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

NICHOLAS A. LEVIS*,⊙, CARLY G. FULLER and DAVID W. PFENNIG

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

Intraspecific competition has long been considered a key driver of evolutionary diversification, but whether it can also promote evolutionary innovation is less clear. Here we examined the interplay between competition and phenotypic plasticity in fuelling 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. Previous research had shown that this species will eat shrimp when detritus is limited, and that these shrimp-fed individuals produce features that are redolent of a rudimentary *Spea* carnivore. In this study, we found that: (1) behavioural and morphological plasticity enabled some individuals 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*.

ADDITIONAL KEYWORDS: competition – diversification – novelty – phenotypic plasticity – plasticity-led evolution.

INTRODUCTION

The contribution of competition 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 & Wilson, 1967; MacArthur, 1972; Roughgarden, 1972) and empirically (e.g. Robinson *et al.*, 1993; Robinson & Wilson, 1994; Swanson *et al.*, 2003). In contrast to this widespread acceptance of the role of competition 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 favour novel phenotypes that exploit unique resources (reviewed by Pfennig & Pfennig, 2012). Such a process

has been demonstrated experimentally in the lab (e.g. Rozen & 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 caecal valve of Italian wall lizards, *Podarcis sicula* (Herrel *et al.*, 2008), and the novel head shape of tiger snakes, *Notechis scutatus* (Aubret & 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 by Pfennig & Pfennig, 2012). Essentially, such plasticity enables individuals to shift their morphology, physiology and/or behaviour *in their lifetime* from resources that are in high demand to

*Corresponding author. E-mail: nicholasalevis@gmail.com

those that are less in demand (Svanbäck & 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 & Pfennig, 2012: 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 & Tilman, 2009) and that niche-width expansion is the intraspecific analogue of character displacement (West-Eberhard, 2003: 397; Dayan & Simberloff, 2005; Pfennig & 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.

Here we sought to examine the interplay between intraspecific competition and plasticity in evolutionary innovation. Specifically, we investigated whether: (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, some proportion of individuals facultatively develop (during ontogeny in a single animal's lifetime) as an alternative 'carnivore' morph (Pfennig, 1990; Levis *et al.*, 2015). The carnivores differ from omnivores in behaviour (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, 1992; Pfennig & 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 by Levis & 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, 2010; Levis *et al.*, 2018; Levis & 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, 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*) (Ledón-Rettig *et al.*, 2008, 2010; Levis *et al.*, 2018). Further evidence from *Scaphiopus couchii* also suggested that cryptic genetic variation in morphological components of the carnivore phenotype was uncovered by shrimp consumption (Ledón-Rettig *et al.*, 2008, 2010). Similarly, a different outgroup species, *Scaphiopus 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, 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* (our personal observations).

In this study, we examined the interplay between competition and phenotypic plasticity in fuelling 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, we designed two experiments to investigate the initial steps in this scenario by using tadpoles of *Scaphiopus holbrookii*. First, we gauged the amount of phenotypic plasticity and phenotypic variation produced by *Scaphiopus holbrookii* tadpoles when reared on live shrimp (the novel diet of the carnivore morph) vs. their standard diet of detritus (the standard diet of most anuran tadpoles). Previous studies found that cryptic genetic variation was uncovered by shrimp consumption in *Scaphiopus couchii*

([Ledón-Rettig *et al.*, 2008, 2010](#)). However, *Scaphiopus couchii* may have secondarily become a detritus specialist as an adaptive response to resource competition with *Spea*, which is a superior competitor for shrimp ([Ledón-Rettig & Pfennig, 2012](#)). If so, then *Scaphiopus couchii* may be less informative regarding the common ancestor with *Spea* ([Ledón-Rettig & Pfennig, 2012](#)). Therefore, characterization of additional species, such as *Scaphiopus holbrookii* (which do not co-occur with *Spea*), is needed to improve our understanding of possible attributes of the last common ancestor with *Spea*. Second, 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 behavioural plasticity in resource use (i.e. greater shrimp consumption) 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 traits among sibships and/or between diets, (2) greater among-sibship 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).

