doi:10.1111/j.1558-5646.2007.00034.x

FIELD AND EXPERIMENTAL EVIDENCE FOR COMPETITION'S ROLE IN PHENOTYPIC DIVERGENCE

David W. Pfennig,[1,2] Amber M. Rice,[1,3] and Ryan A. Martin[1,4]
[1] *Department of Biology, CB#3280, University of North Carolina, Chapel Hill, North Carolina 27599*
[2] *E-mail: dpfennig@email.unc.edu*
[3] *E-mail: arice@email.unc.edu*
[4] *E-mail: martinra@email.unc.edu*

Received August 7, 2006
Accepted October 3, 2006

Resource competition has long been viewed as a major cause of phenotypic divergence within and between species. Theory predicts that divergence arises because natural selection favors individuals that are phenotypically dissimilar from their competitors. Yet, there are few conclusive tests of this key prediction. Drawing on data from both natural populations and a controlled experiment, this paper presents such a test in tadpoles of two species of spadefoot toads (*Spea bombifrons* and *S. multiplicata*). These two species show exaggerated divergence in trophic morphology where they are found together (mixed-species ponds) but not where each is found alone (pure-species ponds), suggesting that they have undergone ecological character displacement. Moreover, in pure-species ponds, both species exhibit resource polymorphism. Using body size as a proxy for fitness, we found that in pure-species ponds disruptive selection favors extreme trophic phenotypes in both species, suggesting that intraspecific competition for food promotes resource polymorphism. In mixed-species ponds, by contrast, we found that trophic morphology was subject to stabilizing selection in *S. multiplicata* and directional selection in *S. bombifrons*. A controlled experiment revealed that the more similar an *S. multiplicata* was to its *S. bombifrons* tankmate in resource use, the worse was its performance. These results indicate that *S. multiplicata* individuals that differ from *S. bombifrons* would be selectively favored in competition. Our data therefore demonstrate how resource competition between phenotypically similar individuals can drive divergence between them. Moreover, our results indicate that how competition contributes to such divergence may be influenced not only by the degree to which competitors overlap in resource use, but also by the abundance and quality of resources. Finally, our finding that competitively mediated disruptive selection may promote resource polymorphism has potentially important implications for understanding how populations evolve in response to heterospecific competitors. In particular, once a population evolves resource polymorphism, it may be more prone to undergo ecological character displacement.

KEY WORDS: **Character displacement, directional selection, disruptive selection, divergent selection, phenotypic plasticity, resource polymorphism, stabilizing selection.**

Competition for resources is ubiquitous. Because competition tends to decrease individual fitness, natural selection is generally thought to favor traits that lessen competition's intensity. One way for selection to do so is to favor evolutionary divergence between initially similar phenotypes.

For instance, theory (Slatkin 1980; Arthur 1982; Taper and Case 1985, 1992; Abrams 1986; Doebeli 1996; Day and Young 2004) suggests that when two species with similar resource requirements coexist, those individuals most dissimilar from the average resource use phenotype of the other species will face the least competition and thereby acquire more resources than other members of their population. Consequently, the most divergent individuals will experience the highest fitness, and each population may evolve to be less like the other (Abrams (1986) has also shown that, under certain circumstances, competition may cause populations to become more (not less) similar; however, there are no examples). In this way, selection acting to minimize resource competition can promote phenotypic divergence between interacting species through a process known as ecological character displacement (the evidence for, and the principles of, ecological character displacement are reviewed in Brown and Wilson 1956; Grant 1972; Slatkin 1980; Taper and Case 1985, 1992; Abrams 1986; Schluter 2001, 2003; Day and Young 2004; Dayan and Simberloff 2005).

Similarly, competition among conspecifics for shared resources can also promote divergence. In a population that exploits a continuously varying resource, individuals that use the most common resource (e.g., intermediate size prey) will initially have a fitness advantage. As more individuals begin to exploit this resource, however, competition becomes increasingly severe and the fitness of these individuals will begin to decline (Rosenzweig 1978; Wilson and Turelli 1986; Milinski and Parker 1991; Day and Young 2004; Rueffler et al. 2006). At the same time, individuals that specialize on less common resources on either end of the resource use spectrum (e.g., very small or very large prey) will have fewer competitors (Rosenzweig 1978; Wilson and Turelli 1986). Eventually, the fitness of these divergent individuals may exceed that of the intermediate individuals as negative frequency-dependent selection driven by resource competition favors these less common, extreme phenotypes (Maynard Smith 1982; Wilson and Turelli 1986). In this way, disruptive selection favors increased phenotypic variation (Eshel 1983; Abrams et al. 1993; Geritz et al. 1998; Day and Young 2004; Dieckmann et al. 2004; Rueffler et al. 2006) and resource polymorphism (Levene 1953; Mather 1955; Maynard Smith 1962; Levins 1968; Wilson 1989; for examples, see Smith 1990; Pfennig 1992a; Hori 1993; Robinson et al. 1993; Benkman 1996; Smith and Skúkason 1996; Maret and Collins 1997; Rainey and Travisano 1998; Maerz et al. 2006).

Competition's role in promoting divergence has proved to be controversial and difficult to demonstrate empirically, however (Conner and Simberloff 1979; Dunham et al. 1979; Schluter and McPhail 1992; Grant 1994; Schluter 1994; and Losos 2000). Some experimental support for competition's role in driving divergence within species comes from studies of bacteria (Rainey and Travisano 1998; Rainey et al. 2000; Friesen et al. 2004; Travisano 2004), fruit flies (Bolnick 2001), fish (Bolnick 2004), and amphibians (Pfennig 1992a; Maret and Collins 1997). Competition's role in species divergence is less clear. Most support comes from correlational studies (but see Pfennig and Murphy 2000, 2002; Grant and Grant 2006), in which natural populations of closely related species differ in characters associated with resource use where they exist together but are indistinguishable where each exists alone (for examples, see Lack 1947; Brown and Wilson 1956; Fenchel 1975; Schluter et al. 1985; Diamond et al. 1989; Schluter and McPhail 1992; Robinson and Wilson 1994; Adams and Rohlf 2000; Schluter 2000; Dayan and Simberloff 2005; Pfennig et al. 2006). Yet, because numerous factors can cause interacting species to diverge in phenotype, such data alone are critical but not necessarily sufficient evidence that competition promotes species divergence (Grant 1972; Arthur 1982; Endler 1986; Schluter and McPhail 1992; Taper and Case 1992).

One way to evaluate competition's role in divergence both within and between species is to test the critical prediction of competition theory that natural selection disfavors those individuals bearing phenotypes most closely resembling their competitor (Slatkin 1980; Abrams 1989; Taper and Case 1992; Doebeli and Dieckmann 2000; Schluter 2000; Day and Young 2004; Dieckmann et al. 2004). Yet, few studies experimentally test this critical prediction in naturally existing populations (but see Pfennig 1992a; Maret and Collins 1997; Gray and Robinson 2002; Schluter 2003; Bolnick 2004).

Here, we use spadefoot toad tadpoles (genus *Spea*) to evaluate competition's role in divergence both within and between species. As we describe below, tadpoles of two species (*S. multiplicata* and *S. bombifrons*) often compete for food in nature and show the hallmark of ecological character displacement: exaggerated divergence in trophic morphology only where they coexist (Pfennig and Murphy 2000, 2002, 2003; Pfennig et al. 2006). Moreover, both species exhibit striking resource polymorphism, which appears to be maintained by negative frequency-dependent selection driven by competition for food (Pfennig 1992a).

This study had two goals. First, we examined whether selection acts against individuals in natural populations that are most similar in trophic morphology to their competitors: conspecifics in pure-species ponds and competitively superior heterospecifics in mixed-species ponds. Second, we used an experimental approach to determine if any such divergent selection is driven by competition. Before outlining our methods, we first describe our study system.

STUDY SYSTEM

Mexican spadefoot toads (*S. multiplicata*) and Plains spadefoot toads (*S. bombifrons*) coexist in the southwestern United States (Stebbins 2003). Within each species, tadpoles are highly variable in resource use and trophic morphology as represented by two extreme morphotypes (Gilmore 1924; Smith 1934; Orton 1954; Bragg and Bragg 1958; Bragg 1965; Pomeroy 1981; Pfennig 1992b; see photos in Pfennig and Murphy 2002): (1) the "omnivore" morph—a relatively small, slowly developing, round-bodied tadpole with a long intestine, small jaw muscles, and smooth mouthparts used for feeding on detritus (60% by gut volume; Pomeroy 1981) and anostracan fairy shrimp (38% by gut volume; Pomeroy 1981); and (2) the "carnivore" morph—a larger, more rapidly developing, narrow-bodied tadpole with a short intestine, greatly enlarged jaw muscles, and notched mouthparts used for feeding on larger anostracan fairy shrimp (85% by gut volume; Pomeroy 1981) and detritus (14% by gut volume; Pomeroy 1981). In some ponds, there is clear dimorphism in trophic morphology (Orton 1954; Bragg and Bragg 1958; Pomeroy 1981; Pfennig 1990). In other ponds, intermediates—both in morphology and in resource use (i.e., specializing on detritus and intermediate size shrimp)—exist between the two extreme forms and may be the most common phenotype present within any given pond (Pomeroy 1981; Pfennig 1990, 1992b).

Shrimp ingestion triggers development of the carnivore morphology (Pomeroy 1981; Pfennig 1990; Frankino and Pfennig 2001; Storz 2004) but heritable variation exists among sibships in propensity to eat shrimp and produce carnivores (Pfennig and Frankino 1997; Pfennig 1999; Pfennig and Murphy 2000, 2002; D.W. Pfennig, unpubl. data). Additionally, the more shrimp a tadpole eats, the more carnivore-like its morphology becomes (Pfennig 1990).

