

A MATERNAL EFFECT MEDIATES RAPID POPULATION DIVERGENCE AND CHARACTER DISPLACEMENT IN SPADEFOOT TOADS

David W. Pfennig^{1,2} and Ryan A. Martin^{1,3}

¹Department of Biology, CB#3280, University of North Carolina, Chapel Hill, North Carolina 27599

²E-mail: dpfennig@unc.edu

³E-mail: martinra@email.unc.edu

Received June 6, 2008

Accepted September 19, 2008

Despite long-standing interest in character displacement, little is known of its underlying proximate causes. Here, we explore the role of maternal effects in character displacement. We specifically investigated whether differences in maternal body condition mediate divergence in the expression of resource-use traits between populations of spadefoot toads (*Spea multiplicata*) that occur in sympatry with a heterospecific competitor and those that occur in allopatry. In sympatry, *S. multiplicata* is forced by its competitor onto a less profitable resource. As a result, sympatric females mature in poorer condition and invest less into offspring. Consequently, their offspring produce a resource-use phenotype that minimizes competition with the other species and that also differs from the phenotype produced in allopatry. These differences in trait expression between allopatry and sympatry disappear once mothers are equilibrated in body condition in the laboratory. Thus, a condition-dependent maternal effect mediates population divergence and character displacement. Such effects potentially buffer populations from extinction (via competitive exclusion) while genetic changes accumulate, which produce divergent traits in the absence of the maternal effect. Maternal effects may therefore often be important in determining the initial direction and rate of evolution during the early stages of character displacement.

KEY WORDS: Ecological character displacement, genetic accommodation, inherited environmental effect, phenotypic plasticity, population divergence, *Spea multiplicata*.

Character displacement—trait evolution stemming from selection to lessen resource competition or reproductive interference between species—has long been viewed as a leading cause of adaptive diversification (Brown and Wilson 1956; Grant 1972; Wilson 1992; Schluter 2000; Coyne and Orr 2004; Dayan and Simberloff 2005). Character displacement arises when individuals most dissimilar from the average resource-use or mate-acquisition phenotypes of a co-occurring, phenotypically similar species are able to procure more resources or matings than other members of their population (Slatkin 1980; Arthur 1982; Taper and Case 1985, 1992; Doebeli 1996; Servedio and Noor 2003). Because such selection acts only where species co-occur, character dis-

placement causes species to become more dissimilar where they are found together (i.e., sympatry) than where each is found alone (i.e., allopatry). Consequently, within each species, sympatric and allopatric populations also diverge. By causing such divergence, character displacement may initiate speciation and even drive adaptive radiation (Schluter 2000; Rundle and Nosil 2005; for possible examples, see Hoskin et al. 2005; Pfennig and Ryan 2006; Pfennig and Rice 2007).

Despite character displacement's potentially critical role in driving population divergence, speciation, and adaptive radiation, little is known about the underlying proximate mechanisms that mediate it. In most cases, phenotypic differences between

populations in sympatry with the heterospecific and those in allopatry are assumed to have a genetic basis (Slatkin 1980; Arthur 1982; Taper and Case 1985, 1992; Doebeli 1996; Servedio and Noor 2003). This assumption has rarely been tested, however (Schluter 2000). At best, we may only know that trait differences between sympatric and allopatric populations are canalized, meaning that these differences between populations persist when individuals are reared under common conditions (reviewed in Schluter 2000). Yet, such data alone are insufficient to demonstrate that underlying genetic differences account for divergence between sympatry and allopatry in trait expression. Elucidating the proximate bases of character displacement is vital, because different proximate mechanisms can influence the speed of character displacement and, therefore, whether character displacement even evolves in the first place. Indeed, any mechanism that facilitates divergence in resource-use or mate-acquisition phenotypes may render character displacement, as opposed to competitive exclusion, more likely to transpire (Rice and Pfennig 2007).

Consider, for instance, that many species respond adaptively to the presence of heterospecifics through phenotypic plasticity (reviewed in Robinson and Wilson 1994; Agrawal 2001; Pfennig and Murphy 2002; Fordyce 2006; Pfennig et al. 2006). When faced with resource competition or reproductive interference from a heterospecific, individuals of these species facultatively express an alternative resource- or mate-acquisition phenotype that lessens competition or reproductive interference (e.g., see Werner and Hall 1976; Pfennig and Murphy 2002). Such shifts have not traditionally been considered character displacement, however, because phenotypic plasticity is often regarded as a nongenetic response that is incapable of mediating adaptive evolution (Grant 1972; Arthur 1982; Schluter 2000). Yet, the magnitude and direction of a plastic response is often genetically variable and, consequently, subject to natural selection and evolutionary change (reviewed in Schlichting and Pigliucci 1998; West-Eberhard 2003; DeWitt and Scheiner 2004).