MATERIAL AND METHODS

DIET-DEPENDENT VARIATION

We bred ten pairs of *Scaphiopus 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 0.01 μg/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 30 tadpoles from each sibship individually into opaque 88-mL cups (56 mm diameter × 55 mm 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 10 mg of detritus on the first day, 10 mg on the third day, 20 mg on the fifth day, 20 mg on the seventh day, 10 mg on the eighth day, 20 mg on the ninth day and 20 mg 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 1 mL of concentrated brine shrimp nauplii on the first day, 4 mL 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. This design was similar to that used previously in this species, but the amount of food given on each diet was greater than those previously used (e.g. [Levis *et al.*, 2018](#)). 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 & 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 serves as a reliable fitness proxy in spadefoots: larval size predicts juvenile survival and adult reproductive success; [Pfennig *et al.*, 2007](#); [Martin & 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 ANOVA (in JMP Pro 15).

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 Ledón-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 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, 2014). This approach cannot rule out the contribution of maternal effects, which can influence carnivore development in some, but not all, *Spea* populations (Pfennig & Martin, 2009, 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 [developmental rate, as with 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 & 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 (in JMP Pro 15) 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 that altered resource-use patterns during intense competition. Specifically, we sought to determine if *Scaphiopus 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 (10 tadpoles per litre) was five to 30 times higher than typical spadefoot tadpole competition experiments (e.g. Pfennig & Murphy, 2000; Pfennig & Rice, 2007; Levis & 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 (~5 tadpoles per litre) seen in natural spadefoot ponds (Newman, 1987; Pfennig *et al.*, 1991) which have a mean density of 0.54 ± 0.91 tadpoles per litre. 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 15 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 7 days we ended the experiment, euthanized tadpoles in MS-222 and preserved them in 95% ethanol. We then measured SVL, Gosner developmental stage, GC, DR, MP and OH of every tadpole as in the first experiment. In addition to these morphological traits, we assessed individual behavioural 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 65 °C 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 behavioural plasticity.

As above, we combined the sibship SVL and Gosner developmental stage (Gosner, 1960) of individuals into a single metric using a principal component analysis. Based on this measure, individuals that were larger and more developmentally advanced were considered to have a selective advantage. We then used a linear mixed effects model to determine the ability of morphological traits, trait plasticities and $\delta^{15}N$ to predict this proxy for fitness (in JMP Pro 15). Specifically, each trait, sibship-level trait plasticity and $\delta^{15}N$ were fixed effect predictor variables, replicate and sibship were random effects, and our fitness proxy was the response variable. We then evaluated if any of the significant predictors of growth and development were also significant predictors of $\delta^{15}N$ using a linear mixed effects model in which $\delta^{15}N$ was the response variable, the significant morphological predictors of growth and development were the fixed effect explanatory variables, and sibship and replicate were random effects. We compared the $\delta^{15}N$ among our control samples using an ANOVA followed by a Tukey HSD in JMP Pro 15.

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; Fig. 1). Specifically, gut length (GC) and jaw muscle width (OH) varied by diet and sibship and mouthpart morphology (MP) varied by sibship. Gut length tended to be shorter and jaw muscle width tended to be larger on a shrimp

Table 1. Results from our assessment of diet-dependent plasticity in trophic traits of *Scaphiopus holbrookii* tadpoles. Rows in bold type indicate that a given variable was a significant predictor of each trait. Rows in italics 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