Although both species grow best on a diet of shrimp when reared alone (D.W. Pfennig 2000; Pfennig and Murphy 2000), natural ponds frequently contain both morphs (Pfennig 1990). Negative frequency-dependent selection maintains both morphs within a pond, such that each is favored whenever it becomes rare. As evidence of such a negative frequency-dependent selection, when the frequency of one morph is increased within different enclosed areas of natural ponds, that morph metamorphoses at a smaller size compared to those in control (unmanipulated) enclosures within the same pond (Pfennig 1992a). Because size at metamorphosis correlates with several fitness components (see Materials and Methods), these data indicate that negative frequency-dependent selection to lessen intraspecific competition for food maintains both morphs within the same pond. Negative frequency-dependent selection is a hallmark of competitively mediated disruptive selection (Day and Young 2004; Rueffler et al. 2006).

Both density-dependent and density-independent disruptive selection may act against individuals with intermediate phenotypes that forage on detritus and intermediate sized shrimp. Density-dependent disruptive selection may favor extreme phenotypes—omnivores, which specialize on small shrimp and detritus, and carnivores, which specialize on large shrimp—because such individuals may have relatively few competitors (within many ponds, individuals with intermediate phenotypes are most abundant; D. Pfennig, unpubl. data). Density-independent disruptive selection might also favor extreme phenotypes if (1) intermediate sized prey become relatively rare, and (2) individuals with intermediate phenotypes are less efficient than omnivores at utilizing small, low nutritive value food particles (e.g., omnivores may be better at processing detritus because of their long intestine) and less efficient than carnivores at handling large food particles (carnivores are better than intermediates at capturing and subduing large shrimp; Frankino and Pfennig 2001).

A pond may contain only one morph, however, if one of the two main resources is uncommon. For ponds in which shrimp are scarce, the entire pond population often consists solely of omnivores, and for ponds in which detritus is scarce, the entire pond population often consists solely of carnivores (Pfennig 1990; Pfennig et al. 2006). Thus, negative frequency-dependent selection can maintain both morphs within any given pond only when both resources are available (Pfennig et al. 2006).

Competition for food also appears to promote divergence between species as evidenced by ecological character displacement in trophic morphology (Pfennig and Murphy 2000, 2002, 2003; Pfennig et al. 2006). When each species exists alone (i.e., in pure-species ponds or allopatry), they tend to produce similar frequencies of both carnivores and omnivores (Pfennig and Murphy 2003; Pfennig et al. 2006). In nearby ponds where the two species exist together (i.e., in mixed-species ponds or sympatry), *S. bombifrons* produces mostly carnivores, whereas *S. multiplicata* produces mostly omnivores (Pfennig and Murphy 2003; Pfennig et al. 2006). Moreover, *S. multiplicata* undergoes an evolutionary shift in developmental mechanism: in allopatry, *S. multiplicata* maintain plasticity to produce both morphs, but in sympatry, they lose this plasticity and become canalized to produce mostly omnivores (Pfennig and Murphy 2000, 2002).

Divergence between species in morph production in sympatry appears to reflect competitive differences between these two species. *Spea multiplicata* is the superior competitor for detritus, whereas *S. bombifrons* is the superior competitor for shrimp (Pfennig and Murphy 2000). These competitive differences apparently stem from species-specific differences in foraging behavior. Because they spend more time actively searching in the water column, *S. bombifrons* forage more efficiently for shrimp (Pfennig and Murphy 2000). In contrast, *S. multiplicata* spend more time grazing on the pond bottom and, thus, forage more efficiently for detritus (Pfennig and Murphy 2000).

Controlled lab experiments reveal that these shifts in morph production have likely evolved because of resource competition imposed by the other species (i.e., ecological character displacement). When allopatric individuals of each species are reared alone under common conditions (i.e., with both shrimp and detritus), they produce similar proportions of carnivores and omnivores (Pfennig and Murphy 2002). When allopatric source populations of each species are experimentally combined, however, *S. multiplicata* are less likely to eat shrimp and thereby experience the cue (shrimp ingestion) that triggers carnivores. As a result, *S. multiplicata* develop mostly into omnivores in the presence of *S. bombifrons* (Pfennig and Murphy 2002). In contrast, *S. bombifrons* are more likely to eat shrimp and thereby experience the cue that triggers carnivores. As a result, *S. bombifrons* develop mostly into carnivores in the presence of *S. multiplicata* (Pfennig and Murphy 2002). Because these experimentally demonstrated niche shifts mirror the canalized niche shifts observed in the field (Pfennig et al. 2006), these data provide experimental support for ecological character displacement in this system.

Materials and Methods

Our study was designed to address the following two questions. First, in natural populations, are individuals that are most similar in trophic morphology to the average phenotype of conspecifics (in pure-species ponds) or heterospecifics (in mixed-species ponds) selected against? Second, is any such divergent selection driven by competition for food? Below, we outline our methods for addressing each of these two questions separately.

TESTING FOR DIVERGENT SELECTION IN NATURAL POPULATIONS

Ideally, to test for divergent selection in natural populations, one would measure the trophic phenotypes of many individuals. One would then determine if those individuals that are most similar in phenotype to the other species have lowest fitness (in mixed-species ponds), and if those individuals with intermediate phenotype have lowest fitness (in pure-species ponds). Unfortunately, we were not able to estimate fitness of individuals directly. Instead, we measured a trait that serves as a proxy for fitness: body size.

Body size is positively correlated with fitness in many species (Kingsolver and Pfennig 2004). In *Spea*, larval size is positively correlated with premetamorphic (Pfennig and Pfennig 2005) and postmetamorphic survival (Pfennig et al. 1991). Moreover, tadpole size correlates positively with adult size in anurans (Smith 1987; Altwegg and Reyer 2003). In *Spea*, adult size is positively correlated with mating success in males (K.S. Pfennig 2000) and fecundity in females (Pfennig and Pfennig 2005). In short, body size predicts several fitness components, suggesting that it is a reliable proxy for fitness.

We were specifically interested in determining whether tadpoles with the most divergent trophic morphology (relative to the rest of the population) tend to have the highest fitness. Based on earlier studies (see Study System), however, we predicted a priori that the mode and direction of selection acting on trophic morphology would differ for each species in pure- versus mixed-species ponds.

In pure-species ponds, we predicted that both density-dependent and density-independent disruptive selection would act against individuals with intermediate phenotypes (see Study System). Such a disruptive selection should be especially pronounced in pure *S. multiplicata* ponds, because these ponds often contain both shrimp and detritus resources (Pfennig et al. 2006). Although similar selection should operate in pure *S. bombifrons* ponds, we predicted that disruptive selection would be weaker than in pure *S. multiplicata* ponds. In pure *S. bombifrons* ponds, omnivores would not likely be as highly favored: detritus is often scarce in pure *S. bombifrons* ponds leaving omnivores with no alternative resource to use (Pfennig et al. 2006). At the same time, extreme carnivore-like individuals are probably selected against because of functional limitations; for example, enlarged jaw muscles and a larger head may impair swimming ability.

We predicted different patterns of selection for the two species in mixed-species ponds. Because *S. bombifrons* is the superior competitor for shrimp (Pfennig and Murphy 2000), and because the most carnivore-like *S. multiplicata* would likely compete more with *S. bombifrons* for food (confirmed in the present study; see Results), we predicted that the most carnivore-like *S. multiplicata* in mixed-species ponds would have lower fitness than conspecifics that are less carnivore-like. At the same time, because *Spea* tadpoles grow better on shrimp than on detritus (D.W. Pfennig 2000; Pfennig and Murphy 2000), and because shrimp tend to be relatively abundant in mixed-species ponds (Pfennig et al. 2006), we predicted that *S. multiplicata* omnivores in mixed-species ponds would have lower fitness than intermediate individuals, because the latter would be able to supplement their diet with larger, more nutritious shrimp. Therefore, we expected to find stabilizing selection favoring phenotypically intermediate individuals among *S. multiplicata* in mixed-species ponds. By contrast, for the *S. bombifrons* in these mixed-species ponds, carnivores should have highest fitness, because *S. bombifrons* is the better competitor for this abundant and superior resource. Thus, we expected directional selection favoring carnivores among *S. bombifrons* in mixed-species ponds. Table 1 summarizes how selection on trophic morphology was expected to vary for each species in the two different types of ponds.

To test our predictions, we sampled tadpoles during summers 1999–2005 from natural ponds throughout southeastern Arizona (AZ) and southwestern New Mexico (NM), United States (for locations of study ponds, see Fig. 2 in Pfennig et al. 2006). In this region, both species generally coexist between 1200 and 1350 m elevation (Pfennig et al. 2006). At lower elevations, ponds typically contain only *S. bombifrons*, whereas at higher elevations, ponds contain only *S. multiplicata* (Pfennig et al. 2006). Tadpoles were sampled from randomly selected sites throughout each pond (10–18 days past pond filling) by using a hand-held dip net. We sampled 1234 tadpoles total from 48 different ponds (mean ± s.e.m. number of tadpoles sampled per pond $= 26.7 \pm 2.2$, range $=$ 8–73).

