

Parallel evolution and ecological selection: replicated character displacement in spadefoot toads

Amber M. Rice, Aaron R. Leichty and David W. Pfennig

Proc. R. Soc. B 2009 **276**, 4189-4196 first published online 2 September 2009
doi: 10.1098/rspb.2009.1337

Supplementary data

["Data Supplement"](#)

<http://rsbp.royalsocietypublishing.org/content/suppl/2009/09/02/rspb.2009.1337.DC1.html>

References

[This article cites 40 articles, 7 of which can be accessed free](#)

<http://rsbp.royalsocietypublishing.org/content/276/1676/4189.full.html#ref-list-1>

Subject collections

Articles on similar topics can be found in the following collections

[ecology](#) (956 articles)

[evolution](#) (1135 articles)

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)

To subscribe to *Proc. R. Soc. B* go to: <http://rsbp.royalsocietypublishing.org/subscriptions>

Parallel evolution and ecological selection: replicated character displacement in spadefoot toads

Amber M. Rice^{*,†}, Aaron R. Leichty and David W. Pfennig

Department of Biology, University of North Carolina, Chapel Hill, NC, USA

Ecological character displacement—trait evolution stemming from selection to lessen resource competition between species—is most often inferred from a pattern in which species differ in resource-use traits in sympatry but not in allopatry, and in which sympatric populations within each species differ from conspecific allopatric populations. Yet, without information on population history, the presence of a divergent phenotype in multiple sympatric populations does not necessarily imply that there has been repeated evolution of character displacement. Instead, such a pattern may arise if there has been character displacement in a single ancestral population, followed by gene flow carrying the divergent phenotype into multiple, derived, sympatric populations. Here, we evaluate the likelihood of such historical events versus ongoing ecological selection in generating divergence in trophic morphology between multiple populations of spadefoot toad (*Spea multiplicata*) tadpoles that are in sympatry with a heterospecific and those that are in allopatry. We present both phylogenetic and population genetic evidence indicating that the same divergent trait, which minimizes resource competition with the heterospecific, has arisen independently in multiple sympatric populations. These data, therefore, provide strong indirect support for competition's role in divergent trait evolution.

Keywords: character displacement; competition; divergent evolution; parallel evolution; population history

1. INTRODUCTION

Ecological character displacement comes about when individuals most dissimilar from the average resource-use phenotype of another species are selectively favoured, thereby causing the species to diverge in resource use and associated traits (Slatkin 1980; Taper & Case 1992). Because this process has long been seen as crucial in explaining how closely related species are able to coexist in the same habitat (Brown 1995), considerable effort has gone into documenting character displacement in natural populations (Brown & Wilson 1956; Grant 1972; Schluter 2000a; Dayan & Simberloff 2005). Much of this evidence is indirect (but see Grant & Grant 2006), in that character displacement is often inferred from patterns of trait expression in different species and populations. For example, because selection to diverge from a heterospecific competitor acts only in areas where the two species actually co-occur (i.e. sympatry), character displacement should produce a distinctive pattern in which potential competitors are more dissimilar in sympatry than in allopatry, and in which sympatric populations within each species differ from conspecifics in allopatric populations (Brown & Wilson 1956; Grant 1972; Endler 1986; Schluter 2000a; but see Goldberg & Lande 2006). These patterns of geographical variation are widely used to infer that

ecological character displacement has taken place (reviewed in Schluter 2000a; Dayan & Simberloff 2005).

Some of the strongest indirect support for character displacement comes from studies showing that divergent phenotypes occur in *multiple* sympatric populations (Schluter & McPhail 1993). Such data are generally taken as evidence of 'parallel' character displacement. Parallel character displacement is a special case of parallel evolution that occurs when a similar trait evolves repeatedly in closely related, independently evolving lineages. Parallel divergence is compelling evidence that selection has promoted trait evolution (Clarke 1975; Endler 1986; Schluter & Nagel 1995). Thus, data showing that a divergent trait has arisen repeatedly in closely related, independently evolving sympatric populations would provide strong support for competition's role in promoting divergence between species and populations.

Demonstrating that alleged instances of parallel character displacement have occurred cannot be accomplished, however, by merely showing that a divergent phenotype is present in multiple sympatric populations. Such a pattern can arise for evolutionary reasons other than selection (Grant 1972; Arthur 1982; Losos 1992). In particular, gene flow may produce a similar pattern, but this possibility is rarely considered (for exceptions, see Hansen *et al.* 2000; Marko 2005; Matocq & Murphy 2007). For example, a divergent phenotype may originate in a single ancestral sympatric population, either because of selection to lessen resource competition (i.e. character displacement) or possibly even because of genetic drift. If high levels of gene flow connect

* Author for correspondence (amber.rice@ebc.uu.se).

† Present address: Department of Animal Ecology, Evolutionary Biology Center, Uppsala University, Uppsala, Sweden.

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2009.1337> or via <http://rsob.royalsocietypublishing.org>.

numerous sympatric populations, or if colonization into sympatry is frequent, then multiple sympatric populations may come to exhibit the divergent phenotype (figure 1*a–c*). However, in all of these derived sympatric populations, the presence of the divergent phenotype would be due to *gene flow* and not to selection promoting parallel evolution. Moreover, if the divergent phenotype arose in the ancestral sympatric population through genetic drift, then character displacement would have played no role in its evolution, despite the presence of this phenotype in multiple, sympatric populations. Thus, in the absence of information on population history, the presence of a divergent phenotype in multiple sympatric populations cannot necessarily be construed as evidence that selection has promoted the repeated evolution of divergent phenotypes in sympatric populations; i.e. that parallel character displacement has occurred. Indeed, as this example illustrates, non-selective processes may ultimately be more important than selection in explaining why species and populations differ.

