition serves as a springboard for the evolution of new features (Pfennig and Pfennig 2012). In particular, 'plasticity-led evolution' (West-Eberhard 1989; West-Eberhard 2003; Schwander and Leimar 2011; Levis and Pfennig 2016; sensu Levis and Pfennig 2019c) begins when a change in the environment triggers a change in phenotype through plasticity and, in doing so, exposes variation in the degree to (or form in) which different genotypes respond to this environmental change (i.e., exposes variation in reaction norms). This evolutionary route continues if selection favors those responses (and, hence, genotypes) that are most well-adapted to the new conditions. Ultimately, this process can cause the extent and/or shape of plasticity to evolve, such that an unrefined, pre-existing plastic response is molded by selection into a well-functioning phenotype (West-Eberhard 2003; Moczek et al. 2011; Levis and Pfennig 2016). The end result is a new phenotype that is part of a 'polyphenism' (when increased plasticity is favored) or a genetically fixed trait (when decreased plasticity is favored) (Waddington 1953).

An untested aspect of plasticity-led evolution is to evaluate how ancestral plasticity might have functioned in the ecological (e.g., competitive) context in which that plasticity was elicited. Since plasticity-led evolution posits that a population (or subpopulation) experiences an environmental change simultaneously, competition among genotypes or individuals that differ in the degree or manner in which they respond to that change is inevitable. Indeed, competitively-mediated natural selection frequently drives intra- and inter-specific diversification (reviewed in Pfennig and Pfennig 2012). Thus, intraspecific competition coupled with pre-existing plasticity could play a powerful role during the initial stages of plasticity-led evolution, as shown in our study. Our study therefore fills a critical gap by not only testing for the existence of ancestral plasticity, but also establishing that some aspects of such plasticity actually confer a selective advantage in a realistic context: namely, during competition.

Previous work on *Spea* carnivores has suggested that intraspecific competition might have promoted the origin of new carnivore variants (Levis et al. 2017). In a similar way, the data presented here hint at a role for intraspecific competition in promoting the origins of the carnivore morph in the first place. Indeed, competition for resources, coupled with new ecological opportunity and plasticity, might explain the origins of novelty in various taxa (e.g., Liem and Kaufman 1984; Bono et al. 2013; Yassin et al. 2016; Levis et al. 2017). Thus, under changing ecological conditions, pre-existing plasticity might foster adaptation, diversification, and evolutionary innovation.

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Table 1. Results from our assessment of diet dependent plasticity in trophic traits of *Sc. holbrookii* tadpoles. Bolded rows indicate that a given variable was a significant predictor of each trait. Italic rows indicate a nearly significant relationship. Most traits showed variation in diet induced morphology and/or variation among sibships in trait values. Only OH muscle width showed nearly significant variation in plasticity among sibships.

GC					DR				
Variable	Sum Sq.	Df	F	*P*	Variable	Sum Sq.	Df	F	*P*
Intercept	**777.60**	**1**	**1075.63**	**2.20E-16**	Intercept	**3904.30**	**1**	**366.14**	**2.00E-16**
Diet	**8.53**	**1**	**11.80**	**6.82E-04**	Diet	2.10	1	0.20	6.55E-01
Sibship	**36.79**	**9**	**5.65**	**3.45E-07**	Sibship	67.60	9	0.70	7.05E-01
Diet*Sibship	8.78	9	1.35	2.10E-01	Diet*Sibship	116.80	9	1.22	2.84E-01
MP					OH				
Variable	Sum Sq.	Df	F	*P*	Variable	Sum Sq.	Df	F	*P*
Intercept	**32.27**	**1**	**213.57**	**2.00E-16**	**Intercept**	**0.06**	**1**	**10.71**	**1.20E-03**
Diet	0.03	1	0.22	6.39E-01	**Diet**	**0.07**	**1**	**11.95**	**6.31E-05**
Sibship	**3.03**	**9**	**2.23**	**2.06E-02**	**Sibship**	**0.13**	**9**	**2.43**	**1.13E-02**
Diet*Sibship	1.50	9	1.10	3.63E-01	*Diet*Sibship*	*0.10*	*9*	*1.91*	*5.07E-02*

Table 2. a) Broad-sense heritability estimated from data and from boot strap replicates (parentheses) for each trait on each diet. ‘Shrimp/Detritus’ indicates the ratio of broad-sense heritability of shrimp to detritus, ‘t’ is the test statistic from a t-test comparing the bootstrap replicate distributions between diets, and ‘*P*’ is the resulting p value from that test. Note that the ratio for DR was modified to 13/0.01 to avoid dividing by zero. (b) Overall phenotypic variance on each diet and results from Levene’s test comparing overall phenotypic variation between diet treatments. Bold values indicate a significant difference in variance between diets.