Table 1. **Summary of predicted mode and direction of selection acting on trophic morphology for two different species of spadefoot toad tadpoles in pure-species ponds (i.e., in the absence of the other species) and mixed-species ponds (i.e., in the presence of the other species).**

Species	Pond type	Mode and direction of selection	Selective mechanism
multiplicata	pure-species	disruptive selection favoring extreme phenotypes	density-dependent disruptive selection should favor individuals with extreme phenotypes, because such individuals should have relatively few competitors
multiplicata	mixed-species	stabilizing selection favoring intermediate phenotypes	*multiplicata* carnivores compete poorly with *bombifrons* for shrimp; also, *multiplicata* omnivores should perform poorer than conspecifics with intermediate phenotypes, because intermediates consume more shrimp (the superior resource), which tends to be abundant in mixed-species ponds
bombifrons	pure-species	weak disruptive selection favoring extreme phenotypes (with carnivores having higher fitness than omnivores)	same as for *multiplicata* in pure-species ponds except that carnivores should be more highly favored than omnivores because detritus tends to be scarce in pure *bombifrons* ponds
bombifrons	mixed-species	directional selection favoring carnivores	*S. bombifrons* carnivores should have highest fitness, because *S. bombifrons* is the better competitor for this abundant and superior resource

Because the two species can be difficult to tell apart, we used genetic markers to determine each tadpole's species identity. Within a few hours of collection, we killed tadpoles by immersion in a 0.1% aqueous solution of tricane methanesulfonate (MS 222) and preserved them in 95% ethanol (in 1999 we first took a tissue sample from each tadpole and froze it at –80°C). In 1999 we used species-specific allozymes (Simovich and Sassaman 1986), and in 2000–2005 we sequenced a 663-bp region of the mtDNA genome to classify each tadpole as *S. bombifrons* or *S. multiplicata*. We also used these data to determine which ponds contained both species (designated as mixed-species ponds) and which ponds contained only *S. multiplicata* or only *S. bombifrons* (designated as pure-species ponds). Based on these analyses, we sampled a total of 800 tadpoles from 33 pure *S. multiplicata* ponds, 164 tadpoles from six pure *S. bombifrons* ponds, and 163 *S. multiplicata* and 107 *S. bombifrons* from nine mixed-species ponds.

We measured each tadpole's overall body size (snout-vent length, SVL) and trophic morphology by using the detailed procedures outlined in Pfennig and Murphy (2002). In particular, we measured three diagnostic characters: (1) width of the orbitohyoideus (OH) muscle, (2) width of the interhyoideus (IH) muscle, and (3) shape of the keratinized mouthparts (MP). The OH muscle is the primary buccal cavity abductor, responsible for opening the mouth cavity; conversely, the IH muscle closes the buccal cavity and forces cavity contents back to the pharynx (Wassersug and Hoff 1979; Cannatella 1999). A relatively large OH increases the tadpoles' ability to create inward suction and thereby capture and subdue larger prey, such as fairy shrimp (Ruibal and Thomas 1988; Pfennig 1992b). A relatively large IH increases the force at which food particles are moved rearward through the buccal cavity, thereby decreasing handling time for large prey (Satel and Wassersug 1981). The MP of carnivore–morph *Spea* are often serrated, suggesting that such serration permits tadpoles to trap, subdue, and cut larger prey efficiently (Turner 1952; Orton 1954; Bragg and Bragg 1958). Overall, for a given body size (SVL), more carnivore-like tadpoles (i.e., those specializing on larger shrimp) have relatively larger OH and IH muscles and MP with more prominent serration. Conversely, more omnivore-like tadpoles (i.e., those specializing on smaller shrimp or detritus) have relatively smaller OH and IH muscles and MP without serration (Pomeroy 1981; Pfennig 1992b).

For each of the four groups of tadpoles separately (i.e., each species in each of the two different types of ponds), we standardized OH and IH for body size (SVL) by regressing log OH and log IH on log SVL; we used the resulting residuals for the subsequent analyses. All regressions were highly significant ($P < 0.0001$) and produced normally distributed residuals. We used the methods in Pfennig et al. (2006) to combine the three trophic characters (MP, residuals of log OH and log IH regressed on log SVL) into a single multivariate shape variable (the "morphological index"). This index was calculated for each tadpole by performing a principal component (PC) analysis of the three characters. We used PC1, which explained >60% of the variance in the three trophic characters.

We tested for selection on trophic morphology by regressing body size (log SVL, a proxy for fitness) on the morphological index. Because we predicted a priori that the four groups would differ in mode and direction of selection, we conducted separate regression analyses on each group. Moreover, because we generally sampled relatively few (<30) tadpoles per pond, we pooled tadpoles from different ponds such that all conspecifics belonging to each pond type (i.e., pure or mixed species) were combined into one analysis. For ponds in which there was a significant regression slope, however, we also performed separate within-pond regressions (using the same procedures as before). All statistical analyses were performed using JMP (SAS Institute, Cary, NC), and all data fit the assumptions of GLM.

We performed separate linear and polynomial regressions on each dataset. Quadratic regression of fitness on morphology indicates disruptive performance when the slope of the quadratic term (γ) is significantly positive (Lande and Arnold 1983; Bolnick 2004). By contrast, quadratic regression of fitness on morphology indicates stabilizing performance when this slope is significantly negative (Lande and Arnold 1983; Bolnick 2004).

We determined which regression model provided the best fit to each dataset by performing stepwise selection procedures and comparing the Akaike's information criterion (AIC) for each model (Burnham and Anderson 2002). We concluded that directional selection might be acting on trophic morphology if the linear model produced the best fit. By contrast, we concluded that disruptive selection might be acting if a quadratic model with an intermediate performance minimum produced the best fit. Finally, we concluded that stabilizing selection might be acting if a quadratic model with an intermediate performance maximum produced the best fit.

We also performed cubic spline analyses ("nonparametric" regression) on each dataset using glmsWIN 1.0 (provided by D. Schluter at http://www.zoology.ubc.ca/∼schluter/splines.html). Cubic spline analysis is less sensitive to outliers and allows estimation of a fitness function without an a priori assumption about the function's shape (Schluter 1988). We used 1000 bootstrap replicates to estimate 95% confidence intervals around each curve and then visually inspected the output to determine if there was a minimum or maximum within the range of the data (Bolnick 2004).

EXPERIMENTAL TEST OF THE ROLE OF COMPETITION IN SPECIES DIVERGENCE

As a follow up to our field estimates of selection, we designed an experiment to determine if divergent selection tends to disfavor individuals that most closely resemble their heterospecific competitor. For this experiment, *S. multiplicata* was treated as the focal species. We specifically investigated whether selection imposed by *S. bombifrons* is most costly to *S. multiplicata* that most closely resemble *S. bombifrons*. Although *S. bombifrons* that resemble *S. multiplicata* also appear to be disfavored (see Results), this selection was not measured here. For this experiment, we used lab microcosms (e.g., see Pfennig and Murphy 2000, 2002; Pfennig et al. 2006). Although such microcosms do not incorporate all of the myriad selective agents that can act on *Spea* in nature (e.g., competition from other species, predation, disease, desiccation), they do provide maximum control over the selective agent (interspecific competition) that we hypothesized is critical for promoting divergent selection on trophic morphology.

To begin, we created 14 full sibships of *S. multiplicata*. To increase the range of variation in carnivore morph expression, we bred both allopatric and sympatric *S. multiplicata* (differences between these two source population types did not drive the patterns observed in the Results). The parents of seven sibships were collected at high elevation sites near Portal, Arizona where *S. bombifrons* are absent (see Study System). The parents of the other seven sibships were collected at low elevation sites near Rodeo, New Mexico where *S. bombifrons* are present (high and low elevation sites were <30 km from each other; see Fig. 2 in Pfennig et al. 2006). In addition, we created three full sibships of *S. bombifrons* by breeding three pairs of *S. bombifrons* that were collected near Rodeo, New Mexico.

Two weeks after the tadpoles hatched, we randomly selected three similar-sized omnivore tadpoles (two *S. multiplicata* and one *S. bombifrons*) and assigned them to tanks as follows: two heterospecifics were placed together in an experimental tank (145 replicate tanks) and one *S. multiplicata* tadpole was placed alone in a control tank (145 replicate tanks). Similar-sized *S. multiplicata* siblings were placed in adjacent experimental and control tanks (Fig. 1A), thereby allowing us to compare the performance of siblings whose rearing environments were similar in all respects except for the presence or absence of an *S. bombifrons* tankmate. All tanks (28 × 18 × 10 cm) were filled with 6 liters of dechlorinated tap water and kept in the same room maintained at 26°C and on a 14 h L: 10 h D light cycle.

Before starting the experiment, we assessed similarity in resource use for each experimental *S. multiplicata* tadpole and its *S. bombifrons* tankmate. We chose not to use the morphological index for this assessment (see Testing for Divergent Selection in Natural Populations) for two reasons. First, trophic characters are most reliably measured on dead tadpoles. Thus, we did not measure them on live tadpoles at the start of the experiment. Second, because *Spea* tadpoles can facultatively adjust their trophic morphology in response to competitors (through phenotypic plasticity; e.g., see Pfennig and Murphy 2002), any measures of trophic characters taken at the end of the experiment might not reflect the tankmates' true similarity during the experiment.