Here, we test for parallel character displacement in spadefoot toads (*Spea multiplicata*). In the San Simon Valley of southeastern Arizona and southwestern New Mexico, USA, this species potentially breeds in the same ponds with a congener, *Spea bombifrons* (figure 2). In this region, both species co-occur below 1350 m in elevation (hereafter termed 'sympatry'). At higher elevations, by contrast, only *S. multiplicata* is present (hereafter termed 'allopatry'; Pfennig *et al.* 2006). Larvae of both species may develop into either a small-headed omnivore morph, which feeds mostly on organic detritus, or a large-headed carnivore morph, which specializes on anostracan fairy shrimp (Pomeroy 1981; Pfennig 1990, 1992). The carnivore morph is induced by ingestion of shrimp (Pomeroy 1981; Pfennig 1990), but heritable variation exists within populations in propensity to respond to the shrimp cue by producing the morphologically and behaviourally distinctive carnivore morph (Pfennig & Frankino 1997; Pfennig 1999).

These two species have undergone ecological character displacement in tadpole morph production (Pfennig & Murphy 2000, 2003; Pfennig *et al.* 2006, 2007). In ponds (populations) where each species occurs alone, both species produce similar, intermediate frequencies of each morph. However, in populations where they co-occur, *S. multiplicata* shift to producing mostly omnivores, whereas *S. bombifrons* shift to producing mostly carnivores (Pfennig & Murphy 2000, 2003; Pfennig *et al.* 2006). Such trait divergence is seen in multiple populations of *S. multiplicata* (Pfennig & Murphy 2000, 2003; Pfennig *et al.* 2006). Moreover, these differences appear to be heritable: *S. multiplicata* tadpoles of parents collected from sympatry are less likely to become carnivores than those of parents collected from nearby allopatry, even when reared under common conditions (Pfennig & Murphy 2000, 2002). Finally, experiments reveal that divergence between species and populations in morph production has probably evolved because of selection acting to lessen interspecific competition for food (Pfennig & Murphy 2000; Pfennig *et al.* 2007).

Here, we test for parallel character displacement in *S. multiplicata*. Our data suggest that the same divergent trait—a more omnivore-like tadpole that minimizes

resource competition with *S. bombifrons*—has arisen independently in multiple sympatric populations.

2. MATERIAL AND METHODS

To assess the relative importance of current competitive environment versus population history in explaining patterns of phenotypic divergence between allopatric and sympatric populations of *S. multiplicata*, we undertook two complementary approaches—a phylogenetic and a population genetic approach. Using these approaches, we evaluated whether variation among populations in trophic morphology is significantly correlated with variation in the competitive environment, even after controlling for either shared phylogenetic history or genetic distance between populations. Finding such an outcome would suggest that the divergent trait has evolved independently in multiple sympatric populations (i.e. the scenario depicted in figure 1*d,e*).

(a) *Sampling and estimation of the intensity of interspecific competition*

For both approaches, we collected *S. multiplicata* tadpoles during summers 1999–2004 in the San Simon Valley. Tadpoles were sampled seven to 15 days post-hatching from random sites throughout natural, temporary ponds using a hand-held dip net. We sampled 10 allopatric populations and seven sympatric populations (figure 2; electronic supplementary material, table S1). Within a few hours of collection, tadpoles were killed by immersion in a 0.1 per cent aqueous solution of tricane methanesulfonate (MS 222) and preserved in 95 per cent ethanol.

We also estimated the intensity of interspecific competition imposed by *S. bombifrons* on *S. multiplicata* in each of the seven sympatric ponds that we sampled. Within natural ponds, the proportion of carnivores produced by *S. multiplicata*, as well as the body size of both tadpoles and adults, is inversely correlated with the frequency of *S. bombifrons* (Pfennig & Murphy 2002; Pfennig & Pfennig 2005). Moreover, experiments have revealed a causal link between the relative abundance of *S. bombifrons* in a population and the degree of character displacement expressed by *S. multiplicata* (Pfennig & Murphy 2002). We therefore estimated the relative abundance of *S. bombifrons* in each of the seven sympatric ponds. To do so, we used the methods in Pfennig & Pfennig (2005). Briefly, for a subset of tadpoles collected in each pond, we sequenced a 663 bp portion of the mitochondrial gene cytochrome *b* and used previously published sequences from each species (*cyt b*, Rice & Pfennig 2008; GenBank accession nos EU285643–EU285652, EU285654, EU285657) to classify each tadpole as either *S. bombifrons* or *S. multiplicata*. We then used these data to estimate the frequency of *S. bombifrons* collected in each pond (electronic supplementary material, table S1), which we calculated as the number of *S. bombifrons* tadpoles sampled divided by the total number of tadpoles of both species sampled. Generally, these frequencies do not vary significantly from year to year (D. Pfennig 1986–2009, unpublished data), suggesting that they are a reliable measure of the recent intensity of competition imposed by *S. bombifrons* on *S. multiplicata* in each pond.

(b) *DNA sequencing and genotyping*

We again sequenced a 663 bp portion of *cyt b* (see above) from a total of 275 individuals (eight to 28 individuals per population; electronic supplementary material, table S1).

