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Intraspecific adaptive radiation: Competition, ecological opportunity, and phenotypic diversification within species

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Intraspecific variation in resource-use traits can have profound ecological and evolutionary implications. Among the most striking examples are resource polymorphisms, where alternative morphs that utilize different resources evolve within a population. An underappreciated aspect of their evolution is that the same conditions that favor resource polymorphism—competition and ecological opportunity—might foster additional rounds of diversification within already existing morphs. We examined these issues in spadefoot toad tadpoles that develop into either a generalist "omnivore" or a specialist "carnivore" morph. Specifically, we assessed the morphological diversity of tadpoles from natural ponds and experimentally induced carnivores reared on alternative diets. We also surveyed natural ponds to determine if the strength of intramorph competition and the diversity and abundance of dietary resources (measures of ecological opportunity) influenced the diversity of within-morph variation. We found that five omnivore and four carnivore types were present in natural ponds; alternative diets led to shape differences, some of which mirrored variation in the wild; and both competition and ecological opportunity were associated with enhanced morphological diversity in natural ponds. Such fine-scale intraspecific variation might represent an underappreciated form of biodiversity and might constitute a crucible of evolutionary innovation and diversification.

KEY WORDS: Competition, diversification, ecological opportunity, intraspecific variation, resource polymorphism.

Among biology's enduring challenges is explaining why living things are so diverse. Ecologists and evolutionary biologists have long recognized that intraspecific competition for resources fosters diversification (Darwin 1859 (2009); Haldane 1932 (1993); Van Valen 1965; MacArthur and Wilson 1967; MacArthur 1972; Roughgarden 1972). Indeed, competitively mediated natural selection can act within a population to: promote increased (or more heterogeneous) phenotypic variation (i.e., character or ecological release; Wilson 1961; Grant 1972; Cox and Ricklefs 1977; Bolnick 2001; Bolnick et al. 2007; Svanbäck and Bolnick 2007); favor the evolution of alternative phenotypes (morphs) that differ in resource use (i.e., resource polymorphism; Smith and Skúlason 1996), including the evolution of novel phenotypes that can exploit unique resources (e.g., Liem and Kaufman 1984; Hori 1993; Carroll et al. 1998; Jones 1998; Bolnick 2001; Benkman 2003; Bono et al. 2013; Yassin et al. 2016); and even facilitate speciation if these morphs become reproductively isolated from each other (i.e., via competitive/adaptive/ecological speciation; Maynard Smith 1966; Rosenzweig 1978; Seger 1985; Dieckmann and Doebeli 1999; Nosil 2012).

Intraspecific competition promotes diversification through frequency-dependent disruptive selection (reviewed in Bolnick 2004; Day and Young 2004; Rueffler et al. 2006; Doebeli 2011; Pfennig and Pfennig 2012; Hendry 2017). To illustrate this process, consider a population in which individuals exploit a normally distributed gradient of resource types (e.g., prey of different sizes) and in which an individual's phenotype determines what prey type it can harvest. Initially, selection should favor individuals that utilize the most common resource type (e.g., prey of intermediate size). As more individuals exploit this resource type, however, it becomes depleted, and these individuals will experience greater competition. Eventually, such individuals will have lower fitness than the other individuals in the population that utilize the extreme ends of the resource gradient (e.g., very small or very large prey). Assuming that these extreme resources are sufficiently numerous to sustain these individuals over time (i.e., assuming sufficient ecological opportunity; sensu Schluter 2000), these fitness differences will generate disruptive selection, in which individuals that specialize on each extreme end of the resource gradient are favored over those that utilize the intermediate resource type. Moreover, because each of these extreme phenotypes will always compete more against itself than against the alternative phenotype, if either extreme phenotype becomes rare, it will be favored by negative frequency-dependent selection until it increases in frequency (e.g., see Pfennig 1992; Hori 1993; Benkman 1996; Maret and Collins 1997; Bolnick 2004). In this way, competitively mediated, frequency-dependent disruptive selection maintains alternative morphs in the same population, thereby promoting the origin and maintenance of a resource polymorphism (reviewed in Smith and Skúlason 1996; Pfennig and Pfennig 2012).

An important consequence of the evolution of a resource polymorphism is increased ecological specialization. Indeed, in most resource polymorphisms, one or both morphs utilize a specific type of resource or a relatively narrow range of resources (e.g., see Liem and Kaufman 1984; Hori 1993; Benkman 1996; Robinson and Wilson 1998; Paull et al. 2012; Bono et al. 2013). Thus, ecological specialization is accompanied by reduced niche breadth (Futuyma and Moreno 1988). Such limitation arises from evolutionary trade-offs between the ability to exploit a range of resources and the capacity to use a specific type of resource (the "jack-of-all-trades is master of none" hypothesis; MacArthur 1972; e.g., see Benkman 1996; Robinson et al. 1996; Bolnick et al. 2003; Martin and Pfennig 2009; Ellerby and Gerry 2011).

Although specialists can benefit by monopolizing a more profitable prey type (e.g., see Liem and Kaufman 1984; Smith 1993), they should also pay an important cost. Namely, because specialists should compete more against fellow specialists than generalists will against fellow generalists (generalists should always have the capability to switch to alternative, underutilized resources), specialists should experience more intense competition than generalists in the same population (Paull et al. 2012). Therefore, resource-use specialists should have lower fitness than generalists if they are forced to switch to another resource for which they are poorly adapted (as might occur, for instance, if their original resource is in short supply or if it is depleted).

This "intramorph" competition experienced by a specialist morph might, in turn, fuel further diversification. Although new specialist morphological variants (in which different individuals specialize on slightly different resources) might often be expected to evolve within a generalist morph, these variants might even evolve within an already existing specialist morph. By subdividing the resource base even further, such increased specialization reduces pair-wise overlap between interacting individuals in resource use, which lessens the intensity of intraspecific competition (Bolnick et al. 2003). Essentially, this process of morphological variant proliferation could be viewed as the intraspecific analog of adaptive radiation (sensu Rainey and Travisano 1998; Schluter 2000).

Yet because such intramorph diversification can be subtle, it might be missed by researchers. However, these "cryptic" morphological variants might represent an underappreciated source of ecologically relevant, phenotypic diversification within species. Moreover, as with resource polymorphism generally, the presence of cryptic morphological variation in a population might have important ecological and evolutionary consequences, such as altering the outcome of interactions with other species (Clark 2010; Bolnick et al. 2011; Turcotte and Levine 2016), shaping ecosystem functioning (Harmon et al. 2009), promoting the origins of novel features (Pfennig and Pfennig 2012), and even enhancing species diversity (West-Eberhard 2003; Mallet 2008; Corl et al. 2010; Pfennig and McGee 2010).