Instead, we assessed similarity between experimental *S. multiplicata* tadpoles and their *S. bombifrons* tankmates by measuring how similar the two tadpoles were in their predilection to eat an equivalent number of anostracan shrimp. Such a

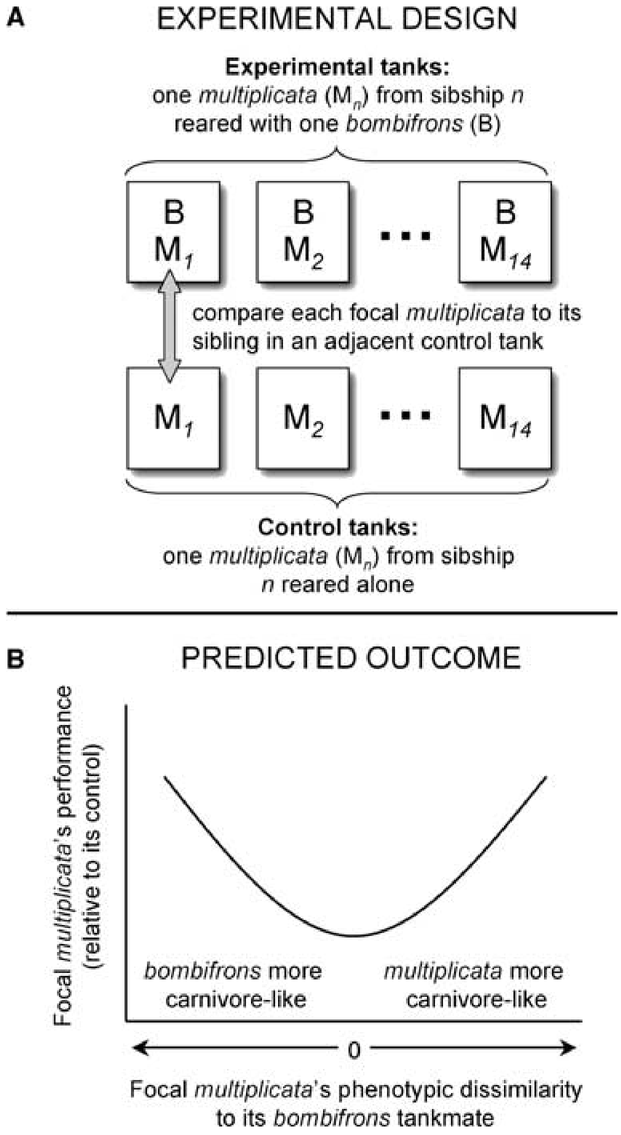

Figure 1. **Diagrammatic representation of the experimental design and predicted outcome. (A) The performance of a focal *S. multiplicata* tadpole (M_n) in each experimental tank was compared to that of its sibling in a neighboring control tank. (B) If divergent natural selection disfavors those individuals with phenotypes most closely resembling their competitor, then the relative performance of the focal *S. multiplicata* tadpole in each experimental tank should be lowest when the focal animal is the least dissimilar to its *S. bombifrons* tankmate in trophic phenotype (indicated here by a dissimilarity value of zero).**

measure is highly repeatable for individual tadpoles (D. Pfennig, unpubl. data), and heritable variation exists among sibships in propensity to consume shrimp (see Study System). Moreover, this measure predicts a tadpole's propensity to ultimately develop the carnivore morphology: control tadpoles that ate shrimp fastest later expressed the most carnivore-like phenotype (the Spearman correlation between mean time to eat three shrimp and morphological index for 130 tadpoles $= -0.36$, $P < 0.001$). Finally, tadpoles do not appear to modify this rate in the presence of competitors (D. Pfennig, unpubl. data).

To conduct these assays, we placed each tadpole alone in a small, round, individually numbered, opaque plastic container (12 cm diameter × 6 cm deep) filled with 600 ml of dechlorinated tap water. Each tadpole acclimated to its new surroundings for 24 h, during which time it was given crushed fish food (Wardley cichlid floating pellets) ad libitum to ensure that all tadpoles were equally satiated. We then placed into each container three live brine shrimp (*Artemia* sp., 10 mm total length). Brine shrimp are similar to the fairy shrimp on which *Spea* prey in natural ponds (Pomeroy 1981). An observer then recorded how long each tadpole took to consume all three shrimp in its container.

We assumed that tankmates that were most similar in time to eat shrimp would compete the most for food. In tanks where both individuals consumed shrimp quickly, the two would likely compete for shrimp, and in tanks where both individuals consumed shrimp more slowly, the two would likely compete for detritus.

We started the experiment when the tadpoles were three weeks old (7–12 mm SVL). Once the experiment began, each experimental tank received 20 live brine shrimp once a day and 20 mg of crushed fish food every other day (fish food simulates in form and nutrition the detritus on which *Spea* feed in natural ponds; see Pfennig et al. 2006). Control tanks received half the amount of food as experimental tanks, such that the per capita amount of food provided to both treatments was identical. In all experimental tanks, shrimp and detritus had disappeared by the time the tadpoles were fed again, ensuring that competition had taken place.

The experiment ended after 17 days, at which point we measured the SVL and wet mass of each tadpole. Each tadpole was then killed by immersion in MS 222 and placed in 95% ethanol. For tadpoles in experimental tanks, we sequenced a 663-bp region of the mtDNA genome to classify each as *S. bombifrons* or *S. multiplicata*.

Our response measure was each tadpole's "condition" (operationally defined as the residual from the regression of wet mass on SVL). Thus, for a given SVL, a lighter individual was considered to be in a poorer condition than a heavier individual. Poor condition at metamorphosis is associated with low postmetamorphic survival in *Spea* (Pfennig 1992a).

We predicted that *S. multiplicata* tadpoles with tankmates most similar in resource use (i.e., shrimp-eating times) would perform the worst in competition (Fig. 1B). The performance of each experimental *S. multiplicata* tadpole was estimated by subtracting from its final condition that of its matched sibling in a neighboring control tank. Thus, performance values less than zero indicated that the focal tadpole reared in competition with *S. bombifrons* was in a worse condition than was its sibling when reared alone. By contrast, performance values greater than zero indicated that the focal tadpole reared in competition with *S. bombifrons* was in a better condition than was its sibling when reared alone.

If divergent natural selection disfavors individuals that most closely resemble their competitor, then the performance of experimental *S. multiplicata* should be lower the more closely they resemble their *S. bombifrons* tankmate in resource use (i.e., shrimp-eating times). To test this prediction, we plotted performance of all experimental *S. multiplicata* as a function of similarity to their *S. bombifrons* tankmate in time to eat three shrimp. As in the field surveys (see Testing for Divergent Selection in Natural Populations), we performed regression analyses to determine both if the best-fit regression model (with the lowest AIC value) and the cubic spline curve were quadratic regressions with intermediate minima where competitors were most similar in shrimp-eating times.

Results

TESTING FOR DIVERGENT SELECTION IN NATURAL POPULATIONS

As predicted (Table 1; see also Materials and Methods), regression estimates of the relationship between the morphological index (a composite shape variable (PC1), where larger values are more carnivore-like) and body size (a proxy for fitness) differed for each species and in pure- and mixed-species ponds (Figs. 2, 3). The best-fit regression model for *S. multiplicata* from pure-species ponds was a quadratic regression with positive slope (Table 2), in which individuals that were the most extreme in trophic morphology were largest (Fig. 2A; the minimum occurred where the morphological index equaled −1.16, with a predicted log SVL (± 95 % CI) at 2.42 ± 0.03, which was within the range of the data). Cubic spline analysis confirmed the presence of an intermediate performance minimum (Fig. 3A). Thus, body size for *S. multiplicata* from pure-species ponds showed a disruptive performance function with respect to trophic morphology. The best-fit regression model for *S. multiplicata* from mixed-species ponds was also a quadratic regression. In this case, however, the regression slope was negative (Table 2), and individuals that were intermediate in trophic morphology were largest (Fig. 2B). Cubic spline analysis confirmed the presence of an intermediate performance maximum (Fig. 3B). Thus, body size for *S. multiplicata* from mixed-species ponds showed a stabilizing performance function with respect to trophic morphology.

In contrast to *S. multiplicata*, the best-fit regression model for *S. bombifrons* from pure-species ponds was a fifth degree polynomial (Table 2). Here, individuals that were extremely omnivore-like, intermediate, or extremely carnivore-like were smallest (Fig. 2C). Cubic spline analysis confirmed this pattern (Fig. 3C). Thus, for *S. bombifrons* from pure-species ponds, body

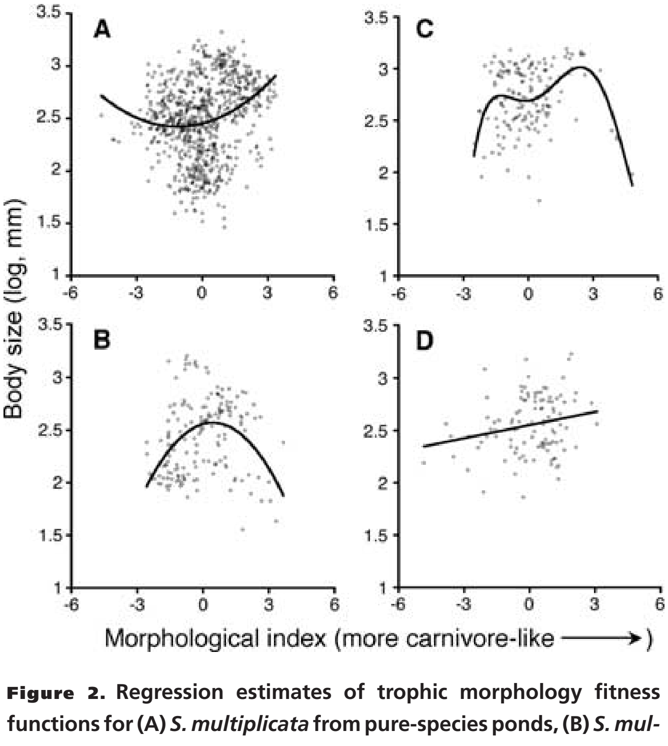

Figure 2. Regression estimates of trophic morphology fitness functions for (A) *S. multiplicata* from pure-species ponds, (B) *S. multiplicata* from mixed-species ponds, (C) *S. bombifrons* from pure-species ponds, and (D) *S. bombifrons* from mixed-species ponds. The fitness proxy is log snout-vent length. The quadratic regression term is significant in (A)–(C). The linear regression term is significant in (D).

