

Disruptive Selection in Natural Populations: The Roles of Ecological Specialization and Resource Competition

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ABSTRACT: Disruptive selection is potentially critical in maintaining variation and initiating speciation. Yet there are few convincing examples of disruptive selection from nature. Moreover, relatively little is known about the causes of disruptive selection. Here, we document disruptive selection and its causes in natural populations of spadefoot toad tadpoles (*Spea multiplicata*), which are highly variable in trophic phenotype and resource use. Using a mark-recapture experiment in a natural pond, we show that selection favors extreme trophic phenotypes over intermediate individuals. We further show that such disruptive selection likely reflects both ecological specialization and resource competition. Evidence for ecological specialization comes from two field experiments, which demonstrate that extreme phenotypes forage more effectively on the main alternative resource types. Support for competition's role in disruptive selection comes from two additional experiments, which demonstrate that intermediate phenotypes, which are often the most common phenotype, compete more with each other than with extreme forms and that the intensity of disruptive selection increases with conspecific density. Generally, ecological specialization and competition are widespread, suggesting that many populations may experience some level of disruptive selection. Thus, disruptive selection may be a more common force contributing to phenotypic variation in natural populations than is currently recognized.

Keywords: density dependence, disruptive selection, ecological specialization, frequency dependence, resource competition, resource polymorphism.

Introduction

Identifying the mechanisms that generate and maintain variation within natural populations has long been a major goal of evolutionary biology. One such mechanism is disruptive selection. Disruptive selection arises when extreme phenotypes have a fitness advantage over more intermediate phenotypes (Mather 1953). By favoring extreme phenotypes, disruptive selection maintains and may even in-

crease variation in natural populations (Rueffler et al. 2006). Moreover, such selection could result in the evolution of discrete phenotypes within populations (Smith and Skúlason 1996), which might trigger speciation if these phenotypes become reproductively isolated from one another (Maynard Smith 1966; Rosenzweig 1978; Dieckmann and Doebeli 1999).

Despite its potential importance in maintaining variation and possibly initiating speciation, disruptive selection has received relatively little empirical attention. Historically, disruptive selection was assumed to be rare (Endler 1986). Yet a recent meta-analysis suggests that disruptive selection may be as common as stabilizing selection in natural populations (Kingsolver et al. 2001). Moreover, recent research has unearthed several convincing examples of disruptive selection in natural populations (Smith 1993; Medel et al. 2003; Bolnick 2004; Pfennig et al. 2007; Bolnick and Lau 2008; Calsbeek and Smith 2008; Hendry et al. 2009). Thus, disruptive selection may be more common than is generally assumed and therefore deserving of greater empirical scrutiny to understand its role in promoting phenotypic diversity.

Because few studies have focused specifically on disruptive selection in natural populations, relatively little is known of its underlying causes. A frequently cited cause of disruptive selection is ecological specialization (Smith and Skúlason 1996). According to this view, disruptive selection arises when individuals with extreme phenotypes are better adapted to alternative niches (e.g., different resource types) than are individuals with intermediate phenotypes (Levene 1953; Mather 1955; Maynard Smith 1962; Levins 1968). Such ecological specialization has been documented in several systems that are experiencing disruptive selection (Smith 1993; Robinson et al. 1996; Medel et al. 2003; Calsbeek 2009; Hendry et al. 2009).

Evolutionary biologists have long known, however, that ecological specialization, by itself, cannot lead to evolutionarily stable disruptive selection. Disruptive selection that is driven by ecological specialization alone should

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quickly break down as the population evolves to utilize a single niche. Indeed, this lack of evolutionary stability is why disruptive selection was assumed to be rare (Endler 1986). However, this view presupposes that each phenotype has a specific fitness determined by an underlying static fitness landscape (Bolnick 2004). If, instead, the fitness of each phenotype depends on its frequency in the population—that is, if fitness is frequency dependent—then the fitness landscape will be dynamic and the mean phenotype will be kept in a fitness valley, allowing for persistent disruptive selection.

A key driver of frequency-dependent fitness is intraspecific competition (Milinski and Parker 1991). Indeed, theory suggests that intraspecific competition can generate disruptive selection (Rosenzweig 1978; Wilson and Turelli 1986; Day and Young 2004; Rueffler et al. 2006). In a population that exploits a continuously varying resource gradient, intraspecific competition should cause selection to favor individuals with extreme resource-use traits, because such individuals specialize on less common but underutilized resources on either end of the resource gradient (fig. 1). This process is driven by negative frequency-dependent selection, in which rare resource-use phenotypes have a fitness advantage because of decreased competition with more common forms (Day and Young 2004; Rueffler et al. 2006).

Despite these long-standing theoretical arguments, competition's role in promoting disruptive selection has proved to be difficult to demonstrate in natural populations (Rueffler et al. 2006). Some support comes from a recent study showing that intraspecific competition for food can favor extreme trophic phenotypes in natural populations of stickleback fish (Bolnick 2004). Moreover, several studies have shown that competition generates negative frequency dependence among different resource-use phenotypes (Pfennig 1992; Hori 1993; Benkman 1996; Maret and Collins 1997), which, as noted above, is a hallmark of competitively mediated disruptive selection. Nevertheless, additional studies are needed to establish the generality of competitively mediated disruptive selection.

Another issue requiring clarification is whether both ecological specialization and intraspecific competition act in generating disruptive selection. Most studies of disruptive selection treat these two agents separately, focusing on one or the other. However, neither agent can drive persistent disruptive selection by acting alone; they must act in concert. Indeed, ecological specialization, caused by functional trade-offs among different resource-use phenotypes, is what allows for frequency-dependent competition, thereby generating disruptive selection. Yet empirical studies are needed to establish that both intraspecific competition and ecological specialization indeed act during disruptive selection.

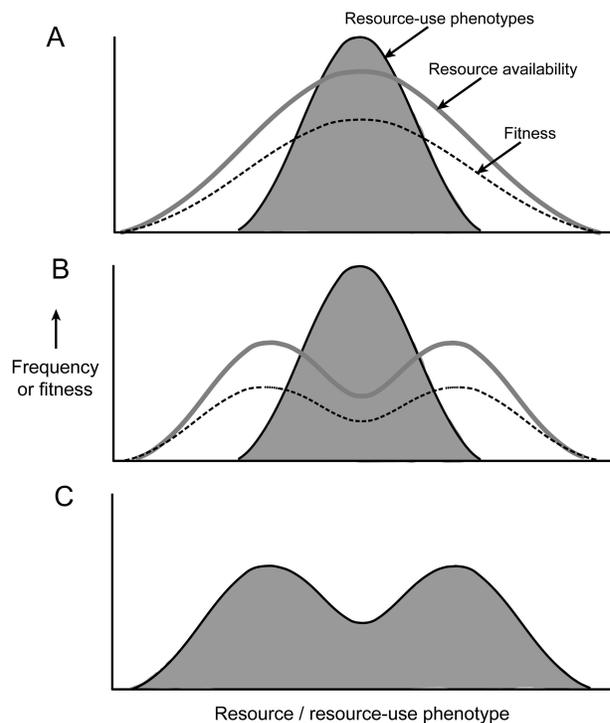


Figure 1: How intraspecific competition for resources promotes disruptive selection and maintains phenotypic variation within populations. In the plots, the shaded area represents a hypothetical population's distribution of resource-use phenotypes (shown as a quantitative trait), the dashed line shows the fitness associated with different phenotypes, and the thick gray line represents the distribution of resource types. *A*, Initially, in a population that exploits a range of resource types that are normally distributed (e.g., a range of seed sizes), selection will favor individuals that utilize the most common resource type (e.g., seeds of intermediate size). *B*, As more individuals exploit this resource type, however, it becomes depleted. Individuals that utilize the intermediate resource type will therefore experience more severe competition than those that utilize more extreme, but underexploited, resource types (e.g., very small or very large seeds). Eventually, individuals that utilize the intermediate resource type will have lower fitness than those that utilize extreme resource types, causing disruptive selection. *C*, Such selection may promote the evolution of alternative phenotypes within the same population that specialize on different resource types, that is, resource polymorphism.

