

# EVALUATING THE TARGETS OF SELECTION DURING CHARACTER DISPLACEMENT

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Ecological character displacement occurs when competition imposes divergent selection on interacting species, causing divergence in traits associated with resource use. Generally, divergence is assumed to occur when selection acts on the same, continuously varying trait in both species. However, selection might target multiple traits, and even closely related heterospecifics involved in character displacement might differ in selective targets. We investigated the targets of selection in a species of spadefoot toad, *Spea multiplicata*, during experimentally imposed competition with a congener, *S. bombifrons*. When examining traits separately, we found significant selection acting on multiple resource-acquisition traits. Yet, controlling for the independent effects of these traits in a multiple regression revealed that direct selection on a single trait might have contributed toward indirect selection on other correlated traits. Moreover, although we found evidence for plasticity in most traits, competition with *S. bombifrons* imposed selection on morphology and not on plasticity. Additional experiments suggest that the selective targets during character displacement might differ between the two species involved in this one instance of character displacement. Identifying the targets of competitively mediated selection is crucial, because whether and how character displacement ultimately unfolds depends on the nature of these targets and correlations among them.

**KEY WORDS:** Competition, divergent selection, phenotypic plasticity, selection differential, selection gradient, *Spea*, targets of selection.

Ecological character displacement arises when selection acting to lessen competition between species causes them to diverge in traits associated with resource use (Grant 1972; Day and Young 2004). This process has long been viewed as being critical in facilitating species coexistence, enhancing phenotypic differences between sympatric species, and even promoting speciation and adaptive radiation (Lack 1947; Brown and Wilson 1956; Grant 1972; Schluter 2000; Dayan and Simberloff 2005; Pfennig and Pfennig 2009; 2010).

Models of character displacement generally assume that competitively mediated selection acts on a single axis of phenotypic variation, such as a resource-acquisition trait, and that the more similar two interacting individuals are in this trait, the greater the competition between them (e.g., see Slatkin 1980; Abrams 1987; Doebeli 1996; Schluter 2000; for exceptions, see

Roughgarden 1972; Taper and Case 1985). This competition imposes selection on sympatric populations of each species to diverge. However, because resource use typically involves a complex suite of traits, competitively mediated selection would likely act on many interacting traits simultaneously. Yet, because few studies have experimentally identified the targets of selection during character displacement, this critical prediction is seldom tested empirically (but see Grant and Grant 2006; Smith and Rausher 2008a).

Identifying the targets of competitively mediated selection is crucial, because the number of traits under selection, and the nature of any correlations among these traits, could influence whether character displacement occurs. Specifically, if selection for character displacement acts on multiple traits that must be functionally coordinated for individuals that express these post

displacement phenotypes to succeed in acquiring resources (e.g., see Schluter 1993), then poor integration among these traits could reduce the bearers' fitness and thereby impede character displacement. Moreover, correlations among different traits could either facilitate (or inhibit) character displacement, depending on whether (or not) these correlations are concordant with the direction of selection acting on each trait (Lande and Arnold 1983).

Establishing the targets of selection is also important for clarifying phenotypic plasticity's role in character displacement. When facing competition, the individuals of many species facultatively express traits that lessen competitive interactions with the members of another species. For example, when confronted with a heterospecific competitor, spadefoot toad tadpoles forage for different prey (Pfennig and Murphy 2000), sunfish exploit different microhabitats (e.g., Werner and Hall 1976), and certain plants facultatively alter the extent of their root system (Nobel 1997). Traditionally, such competitively mediated plasticity has not been regarded as character displacement, because phenotypic plasticity is often viewed as a "nongenetic" response incapable of mediating adaptive evolution (e.g., see Arthur 1982; Schluter and McPhail 1992; Taper and Case 1992). Yet, the tendency to respond facultatively to a competitor, and the nature of these responses, is often genetically variable (Ledón-Rettig et al. 2010). Thus, the reaction norms that influence the expression of resource-use traits could serve as targets of selection that diverge between interacting species during character displacement (Pfennig and Pfennig 2010).

In this study, we investigated the targets of selection during character displacement in a species that has undergone ecological character displacement with a heterospecific competitor. For our study, we used a population of our focal species that is allopatric to the heterospecific competitor and that has therefore not undergone character displacement itself. This allowed us to investigate the selective targets during character displacement's early stages. As we explain below, doing so allowed us to examine how selection might have caused character displacement to unfold in our focal species.

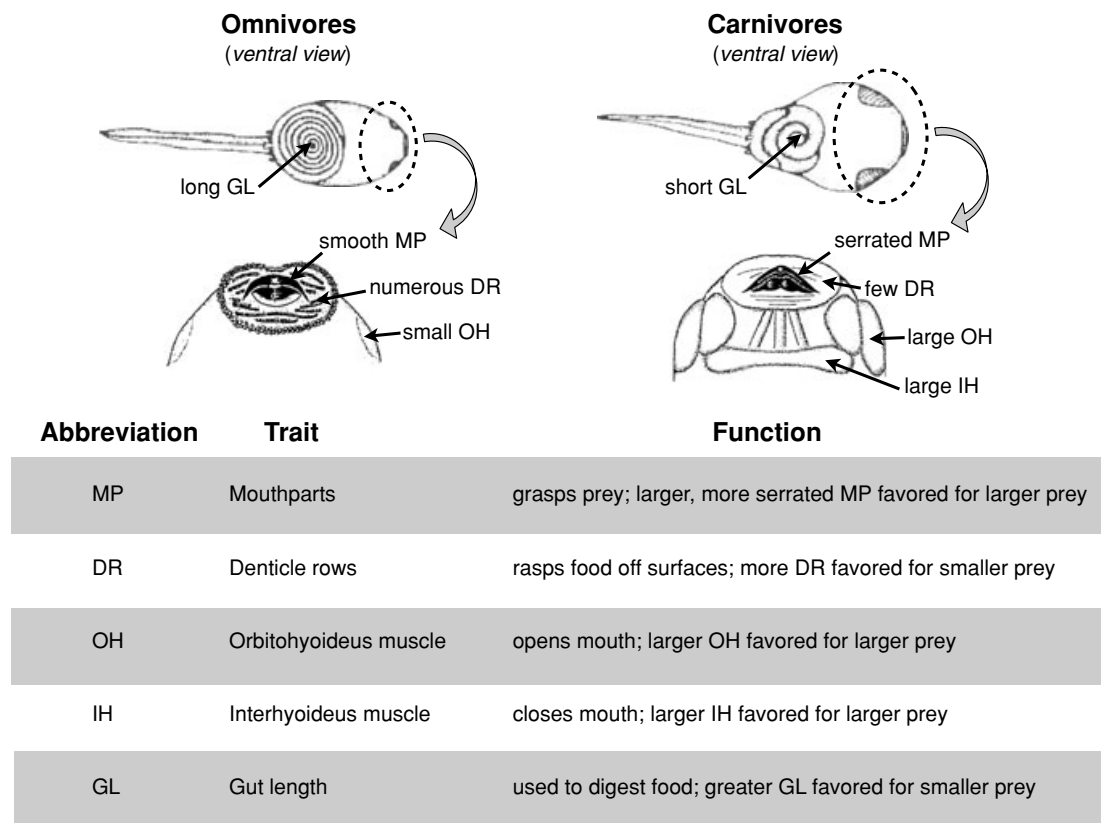
## Study System

Mexican spadefoot toads, *Spea multiplicata*, and Plains spadefoot toads, *S. bombifrons* have undergone ecological character displacement with each other (Pfennig and Murphy 2000, 2002, 2003; Pfennig et al. 2006, 2007; Rice et al. 2009; Pfennig and Martin 2009, 2010). In allopatry, both species produce similar, intermediate frequencies of two larval ecomorphs (Pfennig and Murphy 2002): (1) an "omnivore" ecomorph that specializes on organic detritus; and (2) a larger "carnivore" ecomorph that is induced by, and specializes on, anostrocan fairy shrimp (Pfennig 1992; Fig. 1). In sympatry, by contrast, larvae of each species tend to develop predominantly as a single resource-use pheno-