a. Trait	Detritus	Shrimp	Shrimp/Detritus	t	*P*	CGV on shrimp?
SVL	0.25 (0.38)	0.35 (0.45)	1.40 (1.18)	25.01	2.2E-16	Y
GC	0.47 (0.57)	0.40 (0.48)	0.85 (0.84)	35.96	2.2E-16	N
DR	0.00 (0.12)	0.13 (0.24)	13.00 (2.00)	54.87	2.2E-16	Y
MP	0.16 (0.28)	0.23 (0.35)	1.44 (1.25)	21.86	2.2E-16	Y
OH	0.15 (0.27)	0.31 (0.42)	2.07 (1.56)	58.56	2.2E-16	Y

b. Trait	Detritus	Shrimp	F	*P*
SVL	**0.35**	**0.49**	**4.55**	**0.034**
GC	0.95	0.85	0.05	0.825
DR	8.41	13.59	2.77	0.097
MP	0.16	0.17	0.92	0.337
OH	0.01	0.01	0.83	0.363

Table 3. Results from our test of which variables best explain growth and development under non-competitive, abundant food conditions. Bolded and italic rows indicate that a given variable was a significant or nearly significant, respectively, predictor of growth and development. Note that trait plasticity values were measured at the sibship level for these analyses.

Variable	Estimate	χ^2	*P*
Intercept	-1.53	---	---
GC plasticity	-0.16	0.23	0.634
DR plasticity	0.05	0.01	0.916
MP plasticity	-0.15	1.14	0.286
OH plasticity	*2.24*	*3.38*	*0.066*
GC	**0.14**	**4.40**	**0.036**
DR	-0.01	0.20	0.651
MP	**0.43**	**8.60**	**0.003**
OH	-0.32	0.19	0.665
Diet	*-0.25*	*3.42*	*0.064*

Table 4. Results from competition experiment. $\delta^{15}N$ was used to estimate the amount of resource (shrimp or detritus) an individual consumed and acted as a proxy for behavioral plasticity in resource use. Bolded rows indicate that a given variable was a significant predictor of growth and development during competition within sibships for alternative resources.

Variable	Estimate	χ^2	*P*
Intercept	0.70	---	---
$\delta^{15}N$	**-0.25**	**10.30**	**0.001**
GC	**0.17**	**4.34**	**0.037**
DR	-0.01	1.14	0.285
MP	**0.59**	**15.50**	**8.245E-05**
OH	-0.50	0.45	0.502

Figure 1. Study system. a) *Spea* tadpoles develop as either a typical ‘omnivore’ morph (left) or a novel ‘carnivore’ morph (right). The latter is characterized by larger jaw muscles, notched mouthparts, and a shorter gut. b) Some of these same features can be environmentally induced—in a much-reduced form—in *Scaphiopus* (the sister genus to *Spea*), as in this *Sc. holbrookii* eating another tadpole. c) However, this induced *Scaphiopus* form is not a fully functional carnivore. Indeed, *Scaphiopus* are closer in morphospace to *Spea* omnivores than to carnivores (panel c based on data in, and redrawn from, Levis and Pfennig 2019b).

Figure 2. When in competition, tadpoles that had (a) longer guts, (b) more carnivore-like mouthparts, and/or (c) consumed more shrimp (lower $\delta^{15}N$ values) grew and developed faster than those with shorter guts, less carnivore-like mouthparts and/or that consumed fewer shrimp (higher $\delta^{15}N$ values). Thus, exaggerated morphological features and greater behavioral plasticity (i.e., greater propensity to eat shrimp) was advantageous. Each point is an individual that was corrected for sibship and replicate by adding the residuals of these terms from linear model to the overall mean (Levis et al. 2016). The linear regression lines are for visualization purposes only.