Here we employ an experimental approach to address these issues in a natural population of spadefoot toads. Tadpoles of Mexican spadefoot toads (*Spea multiplicata*) express a striking resource polymorphism, the extremes of which are represented by two distinct ecomorphs: (1) an “omnivore” ecomorph, a round-bodied tadpole with a long intestine, small jaw muscles, numerous labial teeth, and smooth mouthparts used for feeding primarily on organic detritus on the pond bottom; and (2) a “carnivore” ecomorph, a narrow-bodied tadpole with a short intestine, greatly enlarged jaw muscles, few labial teeth, and notched

mouthparts used for feeding on anostracan fairy shrimp in the water column (fig. 2A). In addition, in some ponds, individuals that are intermediate between omnivores and carnivores in both morphology and resource use exist and may be the most common phenotype (Pfennig 1990).

Previous research by Pfennig et al. (2007) suggests that disruptive selection disfavors intermediate phenotypes in this system. Specifically, they sampled numerous natural populations and found that omnivores and carnivores tend to be larger at metamorphosis than intermediate individuals. They speculated that both ecological specialization and competition drive disruptive selection. Ecological specialization may select against intermediate individuals because they likely feed less effectively than extreme forms on the main alternative resource types: fairy shrimp and

detritus (fig. 2B–2E). In addition, intermediate individuals might also suffer from greater intraspecific competition for resources. Intermediates are often the most common phenotype among young tadpoles (D. W. Pfennig, unpublished data). Provided that competition is strongest between phenotypically similar individuals, intermediate individuals should face greater competition than more extreme forms.

In this study, we specifically sought to document disruptive selection and establish its causes. In particular, if both ecological specialization and intraspecific competition for resources generate disruptive selection on trophic morphology in *S. multiplicata*, then we predicted that (1) omnivores and carnivores should have higher fitness than intermediate individuals; (2) the two extreme morphs

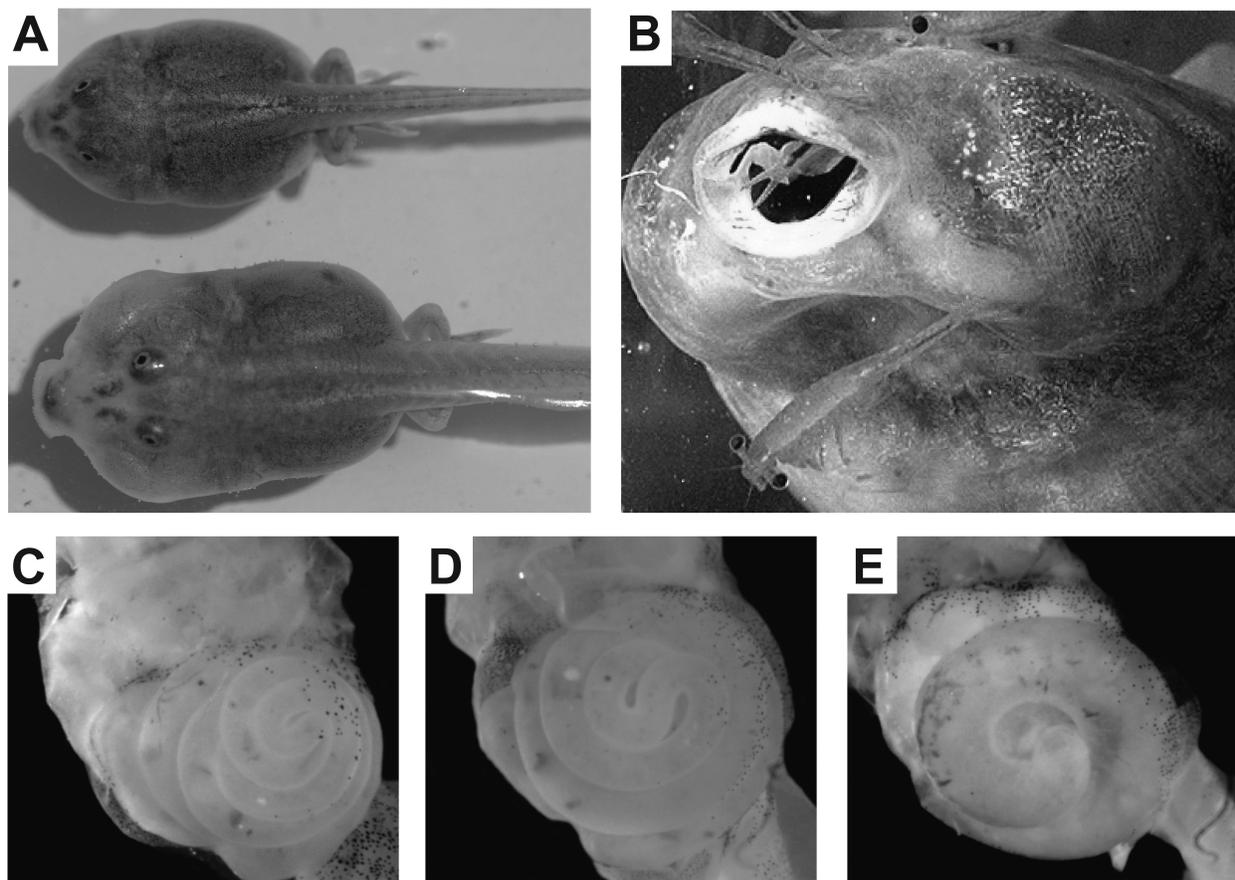


Figure 2: How ecological specialization might promote disruptive selection among spadefoot toad tadpoles (genus *Spea*). *A*, *Spea* tadpoles often occur as two distinct ecomorphs: an omnivore (*upper tadpole*) and a carnivore. In addition, many ponds also contain individuals that are intermediate between these two extreme forms. We hypothesized that intermediate individuals will generally be selected against, in part because they should feed less effectively than extreme forms on the main alternative resource types: fairy shrimp and detritus. Specifically, carnivores (*B*) have notched beaks and large jaw muscles, which should enable them to prey on fairy shrimp more effectively. *C*, Omnivores, by contrast, have longer intestines than either intermediates (*D*) or carnivores (*E*), which should enable them to process detritus more effectively. A color version of this figure is available in the online edition of the *American Naturalist*.

should outperform intermediate individuals on the two alternative resources types: specifically, omnivores should grow more than intermediate individuals when both are forced to feed on detritus, and carnivores should consume fairy shrimp faster than intermediate individuals when both are forced to feed on fairy shrimp; and (3) competition should be strongest between phenotypically similar competitors, and the strength of disruptive selection should increase with the intensity of competition. In the experiments outlined below, we sought to test each of these three predictions.