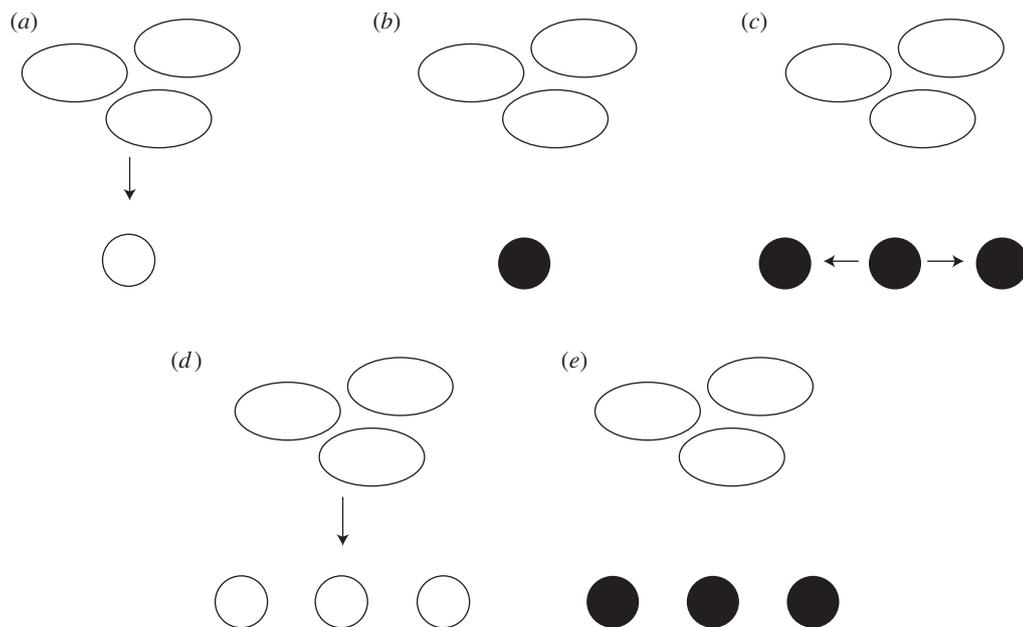


Figure 1. Alternative evolutionary routes by which a divergent trait may occur in multiple populations that are sympatric with a heterospecific competitor. In all panels, ovals denote allopatric populations; circles, sympatric populations; filled circles, sympatric populations in which the divergent phenotype is present. Under one route (*a*), individuals from allopatriy establish a single population in sympatry. (*b*) A divergent phenotype then evolves in this single sympatric population, either because of selection to lessen resource competition (i.e. character displacement) or because of genetic drift. (*c*) Gene flow from the ancestral sympatric population causes this divergent phenotype to become established in multiple, derived sympatric populations. Under the second route (*d*), individuals from allopatriy establish multiple populations in sympatry. (*e*) Selection acting to minimize interspecific competition then favours the parallel evolution of the divergent phenotype in all of these sympatric populations. Note that under both routes, a divergent phenotype becomes established in multiple sympatric populations (compare (*c*) and (*e*)). However, under the first route (*a–c*), divergence occurs *before* invasion into multiple sympatric populations, whereas under the second route (*d,e*), divergence occurs *after* invasion into multiple sympatric populations; i.e. parallel character displacement has occurred.

Additionally, for eight to 10 individuals per population (for sample sizes, see electronic supplementary material, table S1), we amplified eight microsatellite loci using published protocols (Rice *et al.* 2008). The amplified products were genotyped on an ABI 3730 Genetic Analyser. Using standard tests, we established that most microsatellite loci were in Hardy–Weinberg equilibrium in each population, and that none of the loci were in linkage disequilibrium; see electronic supplementary material for additional details.

(c) Assessing population variation in trophic morphology

In order to corroborate that divergent trait evolution (i.e. character displacement) had indeed occurred among the populations of *S. multiplicata* used in our analysis (as predicted by earlier studies; see §1), we began by assessing variation in trophic morphology between multiple allopatric and sympatric populations. Following the methods of Pfennig *et al.* (2007), we measured three diagnostic trophic characters (width of the orbitohyoideus (OH) muscle, width of the interhyoideus (IH) muscle and the shape of the keratinized mouthparts (MP)) on a total of 458 tadpoles (11–86 individuals per pond; see electronic supplementary material, table S1), and standardized the OH and IH measurements by snout–vent length (body size). We then used a principal component analysis (see electronic supplementary material, table S2, for details) to combine these measurements into a single multivariate shape variable, the ‘morphological index’ (Pfennig *et al.* 2006, 2007; Pfennig & Rice 2007). We used PC1, which explained

53.4 per cent of the variance in the three characters, as the morphological index. Larger values of the morphological index indicated a more carnivore-like morphology.

We next characterized the *S. multiplicata* tadpole morphology in each of our populations. Because of ecological character displacement with *S. bombifrons*, we predicted that tadpoles from allopatric populations should generally have higher morphological index scores than tadpoles from sympatric populations (Pfennig *et al.* 2006). We used the mean morphological index score from each population for our comparison because tadpoles within each population are not independent replicates. Because our data did not fit parametric assumptions, we used a non-parametric Wilcoxon rank–sum test to compare the morphological index scores of sympatric versus allopatric populations.

(d) Assessing the relative importance of history versus current competitive environment: phylogenetic approach

Next, we used the method of phylogenetically independent contrasts (Felsenstein 1985) to evaluate whether variation among populations in trophic morphology is significantly correlated with variation in the competitive environment, even after controlling for shared phylogenetic history between populations. Finding such an outcome would suggest that the divergent trait has evolved independently in multiple sympatric populations (i.e. the scenario depicted in figure 1*d,e*).

To undertake this approach, we first constructed a neighbour-joining tree for the 17 populations based on