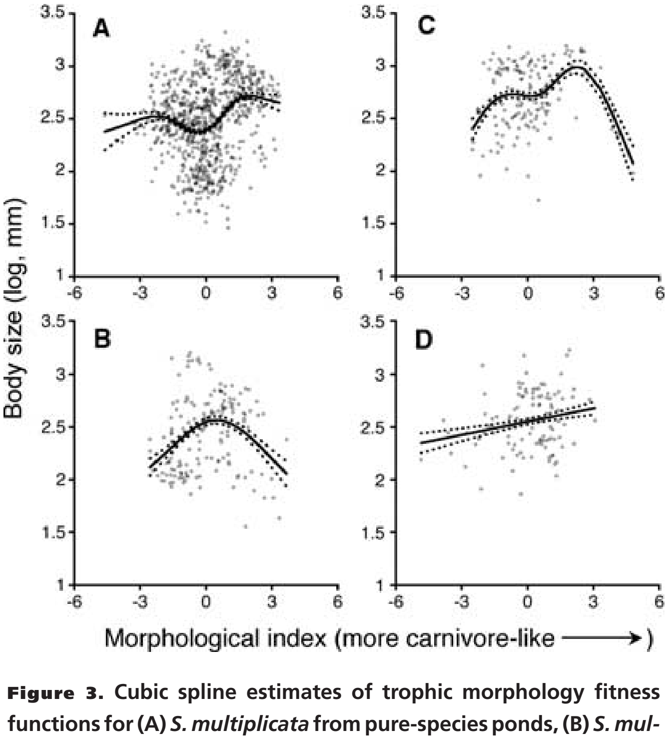

Figure 3. Cubic spline estimates of trophic morphology fitness functions for (A) *S. multiplicata* from pure-species ponds, (B) *S. multiplicata* from mixed-species ponds, (C) *S. bombifrons* from pure-species ponds, and (D) *S. bombifrons* from mixed-species ponds. The fitness proxy is log snout-vent length. Cubic splines (solid lines) are bracketed by 95% confidence intervals (dashed lines) estimated from 1000 bootstrap replicates.

Table 2. **Tests for different modes and patterns of selection on trophic morphology for two species of spadefoot toad tadpoles (*Spea multiplicata* and *S. bombifrons*) from different types of ponds (pure- and mixed-species). PC1 is a composite shape variable (principle component 1) that measures trophic morphology. Regression terms are given for each species and pond type, along with the estimated coefficient for each term, its standard error (SE), *t*-statistic, probability of the null hypothesis that the estimated coefficient is zero, and the Akaike information criterion (AIC). For quadratic regressions, positive coefficients signify disruptive selection, and negative coefficients signify stabilizing selection. Bold rows highlight the statistically significant regression terms. Asterisks signify models with the best fit (i.e., the model with the lowest AIC value).**

	Regression						
Species	Pond type	Term	Coefficient	SE	T	P	AIC
multiplicata	pure-species	**PC1**	**0.056**	**0.016**	**6.12**	**$<$0.0001**	**−1636**
		PC1^2	**0.024**	**0.005**	**4.77**	**$<$0.0001**	**−1657***
multiplicata	mixed-species	**PC1**	**0.060**	**0.020**	**2.94**	**0.0037**	**−335**
		PC1^2	**−0.067**	**0.013**	**−5.36**	**$<$0.0001**	**−359***
bombifrons	pure-species	**PC1**	**0.103**	**0.027**	**3.86**	**0.0002**	**−395**
		PC1^2	−0.004	0.014	−0.32	0.7497	−406
		PC1^3	**−0.009**	**0.004**	**−2.18**	**0.0307**	**−408**
		PC1^4	**−0.008**	**0.002**	**−3.82**	**0.0002**	**−421**
		PC1^5	**0.002**	**0.001**	**−2.07**	**0.04**	**−423***
bombifrons	mixed-species	**PC1**	**0.042**	**0.019**	**2.25**	**0.0263**	**−275***

size showed a disruptive performance function with respect to trophic morphology.

Lastly, the best-fit regression model for *S. bombifrons* from mixed-species ponds was a linear regression (Table 2). Here, individuals that were the most carnivore-like were largest (Figs. 2D, 3D). Thus, for *S. bombifrons* from mixed-species ponds, body size showed a directional positive performance function with respect to trophic morphology.

Although most of the within-pond regressions were not statistically significant because of low sample size, the pattern of selection within ponds generally mirrored that across ponds. For all seven of the 33 pure *S. multiplicata* ponds in which the overall regression model was significant (i.e., $P < 0.05$), there was a significant quadratic term. In these ponds, individuals that were the most extreme in trophic morphology were largest, suggesting disruptive selection, the same conclusion for the analyses across ponds (see above and Fig. 2A). Likewise, for *S. multiplicata* in two of the nine mixed-species ponds, the overall regression model was significant. One of these two ponds had a significant quadratic term. In this pond, individuals that were intermediate in trophic morphology were largest, suggesting stabilizing selection (again, mirroring the pattern across ponds for this species and type of pond). For the remaining mixed-species pond, there was a significant linear term such that *S. multiplicata* that were the most omnivore-like were the largest; thus, *S. multiplicata* that were most divergent from *S. bombifrons* were largest. None of the within-pond regressions for *S. bombifrons* was significant.

Finally, a visual inspection of the pooled analyses (Figs. 2, 3) revealed that tadpoles from the same pond did not tend to cluster. This lack of clustering, coupled with the observation that the pattern of selection within ponds mirrors that across ponds, suggests the trends observed (Figs. 2, 3) result from variation in fitness within populations, and not from variation in both size and morphology across populations.

EXPERIMENTAL TEST OF THE ROLE OF COMPETITION IN SPECIES DIVERGENCE

The *S. multiplicata* that performed the best in the experimental tanks were those that were the most dissimilar to their *S. bombifrons* tankmate in foraging behavior (Fig. 4A). The best-fit regression model was a quadratic regression with a performance minimum (Table 3). Cubic spline analysis confirmed the presence of a performance minimum: *S. multiplicata* that were most similar to their *S. bombifrons* tankmate were smallest (Fig. 4B). Thus, condition showed a disruptive performance function with respect to phenotypic similarity between competitors, suggesting that divergent selection favors individuals with phenotypes most dissimilar from their competitor.

Discussion

In natural ponds containing both species, individuals that were most similar to the most commonly expressed phenotype of the other species appear to be selected against (i.e., carnivores in *S. multiplicata* and omnivores in *S. bombifrons*; Figs. 2, 3). Our experimental results suggest that this selection is likely driven by competition for food. We found that *S. multiplicata* that were the most similar to *S. bombifrons* in resource use performed the worst (Fig. 4). Thus, divergence between *Spea* in trophic morphology (Pfennig and Murphy 2000, 2002, 2003; Pfennig et al.

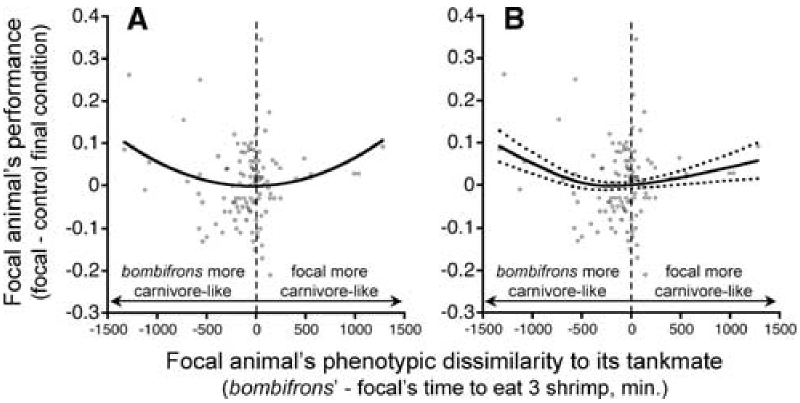

Figure 4. Experimental evidence that divergent natural selection disfavors those individuals with phenotypes most closely resembling their competitor. (A) Quadratic regression estimate of fitness (relative condition) as a function of a focal animal's similarity to its *S. bombifrons* tankmate at the start of the experiment in propensity to consume an identical number of shrimp. The quadratic regression term is significant. (B) Cubic spline estimate of the trophic morphology fitness function (as measured by relative condition) for the experimental animals. The cubic spline (solid line) is bracketed by 95% confidence intervals (dashed lines) estimated from 1000 bootstrap replicates.

2006) appears to reflect selection disfavoring phenotypically similar individuals, because these individuals compete for shared resources. Our data therefore support longstanding theoretical arguments that the intensity of competition between competitors diminishes with declining phenotypic similarity (Roughgarden 1976; Slatkin 1980; Taper and Case 1985; Abrams 1986; Taper and Case 1992; Doebeli 1996). Our results also confirm one of character displacement's central predictions—that the strength of competition between species should decline as divergence between them proceeds (Abrams 1986; Schluter 2003). Although this prediction has been tested in *Anolis* lizards and stickleback fish (Pacala and Roughgarden 1985; Pritchard and Schluter 2001; Gray and Robinson 2002), our results reveal that this declining intensity of competition with decreasing similarity can be remarkably fine-grained (e.g., see Fig. 4; see also Pfennig and Murphy 2002; Schluter 2003; Gray et al. 2005).