Here, we test these ideas by focusing on a well-studied system: the Mexican spadefoot toad, *Spea multiplicata*. As described below, the tadpoles of this species exhibit a striking resource polymorphism consisting of an "omnivore" morph, which is a dietary generalist, and a morphologically distinctive "carnivore" morph, which is a dietary specialist. Our overarching aim was to determine if intramorph competition, coupled with ecological opportunity, has promoted additional rounds of diversification within these two existing morphs. Our specific goals were threefold. First, we measured morphological variation among wild-caught tadpoles to determine if cryptic morphological variation is present within either existing morph: omnivores or carnivores. For example, given that a previous study had shown that, among omnivores, individual variation in trophic morphology correlates with individual variation in diet (Paull et al. 2012), we expected that different morphological variants that specialize on a narrower range of resources might be present within this morph. Similarly, given that the two main prey of carnivores—fairy shrimp and other tadpoles—differ dramatically in size, shape, and behavior (shrimp are small, thread-shaped, slow swimmers; tadpoles, by contrast, are relatively large, round-shaped with distinct body and tail regions, and are rapid swimmers), we expected that different morphological variants that specialize on either shrimp or tadpoles might be present within this morph. Second, having established that previously unrecognized morphological variants were indeed present within both the generalist omnivore morph and the specialist carnivore morph, we conducted an experiment to determine if utilizing alternative diets (i.e., shrimp vs tadpoles) led to morphological differences among carnivores, similar to those observed in the wild. Finally, we explored the relationship between pond-level ecological variables and morphological diversity within the specialist carnivore morph to determine if either intraspecific resource competition, ecological opportunity, or both were associated with the presence of multiple morphological variants.

Materials and Methods

STUDY SYSTEM

Tadpoles of *S. multiplicata* from the southwestern United States develop into ecologically and morphologically divergent alternative morphs: omnivores and carnivores (Bragg 1965; Pomeroy 1981; Pfennig 1990; see photos in Pfennig and Murphy 2002). Omnivores are dietary generalists, feeding on algae, plant material, detritus, and small crustaceans. Carnivores, by contrast, are dietary specialists, feeding almost exclusively on anostracan fairy shrimp and other tadpoles (Pomeroy 1981; Pfennig 1990; Paul et al. 2012).

Whether a tadpole develops into an omnivore or carnivore depends largely on its diet. Omnivores are the default morph; carnivores are induced when a young omnivore eats shrimp or other tadpoles (Pomeroy 1981; Pfennig 1990; Levis et al. 2015). However, heritable variation exists among sibships in their propensity to both eat shrimp and produce carnivores (Pfennig and Frankino 1997; Pfennig 1999; Pfennig and Murphy 2000, 2002; Martin and Pfennig 2011).

As long as the two main resource types (i.e., detritus and shrimp) are available, both morphs are typically present in the same pond (Bragg 1965; Pomeroy 1981; Pfennig 1990; Martin and Pfennig 2010), where they are maintained by negative frequency-dependent selection (Pfennig 1992). Additionally, disruptive selection favoring these morphs over intermediates is widespread (Martin and Pfennig 2012). Indeed, previous experiments have shown that disruptive selection arises because extreme trophic phenotypes (i.e., omnivores and carnivores) forage more effectively than do intermediate phenotypes on the two main resource types present in most ponds: detritus and large animal prey, respectively (Martin and Pfennig 2009). However, presumably because of their narrower niche width, carnivores experience greater intramorph competition than omnivores (Paull et al. 2012).

EVALUATING INTRAMORPH VARIATION IN THE WILD

We began by measuring morphological variation among wild-caught tadpoles to determine if cryptic morphs are present within either existing morph. Given that omnivores have a relatively wide trophic breadth (see Study System) and that a previous study had already shown evidence of individual specialization among omnivores (with variation among individuals in diet being correlated with variation in their trophic morphology; Paull et al. 2012), we anticipated finding subspecialists within omnivores. Therefore, we used omnivores as a baseline for the effects that availability (and presumably, utilization) of alternative resources could have on morphological diversity. We then asked if similar levels of morphological diversity were present within carnivores. Indeed, there were a priori reasons for expecting that carnivores might experience a secondary round of diversification similar to that in omnivores: carnivores compete more with other carnivores than they do with omnivores (Martin and Pfennig 2009; Paull et al. 2012), and alternative resources are available in most ponds for carnivores to consume (shrimp vs other tadpoles). Thus, the combined influence of intense intramorph competition and ecological opportunity might promote diversification even within the specialist carnivore morph.

We analyzed digital photos of ethanol-preserved tadpoles that were collected between 2007 and 2016 from 18 ponds in southeastern Arizona and southwestern New Mexico (*S. multiplicata* was the only species of *Spea* present in these ponds; in most ponds, hundreds of tadpoles were sampled randomly). Initially, we categorized each tadpole as either an omnivore or a carnivore based on a visual inspection of a suite of morphological features that previous studies had shown are relevant for the acquisition of alternative resources and that separate the two morphs: width of jaw muscles, shape of keratinized mouthparts, number of denticle rows, and number of gut coils (Pfennig 1990; Pfennig and Murphy 2000, 2002; Martin and Pfennig 2009, 2011). From these data, we estimated the overall proportion of carnivores in each pond. We then used 11–100 individuals per pond (sampling equal numbers of carnivores and omnivores where possible, and avoiding tadpoles with intermediate phenotypes, see below) to capture the morphological variation within each morph.

To assess fine-scale differences in morphology, we employed landmark-based geometric morphometrics (Bookstein 1991). Tadpole shape was determined using the TPS software suite (Rohlf 2001, 2003, 2013) with seven fixed and 31 sliding landmarks (Fig. 1). Important features captured by these landmarks are: the locations of the eyes and nares; the extent of oral protrusion; the width of the head and body; the width of the body at the base of the tail; and the overall curvature of the tadpole from snout to tail. Following Procrustes superimposition to remove differences in shape due to orientation and size, and after correcting for potential differences in shape due to possible allometric growth during development [i.e., creating Gosner (1960) stage-independent landmarks], we performed a principal component analysis (PCA) on a cross-covariance matrix of the coordinates to reduce our dataset to two principal components. Unless otherwise stated, a matrix of these principal components (i.e., PC1 and PC2) was the response variable for subsequent hypothesis testing using a randomized residual permutation procedure (RRPP; Collyer and Adams 2007; Collyer et al. 2015) in the R package "geomorph" (Adams and Otárola-Castillo 2013).