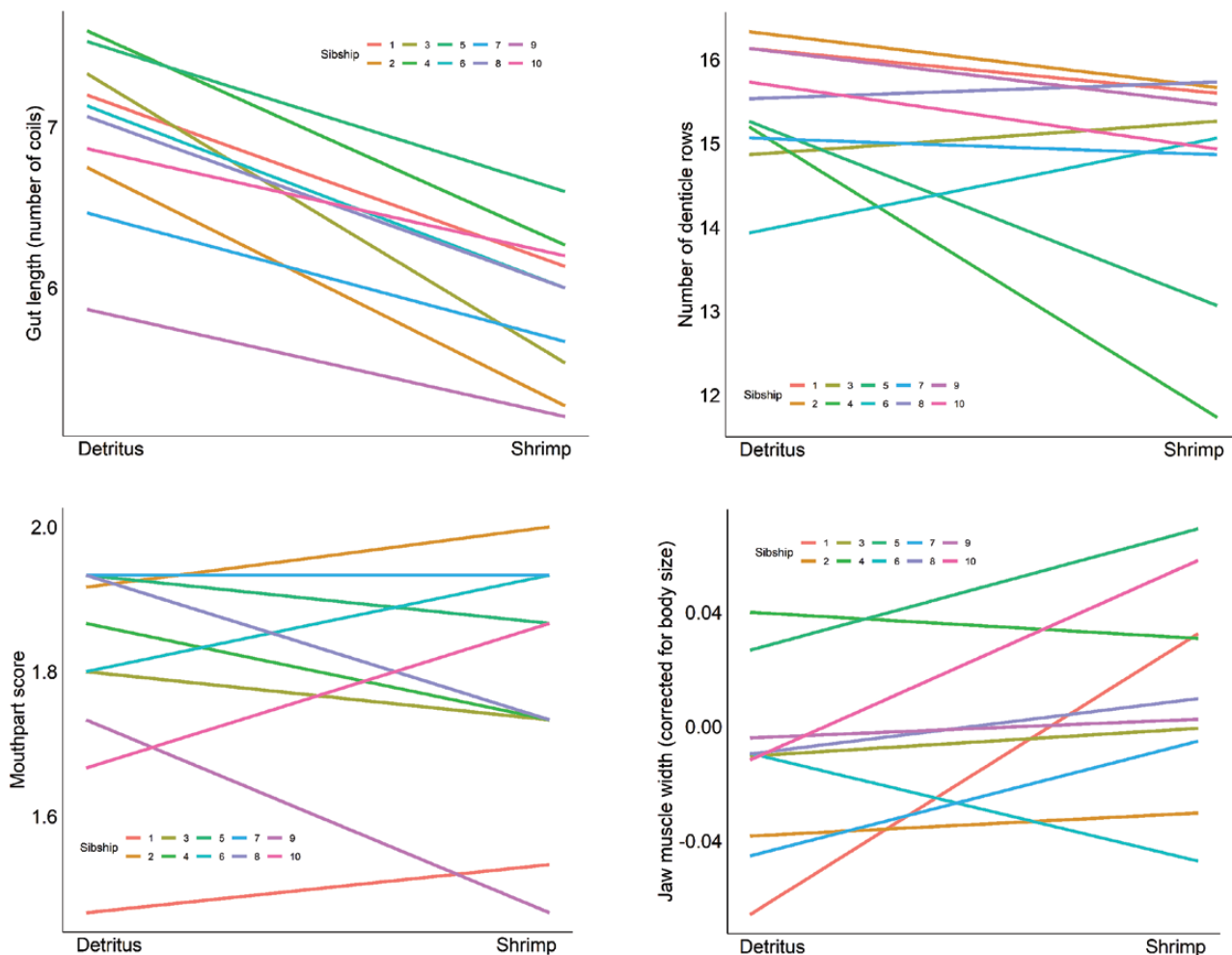| Variable	Sum of squares	d.f.	F	P	Variable	Sum of squares	d.f.	F	P
GC					DR				
Diet	**88.81**	**1**	**112.86**	**0.0001**	Diet	34.25	1	3.21	0.0742
Sibship	**55.44**	**9**	**8.52**	**0.0001**	Sibship	181.36	9	1.89	0.0534
Diet × Sibship	8.78	9	1.35	0.2112	Diet × Sibship	116.79	9	1.22	0.2843
MP					OH				
Diet	0.05	1	0.31	0.5803	**Diet**	**0.05**	**1**	**7.50**	**0.0002**
Sibship	**5.61**	**9**	**4.13**	**0.0001**	**Sibship**	**0.21**	**9**	**3.78**	**0.0066**
Diet × Sibship	1.5	9	1.10	0.3630	*Diet × Sibship*	*0.10*	*9*	*1.91*	*0.0507*