Moreover, plasticity might facilitate adaptive evolution. In particular, transgenerational phenotypic plasticity—in which the environmental stimulus that induces the plasticity is experienced by the parental generation and the phenotypic consequences are expressed by offspring—can constitute an alternative inheritance system by which adaptive evolution can unfold. For example, maternal effects occur when the phenotype of a female influences the phenotype of her offspring, independent of the direct effects of her genes on her offspring's phenotype (Mousseau and Fox 1998). Because these effects can be acted upon by selection (McAdam and Boutin 2004) and then cause information to be conveyed reliably between generations (Rossiter 1996; Agrawal et al. 1999; Plaistow et al. 2006; Allen et al. 2008), they can, by themselves, mediate adaptive evolution (Jablonka and Lamb 1995; Maynard Smith 1998).

Maternal effects may be especially important in mediating character displacement. Ecological character displacement causes interacting species to use different resources (reviewed in Schluter 2000; Day and Young 2003). When resource quality is asymmetric, one species will gain the more profitable resource, whereas the other will be forced onto a less profitable resource (e.g., see Pfennig and Pfennig 2005; Grant and Grant 2006). Consequently, females of the latter species may mature at a smaller body size or in poorer condition (e.g., see Gorbushin 1996; Pfennig and Pfennig 2005; Grant and Grant 2006) and, therefore, produce offspring that are also smaller and in poorer condition. As a result, these offspring may ultimately produce a resource-use phenotype less like that produced by the other species. Moreover, because maternal effects can be transmitted reliably between generations (see above), such differences in trait expression between populations in sympatry with a heterospecific competitor and those in allopatry may persist even when individuals are experimentally reared under common conditions, thereby giving the misleading appearance that genetic differences underlie these trait differences.

Thus, trait differences between populations may be underlain entirely by a maternal effect. Moreover, because they can arise within a single generation (Räsänen and Kruuk 2007), maternal effects potentially buffer populations from extinction (via competitive exclusion) while genetic changes accumulate, which produce the divergent trait even in the absence of the maternal effect through genetic accommodation (*sensu* West-Eberhard 2003; for a possible example, see Badyaev and Oh 2008).

Here, we test whether a maternal effect mediates divergence between conspecific populations that have, and have not, undergone ecological character displacement. We begin by describing our study system, our general hypothesis, and its associated critical predictions.

STUDY SYSTEM

Mexican spadefoot toads (*Spea multiplicata*) potentially develop into either a small tadpole that feeds on detritus on the pond bottom (the “omnivore” morph), or a larger tadpole with greatly enlarged jaw muscles specialized for feeding on anostracan fairy shrimp in open water (the “carnivore” morph; Bragg 1965; Pomeroy 1981; Pfennig 1992). Shrimp ingestion triggers development of carnivores (Pomeroy 1981; Pfennig 1990; Storz 2004), but morph determination is influenced by maternal phenotype. Specifically, female body size is significantly positively correlated with their offspring's propensity to become a carnivore (R. A. Martin and D. W. Pfennig, unpubl. ms). This tendency for large mothers to produce tadpoles with the highest propensity to become carnivores appears to reflect differences in maternal investment. Larger females invest in larger eggs, which produce correspondingly larger tadpoles (R. A. Martin and D. W. Pfennig, unpubl. ms). Larger tadpoles, in turn, handle shrimp more efficiently and consume

them faster and earlier in development than smaller tadpoles (Frankino and Pfennig 2001). Larger tadpoles thereby acquire more of the cue—shrimp ingestion—that induces the carnivore morph.

In southeastern Arizona and southwestern New Mexico, USA, *S. multiplicata* has undergone ecological character displacement with a congener, *S. bombifrons* (Pfennig and Murphy 2000, 2002, 2003; Pfennig et al. 2006, 2007). When each species occurs alone, the two species do not differ in trophic phenotype, and they produce similar frequencies of both resource-use morphs (Pfennig and Murphy 2003; Pfennig et al. 2006). By contrast, when they occur together, *S. multiplicata* produce mostly omnivores, whereas *S. bombifrons* produce mostly carnivores (Pfennig and Murphy 2003; Pfennig et al. 2006). These differences in morph production likely arose because of selection acting to minimize competition between species for food. Experiments have revealed that *S. multiplicata* that are the most similar to *S. bombifrons* in trophic morphology perform the worst when competing with *S. bombifrons* for food, indicating that selection favors divergence between these species when they compete (Pfennig et al. 2007).

Separate experiments have shown that character displacement is mediated in this system through two distinct proximate mechanisms. First, *S. multiplicata* minimize overlap in resource use with *S. bombifrons* by facultatively producing a resource-use phenotype that is distinct from that produced by *S. bombifrons*. In particular, when allopatric individuals of each species are reared alone with both shrimp and detritus, they produced similar proportions of both morphs (Pfennig and Murphy 2002). When allopatric individuals of each species are experimentally combined, however, *S. bombifrons* outcompete *S. multiplicata* for shrimp. As a consequence of consuming less shrimp (the cue that induces carnivores), *S. multiplicata* develop mostly into omnivores in the presence of *S. bombifrons* (Pfennig and Murphy 2002). These sort of facultative shifts in resource-use traits, which are common in nature, have been dubbed “facultative character displacement” by Pfennig and Murphy (2002).