As we had predicted (Table 1), the mode and direction of selection operating on trophic morphology differed for different species and populations. We found evidence of disruptive selection operating in pure-species ponds for both species (Fig. 2A, C), although the pattern of disruptive selection was weaker for *S. bombifrons*. By contrast, we found stabilizing selection acting on *S. multiplicata* in mixed-species ponds (Fig. 2B), and directional selection operating on *S. bombifrons* in mixed-species ponds (Fig. 2D). Although a possible alternative explanation for these results is that there is genetic variation for growth rate and that growth rate per se influences whether individuals are more likely to develop into the carnivore morph (e.g., see Frankino and Pfennig 2001), this alternative hypothesis cannot explain why the relationship between body size and trophic morphology should vary between species and populations. Moreover, some of the smallest individuals in any of the ponds were extreme carnivore-like individuals in pure *S. bombifrons* ponds (Fig. 2C), an observation that runs counter to this alternative hypothesis. Instead, as we explain below, our measures of selection in the field suggest that how competition contributes to phenotypic divergence may be influenced not only by the intensity of competition, but also by the abundance and quality of resources (see also Pfennig et al. 2006).

For pure-species ponds, we found that disruptive selection favors extreme trophic phenotypes (Figs. 2A, C). In these ponds, individuals expressing trophic phenotypes on either end of a

Table 3. Tests for disruptive selection among experimental animals. TIME DIFF refers to the difference between tankmates in time to eat three shrimp (a measure of how similar potential competitors are in resource use). Regression terms are given, along with the estimated coefficient for each term, its standard error (SE), *t*-statistic, probability of the null hypothesis that the estimated coefficient is zero, and the Akaike information criterion (AIC). For the quadratic regression, the positive coefficient signifies disruptive selection. Bold row highlights the statistically significant regression term. Asterisk signifies the model with the best fit (i.e., the model with the lowest AIC value).

	Regression				
Term	Coefficient	SE	*T*	*P*	AIC
TIME DIFF	-5×10^{-6}	2.3×10^{-5}	−0.22	0.8230	−606.7
TIME DIFF^2	$\mathbf{6.31\times10^{-8}}$	$\mathbf{2.5\times10^{-8}}$	**2.50**	**0.0138**	**−610.9***

resource-use spectrum would likely have fewer (and, in the case of extreme omnivores, perhaps lower-quality) resources available. Nevertheless, compared with the majority of the population that may be intermediate in phenotype (and in resource use), individuals on opposite ends of the resource spectrum would also likely have fewer competitors with which to share those resources. Thus, relative to intermediate individuals, the overall fitness of omnivores and carnivores may be high (disruptive selection appears to be weaker in pure *S. bombifrons* ponds than in pure *S. multiplicata* ponds, presumably because there is less detritus in pure *S. bombifrons* ponds for omnivores to use (Pfennig et al. 2006)). Additional support that density-dependent disruptive selection favors extreme phenotypes comes from earlier field experiments, which demonstrated that the two morphs are maintained within ponds by negative frequency-dependent selection (Pfennig 1992a), which is a hallmark of competitively mediated disruptive selection (Maynard Smith 1982; Milinski and Parker 1991; Day and Young 2004; Rueffler et al. 2006). Such selection may fail to produce an evolutionary response if intermediates are recreated each generation by interbreeding between individuals that produce extreme phenotypes. Alternatively, such selection may eventually lead to the evolution of discrete resource use morphs (Smith 1990; Pfennig 1992a; Hori 1993; Robinson and Wilson 1994; Wimberger 1994; Skúkason and Smith 1995; Benkman 1996; Smith and Skúkason 1996; Maret and Collins 1997; Skúkason et al. 1999; Robinson and Parsons 2002). Moreover, the fact that many recent studies (Pfennig 1992a; Hori 1993; Benkman 1996; Robinson and Wilson 1996; Maret and Collins 1997; Kingsolver et al. 2001; Bolnick 2004) have shown that competitively mediated disruptive selection may be more common than was previously assumed (Endler 1986) implies that such selection may be a common means of promoting the evolution of divergent phenotypes.

A different mode of selection was detected among *S. multiplicata* in mixed-species ponds. Here, stabilizing selection appears to favor individuals with intermediate phenotypes (Fig. 2B). Why are extreme trophic phenotypes disfavored among *S. multiplicata* in mixed-species ponds but not in pure-species ponds? Previous work (Pfennig and Murphy 2002), along with our experimental results (Fig. 4), indicates that carnivore phenotypes in these individuals are selectively disfavored because they are competitively inferior to *S. bombifrons*. Yet, why does selection not favor omnivores, which are as distinct as possible from *S. bombifrons*? Presumably, selection acts against *S. multiplicata* omnivores in mixed-species ponds because omnivores metamorphose smaller and later than carnivores (Pomeroy 1981; Pfennig 1992a), possibly owing to their less nutritious detritus diet (recall from the *Study System* that omnivores consume the greatest fraction of detritus in their diet, and that *Spea* tadpoles grow relatively poorly on detritus (D.W. Pfennig 2000; Pfennig and Murphy 2000)). Because mixed-species ponds typically contain relatively high shrimp densities (Pfennig et al. 2006), *S. multiplicata* that express an intermediate trophic phenotype—and that can thereby supplement their detritus diet with, but not specialize on, the more nutritious shrimp resource—may be selectively favored. Thus, selection appears to favor *S. multiplicata* in mixed-species ponds that are as carnivore-like as possible while simultaneously minimizing resource overlap with *S. bombifrons*.

Finally, a third mode of selection was detected for *S. bombifrons* in mixed-species ponds. For these individuals, the most carnivore-like tadpoles were largest (Fig. 2D), indicating that directional selection favors *S. bombifrons* that are the most dissimilar from *S. multiplicata*.

Even in pure *S. multiplicata* ponds, where disruptive selection appears to be strongest, the linear selection term is significant, positive, and larger than the quadratic term (Table 2). Moreover, cubic spline analysis indicates that carnivores are larger (and, thus, perhaps have higher fitness) than omnivores (Fig. 3A). These patterns suggest that, although there is a fitness minimum within the range of the data, the net effect of selection is directional favoring carnivores, implying that disruptive selection might not produce an evolutionarily stable polymorphism as predicted by theory (Levene 1953; Mather 1955; Maynard Smith 1962; Levins 1968; Wilson 1989; Abrams et al. 1993; Rueffler et al. 2006).

Given that the net effect of selection may favor more carnivore-like individuals, why is there not an evolutionary trend toward more extreme carnivores? One answer is that, as carnivores become increasingly common in any given pond, intraspecific competition for large shrimp reduces the fitness of carnivores and increases the fitness of omnivores (Pfennig 1992a). Thus, by favoring the rarer morph, negative frequency-dependent selection maintains both morphs (Pfennig 1992a). Moreover, because mixed- and pure-species ponds exist in the same landscape (Pfennig et al. 2006), *S. bombifrons* migrants may, over a short time period, change a formerly pure *S. multiplicata* pond (where carnivores may have higher fitness than omnivores; Fig. 3A) into a mixed-species pond (where intermediates are favored; Fig. 3B); therefore, selection favoring more carnivore-like individuals may be temporary. Finally, there may be fitness trade-offs associated with producing an extreme carnivore-like phenotype (e.g., individuals with enlarged jaw muscles and heads may be poor swimmers). Indeed, in pure *S. bombifrons* ponds, which harbored the most carnivore-like individuals, extreme carnivores tended to be the smallest individuals in the pond (Fig. 3C), suggesting that selection acts against extreme carnivore-like phenotypes. Generally, frequency-dependent selection, migration, and fitness trade-offs, separately or together, may maintain evolutionarily stable polymorphism despite a seemingly unstable pattern of disruptive selection.

The pattern of selection underlying the evolution of resource polymorphism within species and ecological character displacement between species may not be strictly dependent on simply the degree to which potential competitors overlap in resource use. The quality and abundance of resources for which competition occurs will also dictate the pattern of selection across populations. Our field surveys reveal that the mode and direction of selection may vary among different types of ponds (i.e., mixed-species ponds and pure-species ponds containing each species). Indeed, in the most extreme cases of resource limitation, one species, or one morph within a species, may be eliminated from a population (Pfennig et al. 2006). Thus, variation in interactions among different types of competitors, as well as prevailing resource quality and abundance, may generate a geographical mosaic (sensu Thompson 2005) in the pattern of divergence both within and between species.

Variation among populations in the mode and direction of selection might also help explain why morph determination is mediated in part by phenotypic plasticity (Pomeroy 1981; Pfennig 1990; Pfennig and Frankino 1997; Pfennig and Murphy 2000; Frankino and Pfennig 2001). Theory predicts that natural selection should favor plasticity (as opposed to canalization) whenever external cues are available to an individual that reliably predict the fitness of alterative phenotypes in the individual's particular environment. (Charnov and Bull 1977; Lively 1986; Pfennig 1990; West-Eberhard 2003). In *Spea*, tadpoles can assess the presence of conspecific and heterospecific competitors as well as resource types and levels (e.g., see Pfennig 1990; Pfennig and Murphy 2000; Frankino and Pfennig 2001). Thus, not only might plasticity facilitate competitively mediated divergence (Pfennig and Murphy 2002), it might itself evolve in response to competition. Moreover, although plasticity might slow the evolutionary response to divergent selection under some conditions (Price et al. 2003; Rueffler et al. 2006), under other conditions it might actually hasten the evolution of different canalized phenotypes in different species and populations (West-Eberhard 1989, 2003, 2005; Pfennig et al. 2006). Indeed, different competitively mediated canalized phenotypes appear to have evolved (possibly from initially plastic responses) in different species and populations of *Spea* (Pfennig and Murphy 2002).