Figure 1. Sibship-level reaction norms across diet treatments for four trophic traits. Gut length (top left) varied among sibships and decreased in the shrimp treatment compared to the detritus treatment. Number of denticle rows (top right) did not vary significantly among sibships or across diets. Mouthpart score (bottom left) varied among sibships. Jaw muscle width (bottom right) varied among sibships and tended to be larger on the shrimp diet than on the detritus diet. See Table 1 for statistical analysis.

diet (mean = 5.889 and 0.012, respectively) than on a detritus diet (mean = 6.986 and −0.012, respectively). Jaw muscle width was nearly significant for variation in plasticity (Diet × Sibship; P = 0.0507). 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 (σ^2 = 0.49) than on a detritus diet (σ^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)

were significantly influenced by trophic morphology (Table 3). In particular, our growth and development fitness proxy was higher for individuals with longer guts (GC) and more carnivore-like mouthparts (MP). Diet was a nearly significant predictor of growth and development (P ≈ 0.066) with lower values being associated with a shrimp diet. Based on these results, we then performed a simple Pearson correlation test to see if GC and MP were associated with one another. This was done to help determine if there were alternative strategies to achieving higher growth and development (i.e. no correlation among variables; long guts *or* carnivore-like mouthparts) or if a single strategy was effective (i.e. variables are correlated; long guts *and* carnivore-like mouthparts). We found that the two variables were not significantly correlated (r = 0.0585;

Table 2. A, broad-sense heritability estimated from data and from bootstrap 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. Significant differences are in bold type and indicate uncovering of cryptic genetic variation (CGV) on a shrimp diet. 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 uncovered on shrimp?
SVL	**0.25 (0.38)**	**0.35 (0.45)**	**1.40 (1.18)**	**25.01**	**2.2E-16**	**Yes**
GC	0.47 (0.57)	0.40 (0.48)	0.85 (0.84)	35.96	2.2E-16	No
DR	**0.00 (0.12)**	**0.13 (0.24)**	**13.00 (2.00)**	**54.87**	**2.2E-16**	**Yes**
MP	**0.16 (0.28)**	**0.23 (0.35)**	**1.44 (1.25)**	**21.86**	**2.2E-16**	**Yes**
OH	**0.15 (0.27)**	**0.31 (0.42)**	**2.07 (1.56)**	**58.56**	**2.2E-16**	**Yes**
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. Rows in bold and italic 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	F	P
Intercept	−1.65	–	–
GC plasticity	−0.16	0.23	0.654
DR plasticity	0.06	0.01	0.920
MP plasticity	−0.15	1.14	0.335
OH plasticity	2.21	3.38	0.126
GC	**0.14**	**4.32**	**0.039**
DR	−0.01	0.20	0.652
MP	**0.43**	**8.49**	**0.004**
OH	−0.32	0.19	0.666
Diet	*0.13*	*3.41*	*0.066*

P = 0.3149), 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 δ^{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: (1) having high behavioural plasticity (i.e. consuming more shrimp), (2) having long gut lengths (GC) and (3) having carnivore-like mouthparts (Table 4; Fig. 2).