Second, *S. multiplicata* from different populations appear to possess different inherent propensities to produce carnivores. Sympatric and allopatric populations of *S. multiplicata* differ in morph production even when their tadpoles are produced and reared under common conditions (Pfennig and Murphy 2000, 2002; Pfennig and Rice 2007). Specifically, although *S. multiplicata* from allopatry produce both morphs, those from sympatry produce mostly omnivores, even when fed shrimp (the dietary cue that normally induces carnivores). Thus, population differences in morph production are canalized (sensu Siegal and Bergman 2006), meaning that they appear to be insensitive to environmental perturbation. Such differences in resource-use traits have been dubbed “canalized character displacement” by

Pfennig and Murphy (2002). Here, we investigate the developmental mechanism that mediates this canalized character displacement and explore its evolutionary consequences.

HYPOTHESIS AND PREDICTIONS

We hypothesize that a maternal effect mediates divergence in trophic phenotype and morph production between sympatric and allopatric populations of *S. multiplicata*. As noted in the previous section, *S. multiplicata* produce both morphs in allopatry, but populations in sympatry with *S. bombifrons* appear to be capable of producing only omnivores. These population differences in morph production have also resulted in population differences in adult body size. Partly because they produce only the smaller omnivore morph, sympatric *S. multiplicata* mature as smaller adults than do allopatric *S. multiplicata* (Pfennig and Pfennig 2005). However, sympatric *S. multiplicata* females that developed as omnivores when they were larvae would grow up not only smaller, but—because of a size-dependent maternal effect (see Study System)—they would also produce mostly omnivores in the next generation. Moreover, because enhanced condition should fuel enhanced growth, population differences in body size should reflect population differences in individual condition. A female’s size and condition often impact her ability to invest in offspring (see Bashley 2006 and references therein) and may thereby influence her offspring’s resource-use phenotype (see Introduction). Thus, in sympatric populations of *S. multiplicata*, a condition-dependent maternal effect may promote production of the omnivore phenotype, thereby mediating population divergence and character displacement (Fig. 1).

If the above general hypothesis were true, then five critical predictions should be met:

- (1) The observed population differences in body size should reflect population differences in individual condition (better condition should fuel enhanced growth). Thus, females from sympatry should be in poorer condition than females from allopatry.
- (2) Because they may mature smaller and in poorer condition, females from sympatry should invest less in their offspring than females from allopatry.
- (3) Because they may receive reduced parental investment, tadpoles from females in poorer condition should be less prone to developing into the larger, more robust carnivore-morph tadpole than those from females in better condition.
- (4) Because females from sympatry may be in poorer condition than females from allopatry, and because females in poorer condition may be less prone to produce carnivores, females from sympatry should produce fewer carnivores than females from allopatry. Moreover, because a condition-dependent maternal effect mediates morph production, these population differences should be inherited maternally.