type: *Spea multiplicata* develop mostly as omnivores, whereas *S. bombifrons* develop mostly as carnivores (Pfennig and Murphy 2000, 2002, 2003; Pfennig et al. 2006). This divergence in morph production reflects selection to lessen interspecific competition for food: laboratory experiments have shown that the intensity of competition between *S. multiplicata* and *S. bombifrons* increases with the similarity in resource-use between them, and that competitively mediated selection therefore favors alternative resource-use ecomorphs in each species (Pfennig et al. 2007).

Character displacement in spadefoots can occur in two ways. First, when tadpoles of each species encounter one another, they facultatively produce an ecomorph that is distinct from that produced by the other species, thereby minimizing overlap in resource between them. Specifically, because *S. bombifrons* is a superior competitor for fairy shrimp (and *S. multiplicata* is a superior competitor for detritus), *S. multiplicata* is excluded from the cue (shrimp ingestion) that induces the carnivore morph (Pfennig and Murphy 2000, 2002). Second, sympatric and allopatric populations of both species differ in morph production even when their tadpoles are produced and reared under common conditions (Pfennig and Murphy 2000, 2002; Pfennig and Rice 2007; Pfennig and Martin 2010). Specifically, although *S. multiplicata* from allopatry produce both morphs, those from sympatry produce mostly omnivores, even when fed shrimp. Similarly, sympatric populations of *S. bombifrons* produce more carnivores than allopatric populations when both are fed shrimp (Pfennig and Martin 2010). For sympatric *S. multiplicata* populations in the San Simon Valley of southeastern Arizona, where character displacement in *Spea* has been most thoroughly documented, these differences in carnivore production appear to be partially explained by a condition-dependent maternal effect. Because sympatric females mature in poorer condition, they invest less into their offspring, and, consequently, produce smaller tadpoles that are more likely to develop into the smaller omnivore morph (Pfennig and Martin 2009; Martin and Pfennig 2010). In contrast, there appears to be no relationship between maternal condition and carnivore production within allopatric *S. multiplicata* (R. A. Martin, unpubl. data).

We used allopatric *S. multiplicata* and sympatric *S. bombifrons* in the experiments described below. We did so for two reasons. First, because sympatric *S. bombifrons* are more carnivore-like than allopatric *S. bombifrons* (see above), using sympatric *S. bombifrons* exerted much stronger selection on *S. multiplicata* for character displacement than using allopatric *S. bombifrons* would have. Second, using sympatric *S. bombifrons* with allopatric *S. multiplicata* mimicked the contact between these two species in the San Simon Valley, where we have documented character displacement (see above). In particular, population genetic surveys (Rice and Pfennig 2008) indicate that *S. bombifrons* has undergone a widespread range expansion out of its ancestral



**Figure 1.** Possible morphological targets of selection during character displacement in *Spea*.

range in the southern Great Plains, and that this species is a recent invader into the San Simon Valley, where allopatric *S. multiplicata* was already resident (in contrast to *S. bombifrons*, *S. multiplicata*'s range appears to have been stable; Rice and Pfennig 2008). Because *S. bombifrons* has apparently been expanding its range southwestward, thereby continually invading new habitat formerly occupied solely by *S. multiplicata*, populations of *S. bombifrons* on the wave front of the expansion (e.g., in the San Simon Valley) have likely had long evolutionary contact with *S. multiplicata*. Thus, character displacement has unfolded in the San Simon Valley when sympatric *S. bombifrons* (i.e., *S. bombifrons* that have had long evolutionary contact with *S. multiplicata*) have invaded the habitat of allopatric *S. multiplicata*, just as in our experiments.

## Materials and Methods

We investigated the targets of selection in the spadefoot toad, *S. multiplicata*, during experimentally imposed competition with a congener, *S. bombifrons*. We specifically addressed three questions. First, does specialization on different resource types (onto which interacting species are often shunted following character displacement) involve different traits? Second, during character displacement, does competitively mediated selection act solely on

morphological traits, or does it also act on phenotypic plasticity? Third, how does competitively mediated selection act on complex traits? Specifically, does selection act independently on multiple traits, or does selection also act indirectly on correlated traits? To address these three questions, we conducted three main experiments. To address our first question, we evaluated how individual traits influence performance on alternative resources (Experiment I). To address our second and third questions, we first measured phenotypic variation and plasticity in trophic morphology induced on alternative diets (Experiment II). We then evaluated how competitively mediated selection acted on this variation (Experiment III).

### EXPERIMENT I: HOW DO INDIVIDUAL TRAITS INFLUENCE PERFORMANCE ON ALTERNATIVE RESOURCES?

Martin and Pfennig (2009) evaluated the relationship between a composite measure of trophic morphology (using principal component analysis), and performance on different resource types (i.e., detritus and fairy shrimp) in *S. multiplicata*. Here we use the data from that study to dissect which traits, or trait combinations, are important for feeding on the two resources involved in the character displacement between *S. multiplicata* and *S. bombifrons*. We then interpret these data to make inferences about

whether selection likely acts on the same or on different traits (or sets of traits) during character displacement between the two species of *Spea*. We briefly outline the experimental design and morphological measurements used by Martin and Pfennig (2009) below.

To measure the performance of *S. multiplicata* tadpoles when fed solely on detritus, we placed a total of 117 *S. multiplicata* tadpoles in individual, wire mesh cages, arrayed across nine wading pools (13 cages in each pool). We covered the bottom of each wading pool with soil, previously collected from a single dry pond (this soil forms the detritus upon which experimental tadpoles feed) and filled each pool with dechlorinated water. We captured the tadpoles used in this experiment from a single pond, 11 days after a breeding chorus near Portal, AZ (where *S. multiplicata* is the only *Spea* species found). On the eighth day of the experiment, we removed the tadpoles from the wading pools, sacrificed them by immersion in MS 222, and preserved them in 95% ethanol. Our measure of performance was growth, that is, the change in SVL from the beginning to the end of the experiment. Therefore, we measured each tadpole's snout-vent-length (SVL) at the start of the experiment, and again on the eighth and final day of the experiment.