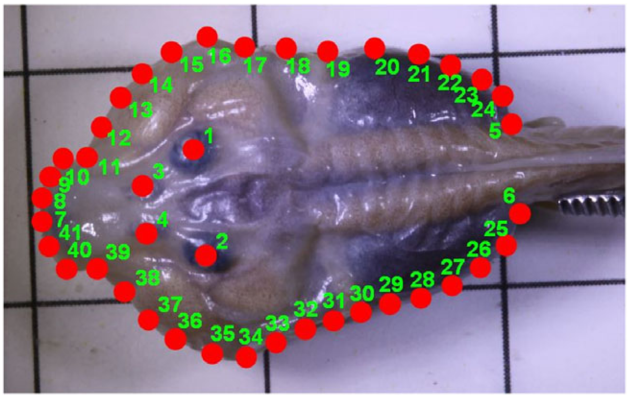

Figure 1. Distribution of landmarks and semi-landmarks. Points 1–7 were fixed landmarks; 8–41 were semi-landmarks that were free to slide between adjacent landmarks.

Using these data, we characterized morphological variation within both carnivores and omnivores. We first performed a discriminant analysis in JMP Pro (12.0.1) to see how the program would categorize each individual based on its PC1 and PC2 values. To focus our analysis on "pure" carnivores or omnivores (i.e., individuals that would be considered carnivores or omnivores in all ponds), we then removed individuals that were classified differently from our initial assessment because their morphology was generally intermediate and/or ambiguous between the two morphs. We excluded these intermediates because they represent a nonstable transitory state between the alternative omnivore and carnivore morphs and do not appear to represent ecologically significant morphological variation (essentially, intermediates appear to be "failed" omnivores or carnivores; Pfennig 1992). Specifically, tadpoles intermediate in trophic morphology have lower performance on shrimp and detritus, and are outcompeted by both omnivores and carnivores for their respective resources (Martin and Pfennig 2009). Consequently, there is strong selection against tadpoles with intermediate trophic morphology (Pfennig et al. 2007; Martin and Pfennig 2009, 2012) and they are generally rare, especially so in populations with abundant ecological opportunity and strong competition (Martin and Pfennig 2010), ecological conditions in which we otherwise expect ecological specialization to be abundant. Removal of these intermediates reduced our total sample size from 1280 to 1064 tadpoles.

Within each morph, we then performed normal mixtures clustering on PC1 and PC2 in JMP to determine how many morphological clusters (i.e., 1–10) best characterized the morphospace. The normal mixtures approach to clustering predicts the proportion of responses expected within each cluster, and it is useful when clusters overlap because it assigns a probability of membership to a cluster rather than clustering based on borders (e.g., k-means clustering; McLachlan and Krishnan 2008). The number of clusters that had the lowest AICc value was chosen as the best if it was at least two units lower than all others (Burnham and Anderson 2002). We also performed this clustering analysis on tadpoles of *Scaphiopus couchii*, a species that does not produce alternative morphs, but occupies the same habitat as *S. multiplicata.* We did so to determine if the degree of clustering into alternative morphological variants within *S. multiplicata* carnivores or omnivores was greater than that observed within a nonpolymorphic species that inhabits the same environment (this analysis on *Sc. couchii* essentially acted as a control for our morphological analyses of *S. multiplicata*). Because our clustering procedure detected two distinct morphological groups within *Sc. couchii* (see *Results*), we performed additional tests (described below) to determine if the variation observed in *S. multiplicata* might be biologically meaningful.

To determine if our carnivore cluster assignments were better than treating each pond as having a single type of carnivore, we performed 1000 iterations of RRPP for each pond individually. If we consistently found that a single carnivore cluster was better than our cluster assignments, then this could suggest that morphological variation between ponds is more important than variation within ponds. Conversely, if the morphological variation in most ponds was better described by our cluster assignments, then this would suggest that our cluster assignments describe relevant morphological differences that repeatedly occur across ponds.

We then determined the morphological diversity of each morph for every pond by using Shannon's diversity index (Shannon 1948) to calculate morphological evenness and multiplying this value by the average least-square mean distance between cluster centroids (hereafter, "morphological diversity") within every pond. Thus, ponds with more clusters, more even representation across clusters, and clusters that are distinct in 2D morphospace had greater morphological diversity.

TESTING THE ROLE OF DIET ON MORPHOLOGICAL VARIATION

Carnivores consume fairy shrimp and other tadpoles. Because ponds vary in the abundance of these two resources (Pfennig 1990; Martin and Pfennig 2010), and because we observed varying degrees of carnivore morphological variation among ponds (see *Results*), we sought to determine experimentally if these different diets alone lead to morphological differences. Note that we do not necessarily expect full recapitulation of wild-caught carnivore morphs since cues other than diet are known to contribute to carnivore morph production in *Spea* (Pfennig and Frankino 1997; Frankino and Pfennig 2001).

On July 20, 2016, we collected 40 carnivore and 40 omnivore *S. multiplicata* tadpoles from a single pond near Portal, Arizona (pond PO2N16; *S. multiplicata* were the only species of *Spea* present in this pond). Tadpoles were approximately 5 days old at the time of collection (typically, *Spea* tadpoles do not differentiate into carnivores until they are 4 days old). We randomly assigned 20 individuals of each morph to one of two diet treatments: one in which the individuals were fed live fairy shrimp (*Thamnocephalus* sp. and *Streptocephalus* sp.) and one in which individuals were fed tadpoles (mostly *Scaphiopus couchii* but occasionally other *S. multiplicata* tadpoles; both species were collected from a different pond than the focal tadpoles). At each feeding, the shrimp-fed group received ~70 moderately sized fairy shrimp (about 12 mm total length), whereas the tadpole-fed group received five small tadpoles (about 5 mm snout-vent length). Both treatments were fed ad lib 2–3 times daily (i.e., they were fed throughout the day whenever all the prey from the previous feeding had been eaten). Tadpoles were reared individually in opaque cups (11.5 cm diameter × 8 cm height) with ~250 mL of dechlorinated water (filled to 6 cm in depth).

After eight days, we euthanized all tadpoles in MS-222, immediately photographed them with a Canon EOS 7D camera (using a 100 mm macro lens) on 1 × 1 cm grid paper, determined their Gosner stage (Gosner 1960), and preserved them in 95% ethanol. Shape was determined as described above. We performed a type III sum of squares MANOVA (in R version 3.1.2) to determine which variables (starting morph, diet, and/or their interaction) were important for describing shape. Since diet was the only variable returned as significant (Table S1), we compared diet groups using 1000 iterations of RRPP.

COMPARING EXPERIMENTALLY INDUCED AND WILD-CAUGHT CARNIVORES

Since we found that diet does significantly influence carnivore morphology, we decided to see how the morphology of our experimentally induced carnivores compared with that of wild-caught carnivores. To do this, we performed RRPP on the shapes of wild-caught carnivores and our experimental tadpoles and looked at their distributions in 2D morphospace. We then used the function "advanced.procD.lm" in the package geomorph to determine if the centroid location of our experimental tadpoles was significantly different from those of the carnivore clusters we identified from the wild. In addition, we tested for a correlation between the PC1 and PC2 loadings (absolute values) of our experimental tadpoles and of our wild-caught carnivores to determine if the same variables were loading similarly between the two groups.