Material and Methods

Testing Prediction 1: Testing for Disruptive Selection

We evaluated the relationship between fitness and trophic morphology in a natural pond by measuring three proxies of fitness: survival, developmental stage, and body size. (The latter two likely serve as reliable proxies for fitness: there is a premium on rapid development in the ephemeral ponds in which *Spea multiplicata* typically breed [Pfennig 1992], and larval body size predicts several fitness components [Pfennig et al. 2007].) We first carried out a mark-recapture study to test whether individuals that were intermediate in trophic morphology were less likely to survive and less developmentally advanced than were individuals that were more extreme in trophic morphology. Second, within the same pond, we asked whether tadpoles intermediate in trophic morphology achieved smaller body size than individuals with more extreme trophic morphology.

To perform the mark-recapture study, we collected 554 *S. multiplicata* tadpoles from an ephemeral pond near Portal, Arizona (31°56'21.27"N, 109°05'09.82"W, 1,325 m elevation) in July 2008. *Spea multiplicata* was the only species of *Spea* present in this pond (i.e., a sympatric congener, *Spea bombifrons*, had not been seen in this pond for at least the past 14 years; D. W. Pfennig, personal observation).

Eleven days after the pond filled with water, tadpoles were collected from randomly selected sites throughout the pond with a handheld dip net. We immediately transported the tadpoles to the nearby Southwestern Research Station (SWRS). While at SWRS, the tadpoles were kept in a wading pool (1.83 m diameter × 0.3 m tall, filled 45 cm deep with dechlorinated well water) and fed ground fish food ad lib.

We identified each tadpole from its overall external morphology as an omnivore, a carnivore, or intermediate (based on the criteria outlined in Pfennig 1990) and gave it a morph-specific mark. We used a 26-gauge hypodermic needle to inject a fluorescent colored elastomer (Northwest

Marine Technology, Shaw Island, WA) into the dorsal tail membrane of each tadpole. Previous studies have shown that these marks do not differentially affect tadpole predation or survival (Pfennig and Murphy 2000; Pfennig and Rice 2007). In total, 303 omnivore tadpoles were marked with yellow elastomer, 113 carnivore tadpoles were marked with orange elastomer, and 138 tadpoles intermediate in trophic morphology were marked with blue elastomer (the three morphotypes were marked according to the proportions in which they were captured from the pond). Two days later, we released the marked tadpoles into their natal pond.

After we released the marked tadpoles, we randomly sampled (and immediately released) about 500 tadpoles twice daily and checked their developmental stage to determine when the first metamorphs were about to appear. When the first metamorphs did appear (8 days after the marked tadpoles were released and 21 days after the pond filled with water), we immediately collected 1,500 tadpoles from randomly selected sites throughout the pond using a handheld dip net. We killed these tadpoles by immersion in a 0.1% aqueous solution of tricane methanesulfonate (MS 222) and preserved them in 95% ethanol. We also searched a 2-m area around the pond's margins for any metamorphs. We recovered six metamorphs, all of which bore the mark of carnivores (it is unlikely that a significant number of metamorphs escaped our detection). We repeated this entire procedure the next day until we had sampled 3,000 tadpoles. We then examined the tail of each individual for the presence of an elastomer mark. We used these data to determine whether individuals that were given a blue elastomer (and were therefore intermediate in trophic morphology as tadpoles) were less likely to survive than individuals that bore yellow or orange marks (and were therefore more extreme in tadpole trophic morphology). We used a χ^2 test to evaluate whether a lower proportion of tadpoles with intermediate trophic morphology were recaptured than were originally marked. In addition, we noted the Gosner developmental stage (Gosner 1960) of each recaptured, marked tadpole. We then used χ^2 tests to evaluate whether marked intermediates differed from either marked omnivores or marked carnivores in developmental stage.

We next asked whether tadpoles intermediate in trophic morphology also achieved smaller body size than individuals with more extreme trophic morphology. We randomly selected 301 unmarked tadpoles from the 1,500 collected on the eighth day of the mark-recapture study and measured each tadpole's body size and trophic morphology. For body size, we measured each tadpole's snout-vent length (SVL), using handheld digital calipers. For trophic morphology, we followed the methods of Pfennig et al. (2007). Briefly, we began by measuring on each tadpole

the width of the orbitohyoideus (OH) muscle and number of rows of labial teeth (LT). We also characterized the shape of each tadpole's keratinized mouthparts (MP). We standardized OH for body size (SVL) by regressing \ln (i.e., natural log) OH on \ln SVL and used the resulting residuals for the subsequent analyses (these residuals were distributed normally). We then combined the MP, the LT, and the residuals of \ln OH regressed on \ln SVL into a single multivariate shape variable (the "morphological index"; see Pfennig et al. 2007) by calculating a principal-component score. We used PC1 (the first principal component), which explained 64% of the variance.

We tested for selection on trophic morphology by regressing body size (\ln SVL, a proxy for fitness; see above) on the morphological index (see Pfennig et al. 2007). We subjected these data to separate linear and polynomial regressions. Quadratic regression of fitness on morphology indicates disruptive performance when double the quadratic term (the selection gradient, 2γ) is significantly positive (Lande and Arnold 1983; Stinchcombe et al. 2008). We determined which regression model provided the best fit by comparing the Akaike information criterion (AIC) for each model (Burnham and Anderson 2002).

We also performed cubic-spline analyses (i.e., "non-parametric" regression), using *glmsWIN* 1.0 (provided by D. Schluter at <http://www.zoology.ubc.ca/~schluter/software.html>). Cubic-spline analysis is less sensitive to outliers than other methods and allows estimation of a fitness function without an a priori assumption about the function's shape (Schluter 1988). We used 1,000 bootstrap replicates to estimate 95% confidence intervals around the curve and then visually inspected the output to determine whether there was a minimum within the range of the data (Bolnick 2004).

Testing Prediction 2: Evaluating the Role of Ecological Specialization

We next asked whether extreme omnivores and carnivores outperform intermediate individuals on the two main resources (detritus and shrimp, respectively). We specifically predicted that omnivores should grow more than intermediate individuals when both are forced to feed on detritus and that carnivores should consume fairy shrimp faster than intermediate individuals when both are forced to feed on fairy shrimp.

We first assessed the relationship between trophic morphology and performance when tadpoles were forced to feed solely on detritus. To do so, we collected soil from the surface of a dry pond near Portal (this soil would form the detritus on which the experimental tadpoles would feed). We then spread the soil evenly across the bottom of nine wading pools (1.52 m diameter \times 0.25 m tall) to

a depth of approximately 1 cm (13 L of soil per pool). The ponds were then filled to a depth of 25 cm with dechlorinated well water. Once the soil had settled to the bottom of the pools, we placed 13 wire mesh cages (20 cm diameter \times 40 cm tall) into each pool (117 cages in total). The cages were arranged so that the bottom of each was placed firmly into the soil and the top protruded roughly 5 cm above the level of the water. Individual tadpoles were placed inside each cage 10 days after the pools were filled.

The study subjects for this experiment were *S. multiplicata* tadpoles that had been collected from a single pond near Portal 11 days after the pond filled with water. After collection and before being used in the experiment, the tadpoles were kept in a wading pool (1.83 m diameter \times 0.3 m tall) filled with dechlorinated well water and fed ground fish food ad lib. Two days after the tadpoles were collected, we selected 117 tadpoles. We specifically chose tadpoles so that the entire range of trophic morphologies (i.e., omnivores, intermediates, and carnivores) was represented. We measured each tadpole's SVL, using handheld digital calipers, and then placed each tadpole into a wire mesh cage (one tadpole per cage; the entire range of trophic morphologies was present in each wading pool). On the fourth day after the experiment began, we moved each cage within each pool to a fresh area of substrate that had not previously been foraged on. We ended the experiment on the eighth day, when we killed the tadpoles by immersion in a 0.1% aqueous solution of MS 222.