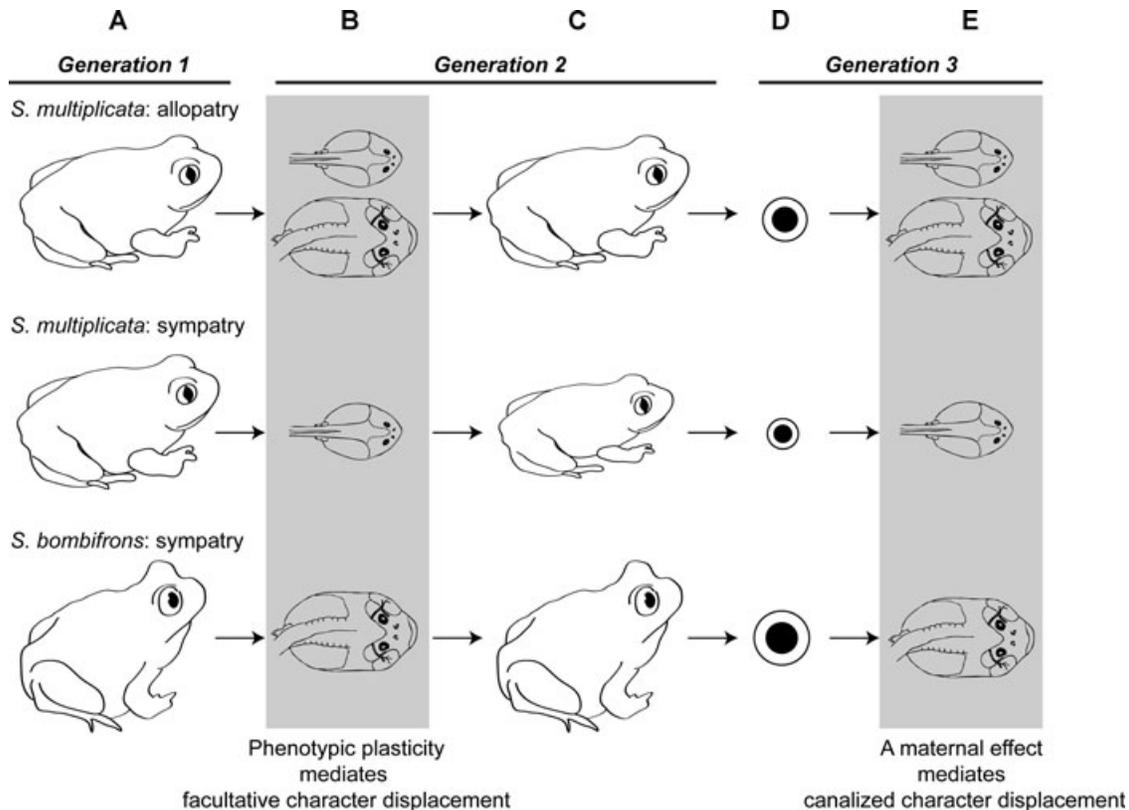


Figure 1. How a maternal effect may mediate rapid population divergence and character displacement. (A) Different populations of *S. multiplicata* occur in allopatry (top row) and sympatry (middle row) with *S. bombifrons* (bottom row). (B) In allopatric populations, *S. multiplicata* produce alternative resource-use morphs: a small, “omnivore” morph, and a large, “carnivore” morph, which is induced by shrimp ingestion. In sympatric populations, however, the two species undergo character displacement in morph production: *S. bombifrons* produce mostly carnivores and *S. multiplicata* produce mostly omnivores. When the two species first come into contact, phenotypic plasticity mediates this divergence between species; i.e., they undergo “facultative character displacement” (sensu Pfennig and Murphy 2002). (C) Because they produce both morphs in allopatry, but only the smaller omnivore morph in sympatry, *S. multiplicata* females from sympatry mature smaller and in poorer condition than *S. multiplicata* females from allopatry. (D) Consequently, sympatric females invest less into offspring by producing smaller eggs. (E) Smaller eggs hatch into smaller tadpoles, which tend to become omnivores. Thus, because of a condition-dependent maternal effect, the tendency to produce different morphs may become developmentally fixed (i.e., canalized) in different species and different populations. “Canalized character displacement” (sensu Pfennig and Murphy 2002) therefore potentially evolves in only two generations.

(5) The reduced propensity of females from sympatry to produce carnivores should disappear once females from sympatry and allopatry are equilibrated in condition.

We sought to test these critical predictions, and thereby evaluate whether a maternal effect mediates character displacement in spadefoot toads.

Materials and Methods

We performed a series of observations and experiments to test the critical predictions outlined in the previous section. Below, we describe the methods aimed at testing each prediction separately.

TESTING PREDICTION 1: COMPARING MATERNAL CONDITION IN SYMPATRY AND ALLOPATRY

To test Prediction (1), we collected adult female *S. multiplicata* at or near breeding aggregations at allopatric and sympatric sites in the San Simon Valley of Cochise County, Arizona and adjacent Hidalgo County, New Mexico (for a map of the study area, see Pfennig et al. 2006). Character displacement has been documented between *S. multiplicata* and *S. bombifrons* from these sympatric populations (Pfennig and Murphy 2000, 2002, 2003; Pfennig et al. 2006, 2007). All females were sexually mature as indicated by the presence of eggs in their body cavity. We measured each female’s body size (snout-vent length, SVL) using hand-held digital calipers and mass using a digital scale. These measures were made within 24 h of capture and before any

female released any eggs. We measured a total of 25 females from seven distinct allopatric sites and 152 females from nine distinct sympatric sites.

We operationally defined maternal “condition” as the residuals of a female’s mass regressed on her SVL (Pfennig 2007). Thus, a female that was heavier for a given SVL than expected was regarded as being in better condition than a female that was lighter (Pfennig 2007). After running the regression analysis to calculate condition, we pooled all female condition values from each population and used these population mean values in the following two analyses.

First, we used a two-tailed *t*-test to contrast mean conditions of females from sympatry with that among females from allopatry. Second, because we found that females from sympatry were in poorer condition than females from allopatry (see Results), we sought to ascertain if the presence of *S. bombifrons* likely caused females in sympatry to have lower condition. To address this issue, we took advantage of the fact that the intensity of competition from *S. bombifrons* is not the same in all populations. In particular, *S. bombifrons* are more abundant at some sites than at others (Pfennig and Murphy 2002). We therefore asked if there was a significant negative relationship between the relative frequency of *S. bombifrons* in a given population and the mean condition of female *S. multiplicata* from that population. To address this question, we used the procedures outlined in Pfennig and Pfennig (2005) to calculate in each pond the proportion of *Spea* tadpoles that were *S. bombifrons*. These data were used as a proxy of the relative frequency of *S. bombifrons* in each population. We then regressed the mean female condition values against the proportion of *S. bombifrons* in each population.

Finally, we asked whether any shift in condition was likely caused by the presence of *S. bombifrons* per se, and not by another environmental variable that differs between sympatric and allopatric sites: elevation. In the San Simon Valley, allopatric sites are generally found at higher elevations than sympatric sites (Pfennig et al. 2006). To determine if different body conditions may be selectively favored at different elevations, we asked if there was a significant relationship between the elevation of a given population and the mean condition of female *S. multiplicata* from that population.

TESTING PREDICTION 2: COMPARING MATERNAL INVESTMENT IN SYMPATRY AND ALLOPATRY

We asked whether females from allopatry invested more into their offspring than did females from sympatry (Prediction (2)), where egg size served as a measure of maternal investment (Bernardo 1996).