Using methods described in detail elsewhere (Pfennig and Murphy 2002; Martin and Pfennig 2009), we measured five morphological characters (Fig. 1), which previous studies suggest are possible morphological targets of selection during character displacement (Pfennig and Murphy 2000; 2002): width of the orbitohyoideus (OH) muscle, width of the interhyoideus (IH) muscle, gut length (GL), shape of the keratinized mouthparts (MP), on an ordinal scale from most omnivore-like (1) to most carnivore-like (5), and number of denticle rows (DRs). We standardized OH, IH, and GL for body size (SVL), by regressing the natural log of each character against the natural log (hereafter, "ln") of SVL, and used the resulting residuals from these regressions in our subsequent analyses. All regressions produced normally distributed residuals.

To measure the performance of *S. multiplicata* tadpoles when fed fairy shrimp, we first collected 181 *S. multiplicata* tadpoles from five different ponds near Portal, AZ. Before starting the experiment, tadpoles were kept for one day in plastic aquaria without food to ensure that they were equally starved, and hence motivated to prey on shrimp. To conduct shrimp-handling trials, we placed a single tadpole in a small, round, opaque plastic container filled with dechlorinated water, and let the tadpole acclimate to its new surroundings for 5 min. We then placed 10 live fairy shrimp into the container and recorded the amount of time elapsed from the moment the tadpole successfully captured a shrimp, until the shrimp was completely swallowed (shorter shrimp handling times should be associated with higher performance). Immediately after these trials, we sacrificed the tadpoles by immersion in MS 222, preserved them in 95% ethanol, and measured their

morphology as described previously (GL was not measured for this experiment).

We used a model selection approach (Burnham and Anderson 2002) separately for each experiment to evaluate which trophic traits best explain performance when feeding on detritus or shrimp. Our initial pool of models used to evaluate the relationship between tadpole growth and trophic morphology in the detritus feeding experiment were based on linear mixed models with wading pool as a random effect, growth on detritus as the response variable, and every linear combination of IH, OH, GL, DR, and MP (transformed and size corrected as described previously) as separate fixed effects. In addition, we standardized each trait to a mean of zero, and a variance of one before model fitting (i.e., *z*-scores).

Our initial pool of models to evaluate the relationship between tadpole feeding performance and trophic morphology in the shrimp feeding experiment was based on linear mixed models with population origin of each tadpole as a random effect, the mean time taken for the first two shrimp to be consumed as the response variable, and every linear combination of IH, OH, DR, and MP as separate fixed effects. The traits were transformed and standardized as described above.

As there was no single best model explaining performance in either experiment, we chose the subset of models with  $\Delta\text{AICc} < 2$  greater than the model with the lowest AICc (sensu Burnham and Anderson 2002). AICc (Akaike Information Criteria corrected for small sample sizes) was used in our analyses because our sample sizes divided by the number of model parameters was  $< 40$  (Burnham and Anderson 2002). We accounted for model uncertainty in the estimated effect of the parameters by performing model averaging (Burnham and Anderson 2002). Model averaging weights the model parameters of each model by the Akaike weights (i.e., model probability) of the models in which they appear.

In addition, we found little evidence that multicollinearity posed a problem in our analyses as variance inflation factors (VIFs) were  $< 4$  for all the terms considered in our models. We used maximum likelihood (ML) to estimate model parameters to compare the models in a model selection framework. In contrast, to obtain unbiased estimates of variance, we used restricted maximum likelihood (REML) to estimate the model-averaged effects.

## EXPERIMENT II: MEASURING PHENOTYPIC VARIATION AND PLASTICITY IN TROPHIC MORPHOLOGY

We characterized the degree of phenotypic variation and plasticity in trophic morphology in allopatric *S. multiplicata* by raising tadpoles from the same sibships on either detritus or fairy shrimp. We then use these data to ask if competitively mediated selection acted on this variation (Experiment III).

To generate tadpoles for this experiment, we collected 15 male:female pairs of *S. multiplicata*, from a breeding chorus near Portal, AZ. We brought the pairs to the nearby Southwestern Research Station (SWRS), where they were allowed to breed. Four days after the eggs hatched, we randomly chose tadpoles from the 15 *S. multiplicata* families and placed groups of four sibs into tanks (28 cm × 18 cm × 10 cm) filled with 600 mL of dechlorinated water. These tanks were assigned to two diet treatments (detritus vs. fairy shrimp), each with seven replicate tanks per family. The replicate tanks were spatially randomized with respect to family and treatment. To supply the detritus diet, three days prior to adding the tadpoles we covered the bottom of half the tanks with 120 mL of soil, collected from a dry pond. We fed tadpoles in the shrimp treatment (tanks lacking soil) approximately 60 live fairy shrimp twice a day for seven days. For the last four days of the experiment, tadpoles in the detritus treatment were supplemented with 20 mg of crushed fish food once a day because the soil-detritus supplied at the beginning of the experiment was becoming exhausted. Tadpoles were sacrificed by immersion in MS 222 on the eighth day and preserved in 95% ethanol. We later measured SVL, OH, MP, GL, and DR on the preserved tadpoles as described previously. Because GL measurements are time intensive, this trait was measured for one randomly chosen individual from each tank. We did not measure IH, because our experiments suggest that it is not an important predictor of feeding performance and growth on shrimp and detritus resources (see Results).

We next estimated the degree of variation in morphology and plasticity in MP, DR, GL, and OH for each family, across the two diets. To reduce skew and increase normality, we first natural log transformed MP, OH, and GL. For the traits DR and MP, we fit separate linear mixed models with diet, family, and the interaction between diet and family as fixed factors. We included tank as a random effect for each model, using REML to calculate unbiased estimates of variance. From these models, we estimated the mean trait values for MP and DR, for each family across the two diets, and separately in each diet. We tested for significant differences in these trait means between families, diet treatments, and between families within each diet treatment. In addition, we derived a measure of plasticity for each family. This measure of plasticity was the slope of the relationship between each family's mean trait values on alternate diets (i.e., a reaction norm).

We used slightly different models to estimate phenotypic variation and plasticity for the traits OH and GL. OH and GL are allometric traits and the slopes of the relationships between both morphological traits and body size differed across the diet treatments (see Results). Thus, these traits could not be corrected for differences in body size to estimate reaction norms (McCoy et al. 2006). We instead fit separate ANCOVAs for each diet

treatment with family, ln (SVL) as a covariate, and the interaction between ln (SVL) and family as fixed factors. We then extracted family means for OH and GL in each diet treatment. To measure plasticity in OH and GL, we converted the slopes of the interaction between ln (SVL) and family in each diet treatment into degree angles, and calculated their difference using the formula  $(\frac{[180 \times \arctan(\beta_{\text{shrimp}})]}{\pi}) - (\frac{[180 \times \arctan(\beta_{\text{detritus}})]}{\pi})$ . Thus, our measure of plasticity was the difference in degree angles between diet treatments, with greater differences indicating greater plasticity.