We then used the methods outlined above (see "Testing Prediction 1") to measure each tadpole's SVL, OH, MP, and LT. In addition, we used the methods of Pfennig and Murphy (2002) to measure on each tadpole the width of the interhyoideus (IH) muscle and the total gut length (GL). We standardized OH, IH, and GL for body size (SVL) by regressing the natural log of each character onto \ln SVL; we used the resulting residuals from these regressions for subsequent analyses. All regressions were significant and produced normally distributed residuals. As before (see "Testing Prediction 1"), we combined these trophic characters into a single morphological index by calculating PC1 from a principal-component analysis (PC1 explained more than 70% of the variation).

Our measure of performance was growth. We calculated each tadpole's growth as the change in body size (i.e., SVL) from the beginning to the end of the experiment. We fit a linear mixed-effect model to evaluate our prediction that individuals with extreme omnivore phenotypes grow the most on detritus. The morphological index was treated as a fixed effect, and growth was the response measure. We included each tadpole's experimental wading pool as a random effect to account for variation between pools. To

test the possibility that trophic morphology changed dramatically via phenotypic plasticity during the course of the experiment, we also asked whether a designation of tadpoles as either more omnivore-like or more carnivore-like at the start of the experiment predicted their subsequent performance, using a two-tailed *t*-test.

We next assessed the relationship between trophic morphology and performance when tadpoles were forced to feed solely on shrimp. We began by collecting 190 *S. multiplicata* tadpoles from five different ponds 8–21 days after the ponds had filled with water (mean number of tadpoles sampled per pond: 38; range: 5–60). After capture but before being used in the experiment, tadpoles were kept for 1 day in plastic aquaria without food to ensure that they were equally satiated and hence motivated to prey on shrimp.

We evaluated performance by measuring the amount of time it took each tadpole to capture and consume a live adult fairy shrimp (*Thamnocephalus* sp.; faster shrimp-handling times should be associated with higher fitness). We used the mean handling time of two trials for each tadpole in our analysis. To conduct shrimp-handling trials, we placed a single tadpole in a small, round, opaque plastic container (12 cm diameter × 6 cm deep) filled with 600 mL of dechlorinated tap water. We let the tadpole acclimate to its new surroundings for 5 minutes. We then placed 10 live fairy shrimp (15–20 mm in total length) into the container and recorded the amount of time elapsed from the moment the tadpole successfully captured a fairy shrimp until the fairy shrimp disappeared from sight inside the tadpole's mouth. In addition, we recorded the number of failed attacks on fairy shrimp for each tadpole until the tadpole successfully captured three shrimp. We recorded a failed attack when a tadpole struck at, but missed (or quickly released), a fairy shrimp.

Immediately after these feeding trials, we killed each tadpole by immersion in MS 222. We then used the methods outlined above (see “Testing Prediction 1”) to measure each tadpole's SVL, OH, IH, MP, and LT. As before (see “Testing Prediction 1”), we combined these trophic characters into a single morphological index by calculating PC1 from a principal-component analysis (PC1 explained more than 72% of the variation). To assess the relationship between trophic morphology and shrimp-handling time, we fitted a linear mixed-effect model. We treated PC1 (the morphological index) as a fixed effect, and the mean shrimp-handling time for each tadpole was the response measure. We included the pond of origin and the date of each tadpole's trial as random effects. To assess the relationship between trophic morphology and the proportion of failed attacks, we used analysis of deviance. We treated PC1 as a fixed effect, and the proportion of failed attacks recorded for each tadpole was the response measure. We

included the pond of origin and the date of each tadpole's trial as random effects.

Testing Prediction 3: Evaluating the Role of Resource Competition

To evaluate the role of intraspecific resource competition on disruptive selection, we addressed two issues. First, we determined whether competition is more intense between phenotypically similar competitors than between dissimilar competitors. Second, we evaluated whether the strength of disruptive selection increases with the intensity of intraspecific competition for resources.

To assess whether competition is more intense between phenotypically similar individuals, we generated five sibships by breeding adult *S. multiplicata* previously collected near Portal and maintained at the University of North Carolina at Chapel Hill. Two weeks after the tadpoles hatched, we randomly selected tadpoles for use in the experiment. We first measured each tadpole's mass with a digital scale and assigned each to one of two treatment groups: (1) an experimental group, in which two unrelated tadpoles were placed together in a tank (192 replicate tanks), and (2) a control group, in which one tadpole was placed alone in a tank (192 replicate tanks). Similar-sized siblings were placed in adjacent experimental and control tanks (see fig. 1A in Pfennig et al. 2007), thereby allowing us to compare the growth of siblings whose rearing environments were similar in all respects except for the presence or absence of a stimulus tankmate. Tadpoles placed together in experimental tanks were marked with differently colored elastomer marks (see “Testing Prediction 1”); tadpoles placed in the control tanks were marked with the same color as their siblings in the experimental tanks. All tanks (28 cm × 18 cm × 10 cm) were filled with 6 L of dechlorinated tap water and kept in the same room maintained at 26°C and on a 14L : 10D cycle.

Before starting the experiment, we assessed similarity in resource use for each experimental tadpole and its stimulus tankmate by following the methods of Pfennig et al. (2007). Specifically, we measured how similar experimental tadpoles and their stimulus tankmates were in their predilection to eat an equivalent number of fairy shrimp. 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 ad lib. 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. An observer then recorded how long

each tadpole took to consume all three shrimp in its container. We predicted that tankmates that were most similar in time to eat shrimp would compete the most for food (see Pfennig et al. 2007).

We started the experiment when the tadpoles were 3 weeks old. 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 *S. multiplicata* feed in natural ponds; see Pfennig et al. 2006). Control tanks received half the amount of food as experimental tanks, so 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 7 days, at which point we again measured the mass of each tadpole.

Our response measure was each tadpole's growth during the course of the experiment (change in mass), controlling for that of its matched control sibling. In particular, we subtracted from each experimental tadpole's change in mass the change in mass of its matched sibling in a neighboring control tank. Thus, values <0 indicated that the focal tadpole reared in competition grew less than did its sibling reared alone. By contrast, values >0 indicated that the focal tadpole reared in competition grew more than did its sibling reared alone.

If competition is more intense between phenotypically similar competitors, then the growth (adjusted change in mass) of experimental tadpoles should be lower the more closely they resemble their tankmate in resource use (i.e., shrimp-eating times). To test this prediction, we plotted the adjusted change in mass for all experimental tadpoles as a function of similarity to their tankmate in time to eat three shrimp. We performed regression analyses to determine whether the best-fit regression model (with the lowest AIC value) was a quadratic regression where competitors who were most similar in shrimp-eating times grew the least (see fig. 1B in Pfennig et al. 2007).

We then performed a separate experiment to assess the effect of competition on the strength of disruptive selection. We manipulated the intensity of competition by varying the density of *S. multiplicata* tadpoles in experimental ponds. The experimental design consisted of 12 wading pools (1.5 m diameter \times 0.25 m high) set up in an open field at SWRS. We assigned each pool to one of two treatments: (1) a low-conspecific-density treatment group (the "low-competition" treatment) or (2) a high-conspecific-density treatment group (the "high-competition" treatment). There were six replicate pools per treatment; different treatments were alternated spatially within the array of experimental pools.