As above, GC and MP were not correlated (r = −0.0289; P = 0.6197). In addition, neither GC nor MP was a significant predictor of δ^{15}N (Table S3). Thus, carnivore-like behaviour (shrimp consumption) and carnivore-like 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 *Scaphiopus holbrookii*, which are members of the sister genus to *Spea* and do not produce carnivores. When we subjected *Scaphiopus holbrookii* to resource competition, we found: (1) heritable variation in morphology, (2) an increase in such heritable variation

Table 4. Results from our test of which variables best explain growth and development under highly competitive conditions. $\delta^{15}N$ was used to estimate the amount of resource (shrimp or detritus) an individual consumed and acted as a proxy for behavioural plasticity in resource use (lower values indicate greater shrimp consumption). Rows in bold type indicate that a given variable was a significant predictor of growth and development during competition within sibships for alternative resources. Note that trait plasticity values were measured at the sibship level for these analyses

Variable	Estimate	*F*	*P*
Intercept	3.49	–	–
GC plasticity	1.01	0.83	0.5272
DR plasticity	0.64	9.12	0.1878
MP plasticity	−9.74	10.72	0.1699
OH plasticity	−8.85	1.51	0.4342
GC	**0.14**	**6.01**	**0.0148**
DR	−0.01	0.99	0.3195
MP	**0.32**	**8.53**	**0.0038**
OH	−0.35	0.42	0.5188
$\delta^{15}N$	**−0.33**	**18.73**	**0.0001**

on the novel shrimp diet and (3) that the expression of some morphological features and behaviours 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 that is found in the sister genus *Spea*. 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 favoured individuals that switched to a novel resource (shrimp). Our competition experiment revealed that individuals that expressed alternative foraging behaviour through plasticity (i.e. lower $\delta^{15}N$ and higher consumption of shrimp) probably had a selective advantage (Fig. 2). Presumably, competition for detritus favoured those individuals that were able to switch to consuming shrimp because these individuals were thereby released from competition; i.e. they were favoured by negative frequency-dependent selection (e.g. see Pfennig, 1992; Hori, 1993; Benkman, 1996; Maret & Collins, 1997; Bolnick, 2004). Such niche-width expansion in the face of intraspecific resource competition has been documented in numerous taxa, in both the lab and the wild. By favouring 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 & 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. Because *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. We observed such a pattern in our *Diet-dependent variation* experiment (Fig. 1) wherein tadpoles fed a shrimp diet had shorter guts than tadpoles fed a detritus diet. 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 (i.e. the traits varied independently), and neither predicted resource use during competition (Table S3). In general, these results point to carnivore-like behaviour (shrimp consumption) and morphology (more carnivore-like mouthparts) potentially being advantageous, and that the adaptive refinement and innovation during the evolution of carnivores in *Spea* might have required breaking developmental constraints among traits (Hallgrímsson *et al.*, 2009, 2012) that were limiting simultaneous specialization on alternative resources.