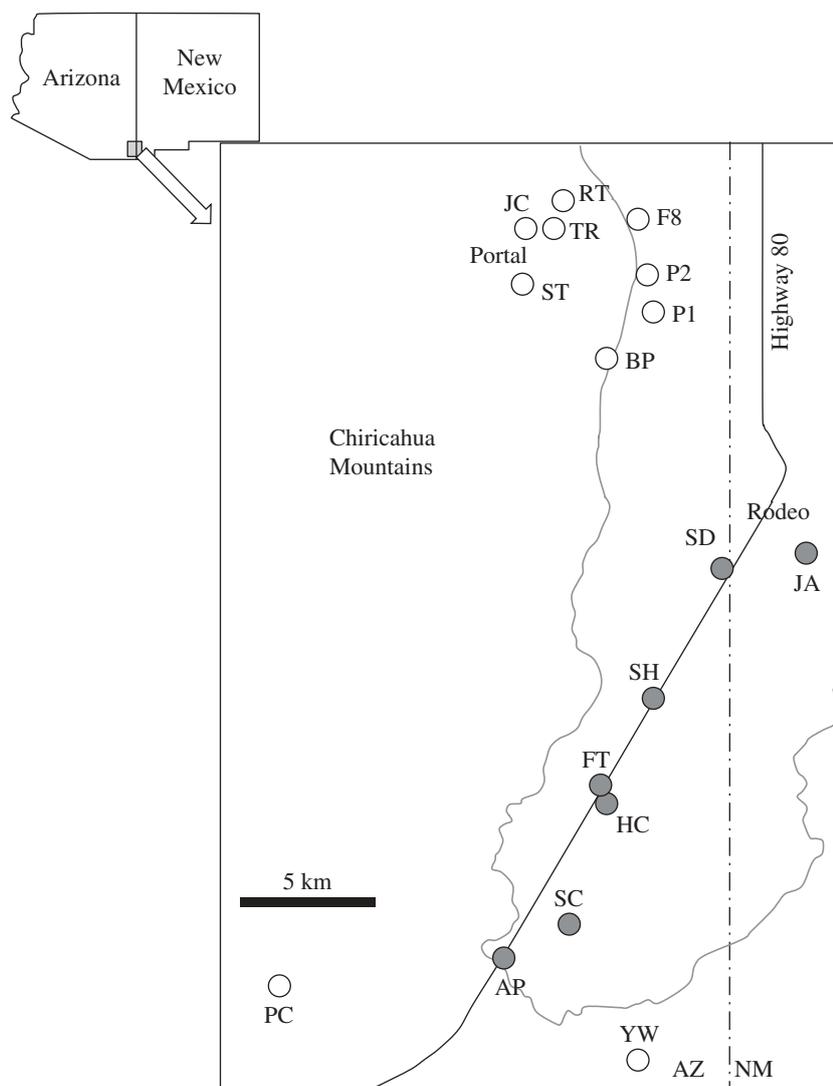


Figure 2. Map of the San Simon Valley showing locations of the 17 sampled populations (10 allopatric populations and seven sympatric populations): Rock Tank (RT), Troller (TR), Figure Eight (F8), John Carron (JC), Peach Orchard 2 (P2), Starview (ST), Peach Orchard 1 (P1), Bull Pond (BP), Javelina (JA), Sulfur Draw (SD), Shrimp Pond (SH), Four Ten (FT), Horned Cow (HC), Skeleton Canyon (SC), Apache (AP), Price Canyon (PC) and Yucca Wash (YW). For specific location information, see electronic supplementary material, table S1. The dashed line is the boundary between the states of Arizona and New Mexico. The solid black line is Highway 80 and the solid grey line follows the foothills of the Chiricahua Mountains; i.e. the 1350 m elevation contour. White circles, allopatric population; grey circles, sympatric population. Scale bar, 5 km.

Nei's D_A genetic distances calculated from the microsatellite genotype data. We used PAUP 4.0d104 (Swofford 2003) to construct the tree, using the BioNJ method with the Fitch–Margoliash criterion, midpoint rooting and branch lengths constrained to be non-negative.

Next, we conducted a phylogenetically independent contrast analysis using the neighbour-joining tree. We imported our tree into MESQUITE 2.6 (Maddison & Maddison 2009) and mapped onto the tree both trophic morphology (positivized population morphological index scores) and interspecific competitive environment (frequency of *S. bombifrons* in each population). We then used the PDAP 1.07 package in MESQUITE (Midford *et al.* 2005) to generate independent contrasts and analyse the relationship between trophic morphology and competitive environment after controlling for population history.

Previous analyses revealed that the true phylogenetic relationships of our populations are unknown (Rice 2008). Specifically, a consensus phylogeny produced from 2500 bootstrap replicates had only two nodes with greater than

50 per cent support. When the true phylogeny remains unknown, statistical analysis using all possible phylogenies may be a viable substitute (Losos 1994). Similarly, we reasoned that statistical analysis using permutations of the population relationships would reveal the robustness of our correlation, even if the true phylogeny is unknown. Using 100 distance matrices previously generated for bootstrap analyses (Rice 2008), we constructed neighbour-joining trees and conducted independent contrast analyses as described above. For additional details of this analysis, see electronic supplementary material.

(e) Assessing the relative importance of history versus current competitive environment: population genetic approach

Because an analysis using TREEFIT (Kalinowski 2009a,b) suggested that the genetic relationships among our populations are not well represented by a phylogeny (see electronic supplementary material), we also used a population genetic approach to disentangle the effects of

population history from those of ongoing selection. For this approach, we specifically asked whether variation among populations in trophic morphology is significantly correlated with variation in the competitive environment, even after controlling for genetic distance between populations. We reasoned that such an outcome would support the hypothesis that the divergent trait has evolved independently in multiple sympatric populations.

To undertake this approach, we ran both Mantel tests (Mantel 1967) and partial Mantel tests (Smouse *et al.* 1986) using ARLEQUIN 3.11 (Schneider *et al.* 2000). These tests assess correlations (or partial correlations) among distance matrices, and they have frequently been used to disentangle the contributions of genetic relatedness and ecology to intraspecific morphological evolution (e.g. Thorpe *et al.* 1995; Thorpe 1996; Langerhans *et al.* 2007).

We generated a dependent matrix of differences in the morphological index between all pairs of populations by taking the absolute value of the difference between each population's mean morphological index score. A larger value in this matrix indicated that two populations differed more in morphology. We then generated two predictor matrices. The first predictor matrix described genetic distances between all pairs of populations. For the microsatellite data, we used either pairwise F_{ST} values or Nei's D_A distances, both generated with MSA 4.05 (Dieringer & Schlötterer 2003). For the *cyt b* data, we used pairwise F_{ST} values calculated with ARLEQUIN 3.11 (Schneider *et al.* 2000). The second predictor matrix described differences in the competitive environment between all pairs of populations. We did this in two ways: (i) we coded the difference in competitive environment categorically (i.e. 0 = both populations either lacked or contained the competitor *S. bombifrons*; 1 = one population lacked *S. bombifrons* while one population contained *S. bombifrons*) and (ii) we calculated the difference between pairs of populations in the frequency of *S. bombifrons* (see above). To estimate statistical significance, we used 100 000 permutations of the data.