Before starting the experiment, we provided each pool

with detritus by collecting soil from four dry ponds in which *S. multiplicata* typically breed. Soil from all four ponds was mixed thoroughly, and 13 L of the mixed soil was spread evenly across the bottom of each wading pool to a depth of approximately 1 cm. The pools were then filled to a depth of 25 cm with dechlorinated well water.

To generate tadpoles for this experiment, we collected 20 amplexed pairs of adult *S. multiplicata* from a breeding chorus near Rodeo, New Mexico (about 18 km from SWRS), and let them oviposit in separate plastic aquaria. Two days after the tadpoles hatched, we mixed 17 of the clutches together into a large wading pool and added handfuls of lettuce for food. Later that same day, we randomly chose and distributed these tadpoles into the separate experimental wading pools. We added 1,080 tadpoles to each high-competition pool and 180 tadpoles to each low-competition pool. The experimental densities lie within the range of densities found in natural ponds (Pfennig 1990).

We fed an equal amount of live, wild-caught fairy shrimp to each pool each morning, starting the day the tadpoles were added. To do so, we collected fairy shrimp each day with dip nets from two adjacent ponds near Rodeo. We then added fairy shrimp to a bucket filled with 13 L of water and, mixing the bucket between wading pools, added 500 mL of shrimp-saturated water to each pool. The amount of shrimp given to each pond was within the range of shrimp densities found in natural ponds. Moreover, the amount of shrimp was limiting; by afternoon, most pools had no shrimp.

We ended the experiment after 12 days. We captured every surviving tadpole from each pool. We killed the tadpoles by immersion in a 0.1% aqueous solution of MS 222 and preserved them in 95% ethanol. We used every tadpole we collected from the low-competition pools in the analysis. However, we randomly selected 500 tadpoles from each high-competition pool for the analysis. Using the methods described above (see "Testing Prediction 1"), we measured each tadpole's body size (SVL) and trophic morphology and recorded its Gosner developmental stage. We also measured each tadpole's mass (mg) with a digital scale. For each wading pool, we combined the trophic characters into a morphological index, as described above. We predicted that tadpoles from the high-competition pools would experience lower survival and smaller body size (ln SVL) than tadpoles from the low-competition pools if our density manipulations affected the intensity of competition for resources. To evaluate this prediction, we used separate two-tailed *t*-tests.

We then tested for selection on trophic morphology in each pool, using three fitness proxies: body size (ln SVL), mass (ln mass), and Gosner developmental stage. We separately regressed each of these three fitness proxies onto the morphological index. We performed linear and qua-

dratic regressions on the tadpoles from each pond separately. We determined which regression model provided the best fit to each data set by comparing the Akaike information criterion (AIC) for each model (Burnham and Anderson 2002). As described above (see “Testing Prediction 1”), we performed cubic-spline analyses to verify the presence of a fitness minimum (Schluter 1988; Bolnick 2004; Pfennig et al. 2007).

Because we found evidence of disruptive selection in every wading pool (table A1 in the online edition of the *American Naturalist*), we asked whether disruptive selection was stronger in high-competition pools than in low-competition pools. To do so, we determined whether the mean disruptive selection gradient, 2γ (Lande and Arnold 1983; Stinchcombe et al. 2008), calculated from the regressions on mass, body size, and developmental stage, was larger in high-competition pools than in low-competition pools, using separate two-tailed t -tests.

In addition, we evaluated whether the variance and range of trophic morphology differed between high- and low-competition pools. We did so because variation in feeding morphology could lead to variation in resource use and thereby affect the strength of competition (Bolnick et al. 2003). Feeding morphology is plastic in spadefoot toad tadpoles (Pfennig 1990), and resource competition has been shown to affect feeding morphology in other anuran tadpoles (Relyea 2004). To determine whether the variance and range of trophic morphology differed between high- and low-competition pools, we first treated individuals from all ponds as a single group and compiled a morphological index, as described above (see “Testing Prediction 1”), so that all pools could be compared on a common morphological index. We then calculated the variance and range of trophic morphology for each pool and used separate two-tailed t -tests to determine whether either the variance or the range of trophic morphology differed between high- and low-competition pools.

Results

Testing Prediction 1: Testing for Disruptive Selection

In our mark-recapture experiment, we recaptured 10% (57 of 554) of the marked tadpoles released. Furthermore, none of these tadpoles had changed to a different trophic phenotype during the course of the experiment, so any disparity between the proportion of each morph recaptured and the proportion marked can be ascribed to differences in survival and not to differences in phenotypic plasticity. As predicted, tadpoles intermediate in trophic morphology had a lower probability of survival than individuals more extreme in trophic morphology ($\chi^2_2 = 6.5$, $N = 57$, $P = .038$; fig. 3A). Moreover, recaptured

marked intermediate individuals were less developmentally advanced than were marked carnivores ($\chi^2_2 = 16.611$, $N = 26$, $P = .018$) and no more advanced than were marked omnivores ($\chi^2_2 = 5.122$, $N = 39$, $P = .1631$). Finally, not only were intermediates less likely to survive, but they were also smaller in body size than were omnivores or carnivores (table 1; fig. 3B).

Testing Prediction 2: Evaluating the Role of Ecological Specialization

As predicted (e.g., see fig. 2), individuals with the most extreme omnivore morphology were the most efficient foragers on detritus. Specifically, the more omnivore-like an individual (i.e., the lower its morphological index value), the greater was its growth when forced to feed on detritus ($F_{1,98.74} = 162.297$, $P < .0001$; fig. 4A). In addition, our original assessment of trophic morphology accurately predicted performance during the experiment. Tadpoles scored as being more omnivore-like grew more during the experiment (6.93 ± 0.19 mm) than did tadpoles scored as being more carnivore-like (3.69 ± 0.21 mm; mean difference in change in SVL between omnivores and carnivores: 3.24 mm, $t_{98} = 11.322$, $P < .0001$).

Also as predicted, individuals with the most extreme carnivore morphology were the most efficient foragers on shrimp. Specifically, the more carnivore-like an individual (i.e., the higher its morphological index value), the better (shorter) was its shrimp-handling time ($F_{1,54.16} = 79.188$, $P < .0001$; fig. 4B). Moreover, more carnivore-like individuals had a lower proportion of failed attacks on fairy shrimp ($\chi^2_1 = 12.376$, $N = 148$, $P < .0001$).

Testing Prediction 3: Evaluating the Role of Resource Competition

As predicted, competition was more intense between phenotypically similar individuals. The best-fit regression model was a quadratic regression with a positive quadratic regression coefficient (table 2). The focal tadpoles that performed the best in the experimental tanks were those that were the most dissimilar to their tankmate in foraging behavior. In contrast, the experimental tadpoles most similar to their tankmate performed the worst (fig. 5).