ASSESSING THE RELATIONSHIP BETWEEN INTRAMORPH DIVERSITY AND ECOLOGICAL VARIABLES

We used model selection and multimodal inferences (Burnham and Anderson 2002; Grueber et al. 2011) to evaluate the predictive power and statistical effects of ecological variables on two metrics of carnivore diversity. Our metrics of diversity were the number of carnivore clusters represented in a pond and morphological diversity (see **EVALUATING INTRAMORPH VARIATION IN THE WILD**). For our possible explanatory variables, we estimated the following ecological variables for each pond: conspecific tadpole density, shrimp density, *Sc. couchii* tadpole density, the proportion of carnivores, and carnivore ecological opportunity ("CEO"; see below). We focused on these variables in particular, because they estimated the degree of competition or ecological opportunity experienced by carnivores, and (as noted in the ***Introduction***) longstanding theory has suggested that competition and ecological opportunity should foster increased diversification in resource-use traits.

We estimated conspecific tadpole, shrimp, and *Sc. couchii* tadpole density by sweeping a net throughout each pond and categorizing densities as “high” (score of 5), “moderate-high” (4), “moderate” (3), “moderate-low” (2), “low” (1), and, “none” (0). These subjective estimates are corroborated by previously published, intensive, quantitative sampling (Pfennig 1990; Pfennig et al. 2006). The proportion of carnivores in each pond (which ranged from 0–1) was estimated based on random sample of tadpoles that we had collected from each pond. Carnivore ecological opportunity (CEO) incorporated the density of potential resources for carnivores weighted by the preference carnivores have for each resource. Specifically, CEO = [*Sc. couchii* density*(1) + omnivore density*(.66) + shrimp density*(.33)]/10. Carnivores prefer to eat tadpoles over shrimp, and they prefer heterospecific tadpoles to conspecifics (Pfennig 2000). Additionally, *S. multiplicata* tadpoles raised on a diet of *Scaphiopus* tadpoles produced ~3 times more carnivores than those raised on shrimp (Levis et al. 2015). Larger CEO values are indicative of a greater number of alternative resources for carnivores and is still applicable even if a particular resource is absent from a given pond.

To perform our analysis, we constructed a global linear model for each of our diversity metrics with conspecific density, shrimp density, *Sc. couchii* density, the proportion of carnivores, and carnivore ecological opportunity (CEO) as predictor variables. We performed model selection on this global model using the R package MuMIn (Bartón 2012). Specifically, we used the "dredge" function to fit all possible permutations of the global model fit using maximum likelihood (ML) to compare and rank models by their information criteria. We subsequently ranked the model pool using AICc. For each response variable, we chose a subset of the most informative models with ΔAICc ≤ 4 (Tables 2 and 3). Here, ΔAICc ≤ 4 was chosen to allow for inclusion of multiple models (and thus explanatory variables) in our model averaging procedure. We carried out model averaging of each model subset using the "model.avg" that uses information criteria (i.e., AICc) to assess the predictive power of explanatory variables (i.e., relative importance values: the sum of the Akaike model weights for

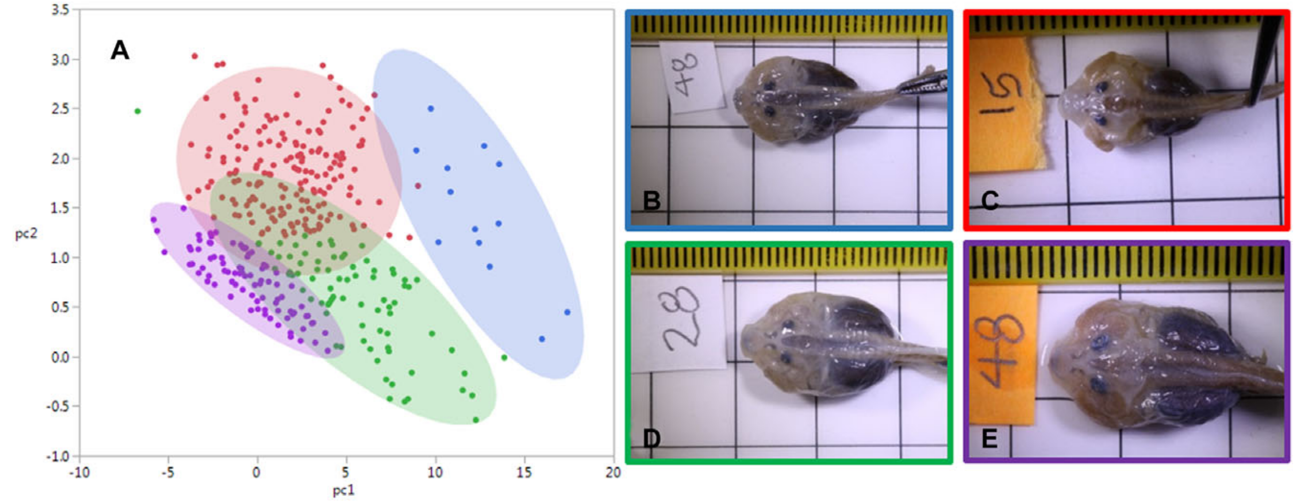

Figure 2. (A) Distribution of carnivore morphological clusters (with 95% confidence ellipses). (B–E) Examples of carnivores from each of the clusters, which we dubbed (B) "oddball," (C), "classic," (D) "bulgy," and (E) "boxcar." The border color of panels B–E matches the color of the cluster from which that individual originates in panel A.

each model the variable occurs in, across the candidate models of the chosen model subset) and obtain averaged parameter estimates and standard errors using the natural averaging method from a set of models when there is no single best-supported model or hypothesis (sensu Burnham and Anderson 2002; reviewed in Grueber et al. 2011).

We performed four additional tests to evaluate the relationships among the intensity of intramorph competition (as estimated by the proportion of carnivores in a pond), CEO, and carnivore morphological diversity. First, we used a Pearson correlation to evaluate the relationship between the proportion of carnivores and CEO. Next, we evaluated the Pearson correlation between proportion of carnivores in a pond and that pond's carnivore morphological diversity for ponds that differ in their CEO. Specifically, we considered all the ponds that had below average levels of CEO as low CEO and those with above average levels of CEO as high CEO and found the Pearson correlation between proportion carnivores and morphological diversity in these two conditions. In addition, we used a linear model to test for an interaction between proportion of carnivores and CEO level (high or low) in explaining carnivore morphological diversity. Finally, because omnivores represent a generalist feeding strategy with relatively wide trophic variation (Paull et al. 2012), we used omnivores to act as a baseline for the effects that availability (and presumably, utilization) of alternative resources can have on morphological diversity. Therefore, we compared the degree of morphological diversity within omnivores to that of carnivores in low CEO and high CEO ponds using a *t*-test and Wilcoxon signed rank test.