3. RESULTS

(a) Assessing population variation in trophic morphology

As we predicted, allopatric populations generally exhibited more carnivore-like trophic morphology than did sympatric populations (mean morphological index \pm s.e.m.; allopatric: 0.243 ± 0.167 ; sympatric: -0.604 ± 0.339 ; $W_{7,10} = 41$, $p = 0.032$; figure 3).

(b) Assessing the relative importance of history versus current competitive environment: phylogenetic approach

Independent contrast analysis of the phylogeny built from the actual distance matrix was statistically significant at the minimum associated degrees of freedom ($r = -0.602$, d.f. = 14, $p = 0.011$), indicating that the variation among populations in trophic morphology is significantly correlated with variation in the competitive environment, independent of shared phylogenetic history.

Moreover, this correlation was robust to uncertainty in the phylogeny. Of the 100 phylogenies produced from the permuted distance matrices, two were removed from the analysis owing to a lack of fit to the evolutionary model. Of the remaining 98, 89 were found to be

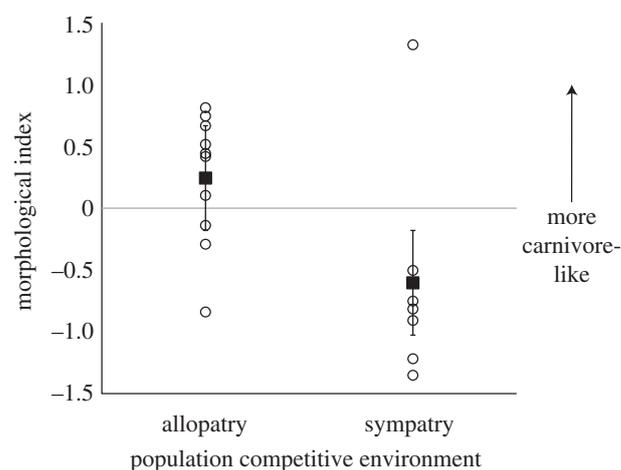


Figure 3. *Spea multiplicata* tadpole trophic morphology in allopatric and sympatric competitive environments. White circles represent mean morphological index scores for each population. The black squares are the mean morphological index scores for each competitive environment, with standard errors.

statistically significant (91%) at their minimum associated degrees of freedom (d.f. = 14, $p \leq 0.05$); see electronic supplementary material for additional details.

(c) Assessing the relative importance of history versus current competitive environment: population genetic approach

Differences between populations in the competitive environment were better than differences in population history (as described by genetic distance) at explaining variation between populations in trophic morphology. Without controlling for genetic distance, the competitive environment matrices were significantly correlated with the morphological matrix (table 1, figure 4a). By contrast, none of the genetic distance matrices were significantly correlated with the morphological matrix (table 1, figure 4b). When we used partial Mantel tests to control for the opposite predictor matrix, the results were qualitatively similar (table 1). Thus, similarity in the competitive environment was a stronger predictor of how similar populations were in trophic morphology than was their genetic distance.

4. DISCUSSION

We evaluated the impact of historical events as opposed to recent selection in generating divergence in trophic morphology between populations of spadefoot toads, *S. multiplicata*. Previous research indicated that this species has undergone character displacement. In particular, *S. multiplicata* in sympatry with *S. bombifrons* has undergone an evolutionary shift towards producing less carnivore-like (more omnivore-like) tadpoles than conspecific populations in allopatry (figure 3, Pfennig & Murphy 2000, 2003; Pfennig *et al.* 2006). This divergence is probably the evolutionary response to selection to avoid resource competition with *S. bombifrons*, which produces competitively superior carnivore-morph tadpoles (Pfennig & Murphy 2000, 2002, 2003; Pfennig *et al.* 2007). However, previous studies did not consider the relative importance of population history versus recent selection on the evolution of trait divergence in this system (figure 1).

Table 1. Mantel and partial Mantel correlation coefficients (and associated *p*-values) indicating the relationships between variation in trophic morphology and either variation in competitive environment or variation in genetic distance. Italic values are statistically significant.

	without controlling for opposite predictor matrix (Mantel test)	controlling for genetic distance (partial Mantel test)			controlling for competitive environment (partial Mantel test)	
		<i>cyt b</i> F_{ST}	microsatellite F_{ST}	microsatellite Nei's D_A	presence/absence of <i>S. bombifrons</i>	relative frequency of <i>S. bombifrons</i>
<i>variation in trophic morphology versus variation in competitive environment</i>						
difference in presence/ absence of <i>S. bombifrons</i>	0.428 (0.002)	0.426 (0.002)	0.426 (0.002)	0.422 (0.002)	—	—
difference in relative frequency of <i>S. bombifrons</i>	0.423 (0.0006) ^a	0.423 (0.0005)	0.422 (0.0006)	0.416 (0.0009)	—	—
<i>variation in trophic morphology versus genetic distance</i>						
<i>cyt b</i> F_{ST}	-0.035 (0.561)	—	—	—	-0.0007 (0.417)	0.033 (0.307)
microsatellite F_{ST}	0.037 (0.351)	—	—	—	0.009 (0.432)	-0.023 (0.538)
microsatellite Nei's D_A	0.132 (0.124) ^a	—	—	—	0.109 (0.170)	0.100 (0.184)

^aCorrelations illustrated in figure 4.