We collected naturally mated pairs from two distinct sites in allopatry (seven pairs) and six distinct sites in sympatry

(19 pairs) in the San Simon Valley. We brought these pairs into the laboratory, placed each pair separately into an 11.3 L tank filled with dechlorinated water, and left them undisturbed overnight. The next morning, we collected 7–25 eggs at random from each clutch. We immediately preserved these eggs in 10% buffered formalin. Later, we placed each egg in a petri dish filled with 40 mL of an isotonic buffer [0.1 × MMR, pH 7.5 (100 mM NaCl, 2 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, 2 mM KCl)] and used a Leica MZ16 dissecting microscope with a Leica DFC480 digital camera (Leica, Wetzlar, Germany) to photograph the eggs at 1.0 × magnification. We determined the developmental stage of each egg (Gosner 1960) and measured its diameter by using ImageJ 1.37v (Rasband 1996–2007). We only included eggs in the same (Gosner 1960) stage in our analysis (stage 12). We then pooled all the egg sizes from each female to create a mean egg size per female. We used a two-tailed *t*-test to contrast mean egg diameter of females from sympatry with that of females from allopatry.

TESTING PREDICTION 3: ASSESSING THE RELATIONSHIP BETWEEN MATERNAL CONDITION AND OFFSPRING MORPH PRODUCTION

To test Prediction (3), we asked whether maternal condition predicts the time it takes the offspring to capture and consume a standard amount of shrimp. Shrimp ingestion induces development of the carnivore morph (Pomeroy 1981; Pfennig 1990; Storz 2004) and serves as a proxy of carnivore induction (Pfennig et al. 2007). In particular, the time to eat a standard number of anostracan shrimp is highly repeatable for individual tadpoles (D. W. Pfennig and R. A. Martin, unpubl. data), and it predicts a tadpole’s propensity to later develop into a carnivore morph: tadpoles that eat shrimp the fastest are ultimately the most likely to express the distinctive large-headed carnivore phenotype (Pfennig et al. 2007; confirmed in this study; see Results).

We paired 10 females and males that had been collected from sympatry. Immediately before breeding, we measured each female’s SVL and mass. We then injected both females and males with 0.07 mL of 0.1 mM gonadotropin releasing hormone agonist. We placed each male–female pair in an 11.3 L tank filled with dechlorinated water and left them undisturbed overnight. Five days after the breeding, we thinned out the tadpoles to approximately 50 tadpoles per 11.3 L plastic tank (we did not measure egg size in this, or in any of the subsequent, experiments). Sibships were kept in separate nursery tanks and fed fish food ad libitum until needed.

Next, using the procedures in Pfennig et al. (2007), we measured the propensity of tadpoles in each sibship to capture and consume three live shrimp. We randomly selected 45 similarly sized, 12-day-old tadpoles from all 10 sibships and placed each

tadpole alone in a small, round, individually numbered, opaque plastic container (12 cm diameter \times 6 cm deep) containing 600 mL of dechlorinated tap water (at 23°C). We arranged the containers on a table randomly with respect to sibship. We allowed the tadpoles to acclimate to their new surroundings for 24 h, during which time they were fed crushed fish food to ensure that all tadpoles were equally satiated. The next day, we placed into each container three, live brine shrimp (*Artemia* sp., 10 mm total length; brine shrimp are similar to the fairy shrimp on which *Spea* tadpoles prey in natural ponds and can induce carnivore-like morphology; Pfennig et al. 2007). We then observed the tadpoles continuously and recorded the time each took to capture and consume all three shrimp (none of the tadpoles used in this experiment had previously encountered shrimp). We pooled shrimp-eating times for the 45 siblings tested from each sibship to obtain a mean shrimp-eating time for each of the 10 sibships (females). To determine if maternal condition predicted offspring shrimp foraging ability, we regressed mean time to eat shrimp (natural log transformed to meet the assumptions of parametric analysis) on maternal condition.

TESTING PREDICTION 4: COMPARING MORPH PRODUCTION IN SYMPATRY AND ALLOPATRY AND ASSESSING ITS MODE OF INHERITANCE

To test Prediction (4), we collected adult *S. multiplicata* from allopatric and sympatric sites in the San Simon Valley. Immediately before breeding them, we measured each female's SVL and mass, and calculated her condition. From this analysis, we confirmed that the mean (\pm SEM) condition of allopatric females (1.78 ± 0.49) was significantly greater than that of sympatric females (-0.31 ± 0.52 , mean difference in condition, allopatry – sympatry = 1.46, $t_{36} = 2.04$, $P = 0.025$).

We created 36 full sibships by pairing adults from allopatry and sympatry according to four different cross types: (1) allopatric female \times allopatric male (“AA” cross type; seven sibships); (2) allopatric female \times sympatric male (“AS” cross type; nine sibships); (3) sympatric female \times allopatric male (“SA” cross type; seven sibships); and (4) sympatric female \times sympatric male (“SS” cross type; 13 sibships). Sibships were kept in separate nursery tanks and fed fish food ad libitum until needed.