Results

EVALUATING INTRAMORPH VARIATION IN THE WILD

For all tadpoles combined, PC1 and PC2 explained 82.39% and 5.54% of the variation, respectively, and the distances between points in two dimensions were highly correlated with distances in all possible dimensions ($R = 0.9915$).

When comparing the centroid locations of pond morphology as a whole, we found that 12 ponds were connected in a complex network, three ponds were only connected to one other pond, and two ponds were significantly different from all the others (Table S4; Fig. S1). The network of similar carnivore morphologies was even more highly connected than that of whole ponds. Fifteen out of seventeen ponds (Richardson pond had no carnivores) were connected either directly or via one intermediate. The remaining two ponds were not significantly different from each other, but did differ from all the rest (Table S5; Fig. S2).

We found that five and four clusters (dubbed "oddball," "classic," "bulgy," and "boxcar") best characterized the morphospace of pure omnivores and carnivores, respectively (Fig. 2; Table S6; Fig. S3). In contrast, we found that only two clusters were detected for *Sc. couchii* (Table S6). Our carnivore cluster assignments better explained the morphological variation than a single cluster for most ponds (Table 1). Furthermore, there were significant differences among clusters in both body size (snout-vent length, SVL) and Gosner (1960) developmental stage (both measures are positively correlated with fitness in *Spea*; Martin and Pfennig 2012; omnivores: SVL, $F_{4,706} = 131.7125$, $P < 0.0001$; stage, $F_{4,706} = 149.5463$, $P < 0.0001$; carnivores: SVL, $F_{3,347} = 34.8295$, $P < 0.0001$; stage, $F_{3,347} = 13.5775$, $P < 0.0001$). We performed

Table 1. Determination of whether a single cluster or carnivore cluster assignments better describe the morphological variation within ponds.

Pond	*N*	Number of carnivore clusters	Morphological diversity	Carnivore clusters better?	*F*-ratio	*P* value
BIP	14	3	1.165808	No	1.6659	0.2125
Bull	7	2	5.092337	Yes	56.712	0.031
Crown Dancer	4	2	3.702147	No	1.9346	0.413
Dead Cow	4	1	NA	—	—	—
Eagles Cry	14	2	3.086877	Yes	15.604	0.003
Good Pond	26	2	0.326859	No	0.2055	0.733
Guy Miller	46	4	3.475216	Yes	13.829	0.001
Hawk Pond	8	3	2.023529	Yes	8.6882	0.0135
Horseshoe	18	3	3.320935	Yes	6.9294	0.005
McBride’s	14	3	5.392734	Yes	27.11	0.001
Peach Orchard	49	3	2.540683	Yes	13.524	0.001
PGN	2	1	NA	—	—	—
PGS	1	1	NA	—	—	—
PO2N09	19	2	2.220691	No	4.8292	0.051
PO2N16	37	3	2.403485	Yes	15.287	0.001
Red Tank	33	4	7.876833	Yes	20.306	0.001
Silver Creek	55	3	0.872946	No	2.7129	0.076

For most ponds, carnivore cluster assignments were better than treating the pond as having a single carnivore type. Ponds in which only a single cluster was better either had low morphological diversity (BIP, Good Pond, Silver Creek), small samples sizes (Crown Dancer), or both (Dead Cow, PGN, PGS).

a Tukey HSD post hoc test to determine which clusters were significantly different from each other in these fitness proxies (Table S7). Additionally, the magnitude of morphological diversity between carnivores and omnivores was not significantly different (morphological diversity: Wilcoxon Signed Rank $S_{12} = 24.500$ $P = 0.0942$).

TESTING THE EFFECT OF DIET ON MORPHOLOGICAL VARIATION

In the diet experiment, PC1 and PC2 explained 96.53% and 1.12% of the variation, respectively, and distances in two dimensions were highly correlated with distances in all dimensions ($R = 0.9993$).

Wild-caught tadpoles raised on either a diet of shrimp or other tadpoles significantly differed in shape ($F_{1,77} = 39.148$, $P = 0.001$; Fig. 3). When we examined the consensus shape for each group, the most notable difference between shrimp-fed and tadpole-fed individuals was the position of the eyes and nares. Shrimp-fed tadpoles tended to have eyes and nares located more anteriorly than tadpole-fed individuals (Fig. 3). In addition, there was more variation in the location of the eyes and nares in tadpole-fed individuals, as indicated by the greater spread of points at these landmarks compared to shrimp-fed individuals. The two groups also differed in the extent of mouthpart protrusion: shrimp-fed tadpoles tended to have a more defined mouthpart protrusion than tadpole-fed tadpoles.

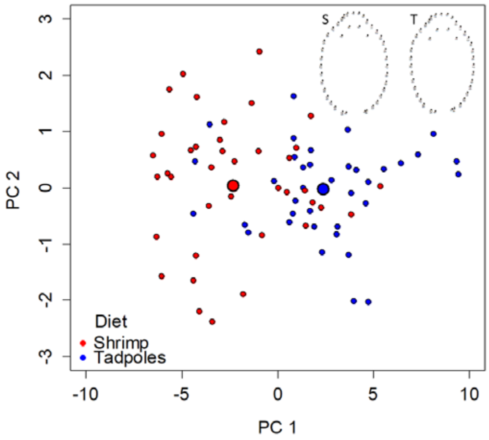

Figure 3. Distribution of experimental tadpole morphology based on diet. Small dots correspond to each individual; large dots are the centroids for each group. Insets in upper right denote the consensus shapes of shrimp-fed (S) and tadpole-fed (T) individuals, respectively.

COMPARING EXPERIMENTALLY INDUCED AND WILD-CAUGHT CARNIVORES

In comparing wild-caught with experimental tadpoles, PC1 and PC2 explained 85.21% and 4.55% of the variation, respectively, and two dimensional distances were highly correlated with distances in the full morphospace ($R = 0.9935$).