### EXPERIMENT III: ESTIMATING SELECTION ON TROPHIC MORPHOLOGY AND PLASTICITY

To evaluate which of the previously measured traits were targets of competitively mediated selection, we ran a separate experiment concurrent with Experiment II, to impose competition between *S. bombifrons* tadpoles (the stimulus species), and additional tadpoles from the 15 *S. multiplicata* sibships (the focal species) used in Experiment II. The *S. bombifrons* tadpoles used in this experiment were generated from two male:female pairs of *S. bombifrons* collected from a breeding chorus near Rodeo, NM (where both *S. multiplicata* and *S. bombifrons* are found). The *S. bombifrons* pairs were collected and bred at SWRS on the same night as the aforementioned *S. multiplicata* pairs.

We covered the bottom of 210 plastic tanks (28 cm × 18 cm × 10 cm) with 120 mL of soil (collected from the same source used in Experiment II), and filled each tank with 600 mL of dechlorinated water. Three days later, we assigned the tanks to two treatments, each with seven replicate tanks per family: (1) an interspecific competition treatment with two *S. multiplicata* tadpoles randomly chosen from the same family, and two stimulus *S. bombifrons* tadpoles, each randomly chosen from two families; and (2) a control, intraspecific competition treatment with four *S. multiplicata* tadpoles chosen from the same family. The replicate tanks were randomized spatially with respect to family and treatment, and all were supplied with detritus (provided by the soil in each tank) and 60 live fairy shrimp twice a day. For the last four days of the experiment, we supplemented the detritus diet with 20 mg of crushed fish food once a day because the soil-detritus supplied at the beginning of the experiment was becoming exhausted. On the eighth day, we sacrificed each tadpole by immersion MS 222, and stored tadpoles from each tank together in 95% ethanol.

We calculated survival for each *S. multiplicata* family in each treatment. We specifically calculated the number of replicate tanks in which at least one *S. multiplicata* tadpole died, out of the total number of replicate tanks for each family in each treatment. First, however, we determined the species identity of each surviving tadpole in the interspecific competition treatment. To do so, we amplified a 663-bp portion of the mitochondrial gene *cytochrome*



*b* using previously published sequences from each species (Rice and Pfennig 2008), and developed a restriction enzyme digest (ACC1) to discriminate tadpoles from the two species on the basis of DNA banding patterns, when run out on an agarose gel by electrophoresis.

We used three separate statistical approaches to evaluate the targets of selection imposed by interspecific competition. First, we used a model selection approach to ask which traits or combination of traits best explained variation in relative fitness (measured by survival) of *S. multiplicata* families in competition with *S. bombifrons*. Second, we calculated standardized selection differentials, and tested for significant selection on each trait. Selection differentials are a measure of total selection on a trait (sensu Conner 1988), that is the sum of selection acting directly on the trait in question, and selection acting indirectly on the trait, via selection on correlated traits. The traits used in these analyses were: (1) family-level measures of plasticity; and (2) the family-means in each diet treatment for all traits except DR. We used a single mean DR for each family, rather than separate measures of DR from each diet treatment because family means of DR from the detritus and shrimp diets were collinear ( $r = 0.90$ ,  $n = 15$ ,  $P < 0.0001$ ). Finally, to evaluate which traits might directly influence fitness, we fit a multiple regression between relative fitness and those traits exhibiting significant selection in the linear regression models, or identified as informative by their relative importance values via model selection. This allowed us to account for potential phenotypic correlations between the traits and estimate the magnitude and direction of direct selection on each trait (i.e., standardized selection gradients sensu Lande and Arnold 1983). We describe these approaches in detail below.

For our model selection approach, we regressed the relative fitness of each *S. multiplicata* family in the interspecific competition treatment against every linear combination of the trait values for each family. We calculated relative fitness in the interspecific competition treatment as the proportional survival of each family, divided by mean proportional survival, and standardized each trait from both diet treatments to a mean of zero and a variance of one (i.e., z-scores). We identified the subset of models with the greatest empirical support ( $\Delta\text{AICc} < 2$ , relative to the model with the lowest AICc), and calculated model-averaged coefficients, and standard errors.

We next estimated standardized selection differentials (i.e., regression coefficients) from individual regressions of relative fitness against the trait values for each family. We tested for significance of each selection differential in separate, generalized linear models (GLMs), (sensu Lande and Arnold 1983; Mitchell-Olds and Shaw 1987). We fit GLMs with binomial distributions and probit link functions because we scored each family's probability of survival as a binary response (each

replicate tank was scored as either zero if at least one tadpole died before the end of the experiment, or one if all tadpoles survived).

Additionally, we asked if the traits identified as targets of selection in the interspecific competition treatment (see Results) were not under selection in the control, intraspecific competition treatment. Finding that selection acted on these traits in the interspecific competition treatment, but not the control, intraspecific competition treatment would suggest that competition with *S. bombifrons*—and not some other, unmeasured factor—caused selection on these traits. Therefore, we estimated standardized selection differentials for each trait and tested for significance in the intraspecific competition treatment, using the same methods described previously.

Finally, to estimate direct selection, we calculated standardized selection gradients for the subset of traits with statistically significant selection differentials in the interspecific competition treatment, or that were identified as informative by model selection (see Results). To do so, we fit a multiple regression of relative fitness on DR, OH, MP, and GL, the latter three traits measured from the detritus diet. The standardized selection gradients are the partial regression coefficients from this model (Lande and Arnold 1983). As before, we tested for significance using a separate GLM with a binomial distribution and a probit link function. We fit the terms simultaneously to account for phenotypic correlations between the trait predictors (Table S3). In addition, we found little evidence that multicollinearity posed a problem in our multiple regression of traits on relative fitness as  $\text{VIF} < 3.5$ . However, one VIF ( $\text{VIF}_{\text{DR}} = 5.1$ ) in the separate GLM used to test significance, lies on a cut-off suggested by some authors to indicate when multicollinearity may begin to influence the error in the estimation of coefficients ( $\text{VIF} > 5$ , see Fox 2008). Although there is no consensus regarding the interpretation of VIFs (e.g., a suggested cut-off of  $\text{VIF} > 10$ ; see Kutner et al. 2004), this finding suggests that we interpret the results of our significance testing (but not our estimation of the selection gradients obtained from a separate model) with some caution.

We also estimated quadratic and correlational selection differentials in this study following the methods of Lande and Arnold (1983), and visualized the selective landscape of any significant nonlinear relationships with cubic splines (sensu Schluter 1988). We did not find any significant correlational selection coefficients and while several quadratic selection coefficients were significant, visual inspection of the fitness surface revealed there was no stable minimum or maximum in the data (Mitchell-Olds and Shaw 1988). Because we did not find evidence for quadratic or nonlinear selection we therefore do not report the nonlinear selection coefficients. All statistical analyses were performed using R (version 2.9.2; R Development Core Team, 2009).

**Table 1.** Summary of the best-fitting models with model-averaged coefficients ( $\beta$ ) and corresponding standard errors (SE) for traits explaining tadpole foraging performance on detritus, and shrimp. See Figure 1 for trait abbreviations.