Also as predicted, both mean body size (ln SVL [high competition: 2.23 ± 0.02 mm, low competition: 2.83 ± 0.02 mm, $t_{10} = 21$, $P < .0001$]) and mean survival (proportion surviving [high competition: 0.69 ± 0.04 , low competition: 0.9 ± 0.04 , $t_{10} = 3.66$, $P = .0044$]; see also table A1) decreased with increased conspecific density, which serves as a measure of the intensity of competition. Furthermore, the intensity of disruptive selection increased with conspecific density (table A1). Specifically, for all three fit-

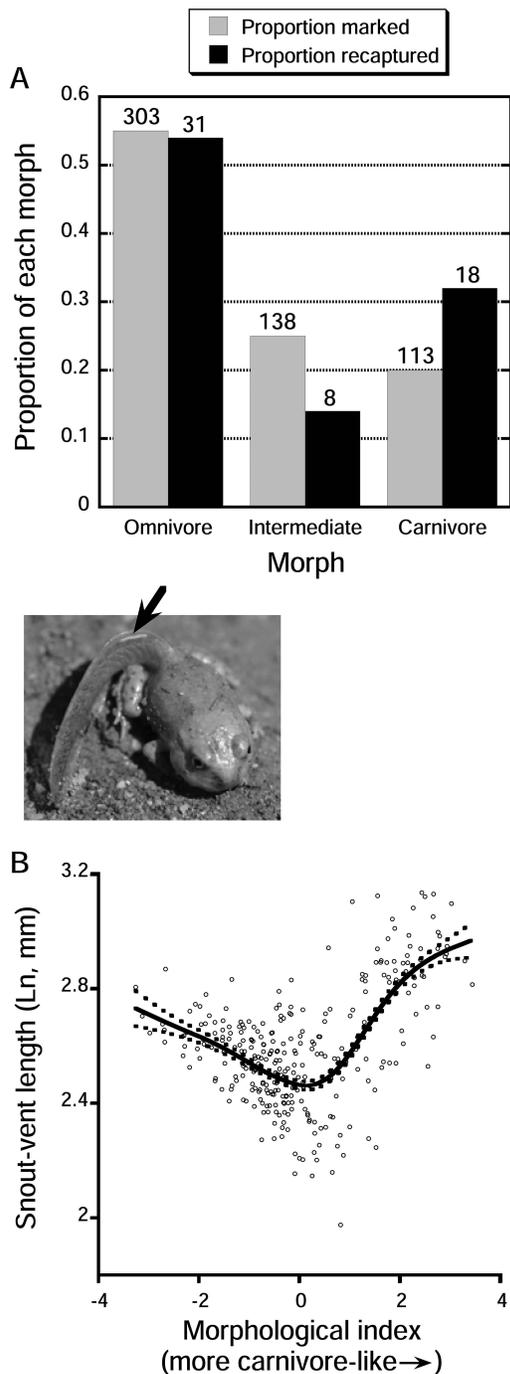


Figure 3: Evidence that disruptive selection disfavors individuals with intermediate trophic phenotypes in a natural pond. *A*, Probability of survival for omnivores, carnivores, and intermediate individuals, based on a mark-recapture experiment. Intermediates had a lower probability of survival than either carnivores or omnivores. In particular, the proportion of intermediates recaptured was lower than expected, while the proportion of omnivores recaptured was statistically equal to the proportion marked and the proportion of carnivores recaptured was

ness proxies, the mean disruptive selection gradient (2γ) was significantly larger in high-competition pools than in low-competition pools: ln SVL (high competition: 0.067 ± 0.005 , low competition: 0.043 ± 0.005 , $t_{10} = -3.254$, $P = .007$), ln mass (high competition: 0.202 ± 0.016 , low competition: 0.119 ± 0.016 , $t_{10} = -3.675$, $P = .0026$), and Gosner developmental stage (high competition: 0.568 ± 0.053 , low competition: 0.243 ± 0.053 , $t_{10} = -4.348$, $P = .0014$). Finally, the mean variance and mean range of trophic phenotypes (as measured by the extremes of the morphological index represented in each pool) were smaller in high-competition pools than in low-competition pools (variance in high-competition pools: 0.98 ± 0.14 , variance in low-competition pools: 2.79 ± 0.14 , $t_{10} = 8.903$, $P < .0001$, range in high-competition pools: 6.99 ± 0.25 , range in low-competition pools: 8.01 ± 0.25 , $t_{10} = 2.929$, $P = .015$ [see also table A1]).

Discussion

Despite its potentially critical role in maintaining variation and possibly initiating speciation, few studies have specifically tested for disruptive selection in natural populations (but see Smith 1993; Medel et al. 2003; Bolnick 2004; Pfennig et al. 2007; Bolnick and Lau 2008; Calsbeek and Smith 2008; Hendry et al. 2009). Even fewer studies have identified the agent(s) of such selection (but see Smith 1993; Medel et al. 2003; Bolnick 2004; Calsbeek 2009). We therefore sought to test for disruptive selection in a natural population of spadefoot toad tadpoles and to identify its likely causes.

Two lines of evidence indicate that selection favors extreme trophic ecomorphs in a natural population of *Spea multiplicata*. First, a mark-recapture experiment revealed that intermediate individuals were less likely to survive to metamorphosis than were either omnivores or carnivores (fig. 3A). Second, intermediates were smaller in body size (table 1; fig. 3B). These results therefore confirm the findings of a previous study, which reported a general pattern of disruptive selection acting on trophic morphology in *S. multiplicata* (Pfennig et al. 2007).

Our results also allow us to reject two alternative hy-

higher than expected (numbers above each bar show sample sizes). The photo shows a marked metamorph (mark indicated by arrow; this individual was marked as a carnivore). *B*, Cubic-spline estimate of body size (a fitness proxy) on a composite shape variable of trophic morphology (morphological index). The cubic spline (solid line) is bracketed by 95% confidence intervals (dashed lines) estimated from 1,000 bootstrap replicates. The presence of an intermediate fitness minimum suggests that disruptive selection acts on trophic morphology. A color version of this figure is available in the online edition of the *American Naturalist*.

Table 1: Tests for the mode of selection operating on trophic morphology among 301 *Spea multiplicata* tadpoles from a natural population

Regression term	Selection gradient			<i>P</i>	AIC
	($\beta/2\gamma$)	SE/2SE	<i>t</i>		
PC1	.059	.009	6.34	<.0001	−991
PC1 ²	.114	.012	9.61	<.0001	−1,088 ^a

Note: The fitness measure is ln SVL (snout-vent length). PC1 is a composite shape variable that measures trophic morphology. Also given are the estimated selection gradient for each term, its standard error (SE), *t* statistic, probability of rejecting the null hypothesis that the estimated coefficient is 0 (*P*), and Akaike information criterion (AIC). For quadratic regressions, positive selection gradients signify disruptive selection, the regression coefficient (γ) is doubled to calculate the selection gradient (2γ), and the associated SE is also doubled.

^a Model with the best fit (i.e., with the lowest AIC value).

potheses. First, because our separate measures of fitness in the mark-recapture experiment produced a remarkably similar pattern (cf. fig. 3A and 3B), we can reject the alternative hypothesis that the differences in body size reflect intrinsic differences in growth rate rather than selection against intermediates (e.g., see Pfennig et al. 2007). In particular, given that an individual's size influences its likelihood of developing into an omnivore or a carnivore (Frankino and Pfennig 2001), the fact that extreme forms are larger could be interpreted as evidence of underlying variation for growth rate (i.e., size may determine morphology, rather than the reverse). However, our discovery that intermediate individuals were also less likely to survive (fig. 3A) mitigates against this alternative hypothesis. Second, we can also reject the alternative hypothesis that intermediate tadpoles compensate for smaller size by developing faster (growth and developmental rate are often inversely correlated in larval anurans). At the end of the mark-recapture experiment, marked intermediate individuals were less developmentally advanced than were marked carnivores and no more advanced than were marked omnivores (see “Results”); it is unlikely that any developmentally advanced intermediates escaped our detection, because we sampled the pond multiple times a day throughout the course of the experiment). In addition, our wading-pool experiment revealed that disruptive selection also acts on developmental stage (see “Results”), with intermediate individuals being the least developmentally advanced. Thus, taken together, these data support the hypothesis that disruptive selection acts on trophic phenotypes in *S. multiplicata*.