Our finding that selection favors *S. multiplicata* in mixed-species ponds that are as carnivore-like as possible, while minimizing overlap in resource use with *S. bombifrons*, suggests that *S. bombifrons* has "won" the competitive interaction with *S. multiplicata* in that it dominates the superior resource (i.e., large shrimp). Although the outcome of ecological character displacement is not typically framed in terms of a "winner" and a "loser," when asymmetries exist in resource quality, individuals that monopolize the higher quality resource will potentially have higher fitness (and may therefore be deemed "winners") compared to those displaced from the resource (Pfennig and Pfennig 2005). Why one species wins or loses by being displaced onto the higher or lower quality resource respectively may depend on a variety of factors, including, but not limited to, historical patterns of selection on behavior or morphology that pre-adapt individuals for resource acquisition; relative abundance of the two species; or genetic factors that limit or enable one species to evolve phenotypes capable of monopolizing the higher quality resource. In the case of spadefoots, why *S. bombifrons* are more likely to develop into carnivores is unclear. At the proximate level, they do so because they are more active than *S. multiplicata* and spend more time foraging in the water column (Pfennig and Murphy 2000). Thus, they are more likely to experience the cue—shrimp ingestion—that triggers development of the carnivore phenotype.

Finally, our finding that competitively mediated disruptive selection may promote the evolution of resource polymorphism in pure-species ponds has potentially important implications for understanding how populations evolve in response to heterospecific competitors. In particular, once a population evolves resource polymorphism, it may be more prone to undergo ecological character displacement (Pfennig and Murphy 2002; Rice and Pfennig 2007). When a population consisting of polymorphic individuals is confronted with a heterospecific competitor, it may undergo character displacement by shifting from producing alternative resource-use morphs to producing solely the morph that is less like its heterospecific competitor. In the absence of resource polymorphism, encountering a new superior competitor may drive a population extinct before it can evolve new resource-use phenotypes that lessen competition. In such a case, competitive exclusion rather than character displacement may transpire. In essence, if a population already uses an alternative resource, even at low frequencies, it may be better poised to take advantage of that resource when faced with competition for its primary resource (Rice and Pfennig 2007). The evolution of resource polymorphism (coupled with a flexible developmental system) may therefore pre-adapt populations to undergo character displacement (for a similar argument, see Robinson and Parsons 2002). Ultimately, whether and how evolutionary divergence arises may depend on a complex interplay of intra- and interspecific competition.

ACKNOWLEDGMENTS

We thank K. Pfennig and M. McGee for lab and field assistance and K. Pfennig, J. Kingsolver, B. Robinson, T. Day, and two anonymous referees for many helpful comments on the manuscript. We are also grateful to the American Museum of Natural History's (AMNH) Southwestern Research Station (SWRS) for furnishing housing and logistical support, the many landowners for allowing us to collect on their property, the Game and Fish Departments of Arizona and New Mexico for providing scientific collecting permits, and the National Science Foundation, Sigma Xi, the AMNH, and SWRS for funding.

LITERATURE CITED

Abrams, P. A. 1986. Character displacement and niche shift analyzed using consumer-resource models of competition. Theor. Pop. Biol. 29:107–160.

Abrams, P. A. 1989. The importance of intraspecific frequency-dependent selection in modeling competitive coevolution. Evol. Ecol. 3:215–220.

Abrams, P. A., H. Matsuda, and Y. Harada. 1993. Evolutionarily unstable fitness maxima and stable fitness minima of continuous traits. Evol. Ecol. 7:465–487.

Adams, D. C., and F. J. Rohlf. 2000. Ecological character displacement in *Plethodon*: biomechanical differences found from a geometric morphometric study. Proc. Natl. Acad. Sci. U.S.A. 97:4106–4111.

Altwegg, R., and H.-U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57:872–882.

Arthur, W. 1982. The evolutionary consequences of interspecific competition. Adv. Ecol. Res. 12:127–187.

Benkman, C. W. 1996. Are the bill crossing morphs in crossbills the result of frequency-dependent selection? Evol. Ecol. 10:119–126.

Bolnick, D. I. 2001. Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. Nature 410:463–466.

———. 2004. Can intraspecific competition drive disruptive selection? An experimental test in natural populations of sticklebacks. Evolution 58:608–618.

Bragg, A. N. 1965. Gnomes of the night: the spadefoot toads. Univ. of Pennsylvania Press, Philadelphia, PA.

Bragg, A. N., and W. N. Bragg. 1958. Variations in the mouth parts in tadpoles of *Scaphiopus* (*Spea*) *bombifrons* Cope (Amphibia: Salientia). Southwest. Nat. 3:55–69.

Brown, W. L., and E. O. Wilson. 1956. Character displacement. Syst. Zool. 5:49–64.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and inference: a practical information-theoretic approach. Springer-Verlag, New York.

Cannatella, D. 1999. Architecture: cranial and axial musculoskeleton. Pp. 52–91 *in* R. W. McDiarmid and R. Altig, eds. Tadpoles: the biology of anuran larvae. Univ. of Chicago Press, Chicago.

Charnov, E. L., and J. J. Bull. 1977. When is sex environmentally determined? Nature 266:828–830.

Conner, D. F., and D. S. Simberloff. 1979. The assemby of species communities: chance or competition? Ecology 60:1132–1140.

Day, T., and K. A. Young. 2004. Competitive and facilitative evolutionary diversification. BioScience 54:101–109.

Dayan, T., and D. Simberloff. 2005. Ecological and community-wide character displacement: the next generation. Ecol. Lett. 8:875–894.

Diamond, J., S. L. Pimm, M. E. Gilpin, and M. LeCroy. 1989. Rapid evolution of character displacement in Myzomelid honeyeaters. Am. Nat. 134:675–708.

Dieckmann, U., M. Doebeli, J. A. J. Metz, and D. Tautz. 2004. Adaptive speciation. Pp. 460 *in* U. Dieckmann and J. A. J. Metz, eds. Cambridge Studies in Adaptive Dynamics. Cambridge Univ. Press, Cambridge, U.K.

Doebeli, M. 1996. An explicit genetic model for ecological character displacement. Ecology 77:510–520.

Doebeli, M., and U. Dieckmann. 2000. Evolutionary branching and sympatric speciation caused by different types of ecological interactions. Am. Nat. 156:S77–S101.

Dunham, A. E., G. R. Smith, and J. N. Taylor. 1979. Evidence for ecological character displacement in western American catostomid fishes. Evolution 33:877–896.

Endler, J. A. 1986. Natural selection in the wild. Princeton Univ. Press, Princeton, NJ.

Eshel, I. 1983. Evolutionary and continuous stability. J. Theor. Biol. 103:99–111.

Fenchel, T. 1975. Character displacement and coexistence in mud snails (Hydrobiidae). Oecologia 20:19–32.

Frankino, W. A., and D. W. Pfennig. 2001. Condition-dependent expression of trophic polyphenism: effects of individual size and competitive ability. Evol. Ecol. Res. 3:939–951.

Friesen, M. L., G. Saxer, M. Travisano, and M. Doebeli. 2004. Experimental evidence for sympatric ecological diversification due to frequency-dependent competition in *Escherichia coli*. Evolution 58:245–260.

Geritz, S. A., E. Kisdi, G. Meszena, and J. A. J. Metz. 1998. Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. Evol. Ecol. 12:35–57.

Gilmore, R. J. 1924. Notes on the life history and feeding habits of the spadefoot toad of the western plains. Colorado Coll. Pub. Sci. Ser. 13:1–12.

Grant, P. R. 1972. Convergent and divergent character displacement. Biol. J. Linn. Soc. Lond. 4:39–68.

———. 1994. Ecological character displacement. Science 266:746–747.

Grant, P. R., and B. R. Grant. 2006. Evolution of character displacement in Darwin's finches. Science 313:224–226.

Gray, S. M., and B. W. Robinson. 2002. Experimental evidence that competition between stickleback species favours adaptive character divergence. Ecol. Lett. 5:264–272.

Gray, S. M., B. W. Robinson, and K. J. Parsons. 2005. Testing alternative explanations of character shifts against ecological character displacement in brook sticklebacks (*Culaea inconstans*) that coexist with ninespine sticklebacks (*Pungitius pungitius*). Oecologia 146: 25–35.

Hori, M. 1993. Frequency-dependent natural selection in the handedness of scale-eating cichlid fish. Science 260:216–219.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, N. Vignieri, C. E. Hill, A. Hoang, P. Gilbert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. Am. Nat. 157:245–261.

Kingsolver, J. G., and D. W. Pfennig. 2004. Individual-level selection as a cause of Cope's rule of phyletic size increase. Evolution 58:1608–1612.

Lack, D. 1947. Darwin's Finches. Cambridge Univ. Press, Cambridge, U.K.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1220–1226.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. Am. Nat. 87:331–333.

Levins, R. 1968. Evolution in changing environments. Princeton Univ. Press, Princeton, NJ.

Lively, C. M. 1986. Canalization versus developmental conversion in a spatially variable environment. Am. Nat. 128:561–572.