This study corroborates previous work that found evidence of ancestral plasticity (Ledón-Rettig *et al.*, 2008; Levis *et al.*, 2018). Similar to previous work on this species (Levis *et al.*, 2018), we found that gut length showed an adaptive response by becoming shorter when tadpoles were fed an exclusive diet of shrimp. However, other patterns of dietary plasticity were mixed. In the previous study, mouthpart morphology showed a putatively maladaptive response to diet with more carnivore-like mouthparts developing on a detritus diet. Here, we found no such change. Similarly, denticle row number showed an adaptive response in the previous study, but showed no change here. Interestingly, the previous study did not find evidence of jaw muscle width plasticity, but we found a significant adaptive response to shrimp consumption. The reasons for this variation between studies is unclear, but it might reflect subtle differences in the feeding regimes (we supplied more food in this study) and/or differences among genotypes used in the two studies. Nonetheless, the key point remains: *Scaphiopus holbrookii* tadpoles exhibit plasticity – some of which may be adaptive – when fed a novel
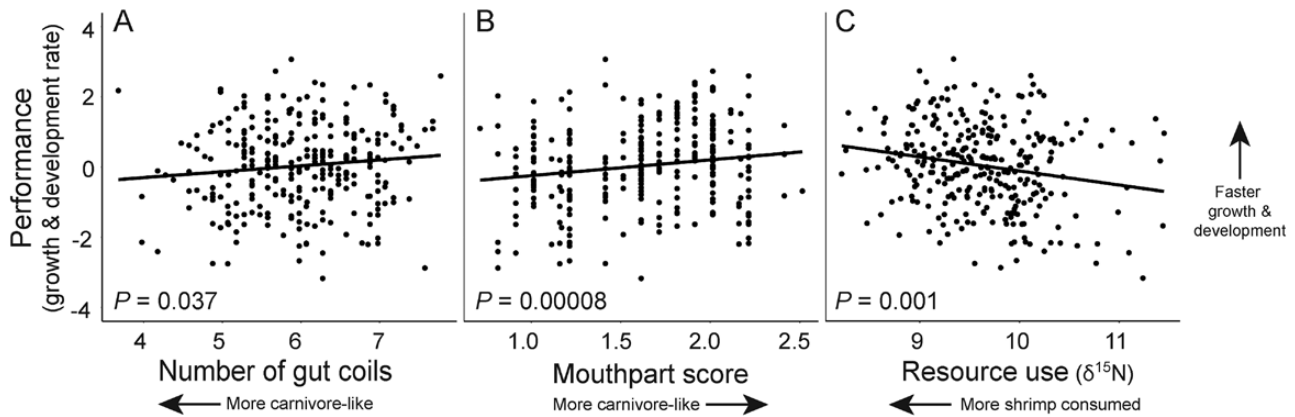
Figure 2. When competing, tadpoles that had (A) longer guts, (B) more carnivore-like mouthparts and/or (C) consumed more shrimp (lower δ^{15}N values) grew and developed faster than those with shorter guts, less carnivore-like mouthparts and/or that consumed fewer shrimp (higher δ ^{15}N values). Each point is an individual that was corrected for sibship and replicate by adding the residuals of these terms from the linear model to the overall mean (Levis *et al.*, 2016). The linear regression lines are for visualization purposes only.

shrimp diet. This suggests that the common ancestor with *Spea* probably did as well.

Our finding of heritable and cryptic variation in various traits corroborates 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 & Pfennig, 2019a) points to the possibility that the common ancestor of *Scaphiopus* and *Spea* (before the evolution of the carnivore morph) may have also harboured 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 & Dworkin, 2004; Paaby & 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 & Bolnick, 2007).

Our results have implications for understanding how novel, complex phenotypes originate (Mayr, 1959; West-Eberhard, 2003; Wagner & Lynch, 2010), and support the idea that an adaptive response to intraspecific competition serves as a springboard for the evolution of

new features (Pfennig & Pfennig, 2012). In particular, 'plasticity-led evolution' (West-Eberhard, 1989, 2003; Schwander & Leimar, 2011; Levis & Pfennig, 2016; *sensu* Levis & 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 favours 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 moulded by selection into a well-functioning phenotype (West-Eberhard, 2003; Moczek *et al.*, 2011; Levis & Pfennig, 2016). The end result is a new phenotype that is part of a 'polyphenism' (when increased plasticity is favoured) or a genetically fixed trait (when decreased plasticity is favoured) (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. Because 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 by Pfennig & 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 & 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.

Finally, studies of competition-induced plasticity have implications for conservation biology. Indeed, the capacity of individual organisms to respond adaptively to the presence of new competitors via plasticity, coupled with the capacity of populations to undergo adaptation, will probably determine which species will win and which will lose under anthropogenic environmental change (Diamond & Martin, 2016).

ACKNOWLEDGMENTS

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SUPPORTING INFORMATION

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

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).

Table S2. A, ANOVA summary from $\delta^{15}N$ 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.

Table S3. The ability of gut length (GC) and mouthpart score (MP) to predict resource use ($\delta^{15}N$) during intense competition. Lower values of $\delta^{15}N$ indicated greater consumption of shrimp.