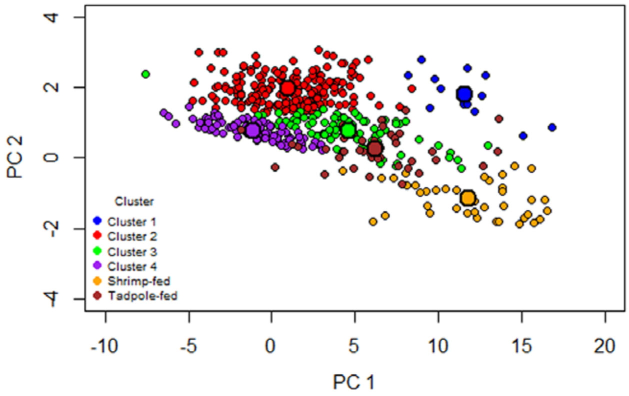

Figure 4. Distribution of wild-caught carnivores and experimentally fed tadpoles in two-dimensional morphospace. Small dots are individuals; large dots are the centroids of each group. The centroid of shrimp-fed individuals was significantly different from all others groups; the centroid of tadpole-fed individuals, by contrast, was not significantly different from carnivore cluster 3.

Whereas shrimp-fed tadpoles were significantly different from all wild-caught carnivores, tadpole-fed tadpoles were not significantly different from our "bulgy" carnivore cluster (Cluster 3; Fig. 4; Table S8). Two ponds (Eagles Cry and Red Tank) accounted for 36.7% of the individuals in this carnivore cluster and had moderate or moderate/high *Sc. couchii* densities.

The loadings of variables on PC1 and PC2 for our experimental tadpoles was significantly correlated with the loadings of these variables on PC1 and PC2 of our wild-caught carnivores (PC1: $R = 0.9587$, $P < 0.0001$; PC2: $R = 0.3114$, $P = 0.0044$; Table S9).

ASSESSING THE RELATIONSHIP BETWEEN INTRAMORPH DIVERSITY AND ECOLOGICAL VARIABLES

Table 2 summarizes the power of different ecological variables to explain the number of carnivore clusters and carnivore morphological diversity in each pond. With the exception of shrimp density describing carnivore morphological diversity, all variables were included in the final averaged model. The proportion of carnivores in a pond (a proxy for the potential intensity of competition among carnivores) was positively associated with the number of carnivore clusters in a pond and had the greatest explanatory power for predicting the number of carnivore clusters in a pond. The proportion of carnivores was also considerably more important than all other variables. For morphological diversity, the proportion of carnivores was also the most important variable. CEO was also a strong predictor that positively associated with diversity. The importance of both of these variables on carnivore morphological diversity was confirmed using standard least squares regression (Table S10). Shrimp density had the weakest explanatory power for the number of carnivore clusters and was the only variable not included in the averaged model for carnivore morphological diversity.

CEO was significantly negatively correlated with the proportion of carnivores in a pond ($R = -0.5176807$; $P = 0.02777$). That is, greater carnivore ecological opportunity reduced the potential for competition among carnivores in a pond. In low CEO ponds, the correlation between the proportion of carnivores and morphological diversity was not significant ($R = 0.6526$; $P = 0.1120$) and omnivores had greater morphological diversity (4.03) than carnivores (2.11; $t_5 = 3.1650$, $S_5 = 10.5$; $P = 0.025$, $P = 0.0313$, respectively). In contrast, high CEO ponds had a significantly positive relationship between proportion of carnivores and carnivore morphological diversity ($R = 0.8959$; $P = 0.0063$) and showed equivalent levels of morphological diversity within omnivores (4.07) and carnivores (3.63; $t_6 = 0.3182$, $S_6 = 2$; $P = 0.7611$, $P = 0.8125$, respectively). The slope of the relationship between the proportion of carnivores and morphological diversity was steeper (and the fit was better) in high CEO ponds than in low CEO ponds (9.606 vs 3.815; $R^2 = 0.80$ vs $R^2 = 0.43$), and the interaction between the proportion of carnivores and CEO level was nearly significant ($P = 0.07608$). Thus, when there was

Table 2. Summary of results from our model selection and averaging analysis of carnivore diversity.

	Number of clusters			Morphological diversity		
Explanatory variables	Model averaged coefficients (±SE)	Mean effect size (Z)	Relative importance	Model averaged coefficients (±SE)	Mean effect size (Z)	Relative importance
Shrimp density	0.0004 (0.032)	0.013	0.09	—	—	0.00
Sc. couchii density	0.007 (0.037)	0.168	0.12	0.291 (0.408)	0.696	0.39
CEO	**0.122 (0.484)**	**0.237**	**0.14**	**4.645 (5.074)**	**0.897**	**0.50**
Tadpole density	0.007 (0.042)	0.165	0.12	–0.199 (0.375)	0.514	0.30
Proportion carnivores	**2.613 (0.526)**	**4.567**	**1.00**	**3.603 (3.167)**	**1.112**	**0.65**

The bolded values indicate the proportion of carnivores in a pond and carnivore ecological opportunity (CEO). These two variables were the most important predictors for both metrics of carnivore diversity.

abundant ecological opportunity, competition promoted increased morphological diversity.

Discussion

Competitively mediated disruptive selection has long been viewed as a driver of resource polymorphism (see citations in the ***Introduction***), but the notion that it can also foster repeated rounds of diversification, including that within already existing resource-use morphs, has seldom been explored. We sought to determine if competition and ecological opportunity have given rise to a secondary round of diversification in a resource polymorphism consisting of alternative omnivore (a dietary generalist) and carnivore (a dietary specialist) morph tadpoles of the spadefoot toad, *Spea multiplicata*. We found that there are: two "types" of *Sc. couchii* morphological variants, at least five "types" of omnivore morphological variants and, even more surprisingly, at least four "types" of carnivore morphological variants in natural populations (Table 1, Fig. 2). Although we detected only one carnivore type in three of the 17 ponds that had carnivores, there were at least two types of carnivores in the vast majority (14 of 17) of ponds. Furthermore, we found that alternative carnivore diets produced different morphologies (Fig. 3); tadpole-fed tadpoles were more similar to wild-caught carnivores than shrimp-fed tadpoles were (Fig. 4); and the intensity of intramorph competition and ecological opportunity within a pond predicted the morphological diversity of carnivores in that pond (Table 2).

We expected to find evidence of tadpole- and shrimp-eating specialists that adopt different morphologies, and the results from our experiment supported this notion (Fig. 3). The anterior shift of eyes and nares in shrimp-fed individuals is consistent with the forward shift that occurs during metamorphosis when, almost universally, amphibians feed on animal prey (Stebbins and Cohen 1995). The overlap of visual fields of the two eyes aids in depth perception in targeting prey (Stebbins and Cohen 1995). Similarly, binocular vision of fish improves with more anteriorly positioned eyes (Gerking 1994). The improved depth perception owed to increased binocular vision might be important for acquiring prey from the water column (where shrimp are often located). However, the patterns of morphology from wild-caught tadpoles did not completely match those produced in our experiment. Instead, our cluster analysis of carnivores revealed at least four types of carnivores: “oddball,” “classic,” “bulgy,” and “boxcar” (Fig. 2). One of these carnivore types may be considered a tadpole-specialist (bulgy) because it was not significantly different from our tadpole-fed experimental animals.