Given evidence of disruptive selection, we next asked what agents drive this selection. First, we found that ecological specialization was, in part, responsible. Specifically, compared to individuals with extreme trophic phenotypes, intermediate individuals performed relatively poorly on both detritus and fairy shrimp (fig. 4). As we had predicted (fig. 2), tadpoles with the most extreme omnivore morphology grew best on detritus, whereas tadpoles with the

most extreme carnivore morphology ate fairy shrimp the fastest. Therefore, individuals expressing intermediate trophic phenotypes experience low fitness in natural ponds (fig. 3), in part because such individuals are less efficient at utilizing detritus and fairy shrimp (fig. 4), the main alternative resource types found in natural ponds (Pfennig et al. 2006). Generally, disruptive selection may often arise when individuals with extreme phenotypes are best adapted for the available niches in a particular habitat (for other possible examples of such ecological specialization, see Smith 1993; Robinson et al. 1996; Calsbeek 2009; Hendry et al. 2009).

As we noted in the “Introduction,” however, theory predicts that ecological specialization cannot drive persistent disruptive selection. As predicted by theory (fig. 1), intraspecific competition for resources also promotes disruptive selection in *S. multiplicata*. In particular, two lines of evidence implicate intraspecific competition as an agent of disruptive selection in our system. First, a critical assumption behind the theory of competitively mediated disruptive selection (see “Introduction”) is that competition should be most intense between phenotypically similar individuals (Rosenzweig 1978; Wilson and Turelli 1986; Doebeli 1996). Our results revealed that focal tadpoles that were the most similar to competitors grew the least (table 2; fig. 5). Thus, given that intermediate phenotypes are often the most prevalent phenotype among young tadpoles (D. W. Pfennig, unpublished data), intermediates should experience the greatest competition for resources. Our data therefore corroborate long-standing theory suggesting that the intensity of competition between individuals is greater the more similar they are in resource use and in phenotypes associated with resource use (Roughgarden 1976; Slatkin 1980; Abrams 1986).

A second line of evidence indicating that resource competition drives disruptive selection comes from our experimental manipulation of tadpole density, which demonstrated that the strength of disruptive selection increases with the intensity of competition. Specifically, the disrupt-

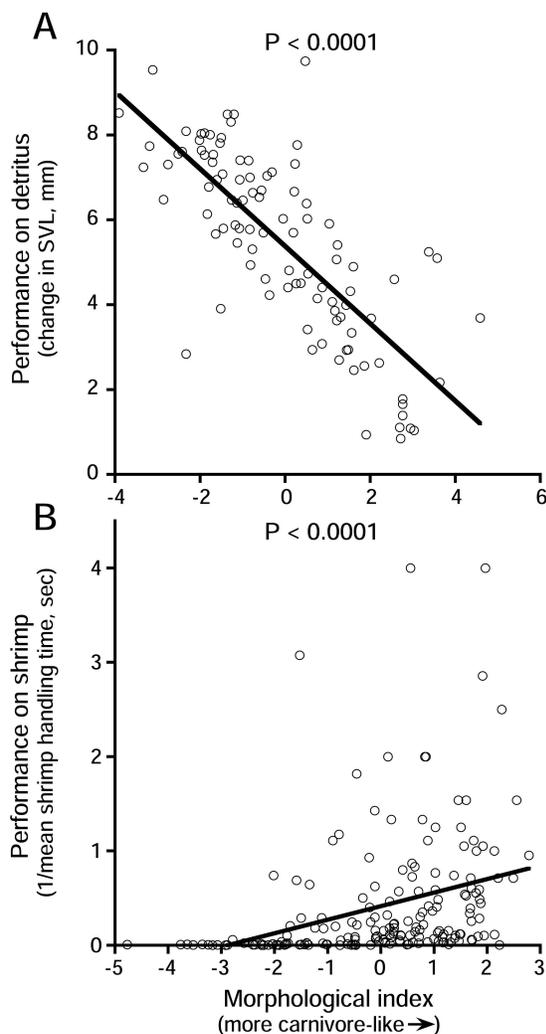


Figure 4: Experimental evidence suggesting that ecological specialization can promote disruptive selection. *A*, Trophic morphology (morphological index) predicts performance on detritus (change in snout-vent length [SVL]). In particular, there is a significant, positive relationship between the degree of omnivore morphology and growth on detritus. *B*, Trophic morphology (morphological index) predicts performance on shrimp. In particular, there is a significant, positive relationship between the degree of carnivore morphology and performance on fairy shrimp. Performance is shown as the inverse of mean shrimp-handling time. Larger performance values indicate shorter mean shrimp-handling times.

tive selection gradients were positive and significantly larger in the high-competition pools than in the low-competition pools (see “Results”). Thus, these data provide a causal link between competition and disruptive selection (for a similar finding in sticklebacks, see Bolnick 2004).

Our results therefore suggest that competition mediates disruptive selection in *S. multiplicata*. Additional support for the role of competition in generating disruptive selec-

tion in this system comes from an earlier study, which demonstrated that the two extreme ecomorphs are subject to negative frequency-dependent selection (Pfennig 1992). In particular, as carnivores become increasingly common in any given pond, intraspecific competition for shrimp reduces the fitness of carnivores and increases the fitness of omnivores (Pfennig 1992). Such frequency dependence is expected whenever individuals compete more with their own kind than with the alternative morph, as we found in this study (table 2; fig. 5). Negative frequency dependence is a hallmark of competitively mediated disruptive selection (Day and Young 2004; Rueffler et al. 2006).

Although we explicitly considered only ecological specialization and competition as agents of disruptive selection in our system, predation (specifically, cannibalism) may have also been a contributing factor. Predation’s role was suggested by the results of our wading-pool experiment. In particular, although extreme carnivores were present by the end of the experiment in both low- and high-competition wading pools, extreme omnivores were present in low-competition pools only (see “Results” and table A1). This result was surprising, because disruptive selection was stronger in high-competition pools, and therefore one would expect more extreme morphs in these pools (because extreme morphs were more highly favored under high competition). However, the absence of extreme omnivores in high-competition pools likely reflects greater predation on omnivores in these pools. Survival was lower in high-competition pools (see “Results”), and we observed frequent acts of cannibalism in these pools. *Spea* carnivores are highly cannibalistic (Bragg 1965), and they often target omnivores (D. W. Pfennig, personal observation), presumably because they are relatively slow swimmers. More importantly, because of this differential predation on omnivores, a reduced range of phenotypes was present in high-competition pools, which would have further intensified competition and disruptive selection in these pools. In general, predation and competition might often interact to promote disruptive selection.