Diet	Model no. (i)	$\Delta AICc$	Model wt. ( $w_i^1$ )	DR	MP	OH	IH	GL
Detritus								
	1	0.00	0.39	•		•		•
	2	0.96	0.24	•	•	•		•
	3	1.23	0.21	•		•	•	•
	4	1.76	0.16	•	•	•	•	•
	$w_{+i}^2$		1.00 (cum. $w_i^1$ )	1.00	0.40	1.00	0.37	1.00
	$\beta$			0.12	-0.05	-0.14	-0.06	0.12
	SE			0.05	0.04	0.06	0.05	0.05
Shrimp								
	1	0.00	0.65	•	•	•		—
	2	1.26	0.35		•	•		—
	$w_{+i}^2$		1.00 (cum. $w_i^1$ )	0.65	1.00	1.00	0	—
	$\beta$			0.30	-0.47	-0.71	0.00	—
	SE			0.16	0.15	0.18	0.00	—

• indicates a trait appears in a model (model no. [i]). Blank indicates absence.

<sup>1</sup> The Akaike model weight ( $w_i$ ) is the probability that  $i$  is the best model among the subset of best fitting models.

<sup>2</sup> The relative importance value ( $w_{+i}$ ) is the sum of the Akaike model weights ( $w_i$ ) for each model the term occurs in, across the candidate models of the model subset. Relative importance values range from 0 to 1, with values closer to 1 being more important in explaining performance on detritus.

## Results

### EXPERIMENT I: HOW DO INDIVIDUAL TRAITS INFLUENCE FEEDING PERFORMANCE ON ALTERNATIVE RESOURCES?

Our results indicate that traits differentially contributed to performance when *S. multiplicata* tadpoles fed on detritus, as opposed to shrimp. When tadpoles were forced to feed on detritus, model selection revealed that the strongest predictors of performance were DR, OH, and GL, which appeared in all the best-fitting models, and shared the highest relative importance values (Table 1). Furthermore, all three traits showed model-averaged effects of similar magnitude on tadpole growth. Specifically tadpoles with a greater number of DR, smaller OH muscles, and longer guts achieved higher growth on detritus (Table 1). In contrast, IH and MP occurred in only two of the selected models, had lower relative importance values, and model averaging revealed that their effects on growth were small (Table 1).

When tadpoles were forced to feed on shrimp by contrast, the strongest predictors of performance were MP and OH that appeared in all of the best-fitting models, and shared the highest relative importance values (Table 1). Furthermore, OH and MP had the strongest model-averaged effects on tadpole foraging (Table 1). Specifically, tadpoles with larger MP scores and relatively larger OH muscles had faster handling times when foraging for fairy shrimp. In contrast, DR appeared in only one model, had a lower relative importance value, and had a smaller model averaged effect on handling time than MP and OH (Table 1). IH did not appear in the subset of best-fitting models (Table 1).

### EXPERIMENT II: MEASURING PHENOTYPIC VARIATION AND PLASTICITY IN TROPHIC MORPHOLOGY

Families varied in mean morphology and plasticity in some traits (but not all traits) across the two diet treatments. Therefore, competitively mediated selection could potentially act on both morphology and plasticity in our competition experiments. Specifically, tadpoles reared on shrimp expressed a greater number of DR compared to tadpoles reared on detritus ( $F_{1,180} = 9.64$ ,  $P = 0.002$ ), suggesting that there was diet-induced plasticity for DR. Additionally, families differed in mean number of DR across both diets ( $F_{14,180} = 18.93$ ,  $P < 0.0001$ ). However, the interaction between diet and family was not significant, suggesting that there was not significant variation among families in the degree of plasticity across diets ( $F_{14,180} = 1.05$ ,  $P = 0.409$ ). Furthermore, measures of DR from the detritus and shrimp diets were collinear ( $r = 0.90$ ,  $n = 15$ ,  $P < 0.0001$ ).

Tadpoles reared on shrimp expressed more carnivore-like MP compared to tadpoles reared on detritus ( $F_{1,180} = 158.44$ ,  $P < 0.0001$ ) suggesting that there was also diet-induced plasticity for MP. As with DR, families differed in mean MP score expressed across both diets ( $F_{14,180} = 2.46$ ,  $P = 0.003$ ). Unlike with DR, the interaction between diet and family was significant for MP, suggesting that there was significant variation among families in the degree of plasticity across diets in MP phenotype ( $F_{14,180} = 2.49$ ,  $P = 0.003$ ).

The allometry of OH differed between the detritus and shrimp treatments ( $F_{1,614} = 34.63$ ,  $P < 0.0001$ ), suggesting the

**Table 2.** Summary of best-fitting models with model-averaged coefficients ( $\beta$ ) and corresponding standard errors (SE) for traits explaining relative fitness of *S. multiplicata* in competition with *S. bombifrons*. See Figure 1 for trait abbreviations.

Model no. (i)	$\Delta AIC_c$	Model wt. ( $w_i^1$ )	DR	DR <sup>5</sup>	GL <sup>3</sup>	GL <sup>4</sup>	GL <sup>5</sup>	OH <sup>3</sup>	OH <sup>4</sup>	OH <sup>5</sup>	MP <sup>3</sup>	MP <sup>4</sup>	MP <sup>2</sup>
1	0.00	0.41	•		•								
2	0.37	0.34	•		•						•		
3	0.25	0.25	•										
$W_{+i}^2$		1.00 (cum. $w_{+i}$ )	1	0	0.75	0	0	0	0	0	0.34	0	0
$\beta$			0.22	—	−0.09	—	—	—	—	—	−0.06	—	—
SE			0.04	—	0.04	—	—	—	—	—	0.03	—	—

\*indicates a trait appears in a model (model no. [i]). Blank indicates absence.

<sup>1</sup>The Akaike model weight ( $w_i$ ) is the probability that  $i$  is the best model among the subset of best fitting models.

<sup>2</sup>The relative importance value ( $w_{+i}$ ) is the sum of the Akaike model weights ( $w_i$ ) for each model the term occurs in, across the candidate models of the model subset. Relative importance values range from 0 to 1, with values closer to 1 being more important in explaining in competition with *S. bombifrons*.

<sup>3</sup>Family means from detritus diet.

<sup>4</sup> Family means from fairy shrimp diet.

<sup>5</sup> Family plasticity.

expression of diet-induced plasticity. Specifically, the slope of the relationship between OH and SVL was steeper in the shrimp diet. We therefore evaluated variation in size-corrected OH separately in each diet treatment. Families did not significantly differ in size-corrected mean OH in either the detritus ( $F_{14,90} = 0.722$ ,  $P = 0.747$ ), or shrimp diets ( $F_{14,90} = 1.18$ ,  $P = 0.307$ ).

Similarly, the allometry of GL differed between the diet treatments ( $F_{1,614} = 4.24$ ,  $P = 0.04$ ). The slope of the relationship between GL and SVL was less steep in the shrimp diet. As with OH, we therefore evaluated variation in size-corrected GL separately in each diet treatment. In addition, size-corrected mean GL in the detritus treatment did not significantly differ between families ( $F_{14,75} = 1.20$ ,  $P = 0.292$ ), but did in the shrimp treatment ( $F_{14,75} = 3.88$ ,  $P < 0.0001$ ).