We randomly selected 9–21 similarly sized, 12-day-old tadpoles from each of the 36 sibships (mean \pm SEM = 16.8 ± 0.8 tadpoles per sibship; 623 tadpoles total were tested). Using the same procedures as before, we measured how long each tadpole took to consume three shrimp. These data were transformed (natural log) to meet the assumptions of parametric analysis. We then calculated the mean time that tadpoles from each sibship took to consume shrimp and used these sibship mean shrimp-eating times in two separate analyses. First, we used a two-tailed t -test to determine if the sibships with an allopatric mother (i.e., AA and AS cross types) differed from sibships with a sympatric mother (i.e.,

SS and SA cross types) in time to eat shrimp (where faster shrimp-eating times are associated with a higher propensity to produce carnivores; see Testing Prediction 3). Based on previous research (Pfennig and Murphy 2002), we predicted that sibships with sympatric mothers should take significantly longer than sibships with allopatric mothers to capture and consume shrimp (see Prediction (4)). Second, because we confirmed that sibships with an allopatric mother ate shrimp significantly faster than did sibships with a sympatric mother (see Results), we used two-tailed t -tests to determine if differences in foraging behavior between offspring of allopatric and sympatric females were inherited maternally (see Prediction (4)). We specifically predicted that, in terms of shrimp-eating time, AS sibships would not differ significantly from AA sibships (both cross types with mothers from allopatry), and that SA sibships would not differ significantly from SS sibships (both cross types with mothers from sympatry).

Finally, to corroborate these shrimp-eating time data, we also evaluated whether sibships with an allopatric mother differed from those with a sympatric mother in actual carnivore production. We randomly selected 56 similarly sized, seven-day-old tadpoles from each of 15 sibships (three AA sibships, four AS sibships; four SA sibships; four SS sibships; the choice of which of the original 36 sibships to use was random). We placed these tadpoles individually in replicate tanks (28 \times 18 \times 10 cm) filled with 6 L of dechlorinated water (these were different tadpoles than those used for the shrimp feeding trials described in the previous paragraph). We fed tadpoles live brine shrimp ad libitum. After seven days, we categorized each tadpole as an omnivore or a carnivore based on overall morphology. Tanks were coded by number, and, without knowledge of which sibship or cross type was in a tank, one person scored each animal as being omnivore or carnivore using criteria in Pfennig (1990). Morph assignments were always unambiguous. Using these data, we then calculated the probability of each sibship producing a carnivore by dividing the number of tanks that produced a carnivore by 56, which was the total number of replicate tanks for each sibship (e.g., see Pfennig and Frankino 1997). We then used a two-tailed, nonparametric Mann–Whitney U -test to determine if the sibships with an allopatric mother (i.e., AA and AS cross types) differed from sibships with a sympatric mother (i.e., SS and SA cross types) in terms of proportion of carnivores produced per sibship.

TESTING PREDICTION 5: COMPARING MORPH PRODUCTION IN SYMPATRY AND ALLOPATRY WHEN MATERNAL CONDITION IS EQUILIBRATED

Finally, we asked whether sympatric females produced less carnivore-like tadpoles relative to allopatric females, even when the two groups of females were equilibrated in condition. We predicted (see Prediction (5)) that the reduced propensity of females from sympatry to produce carnivores (see Pfennig and Murphy

2000 and the Results of the present study) would disappear once females from sympatry and allopatry were similar in condition.

For this experiment, we used adults from the same populations as used in the previous experiment. Following their use in the previous experiment, females were maintained on high food levels (crickets ad libitum) for one year to equilibrate sympatric and allopatric females in condition (recall that, when they were used in the previous experiment, allopatric females were in significantly better condition than sympatric females). However, because females were not individually marked, we could not be certain which of the females used in the previous experiment were also used in the present experiment. From this group, we selected at random six allopatric females and 11 sympatric females. Immediately before breeding them, we measured each female's SVL and mass, and calculated her condition. From this analysis, we confirmed that allopatric females and sympatric females did not differ significantly in condition. Indeed, in contrast to their condition in the previous experiment, the mean (\pm SEM) condition of sympatric females (0.19 ± 0.36) was actually greater than that of allopatric females (-0.31 ± 0.49), although not significantly so (mean difference in condition, allopatry – sympatry = -0.50 , $t_{15} = 0.82$, $P = 0.42$).

We paired each female with a randomly chosen male from her population to obtain 17 sibships of tadpoles. Sibships were kept separately and fed fish food ad libitum until needed. Using the same procedures as in the previous experiment, we randomly selected 25 similarly sized, 12-day-old tadpoles from each of the 17 sibships and measured how long each tadpole took to consume three shrimp. We tested 175 tadpoles of allopatric mothers, and 274 tadpoles of sympatric mothers. Shrimp-eating times were transformed (natural log) to meet the assumptions of parametric analysis. We then calculated the mean time that tadpoles from each sibship took to consume shrimp and used these sibship mean shrimp-eating times in the analyses. In particular, we used a two-tailed t -test to determine whether sympatric and allopatric sibships differed in shrimp-eating times.