Interestingly, the two extreme ecomorphs do not appear to be equally favored by disruptive selection. Carnivores were larger and had higher survival than either intermediates or omnivores (fig. 3). Thus, although intermediates reside at a fitness minimum (indicating disruptive selection), there also appears to be significant directional selection favoring carnivores. This raises the question of how the resource polymorphism is maintained. After all, if carnivores generally have higher fitness, selection should favor the evolution of all carnivores. Two factors appear to maintain both morphs within the same population. The first is that negative frequency-dependent competition should cause the higher fitness of carnivores to vanish (or even be reversed) if they occurred at higher frequencies than

Table 2: Tests for the effect of phenotypic similarity on the strength of competition

Regression term	Regression coefficient (β/γ)	SE	t	P	AIC
Time diff	-2.87×10^{-4}	1.89×10^{-4}	-1.52	.132	-248
Time diff ²	2.08×10^{-6}	1.02×10^{-6}	2.04	.044	-250 ^a

Note: The performance measure is Δ mass. "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). Also given are the estimated coefficient for each term, its standard error (SE), t statistic, probability of rejecting the null hypothesis that the estimated coefficient is 0 (P), and Akaike information criterion (AIC). For the quadratic regression, the positive coefficient signifies that individuals most similar to their competitor in resource use performed the worst.

^a Model with the best fit (i.e., with the lowest AIC value).

were present in our study pond (see Pfennig 1992). Second, fitness trade-offs are associated with each trophic phenotype. Compared to carnivores, omnivores invest in larger abdominal fat bodies, which increases postmetamorphic resistance to starvation (Pfennig 1992). Thus, although carnivores may often have higher survival before metamorphosis, omnivores may generally have higher survival immediately after metamorphosis (Pfennig 1992). Such fitness trade-offs likely contribute to the evolutionary maintenance of many resource polymorphisms.

Given that disruptive selection appears to be common in *S. multiplicata* (Pfennig et al. 2007) and given that heritable variation in propensity to produce different trophic phenotypes exists within natural populations (Pfennig 1999), why has the tendency to produce intermediate individuals not been eliminated? Intermediate phenotypes may be recreated in each generation if adults do not mate assortatively on the basis of larval trophic phenotype. Theoretically, such assortative mating should be possible. Carnivores and omnivores likely mature at different sizes as adults (Pfennig and Pfennig 2005), and females could potentially assess a male's size (and, hence, his probable larval phenotype) by his call. Why has the intermediate phenotype not been eliminated through such assortative mating in our populations?

One answer is that selection appears to favor intermediate individuals in some ponds. Specifically, in ponds in which *S. multiplicata* co-occurs with a congener, *Spea bombifrons*, stabilizing selection favors *S. multiplicata* individuals with intermediate phenotypes (Pfennig et al. 2007). Such mixed-species ponds may be within a few kilometers of pure *S. multiplicata* ponds in the San Simon Valley of southeastern Arizona, where our study was conducted (e.g., see Pfennig et al. 2006). Thus, *S. bombifrons* migrants may occasionally colonize nearby pure *S. multiplicata* ponds, thereby changing the selective regime within the pond to one in which intermediate phenotypes are favored. Alternatively, *S. multiplicata* may disperse into pure-species ponds from nearby mixed-species ponds, thereby carrying into these pure-species ponds their in-

herent tendencies to produce intermediate phenotypes. Either reason may explain the lack of assortative mating on larval trophic phenotype and the persistence of intermediate phenotypes in our populations. Generally, intermediate phenotypes may be difficult to eradicate via disruptive selection whenever the mode and direction of selection is spatially or temporally variable.

Although we have focused on spadefoot toads, our results have implications beyond this one system. One implication has to do with the debate over how common disruptive selection is in nature. As noted in the "Introduction," evolutionary biologists have regarded disruptive selection as

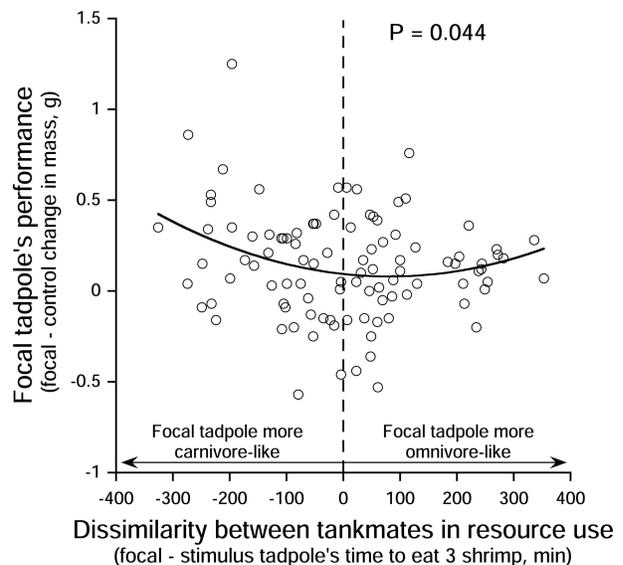


Figure 5: Experimental evidence that competition is stronger between phenotypically similar competitors than between phenotypically dissimilar competitors. Shown is the quadratic regression estimate of performance (focal tadpole's change in mass minus control tadpole's change in mass [g]) as a function of a focal animal's similarity to its tankmate in its propensity to consume three shrimp, measured before the start of the experiment. The quadratic regression term is significant and is the best-fit model.

rare because it was thought to be evolutionarily unstable (Endler 1986). However, this view presupposes a static fitness landscape (Bolnick 2004). If, instead, intraspecific competition acts to promote frequency dependence, then the fitness landscape will be dynamic and thereby allow for persistent disruptive selection (Rosenzweig 1978). Our study demonstrates that frequency-dependent competition for resources may indeed promote disruptive selection (fig. 5; see also Pfennig 1992). Given that intraspecific competition for resources is common and frequently strong in nature (Gurevitch et al. 1992), disruptive selection may be more stable, and hence more widespread, than is generally assumed (e.g., see Kingsolver et al. 2001).

An additional implication of our study concerns disruptive selection's efficacy in promoting intraspecific divergence and speciation. Generally, disruptive selection may be more effective at promoting such divergence when intraspecific competition and ecological specialization both play a major role, for at least three reasons. First, frequency-dependent competition may itself be more intense when the resource base is bimodal (Doebeli 1996), as is often the case when there is strong selection for ecological specialization (Schluter 2000). Second, when disruptive selection is driven by ecological specialization, intermediate phenotypes will be selected against even when their frequency in the population is low and competition for resources is weak. In contrast, when disruptive selection is driven primarily by frequency-dependent competition, intermediate phenotypes will be favored when they are rare. Third, assortative mating, which is necessary for the completion of speciation, is more likely to evolve when incipient species occupy discrete niches (Ripa 2009). Our finding that both ecological specialization and intraspecific competition play major roles in promoting disruptive selection in *S. multiplicata* demonstrates that both agents can act in concert, as predicted by theory (see "Introduction"). By demonstrating that these selective agents act together, our study increases the plausibility of long-standing theory (Maynard Smith 1966; Rosenzweig 1978; Dieckmann and Doebeli 1999; Ripa 2009), which holds that disruptive selection can play a general and important role in promoting the evolution of alternative phenotypes within populations and in facilitating the process of speciation.

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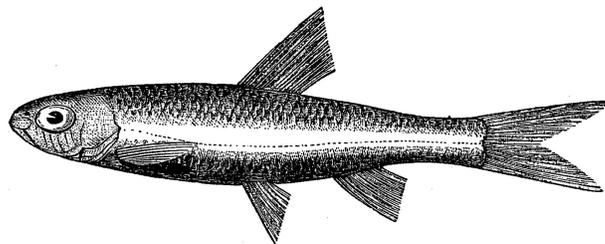
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Hybognathus osmerinus, from “Further Notes on New Jersey Fishes” by Charles C. Abbott, M.D. (*American Naturalist*, 1870, 4:717–720)