**EXPERIMENT III: ESTIMATING SELECTION ON TROPHIC MORPHOLOGY AND PLASTICITY**

Interspecific competition was a significant source of mortality, relative to only intraspecific competition in the control treatment. Specifically, overall mortality was more than twice as high in the interspecific competition treatment (of both *S. multiplicata* and *S. bombifrons*: 8%; 95% C.I. = 5.6–10), than mortality in the intraspecific competition treatment (3%; 95% C.I. = 2–5.5). We did not observe any cannibalism in the experiment, suggesting that the differences in survival were driven by exploitative and/or interference competition between *S. multiplicata* and *S. bombifrons*.

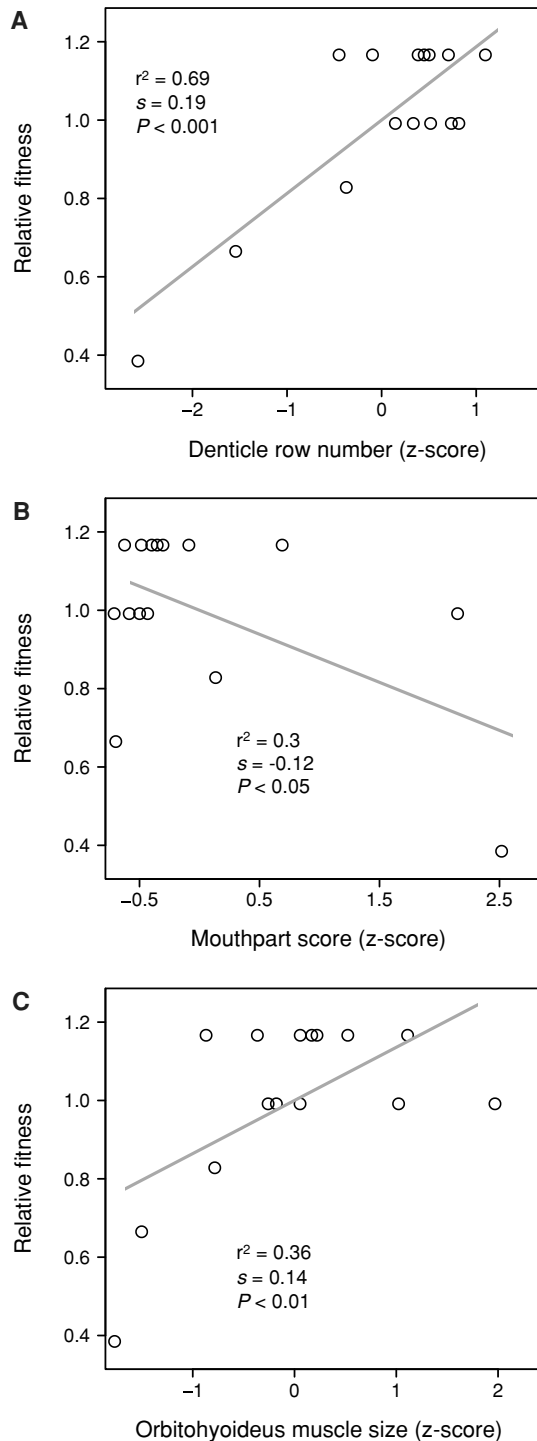
The results of our selection analyses suggest that selection imposed by competition with *S. bombifrons* acted on multiple morphological traits, and of those traits DR explained the greatest amount of variation in survival during selection. Furthermore, our results suggest that direct selection on DR may have contributed toward indirect selection on other correlated traits. We describe the results of our selection analyses in detail below.

First, using a model selection approach, we found that DR was the best predictor of survival of *S. multiplicata* in competition with *S. bombifrons*. Although GL and MP (both measured in the detritus treatment) were strong predictors DR appeared in all three of the best-fitting models, and had the highest relative importance value and greatest model-averaged effect size (Table 2).

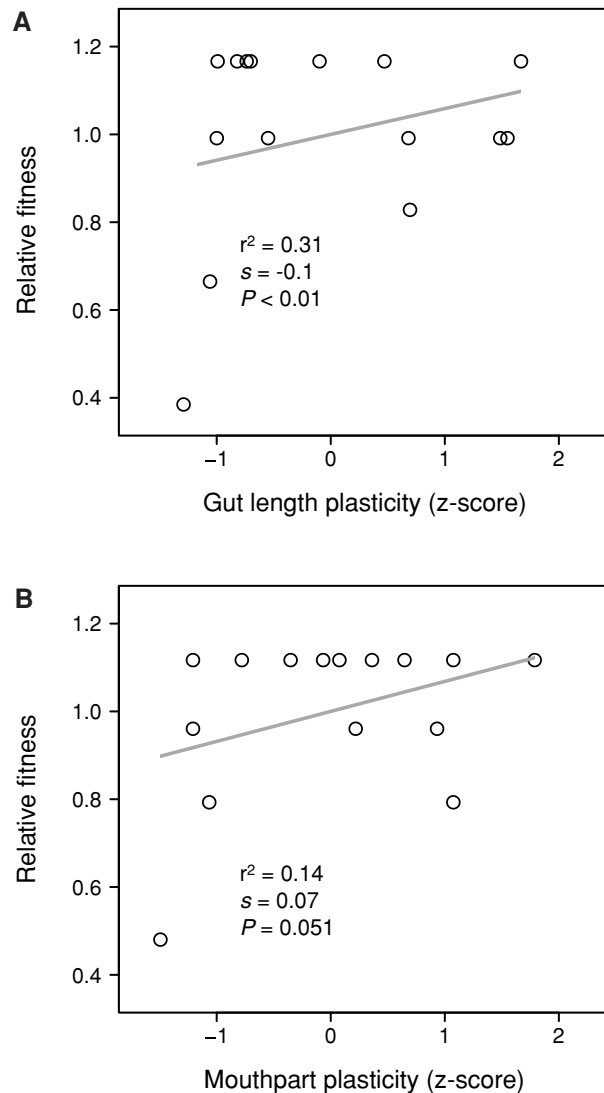
Next, our estimates of total selection (i.e., selection differentials) show evidence for selection on three traits: DR, MP (measured from the detritus treatment), and OH (measured from the detritus treatment; Fig. 2, Table S1). Specifically, families with greater mean DR experienced greater survival in the interspecific competition treatment than families with lower mean DR (Fig. 2A). MP displayed the opposite pattern: families with smaller mean MP scores (more omnivore-like) on the detritus diet experienced greater survival in the interspecific competition treatment than families with higher mean MP on the detritus diet (Fig. 2B). Surprisingly, families with larger mean OH widths on the detritus diet experienced greater survival in the interspecific competition treatment than families with smaller mean OH widths on the detritus diet (Fig. 3C). This result was unexpected, because (1) “larger” OH widths are found in carnivore-like tadpoles, whereas a greater number of DR and lower MP scores are found in omnivore-like tadpoles, and (2) tadpoles with “smaller” OH had higher performance when fed only detritus (Table 1).