Results

TESTING PREDICTION 1: COMPARING MATERNAL CONDITION IN SYMPATRY AND ALLOPATRY

As we had predicted (see Prediction (1) under Hypothesis and Predictions above), freshly captured females from sympatry were in poorer condition than freshly captured females from allopatry. The mean (\pm SEM) condition (residuals of mass regressed on SVL) of allopatric females (1.61 ± 0.48) was significantly greater than that of sympatric females (-0.32 ± 0.43 , mean difference in condition, allopatry – sympatry = 1.93 , $t_{14} = 3.00$, $P = 0.009$).

Although allopatric sites tend to be at higher elevations than sympatric sites (Pfennig et al. 2006), the elevation at which a fe-

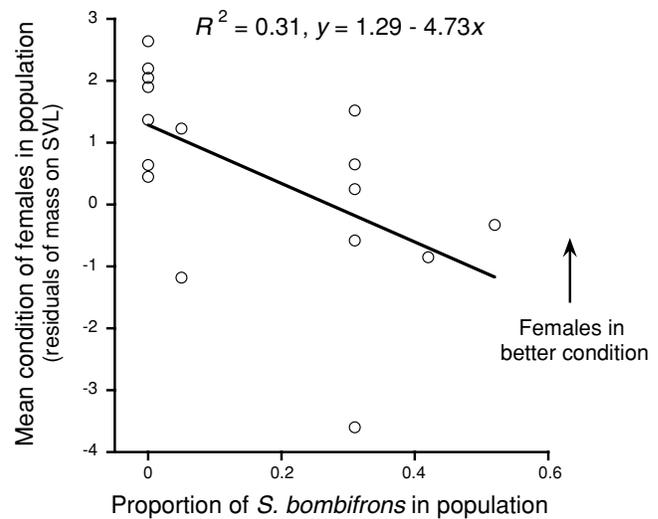


Figure 2. Proportion of *S. bombifrons* in a population predicts mean condition (residuals of mass regressed on SVL) of *S. multiplicata* females. In particular, mean condition of *S. multiplicata* females is significantly, negatively related to the proportion of *S. bombifrons* in the population (the datapoint on the bottom is not an outlier, and removal of this point does not affect overall significance).

male was collected was not significantly correlated her condition ($F_{1,14} = 1.76$, $P = 0.21$). In contrast, the relative abundance of *S. bombifrons* at any given site was significantly correlated with the mean condition of female *S. multiplicata* at those sites ($F_{1,14} = 6.17$, $P = 0.026$). In particular, there was a significant, negative relationship between the frequency of *S. bombifrons* in a population and the mean condition of female *S. multiplicata* in that population (Fig. 2). Thus, *S. multiplicata* that faced the most severe competition from *S. bombifrons* were in the poorest condition (see also Pfennig and Pfennig 2005).

TESTING PREDICTION 2: COMPARING MATERNAL INVESTMENT IN SYMPATRY AND ALLOPATRY

As we had predicted (see Prediction (2)), freshly captured females from sympatry produced smaller eggs than freshly captured females from allopatry. The mean (\pm SEM) egg diameter of allopatric females (1.65 ± 0.03 mm) was significantly greater than that of sympatric females (1.57 ± 0.02 mm, mean difference in egg size, allopatry – sympatry = 0.08 mm, $t_{24} = 2.34$, $P = 0.028$).

TESTING PREDICTION 3: ASSESSING THE RELATIONSHIP BETWEEN MATERNAL CONDITION AND OFFSPRING MORPH PRODUCTION

In accord with Prediction (3), maternal condition significantly predicted offspring foraging behavior ($F_{1,8} = 6.39$, $P = 0.035$). In particular, there was a significant, negative relationship between

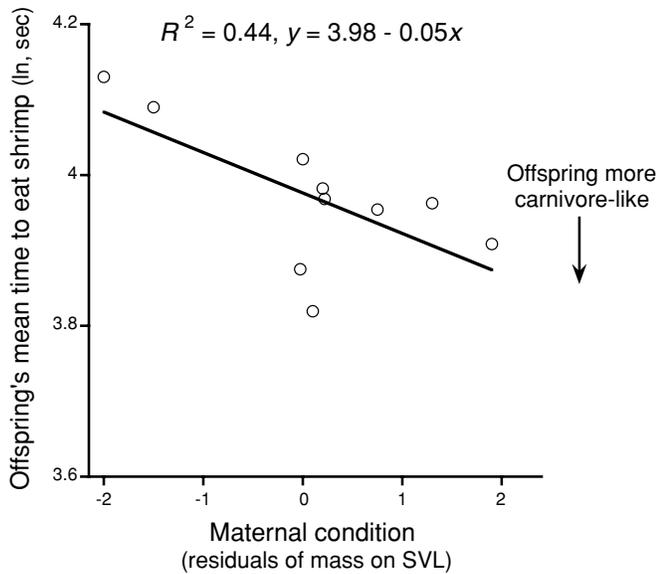


Figure 3. Maternal condition (residuals of mass regressed on SVL) predicts their offspring's time to eat three shrimp, a proxy for carnivore morph production, where faster shrimp-eating times predict higher probabilities of the tadpole becoming a carnivore. In particular, offspring shrimp-eating time is significantly, negatively related to maternal condition.

maternal condition and the time it took her offspring to capture and consume a standard quantity of shrimp (Fig. 3). Thus, females in the best condition produced the most carnivore-like tadpoles.