The adaptive significance of the observed morphological variation is unclear. However, at least six lines of evidence suggest that individuals expressing different morphologies utilize slightly different resources and/or habitats: (1) some of the variation that we measured is known to affect resource acquisition (e.g., the shape of the mouth; Fig. 1); (2) there were differences in fitness proxies (growth and development) among carnivore morphological variants (see ***Results***) and the exact ranking in fitness of these variants differed among ponds; (3) one of the morphological variants is a possible tadpole-specialist; (4) there was a significant correlation between variable loadings on PC1 and PC2 in experimental tadpoles and wild-caught carnivores; (5) the morphological diversity within a pond was associated with ecological opportunity and the intensity of intramorph competition (the latter as approximated by the proportion of carnivores in a pond; Table 2); and (6) previous work on *S. multiplicata* has established that, within omnivores at least, fine-scale variation among individuals in trophic morphology is significantly correlated with variation in diet (Paull et al. 2012). At the same time, we cannot entirely rule out the possibility that at least some of the variation might have arisen from other sources of selection—such as predation (e.g., see Langerhans et al. 2004)—or that it might not be adaptive at all. Further study is needed to determine degree to which the observed morphological variation maps onto differences in ecology.

Assuming that the morphological variation is adaptive, another matter to resolve is whether it represents an adaptive response to ecological differences between ponds vs within. Finding evidence of local adaptation to different pond conditions––i.e., between-pond morphological variation—would not be surprising. After all, local adaptation is ubiquitous (Hereford 2009). Although some of the morphological variation was present between ponds—providing possible evidence of such local adaptation—much of the morphological variation was present within ponds. Indeed, as noted above, the vast majority of ponds sampled (82%) contained more than one carnivore morphological variant (Table 1). Thus, while some of the morphological variation might reflect local adaptation to slightly different conditions in the different ponds sampled, much of it might have arisen as an adaptive response to intraspecific competition for resources. As noted in the ***Introduction***, competitively mediated, frequency-dependent disruptive selection—acting within populations—can foster morphological diversification.

We found that cryptic (i.e., previously unrecognized) morphological variation was present within both omnivores and carnivores. The existence of similar levels of diversity within both the generalist omnivore morph and within the specialist carnivore morph—where we found four distinct morphological variants (Fig. 2)—was somewhat unexpected. However, as noted in the ***Introduction***, as an adaptive response to intramorph resource competition, new specialist morphological variants might evolve that subdivide the resource base further, even within an already existing specialist morph. Finding that one of our morphological variants was not different from tadpole-fed individuals suggests that this might be the case. Such individual specialization reduces overlap between individuals in resource use, thereby lessening the intensity of competition (Bolnick et al. 2003). More generally, our data suggest that existing morphs might often consist of a heterogeneous collection of relatively specialized individuals. Eco-morphological "heterogeneity" can therefore vary, depending on the level at which one is examining it.

Most examples of alternative phenotypes are thought to consist of only two morphs (dimorphism) or (at most) three morphs (trimorphism). Although dimorphism appears to be the norm for most resource polymorphisms, trimorphisms are common in animal and plant species that produce alternative mating types (e.g., Sinervo and Lively 1996; Barrett et al. 2000; Svensson et al. 2005; Painting et al. 2015). Such polymorphisms are thought to be maintained in an evolutionary stable strategy by temporal or spatial variation in directional selection or (as noted in the ***Introduction***) via negative frequency dependent selection in which a rarer morph is always favored (Sinervo and Calsbeek 2006).

Contrary to this prevailing view that alternative phenotypes are nearly always dimorphic or trimorphic, we found an unusual amount of morphological variation in our study populations (Table 1; Fig. 2). Whether such pronounced variation is atypical is unclear; few studies of resource or mating polymorphisms have specifically sought to identify submorphotypes. Exceptions include several species of *Pristionchus* nematodes that are symbionts of figs, in which up to five discrete morphotypes are present within a species (Susoy et al. 2016), and social insects (ants, bees, wasps, and termites), many of which produce several distinct forms (castes) within a single colony (e.g., termites produce up to seven distinct castes; Wilson 1971). Some social insects are even known to produce subcastes that utilize different resources, and these subcastes might be the ecological equivalent of the morphological variants that we found in *Spea*. For example, several species of *Pheidole* ants from the southwestern United States produce worker subcastes that specialize on different food resources (e.g., different-sized seeds) that species of *Pheidole* lacking these subcastes are unable to access (Wilson 2003). More studies are needed to determine if di- or trimorphism is the exception rather than the rule.

Both ecological and developmental factors appear to be vital for promoting pronounced diversity within populations. Regarding the former, the importance of both competition and ecological opportunity was reaffirmed in our study. There was a strong, positive relationship between the proportion of carnivores in a pond (a measure of the intensity of competition among carnivores; Pfennig 1992; Paull et al. 2012) and two measures of diversity: the number of carnivore clusters in the pond and carnivore morphological diversity. Similarly, carnivore ecological opportunity (CEO) was positively associated with carnivore morphological diversity (Table 2). Furthermore, there was a significant correlation between the proportion of carnivores and carnivore morphological diversity in high CEO ponds, and, across all ponds, CEO was negatively correlated with the proportion of carnivores. Taken together, this suggests that abundant and diverse resources favor increased morphological variation, presumably by relaxing selection imposed by conspecifics with similar morphology. This process is akin to interspecific character release, in which the absence of a heterospecific competitor leads to an expansion of a species niche and phenotype (Grant 1972; Bolnick 2001; Svänback and Bolnick 2007).

Developmental factors are also vital for promoting phenotypic diversity within populations. Specifically, phenotypic plasticity is likely critical in fostering the remarkably diverse phenotypes that we observed. Given that carnivores can be induced by the ingestion of shrimp or other tadpoles (Pomeroy 1981; Pfennig 1990; Levis et al. 2015), the observed differences in shape among carnivore morphological clusters might have arisen as plastic responses to subtle differences in the types of prey that the members of different clusters consumed. Such diet-induced plasticity enables individuals to produce resource-use phenotypes that are less like the phenotypes expressed by their competitors, thereby reducing the frequency and intensity of competitive interactions. Indeed, many species have evolved the ability to facultatively adjust their resource-use traits when faced with competition (reviewed in Pfennig and Pfennig 2012; Hendry 2017).