In contrast, DR, MP, and OH in the detritus treatment did not have significant selection differentials in the control, intraspecific competition treatment (Table S2), suggesting that selection on these traits in the interspecific competition treatment was caused by competition with *S. bombifrons*. In fact, we found a significant selection differential for only one trait, plasticity in GL, in the control competition treatment (Table S2). Specifically, we found that families that expressed longer guts when fed shrimp, experienced lower survival than families that expressed shorter guts





**Figure 2.** *Spea multiplicata* families with greater numbers of denticle rows (A), less-carnivorous mouthparts (B), and larger orbitochoideus muscles (C) attained higher relative fitness during competition with a heterospecific competitor, *S. bombifrons*. Family means are plotted for each trait (open circles). The standardized selection differentials,  $s$ , (solid lines) are regression coefficients from linear regressions of relative fitness against each trait, standardized to a mean of one and a variance of zero. Data are jittered (by adding small, random values to the original data along the x-axis for presentation only).



**Figure 3.** *Spea multiplicata* families expressing shorter guts (A), and more-carnivorous mouthparts (B) when fed shrimp attained higher relative fitness during intraspecific competition. The relationship between relative fitness and mouthpart plasticity is marginally nonsignificant. Family means are plotted for each trait (open circles). The standardized selection differentials,  $s$ , (solid lines) are regression coefficients from linear regressions of relative fitness against each trait, standardized to a mean of one and a variance of zero. Data are jittered (by adding small, random values to the original data along the x-axis for presentation only).

when fed shrimp (Fig. 3A). In addition, we found a marginally nonsignificant trend for selection acting on plasticity in MP (Table S2). Specifically, families that expressed less carnivore-like MP when fed shrimp, tended to experienced lower survival than families that expressed more carnivore-like MP when fed shrimp (Fig. 3B)

Finally, a multiple regression with OH, MP, GL (all measured in the detritus treatment), and DR explained a large proportion of

**Table 3.** Standardized selection gradients ( $\beta$ ) and corresponding standard errors (SE) estimating direct selection on morphological traits in *S. multiplicata* tadpoles experiencing competition from *S. bombifrons*. Significance is indicated by bold face type. See Figure 1 for trait abbreviations.

Trait	df	Likelihood ratio	$[P(> \chi^2 )]$	$\beta$ (SE)
DR	1	5.78	<b>0.02</b>	0.21 (0.05)
MP <sup>1</sup>	1	0.55	0.45	-0.06 (0.03)
OH <sup>1</sup>	1	0.11	0.74	0.00 (0.04)
GL <sup>1</sup>	1	2.79	0.10	-0.1 (0.04)

<sup>1</sup>Family means from detritus diet.

variation in fitness ( $r^2 = 0.83$ ). The estimated selection gradients from this model revealed that DR had the largest individual effect on relative fitness in comparison to OH, MP, and GL (Table 3). Moreover, a GLM testing the significance of these selection gradients, found the selection gradient for DR to be significant. In contrast, the selection gradients of OH, MP, and GL were not significant (Table 3).

## Discussion

Despite character displacement's longstanding importance (Lack 1947; Brown and Wilson 1956; Grant 1972; Schluter 2000; Dayan and Simberloff 2005; Pfennig and Pfennig 2009; 2010), few studies have investigated the targets of selection during character displacement. Yet, whether and how character displacement ultimately occurs depends on the nature of these targets (e.g., whether they are morphological, developmental, or behavioral), the variation in them (e.g., whether the traits show underlying heritable variation), and the correlations between these targets and other traits (e.g., whether any such correlations are concordant with the direction of selection acting on each trait; Smith and Rausher 2008b; Pfennig and Pfennig 2009). Moreover, although models of character displacement typically assume selection acts on a single trait (see Introduction), character displacement likely encompasses a complex suite of traits that must be functionally coordinated for successful resource use. Here, we investigated the targets of selection in a spadefoot toad, *S. multiplicata*, during experimentally mediated competition with a congener, *S. bombifrons*, with which it has undergone character displacement (see Study System).

We first asked if specialization on each of two different resource types (onto each of which a different species is shunted following character displacement) favors different traits in *S. multiplicata*. Although some traits affected performance on both resources, we found that different suites of traits were important in explaining performance on the two resource types.

For example, a detritus diet favored tadpoles with a greater number of DRs, smaller OH, and longer guts (GL), whereas variation in MP, and IH had little effect (Table 1). By contrast, a shrimp diet favored tadpoles with more-carnivorous MP and larger OH (Table 1). Expressing fewer DRs also contributed toward performance on shrimp, although less so than variation in MP and OH (Table 1). Additionally, our results point to functional trade-offs between foraging on shrimp and detritus (see also Martin and Pfennig 2009). Specifically, traits that contributed toward performance on both shrimp and detritus had opposite effects on the two resources (Table 1).

We used these results to ask if selection for resource specialization might favor different traits in each of the *Spea* species involved in this instance of character displacement. Our results suggest that selection likely does target different traits in different species. Because different traits were favored when eating detritus than when eating shrimp, different traits are likely targeted in *S. multiplicata* (which specializes on detritus and produces mostly omnivores when sympatric with *S. bombifrons*) compared to *S. bombifrons* (which specializes on shrimp and produces mostly carnivores when sympatric with *S. multiplicata*). Generally, interspecific competition might not only drive divergent selection on a single trait, but it might also often drive selection on different traits in competing species, especially when competition favors the use of dissimilar resources.

We also evaluated the nature of the traits under selection during character displacement in *S. multiplicata* with *S. bombifrons*. We began by asking if competitively mediated selection acts solely on morphology, or whether it also acts on phenotypic plasticity. We found evidence of possibly heritable variation in reaction norms that influence the expression of resource-use traits. We further found that selection could indeed act on this plasticity, but only when individuals experienced intraspecific competition (Table S2). Specifically, families that expressed longer GL, and more omnivore-like MP when fed shrimp had tended to have lower survival than families that expressed shorter GLs, and more carnivore-like MP (Fig. 3). By contrast, there was no evidence that plasticity was a target of selection when individuals experienced interspecific competition (Table S1). Thus, although plasticity served as a target of selection during intraspecific competition, it did not serve as a target during interspecific competition.

Presumably, plasticity was not a target of selection in the interspecific competition treatment because there was little, if any, variation expressed during competition with *S. bombifrons* on which selection could act. In particular, because the sympatric *S. bombifrons* used in our experiment were so effective at eating shrimp and expressing the carnivore phenotype (see Study System), the *S. multiplicata* were precluded from experiencing the cue (shrimp ingestion) that induces carnivores. Thus, all *S. multiplicata* responded similarly to the presence of

*S. bombifrons* by producing few carnivores (just as *S. multiplicata* do in natural sympatric populations; see Study System).

In the Introduction, we suggested that reaction norms that influence the expression of resource-use traits could become targets of selection during character displacement. Yet, we found no evidence of this in the present study. If plasticity is not a target of selection, then how has plasticity diverged between sympatric and allopatric populations of *S. multiplicata* (Pfennig and Murphy 2002)? In particular, whereas *S. multiplicata* maintain plasticity to produce both ecomorphs in allopatry, in sympatry they have lost plasticity and have become fixed for producing only the omnivore ecomorph.