Losos, J. B. 2000. Ecological character displacement and the study of adaptation. Proc. Natl. Acad. Sci., U.S.A. 97:5693–5695.

Maerz, J. C., E. M. Myers, and D. C. Adams. 2006. Trophic polymorphism in a terrestrial salamander. Evol. Ecol. Res. 8:23–35.

Maret, T. J., and J. P. Collins. 1997. Ecological origin of morphological diversity: a study of alternative trophic phenotypes in larval salamanders. Evolution 51:898–905.

Mather, K. 1955. Polymorphism as an outcome of disruptive selection. Evolution 9:52–61.

Maynard Smith, J. 1962. Disruptive selection, polymorphism and sympatric speciation. Nature 195:60–62.

———. 1982. Evolution and the theory of games. Cambridge Univ. Press, Cambridge, U.K.

Milinski, M., and G. A. Parker. 1991. Competition for resources. Pp. 137–168 *in* J. R. Krebs and N. B. Davies, eds. Behavioural ecology: an evolutionary approach. Blackwell, Oxford, U.K.

Orton, G. L. 1954. Dimorphism in larval mouthparts in spadefoot toads of the *Scaphiopus hammondi* group. Copeia 1954:97–100.

Pacala, S. W., and J. Roughgarden. 1985. Population experiments with the *Anolis* lizards of St. Maarten and St. Eustacius. Ecology 66:129–141.

Pfennig, D. W. 1990. The adaptive significance of an environmentally cued developmental switch in an anuran tadpole. Oecologia 85:101–107.

———. 1992a. Polyphenism in spadefoot toads as a locally adjusted evolutionarily stable strategy. Evolution 46:1408–1420.

———. 1992b. Proximate and functional causes of polyphenism in an anuran tadpole. Func. Ecol. 6:167–174.

———. 1999. Cannibalistic tadpoles that pose the greatest threat to kin are most likely to discriminate kin. Proc. R. Soc. Lond. B 266:57–81.

———. 2000. Effect of predator-prey phylogenetic distance on the fitness consequences of predation: a tradeoff between nutrition and disease? Am. Nat. 155:335–345.

Pfennig, D. W., and W. A. Frankino. 1997. Kin-mediated morphogenesis in facultatively cannibalistic tadpoles. Evolution 51:1993–1999.

Pfennig, D. W., and P. J. Murphy. 2000. Character displacement in polyphenic tadpoles. Evolution 54:1738–1749.

———. 2002. How fluctuating competition and phenotypic plasticity mediate species divergence. Evolution 56:1217–1228.

———. 2003. A test of alternative hypotheses for character divergence between coexisting species. Ecology 84:1288–1297.

Pfennig, D. W., A. Mabry, and D. Orange. 1991. Environmental causes of correlations between age and size at metamorphosis in *Scaphiopus multiplicatus*. Ecology 72:2240–2248.

Pfennig, D. W., A. M. Rice, and R. A. Martin. 2006. Ecological opportunity and phenotypic plasticity interact to promote character displacement and species coexistence. Ecology 87:769–779.

Pfennig, K. S. 2000. Female spadefoot toads compromise on mate quality to ensure conspecific matings. Behav. Ecol. 11:220–227.

Pfennig, K. S., and D. W. Pfennig. 2005. Character displacement as the 'best of a bad situation': fitness trade-offs resulting from selection to minimize resource and mate competition. Evolution 59:2200–2208.

Pomeroy, L. V. 1981. Developmental polymorphism in the tadpoles of the spadefoot toad *Scaphiopus multiplicatus*. Univ. of California, Riverside, CA.

Price, T. D., Qvarnström, and D. E. Erwin. 2003. The role of phenotypic plasticity in driving genetic evolution. Proc. R. Soc. Lond. B 270:1433–1440.

Pritchard, J. R., and D. Schluter. 2001. Declining interspecific competition during character displacement: summoning the ghost of competition past. Evol. Ecol. Res. 3:209–220.

Rainey, P. B., A. Buckling, R. Kassen, and M. Travisano. 2000. The emergence and maintenance of diversity: insights from experimental bacterial populations. Trends Ecol. Evol. 15:243–247.

Rainey, P. B., and M. Travisano. 1998. Adaptive radiation in a heterogeneous environment. Nature 394:69–72.

Rice, A. M., and D. W. Pfennig. 2007. Character displacement: *in situ* evolution of novel phenotypes or sorting of pre-existing variation? J. Evol. Biol. DOI 10.1111/j.1420-9101.2006.01187.x.

Robinson, B. W., and K. J. Parsons. 2002. Changing times, spaces, and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. Can. J. Fish. Aquat. Sci. 59:1819–1833.

Robinson, B. W., and D. S. Wilson. 1994. Character release and displacement in fish: a neglected literature. Am. Nat. 144:596–627.

Robinson, B. W., and D. S. Wilson. 1996. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). Evol. Ecol. 10:631–652.

Robinson, B. W., D. S. Wilson, A. S. Margosian, and P. T. Lotito. 1993. Ecological and morphological differentiation of pumpkinseed sunfish in lakes without bluegill sunfish. Evol. Ecol. 7:451–464.

Rosenzweig, M. L. 1978. Competitive speciation. Biol. J. Linn. Soc. Lond. 10:274–289.

Roughgarden, J. 1976. Resource partitioning among competing species: a coevolutionary approach. Theor. Pop. Biol. 9:388–424.

Rueffler, C., T. J. M. Van Dooren, O. Leimar, and P. A. Abrams. 2006. Disruptive selection and then what? Trends Ecol. Evol. 21:238–245.

Ruibal, R., and E. Thomas. 1988. The obligate carnivorous larvae of the frog *Lepidobatrachus laevis* (Leptodactylidae). Copeia 1988:591–604.

Satel, S. L., and R. J. Wassersug. 1981. On the relative sizes of buccal floor depressor and elevator musculature in tadpoles. Copeia 1981:129–137.

Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. Evolution 42:849–861.

Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. Science 266:798–801.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford Univ. Press, Oxford, U.K.

Schluter, D. 2001. Ecology and the origin of species. Trends Ecol. Evol. 16:372–380.

Schluter, D. 2003. Frequency dependent natural selection during character displacement in sticklebacks. Evolution 57:1142–1150.

Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. Am. Nat. 140:85–108.

Schluter, D., T. D. Price, and P. R. Grant. 1985. Ecological character displacement in Darwin's finches. Science 227:1056–1059.

Simovich, M. A., and C. A. Sassaman. 1986. Four independent electrophoretic markers in spadefoot toads. J. Hered. 77:410–414.

Skúkason, S., and T. B. Smith. 1995. Resource polymorphisms in vertebrates. Trends Ecol. Evol. 10:366–370.

Skúkason, S., S. S. Snorrason, and B. Jónsson. 1999. Sympatric morphs, populations and speciation in freshwater fish with emphasis on arctic charr. Pp. 70–92 *in* A. E. Magurran and R. M. May, eds. Evolution of biological diversity. Oxford Univ. Press, Oxford, U.K.

Slatkin, M. 1980. Ecological character displacement. Ecology 61:163–177.

Smith, D. R. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology 68:344–350.

Smith, H. M. 1934. The amphibians of Kansas. Am. Midl. Nat. 15:377–528.

Smith, T. B. 1990. Resource use by bill morphs of an African finch: evidence for intraspecific competition. Ecology 71:1246–1257.

Smith, T. B., and S. Skúkason. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annu. Rev. Ecol. Syst. 27:111–133.

Stebbins, R. C. 2003. A field guide to western reptiles and amphibians: 3d ed. Houghton Mifflin, Boston, MA.

Storz, B. L. 2004. Reassessment of the environmental mechanisms controlling developmental polyphenism in spadefoot toad tadpoles. Oecologia 141:402–410.

Taper, M. L., and T. J. Case. 1985. Quantitative genetic models for the coevolution of character displacement. Ecology 66:355–371.

Taper, M. L., and T. J. Case. 1992. Coevolution among competitors. Oxf. Surv. Evol. Biol. 8:63–109.

Thompson, J. N. 2005. The geographic mosaic of coevolution. Univ. of Chicago Press, Chicago.

Travisano, M. 2004. Experiments on adaptation and divergence in bacterial populations. Pp. 278–300 *in* U. Dieckmann, M. Doebeli, J. A. J. Metz and D. Tautz, eds. Adaptive speciation. Cambridge Univ. Press, New York.

Turner, F. B. 1952. The mouth parts of tadpoles of the spadefoot toad, *Scaphiopus hammondii*. Copeia 1952:172–175.

Wassersug, R. J., and K. Hoff. 1979. A comparative study of the buccal pumping mechanism of tadpoles. Biol. J. Linn. Soc. Lond. 12:225–259.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Annu. Rev. Ecol. Syst. 20:249–278.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford Univ. Press, New York.

West-Eberhard, M. J. 2005. Developmental plasticity and the origin of species differences. Proc. Natl. Acad. Sci. USA 102:6543–6549.

Wilson, D. S., and M. Turelli. 1986. Stable underdominance and the evolutionary invasion of empty niches. Am. Nat. 127:835–850.

Wilson, D. S. 1989. The diversification of single gene pools by density- and frequency-dependent selection. Pp. 366–383 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.

Wimberger, P. H. 1994. Trophic polymorphisms, plasticity, and speciation in vertebrates. Pp. 19–43 *in* D. J. Stouder, K. L. Fresh, and R. J. Feller, eds. Theory and application in fish feeding ecology. Univ. of South Carolina Press, Columbia, SC.

Associate Editor: T. Day