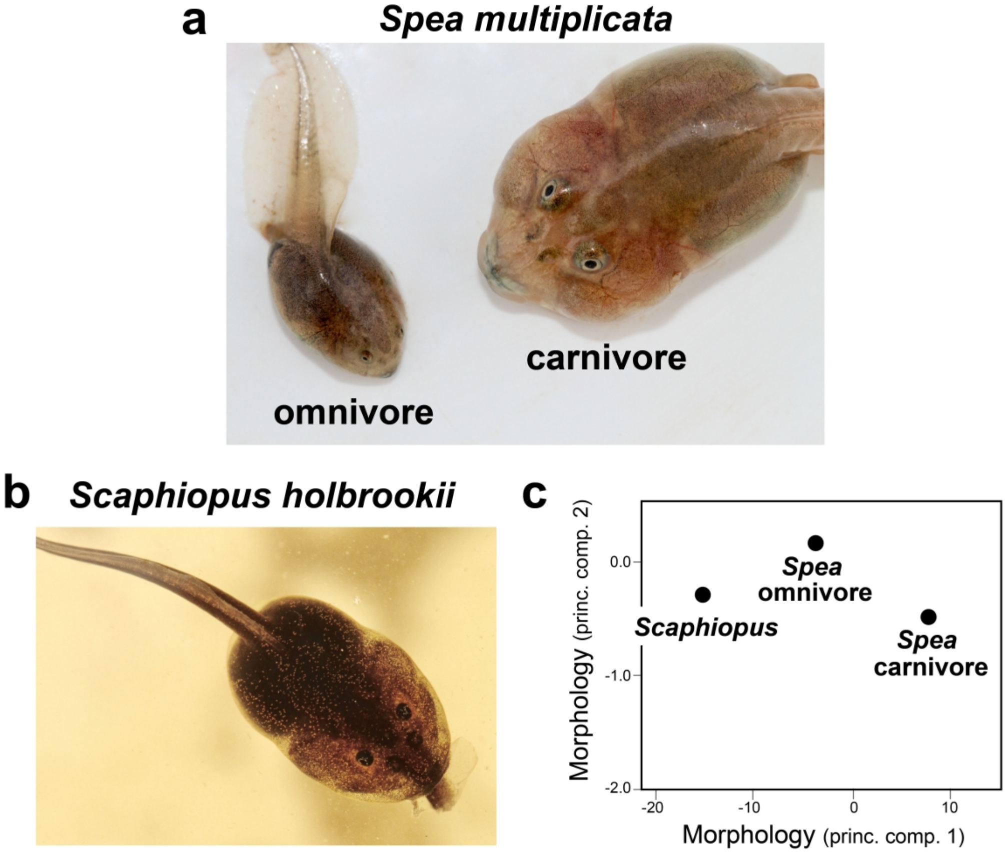
Table S1. Trophic traits, their functions, and their adaptive pattern of plasticity in the *Spea* omnivore-carnivore polyphenism. Adaptive pattern of plasticity refers to the direction the trait values change from a detritus (D) diet to a shrimp (S) diet (e.g., there are fewer denticle rows on a shrimp diet than on a detritus diet).

Trait (abbreviation)	Function	Reference	Adaptive pattern of plasticity (D → S)
Denticle rows (DR)	rasps food off surfaces; more DR favored for smaller prey	(Altig and McDiarmid 1999; Martin and Pfennig 2011)	Negative slope
Gut coils (GC)	used to digest food; more GC favored for more plants or bacteria in diet	(McDiarmid and Altig 1999; Ledón-Rettig et al. 2008)	Negative slope
Keratinized mouthparts (MP)	grasps prey; larger, more serrated MP favored for larger prey	(Altig and McDiarmid 1999; Martin and Pfennig 2011)	Positive slope
Orbitohyoideus muscle (OH)	opens mouth; larger OH favored for larger prey	(DeJongh 1968; Cannatella 1999; Martin and Pfennig 2011)	Positive slope

Table S2. A) ANOVA summary from δ15N analysis of control samples (detritus, shrimp, detritus-fed tadpoles, and shrimp-fed tadpoles). B) Tukey HSD post-hoc test results with differences in sample means above the diagonal and the corresponding p values below the diagonal.

A. Source	df	Sum of squares	Mean square	F	*P*
Group	3	250.65	83.55	1715.24	0.0001
Error	20	0.97	0.05		

B. Group	Detritus	Shrimp	Detritus-fed	Shrimp-fed
Detritus	---	6.43	1.29	4.89
Shrimp	0.0001	---	7.72	1.54
Detritus-fed	0.0001	0.0001	---	6.18
Shrimp-fed	0.0001	0.0001	0.0001	---



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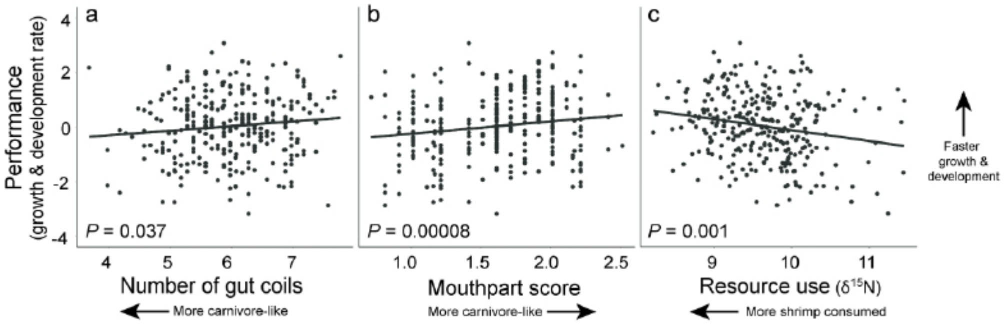

Figure 2. When in competition, tadpoles that had (a) longer guts, (b) more carnivore-like mouthparts, and/or (c) consumed more shrimp (lower □15N values) grew and developed faster than those with shorter guts, less carnivore-like mouthparts and/or that consumed fewer shrimp (higher □15N values). Thus, exaggerated morphological features and greater behavioral plasticity (i.e., greater propensity to eat shrimp) was advantageous. Each point is an individual that was corrected for sibship and replicate by adding the residuals of these terms from linear model to the overall mean (Levis et al. 2016). The linear regression lines are for visualization purposes only.