There are at least three possible explanations for how plasticity has diverged between sympatric and allopatric populations of *S. multiplicata*. First, the reaction norms that influence the expression of ecomorph production might have served as targets of selection, but we failed to detect this in the present study because we used sympatric *S. bombifrons*, which have already evolved to be different from *S. multiplicata* in morph production (note, however, that we simulated the first contact that has likely occurred between these two species in the San Simon Valley, where we have documented character displacement; see Study System). Second, plasticity might have been lost in sympatric populations through the nonselective processes of mutational degradation or genetic drift (Masel et al. 2007). Specifically, if only the omnivore ecomorph is produced in sympatric populations (because sympatric individuals do not get access to the shrimp that induces the alternative carnivore ecomorph), then any alleles that regulate the expression of the unexpressed carnivore ecomorph would not be exposed to selection and would therefore be at greater risk of chance loss (Lahti et al. 2009).

Third, a condition-dependent maternal effect might mediate the divergence in plasticity between allopatric and sympatric populations of *S. multiplicata* (Pfennig and Martin 2009). Specifically, because *S. multiplicata* are forced by *S. bombifrons* onto the less-profitable detritus resource (Pfennig and Murphy 2000), sympatric *S. multiplicata* tend to express only the smaller omnivore ecomorph, which matures as a smaller (Pfennig and Pfennig 2005) and poorer condition adult (Pfennig and Martin 2009). Presumably because of their reduced size and/or condition, female *S. multiplicata* in sympatry invest less into offspring than do female *S. multiplicata* in allopatry (Martin and Pfennig 2010). Because smaller, poorer condition females produce mostly omnivores (Pfennig and Martin 2009; Martin and Pfennig 2010), these females create offspring with a phenotype that minimizes competition with *S. bombifrons* and that thereby differs from the frequency of omnivores produced in allopatric populations of *S. multiplicata*. However, these population differences in morph production disappear once mothers are equilibrated in body condition (Pfennig and Martin 2009). Thus, a condition-dependent

maternal effect mediates the early stages of character displacement, which could explain how plasticity has diverged between sympatric and allopatric populations of *S. multiplicata*.

Given that selection for character displacement in *S. multiplicata* acts on morphological traits, we were interested in determining whether such selection targets a single trait or multiple traits. Moreover, if selection does act on multiple traits, does each trait contribute equally toward fitness? Although our data suggest that selection mediated by interspecific competition did indeed act on multiple traits, two lines of evidence suggest that a single trait—DR number—explained the greatest variation in relative fitness. First, a model selection approach suggested that DR number best predicted the relative fitness of *S. multiplicata* families when experiencing competition from *S. bombifrons* (Table 2). Second, although our estimates of total selection (i.e., the sum of direct and indirect selection acting on a trait; sensu Conner 1988) indicated significant selection for greater numbers of DRs, less-carnivorous MP, and larger OH (Fig. 2, Table S1), further analysis revealed that DR number explained the majority of variation in relative fitness when controlling for the independent effects of each trait (Table 3). Moreover, our results suggest that direct selection on DR number might have contributed to indirect selection on other, phenotypically correlated traits. As evidence of such indirect selection, we found statistically significant “direct selection” acting on DR number, but not on MP shape and OH size (Table 3).

We cannot say with certainty that the number of DRs was the only direct target of selection. Although the Lande and Arnold (1983) multiple regression approach is a powerful tool for identifying targets of selection, there are problems with this approach. For example, multicollinearity between measured traits (see Results), the failure to measure all traits affecting fitness, and low statistical power can all reduce one’s ability to identify the direct targets of selection (see Mitchell-Olds and Shaw 1987). These limitations might also have affected our ability to identify the targets of selection in this study. Experimentally manipulating traits is the best method for identifying direct targets of selection, but doing so is not always feasible (Mitchell-Olds and Shaw 1987). Nevertheless, our results suggest that selection imposed by interspecific resource competition acted on multiple traits, but that DR number was likely the “primary” target of selection.

That selection mediated by interspecific competition acts on correlated traits has implications for the speed and direction of evolution during character displacement. For example, our finding that selection mediated by interspecific competition favored larger OH in *S. multiplicata* contrasted with our a priori prediction; we had predicted that such selection would favor “smaller” OH in *S. multiplicata*. This prediction was based on our understanding of the functional morphology of this muscle (Pfennig and Murphy 2000) as well as the prior observation that *S. multiplicata* evolve to become more omnivore-like (i.e., have “smaller” OH)

when experiencing competition from *S. bombifrons* (Pfennig and Murphy 2000; 2002). One possible explanation for the discrepancy between our a priori prediction and our results is that direct selection on DR number might have contributed to “indirect” selection for larger OH in our experiment (Fig. 2C). However, because we did not measure either the heritability of these traits or the genetic correlations among them, we cannot predict the evolutionary response to this selection. Theoretically, if a population with such a correlation underlain by heritable variation were to experience competition from *S. bombifrons*, character displacement might evolve more slowly, or even be less likely to evolve in the first place. Over time, however, genetic correlations that impede character displacement should be broken down by correlational selection, (i.e., selection acting on the covariance between two traits, e.g., Brodie 1992). Although we did not find evidence of correlational selection in this study, detecting nonlinear selection generally requires large sample sizes (Kingsolver et al. 2001).

That direct selection targeted one trait primarily is surprising, however, when one considers that character displacement often generates divergent ecomorphs that differ in entire suites of characters (e.g., see Schluter 1993; Martin and Pfennig 2009). Yet, competitively mediated selection primarily acting directly on a single trait might facilitate the production of complex ecomorphs through correlated evolution. Indeed, direct selection acting primarily on a single trait that is correlated with numerous other traits might occur more readily than independent selection acting on multiple traits that must also become coordinated in function. In other words, divergence (and the origin of novel, integrated traits) might be fostered by selection acting both directly and indirectly: directly on a key trait involved in resource acquisition, and indirectly on already associated traits that can be pulled along (e.g., Pfennig and Pfennig 2005).

In conclusion, identifying the targets of character displacement is crucial because the types of traits under selection, the amount of variation in them, and their underlying genetic architecture can influence whether and how character displacement occurs (Smith and Rausher 2008b; Pfennig and Pfennig 2009). Moreover, because character displacement often occurs independently in separate populations (Schluter and McPhail 1992; Rice et al. 2009; Adams 2010), character displacement could potentially unfold differently in different populations, thereby further enhancing diversity.

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## Supporting Information

The following supporting information is available for this article:

**Table S1.** Standardized selection differentials ( $s$ ) and corresponding standard errors (SE) estimating total selection on morphological traits in *S. multiplicata* tadpoles experiencing competition from *S. bombifrons*.

**Table S2.** Standardized selection differentials ( $s$ ) and corresponding standard errors (SE) estimating total selection on morphological traits in *S. multiplicata* tadpoles experiencing intraspecific competition.

**Table S3.** Pearson correlation coefficients of the phenotypic correlations between morphological traits measured from 15 *S. multiplicata* families.

Supporting Information may be found in the online version of this article.

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