In the present study, the results of two complementary approaches suggest that population history does not explain the observed trait divergence between sympatric and allopatric populations of *S. multiplicata*. First, phylogenetically independent contrasts (Felsenstein 1985) reveal that variation among populations in trophic morphology is significantly correlated with variation in the competitive environment, even after controlling for shared phylogenetic history between populations. These results suggest that the divergent trait has arisen independently in multiple sympatric populations because of recent selection; i.e. they support the scenario depicted in figure 1*d,e*. Moreover, even though the phylogeny has a high degree of uncertainty, the correlation between competitive environment and trophic morphology is robust. Second, a population genetic analysis also reveals that variation between populations in tadpole trophic morphology is significantly correlated with variation between populations in the competitive environment, but not with genetic distance (table 1, figure 4). Once again, such a result strongly implicates recent selection as the cause of divergence between populations (see also Langerhans *et al.* 2007).

Our data therefore imply that selection to avoid interspecific resource competition has acted independently within each of several sympatric populations. However, one might contend that variation among populations in the competitive environment should necessarily account for variation in trophic morphology in a system such as *Spea*, where individuals use phenotypic plasticity to adjust their feeding morphology facultatively depending on their current environment (e.g. see Pfennig & Murphy 2000, 2002). Yet, phenotypic plasticity does not completely explain the divergence in trophic phenotype observed between sympatric and allopatric populations of *S. multiplicata*. In particular, Pfennig & Murphy (2002) found that these differences are, at least

in part, canalized, meaning that they persist even when tadpoles are reared under common conditions in the laboratory. Therefore, selection to minimize resource competition has apparently generated divergent trait evolution; i.e. ecological character displacement (Schluter 2000*b*).

Although we have posed gene flow and selection as alternative explanations for divergent trait evolution in sympatric populations, the two mechanisms are not mutually exclusive. In particular, character displacement could arise through a selective filtering of divergent phenotypic variants that were already present in allopatry (Rice & Pfennig 2007). This process would come about when gene flow and selection act together to selectively spread a pre-existing divergent trait (i.e. one that arose in a single allopatric population for any of a number of reasons) into multiple sympatric populations. Such sorting of pre-existing variation would produce a pattern similar to that expected under the non-selective hypothesis (figure 1*a-c*). However, our data are not consistent with this explanation. Instead, our data are consistent with the hypothesis that divergent traits arose and spread within each sympatric population following contact with the competitor species. In particular, the fact that genetic distance was relatively unimportant in explaining sympatric trait divergence suggests that character displacement has probably arisen through *in situ* evolution of novel phenotypes, and not through any type of sorting. Our findings, therefore, suggest that *S. multiplicata* have undergone repeated evolution of divergent traits in multiple sympatric populations.

Has parallel character displacement occurred? As noted in §1 parallel character displacement can be viewed as a special case of parallel evolution, which occurs when a similar trait evolves repeatedly in closely related, independently evolving lineages. To demonstrate parallel character displacement, one would ideally present

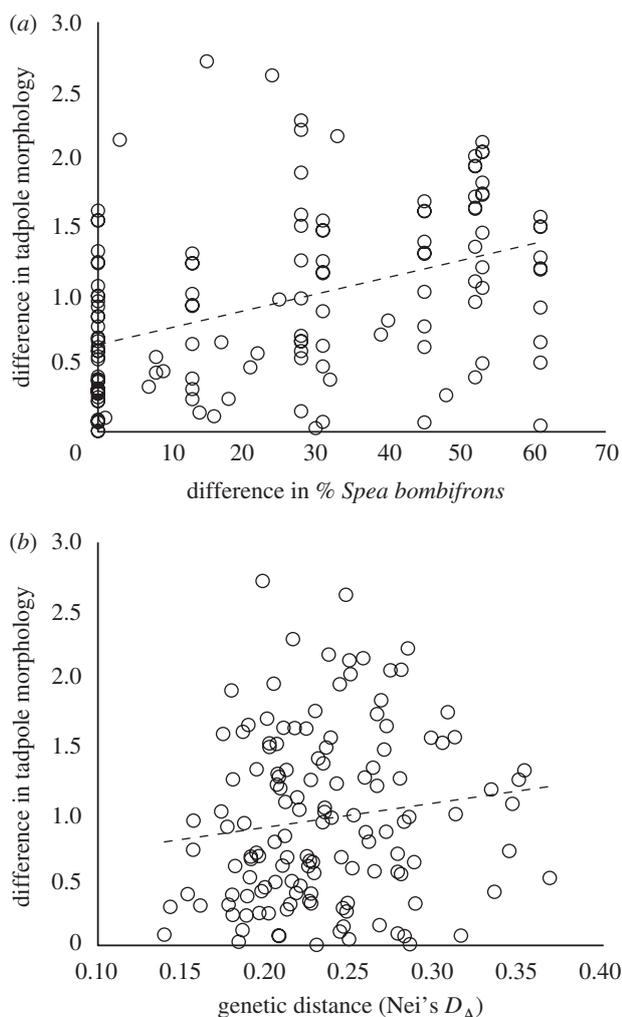


Figure 4. Representative Mantel test results showing correlations between population variation in *S. multiplicata* tadpole trophic morphology and either (a) population variation in the relative presence of *S. bombifrons* ($r = 0.423$, $p = 0.0006$; table 1) or (b) pairwise genetic distance (Nei's D_A) between populations based on eight microsatellite loci ($r = 0.132$, $p = 0.124$; table 1). Difference in tadpole morphology is the absolute value of the difference in morphological index scores between all pairs of populations. Each white circle indicates one pairwise population comparison. The dashed lines represent least-squares regression lines, which are provided for illustrative purposes only.

evidence of replicated phenotypic divergence between pairs of allopatric and sympatric sister populations. Additionally, there should be statistical evidence that this phenotypic divergence is associated with the move into an environment with strong interspecific resource competition (i.e. sympatry, see Revell *et al.* (2007) for such an analysis investigating associations of morphological change with transitions to rock-dwelling in lizard species). Unfortunately, because relationships among populations are often complex such that they cannot be accurately depicted in a phylogeny (Kalinowski 2009a), it is very difficult to identify sister populations with confidence, making such an analysis often untenable at the intraspecific level. Indeed, the high level of uncertainty in our phylogeny precluded us from performing this analysis. Instead, we employed two complementary methods to evaluate whether the presence of similar trophic morphology in multiple populations of

S. multiplicata has probably resulted in greater part from shared competitive environments (as predicted by parallel character displacement) versus shared history. Our results are consistent with the pattern predicted by parallel character displacement. Although indirect, these and other similar methods (Hansen *et al.* 2000; Marko 2005; Matocq & Murphy 2007) may often be the best options for investigating the role of ecological selection versus population history in trait evolution at the intraspecific level.