The occurrence of such pronounced phenotypic variation within populations has potentially important ecological and evolutionary implications. Regarding ecological ramifications, pronounced intraspecific trait variation is thought to enhance species coexistence (reviewed in Bolnick et al. 2011; Pfennig and Pfennig 2012; Violle et al. 2012; Turcotte and Levine 2016). Specifically, individual niche variation *within* species can promote coexistence between species that overlap in resource use, depending on how that variation is distributed within each species. A long-standing principle in ecology is that species with extensive niche overlap are not expected to coexist stably (Gause 1934; Hardin 1960). However, whether one species excludes the other depends on interactions among *individuals*. When interacting heterospecific individuals occupy slightly different niches (e.g., resources), competition will be less severe, and the two species might thereby coexist (Turcotte and Levine 2016). Essentially, greater trait variation within species increases the chances that any two interacting individuals will differ and therefore experience lower competition. Thus, two species that display extensive niche overlap at the *population* level might stably coexist if they harbor substantial variation at the *individual* level (e.g., see Clark 2010).

The occurrence of pronounced phenotypic variation within populations also has potentially important *evolutionary* consequences. The evolution of resource polymorphisms has long been thought to represent an early stage in the speciation process (West-Eberhard 1986; Wimberger 1994; Smith and Skúlason 1996; Skúlason et al. 1999; West-Eberhard 2003; Adams and Huntingford 2004; West-Eberhard 2005; Pfennig and McGee 2010). Indeed, the presence of a resource polymorphism in a species is associated with enhanced species diversity: clades in which a resource polymorphism has evolved are more species rich than are their sister clades in which a resource polymorphism has not evolved (Pfennig and McGee 2010). Resource polymorphism might enhance species diversity by increasing the likelihood that new species will form, by decreasing the chances that existing species will go extinct, or by both pathways (Pfennig and Pfennig 2012). Regarding the former pathway, speciation might occur between populations that have evolved a resource polymorphism and those that have not if these two different types of populations come to occupy different niches and thereby are unable to exchange genes with each other. Alternatively, the morphs that constitute a resource polymorphism might separate into distinct species if they each occupy different niches and therefore diverge from one another owing to contrasting selective pressures (Skúlason et al. 1999). Regarding the latter pathway (reducing extinction risk), clades in which resource polymorphism has evolved occupy more diverse habitats and possess wider geographical ranges than sister clades lacking resource polymorphism (Pfennig and McGee 2010). Consequently, resource polymorphic species may be less restrictive in their habitat requirements and therefore less likely to become extinct owing to habitat change or loss (species with broader geographical ranges are generally less likely to go extinct; Jablonski 1986). In short, pronounced resource polymorphism, such as that documented here in *Spea*, might serve as an important starting point for macroevolutionary diversification.

Our results help to clarify the causes of adaptive radiation. Traditionally, adaptive radiation is defined as a single evolutionary lineage diversifying rapidly into a large number of descendant lineages that occupy a wide variety of ecological niches (Simpson 1953; Schluter 2000). We found that enhanced ecological opportunity appears to be associated with niche-width expansion, whereby individuals have diverged from each other morphologically—and presumably also ecologically—to minimize resource use overlap and competition. Although this diversification was not accompanied by speciation, the proliferation of new morphological variants that (potentially) occupy diverse niches suggests the *intraspecific* analog of adaptive radiation. Moreover, finding that resource competition and ecological opportunity were associated with morphological diversity (Table 2) supports the longstanding idea that both factors spur adaptive radiation (Simpson 1953; Schluter 2000). In addition, it is unclear how the phenomena associated with ecological release (e.g., increased trait variation in released populations) ultimately lead to the rapid speciation and increased trait variation that characterize adaptive radiation (Yoder et al. 2010). Our results indicate that increased trait variation can be achieved when each individual (or a small group of individuals) in a population uses a narrow range of resources and thereby diverges from other similar conspecifics to reduce resource overlap and competition. Such diversification within a morphotype might constitute the first step toward an adaptive radiation.

Finally, our findings have implications for understanding the origins of novel complex phenotypes, which remains a major, unresolved problem in evolutionary biology (Mayr 1959; West-Eberhard 2003; Wagner and Lynch 2010). Many novel features—especially those involved in resource acquisition—might have arisen as an adaptive response to intraspecific competition (Pfennig and Pfennig 2012). In *Spea*, intraspecific competition has promoted the origins of a novel, resource use phenotype not found in other species of frogs—the distinctive carnivore morph (Ledón-Rettig et al. 2008). However, competition among carnivores might have promoted the origin of new morphological variants, some of which are themselves unique. For example, a novel tadpole-specialist morph might have arisen from a pre-existing shrimp-specialist morph as an adaptive response to competition for shrimp. Essentially, eating shrimp might have served as a "stepping stone" for a new morphological variant that could specialize on tadpoles, which, because of their larger size and greater mobility, are more challenging to eat [indeed, many species of tadpoles consume shrimp opportunistically (Altig et al. 2007), but very few species have evolved distinct tadpole specialists (Ruibal and Thomas 1988)]. Such competition among existing resource-use specialists, coupled with ecological opportunity, might explain the origins of novel resource-use variants in numerous taxa (e.g., Liem and Kaufman 1984; Hori 1993; Carroll et al. 1998; Jones 1998; Bolnick 2001; Benkman 2003; Bono et al. 2013; Yassin et al. 2016).

In sum, we found evidence that competition and ecological opportunity have promoted a secondary round of diversification in a resource-use specialist. Such variation within species, although seemingly cryptic, might have important ecological and evolutionary implications. Indeed, this variation may subsequently form a substrate upon which natural selection can act to promote macroevolutionary change through differential speciation, coevolutionary interactions, and/or extinction. Ultimately, untangling the conditions and mechanisms that foster diversification at all levels—including that within populations—will contribute to our understanding of how biodiversity is generated and maintained.

AUTHOR CONTRIBUTIONS

NAL and DWP designed the study. NAL and KAO collected data from wild-caught animals. RAM and DWP performed the diet experiment. NAL analyzed the data. NAL, RAM, and DWP were involved in writing the manuscript.

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DATA ARCHIVING

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.r3m37

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Supporting Information

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

Table S1. Summary statistics from MANOVA to determine which variables significantly describe tadpole morphology in our diet manipulation experiment.
Table S2. Results of model selection procedure on how well different ecological variables describe carnivore morphological diversity.
Table S3. Results of model selection procedure on how well different ecological variables describe the number of carnivore clusters in a pond.
Table S4. Pairwise p-values from 1000 iterations of RRPP on centroid location of whole-pond tadpole morphology.
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Table S6. Ability of various numbers of clusters to describe phenotypic variation among omnivores (top), carnivores (middle), and *Sc. couchii* (bottom).
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Figure S2. Distribution of carnivore morphology centroids in two-dimensional morphospace with lines connecting ponds that had the same morphology.
Figure S3. Distribution of omnivore morphological clusters (with 95% confidence ellipses.