As genetic data from natural populations become increasingly accessible, additional studies should evaluate the role of selective and non-selective processes in putative cases of parallel character displacement. Such studies will be important for demonstrating parallel character displacement, for identifying the evolutionary route by which character displacement most frequently evolves, and for establishing the general importance of interspecific competition in trait evolution.

All procedures complied with applicable state and US federal regulations.

We thank K. Pfennig, P. Marko, C. Cunningham, C. Burch, M. Servedio, D. Kikuchi, R. Martin, C. Ledón-Rettig, A. Gonzalez-Voyer and three anonymous referees for helpful discussions and comments; R. Martin and K. Pfennig for help with collections; M. McGee for help with tadpole measurements; R. Bernstein, S. Jaryan and J. Copp for help with microsatellite genotyping; the American Museum of Natural History's (AMNH) Southwestern Research Station (SWRS) for housing and logistical support; and the Game and Fish Departments of Arizona and New Mexico for providing scientific collecting permits. This work was funded by the National Science Foundation, Sigma Xi, SWRS and the AMNH.

REFERENCES

- Arthur, W. 1982 The evolutionary consequences of interspecific competition. *Adv. Ecol. Res.* **12**, 127–187. (doi:10.1016/S0065-2504(08)60078-1)
- Brown, J. H. 1995 *Macroecology*. Chicago, IL: University of Chicago Press.
- Brown Jr, W. L. & Wilson, E. O. 1956 Character displacement. *Syst. Zool.* **5**, 49–64. (doi:10.2307/2411924)
- Clarke, B. C. 1975 The contribution of ecological genetics to evolutionary theory: detecting the direct effects of natural selection on polymorphic loci. *Genetics* **79**, 101–113.
- Dayan, T. & Simberloff, D. 2005 Ecological and community-wide character displacement: the next generation. *Ecol. Lett.* **8**, 875–894. (doi:10.1111/j.1461-0248.2005.00791.x)
- Dieringer, D. & Schlötterer, C. 2003 Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Mol. Ecol. Notes* **3**, 167–169. (doi:10.1046/j.1471-8286.2003.00351.x)
- Endler, J. A. 1986 *Natural selection in the wild*. Monographs in Population Biology. Princeton, NJ: Princeton University Press.
- Felsenstein, J. 1985 Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15. (doi:10.1086/284325)
- Goldberg, E. E. & Lande, R. 2006 Ecological and reproductive character displacement on an environmental gradient. *Evolution* **60**, 1344–1357.
- Grant, P. R. 1972 Convergent and divergent character displacement. *Biol. J. Linn. Soc.* **4**, 39–68. (doi:10.1111/j.1095-8312.1972.tb00690.x)
- Grant, P. R. & Grant, B. P. 2006 Evolution of character displacement in Darwin's finches. *Science* **313**, 224–226. (doi:10.1126/science.1128374)

- Hansen, T. F., Armbruster, W. S. & Antonson, L. 2000 Comparative analysis of character displacement and spatial adaptations as illustrated by the evolution of *Dalechampia* blossoms. *Am. Nat.* **156**, S17–S34. (doi:10.1086/303413)
- Kalinowski, S. T. 2009a How well do evolutionary trees describe genetic relationships among populations? *Heredity* **102**, 506–513. (doi:10.1038/hdy.2008.136)
- Kalinowski, S. T. 2009b TREEFIT: a computer program for evaluating how well evolutionary trees fit genetic distance data. See <http://www.montana.edu/kalinowski>.
- Langerhans, R. B., Gifford, M. E. & Joseph, E. O. 2007 Ecological speciation in *Gambusia* fishes. *Evolution* **61**, 2056–2074. (doi:10.1111/j.1558-5646.2007.00171.x)
- Losos, J. B. 1992 The evolution of convergent structure in Caribbean *Anolis* communities. *Syst. Biol.* **41**, 403–420.
- Losos, J. B. 1994 An approach to the analysis of comparative data when a phylogeny is unavailable or incomplete. *Syst. Biol.* **43**, 117–123.
- Maddison, W. P. & Maddison, D. R. 2009 MESQUITE: a modular system for evolutionary analysis, Version 2.6. See <http://mesquiteproject.org>.
- Mantel, N. A. 1967 The detection of disease clustering and a generalized regression approach. *Cancer Res.* **27**, 209–220.
- Marko, P. B. 2005 An intraspecific comparative analysis of character divergence between sympatric species. *Evolution* **59**, 554–564.
- Matocq, M. D. & Murphy, P. J. 2007 Fine-scale phenotypic change across a species transition zone in the genus *Neotoma*: disentangling independent evolution from phylogenetic history. *Evolution* **61**, 2544–2557. (doi:10.1111/j.1558-5646.2007.00215.x)
- Midford, P. E., Garland Jr, T. & Maddison, W. P. 2005 PDAP package of MESQUITE. Version 1.07. See http://mesquiteproject.org/pdap_mesquite/index.html.
- Pfennig, D. W. 1990 The adaptive significance of an environmentally-cued development switch in an anuran tadpole. *Oecologia* **85**, 101–107. (doi:10.1007/BF00317349)
- Pfennig, D. W. 1992 Proximate and functional causes of polyphenism in an anuran tadpole. *Funct. Ecol.* **6**, 167–174. (doi:10.2307/2389751)
- Pfennig, D. W. 1999 Cannibalistic tadpoles that pose the greatest threat to kin are most likely to discriminate kin. *Proc. R. Soc. Lond. B* **266**, 57–61. (doi:10.1098/rspb.1999.0604)
- Pfennig, D. W. & Frankino, W. A. 1997 Kin-mediated morphogenesis in facultatively cannibalistic tadpoles. *Evolution* **51**, 1993–1999. (doi:10.2307/2411019)
- Pfennig, D. W. & Murphy, P. J. 2000 Character displacement in polyphenic tadpoles. *Evolution* **54**, 1738–1749.
- Pfennig, D. W. & Murphy, P. J. 2002 How fluctuating competition and phenotypic plasticity mediate species divergence. *Evolution* **56**, 1217–1228.
- Pfennig, D. W. & Murphy, P. J. 2003 A test of alternative hypotheses for character divergence between coexisting species. *Ecology* **84**, 1288–1297. (doi:10.1890/0012-9658(2003)084[1288:ATOAHF]2.0.CO;2)
- Pfennig, D. W. & Rice, A. M. 2007 An experimental test of character displacement's role in promoting postmating isolation between conspecific populations in contrasting competitive environments. *Evolution* **61**, 2433–2443. (doi:10.1111/j.1558-5646.2007.00190.x)
- Pfennig, D. W., Rice, A. M. & Martin, R. A. 2006 Ecological opportunity and phenotypic plasticity interact to promote character displacement and species coexistence. *Ecology* **87**, 769–779. (doi:10.1890/05-0787)
- Pfennig, D. W., Rice, A. M. & Martin, R. A. 2007 Field and experimental evidence for competition's role in phenotypic divergence. *Evolution* **61**, 257–271. (doi:10.1111/j.1558-5646.2007.00034.x)
- Pfennig, K. S. & Pfennig, D. W. 2005 Character displacement as the 'best of a bad situation': fitness trade-offs resulting from selection to minimize resource and mate competition. *Evolution* **59**, 2200–2208.
- Pomeroy, L. V. 1981. Developmental polymorphism in the tadpoles of the spadefoot toad *Scaphiopus multiplicatus*. PhD dissertation, University of California, Riverside, CA.
- Revell, L. J., Johnson, M. A., Schulte II, J. A., Kolbe, J. J. & Losos, J. B. 2007 A phylogenetic test for adaptive convergence in rock-dwelling lizards. *Evolution* **61**, 2898–2912. (doi:10.1111/j.1558-5646.2007.00225.x)
- Rice, A. M. 2008. Ecological character displacement and its consequences: population genetic analyses in spadefoot toads. PhD dissertation, University of North Carolina, Chapel Hill, NC.
- Rice, A. M. & Pfennig, D. W. 2007 Character displacement: *in situ* evolution of novel phenotypes or sorting of pre-existing variation? *J. Evol. Biol.* **20**, 448–459. (doi:10.1111/j.1420-9101.2006.01187.x)
- Rice, A. M. & Pfennig, D. W. 2008 Analysis of range expansion in two species undergoing character displacement: why might invaders generally 'win' during character displacement? *J. Evol. Biol.* **21**, 696–704. (doi:10.1111/j.1420-9101.2008.01518.x)
- Rice, A. M., Pearse, D. E., Becker, T., Newman, R. A., Lebonville, C., Harper, G. R. & Pfennig, K. S. 2008 Development and characterization of nine polymorphic microsatellite markers for Mexican spadefoot toads (*Spea multiplicata*) with cross-amplification in plains spadefoot toads (*S. bombifrons*). *Mol. Ecol. Res.* **8**, 1386–1389.
- Schluter, D. 2000a *The ecology of adaptive radiation*. New York, NY: Oxford University Press.
- Schluter, D. 2000b Ecological character displacement in adaptive radiation. *Am. Nat.* **156**, S4–S16. (doi:10.1086/303412)
- Schluter, D. & McPhail, J. D. 1993 Character displacement and replicate adaptive radiation. *Trends Ecol. Evol.* **8**, 197–200. (doi:10.1016/0169-5347(93)90098-A)
- Schluter, D. & Nagel, L. M. 1995 Parallel speciation by natural selection. *Am. Nat.* **146**, 292–301. (doi:10.1086/285799)
- Schneider, S., Roessli, D. & Excoffier, L. 2000 ARLEQUIN ver. 2.000: a software for population genetics data analysis: genetics and Biometry Laboratory. Switzerland: University of Geneva.
- Slatkin, M. 1980 Ecological character displacement. *Ecology* **61**, 163–177. (doi:10.2307/1937166)
- Smouse, P. E., Long, J. C. & Sokal, R. R. 1986 Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Syst. Zool.* **35**, 627–632. (doi:10.2307/2413122)
- Swofford, D. L. 2003 *PAUP**. *Phylogenetic analysis using parsimony (*and other methods)*. Version 4. Sunderland, MA: Sinauer Associates.
- Taper, M. L. & Case, T. J. 1992 Coevolution among competitors. In *Oxford surveys in evolutionary biology*, vol. 8 (eds D. Futuyma & J. Antonovics), pp. 63–109. Oxford, UK: Oxford University Press.
- Thorpe, R. S. 1996 The use of DNA divergence to help determine the correlates of evolution of morphological characters. *Evolution* **50**, 524–531. (doi:10.2307/2410828)
- Thorpe, R. S., Malhotra, A., Black, H., Daltry, J. C. & Wuster, W. 1995 Relating geographic pattern to phylogenetic process. *Phil. Trans. R. Soc. Lond. B* **349**, 61–68. (doi:10.1098/rstb.1995.0091)