TESTING PREDICTION 4: COMPARING MORPH PRODUCTION IN SYMPATRY AND ALLOPATRY AND ASSESSING ITS MODE OF INHERITANCE

As predicted (Prediction (4)), females from sympatry produced less carnivore-like tadpoles than did females from allopatry. Sibships with a mother derived from sympatry took significantly longer to capture and consume a standard quantity of shrimp (3.81 ± 0.06 ln sec) than did sibships with a mother derived from allopatry (3.58 ± 0.07 ln sec, mean difference in time to eat shrimp, allopatry – sympatry = -0.23 ln sec, $t_{34} = 2.38$, $P = 0.024$; Fig. 4A). Thus, allopatric females produced the most carnivore-like tadpoles.

Differences in foraging behavior between offspring of allopatric and sympatric females appear to have been inherited maternally. Specifically, in terms of shrimp-eating time, AS sibships (3.59 ± 0.04 ln sec) did not differ significantly from AA sibships (3.56 ± 0.04 ln sec, mean difference in time to eat shrimp, AA – AS = -0.02 ln sec, $t_{14} = 0.42$, $P = 0.678$; Fig. 4A). Similarly, SA sibships (3.80 ± 0.14 ln sec) did not differ significantly from SS sibships (3.81 ± 0.11 ln sec, mean difference in time to eat shrimp, SS – SA = -0.001 ln sec, $t_{18} = 0.04$, $P = 0.972$; Fig. 4A).

Finally, not only did offspring of allopatric and sympatric females differ in foraging behavior, but they also differed in morph production. Moreover, as with differences in foraging behavior, differences in morph production appeared to be inherited maternally. Specifically, of 56 tadpoles in each cross type, six AA tadpoles, seven AS tadpoles, zero SA, and zero SS tadpoles developed into carnivores. Overall, sibships with a mother derived

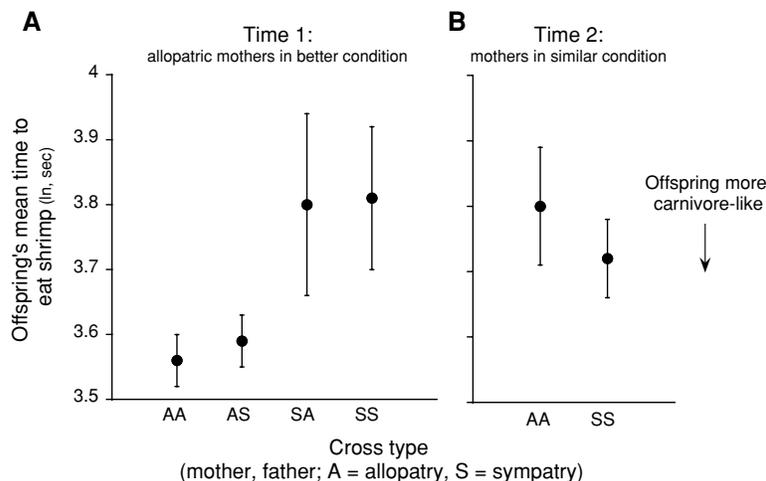


Figure 4. (A) Morph production when females were recently captured, and, hence, when sympatric mothers were in poorer condition than allopatric mothers. Shown is a comparison of sibship mean time to eat three shrimp, a proxy for carnivore morph production, where faster shrimp-eating times predict higher probabilities of the tadpole becoming a carnivore, for four cross types: (1) allopatric female \times allopatric male ("AA" cross type); (2) sympatric female \times sympatric male ("SS" cross type); (3) allopatric female \times sympatric male ("AS" cross type); (4) sympatric female \times allopatric male ("SA" cross type). (B) Morph production (as measured by the offspring's shrimp-eating time) when females had been maintained in the laboratory, during which time sympatric and allopatric mothers were equilibrated in condition before being bred.

from sympatry produced a significantly lower proportion of carnivores (0) than did sibships with a mother derived from allopatry (0.033 ± 0.002 carnivores per sibship, $U = 56$, $P = 0.0003$). Thus, allopatric females produced the most carnivores.

TESTING PREDICTION 5: COMPARING MORPH PRODUCTION IN SYMPATRY AND ALLOPATRY WHEN MATERNAL CONDITION IS EQUILIBRATED

In accord with Prediction (5), when allopatric and sympatric females were equilibrated in condition, the previously observed population differences in shrimp-eating times (Fig. 4A) disappeared. Indeed, when females were similar in condition, sibships with a sympatric mother (3.72 ± 0.06 ln sec) did not differ in shrimp-eating time from sibships with an allopatric mother (3.80 ± 0.09 ln sec, mean difference in time to eat shrimp, allopatry – sympatry = 0.08 ln sec, $t_{16} = 0.78$, $P = 0.445$; Fig. 4B).

Discussion

Maternal effects may often play an important role in ecological character displacement. As with other forms of phenotypic plasticity, maternal effects can instigate immediate phenotypic responses to changing environmental circumstances, such as the presence (or absence) of a heterospecific competitor. Maternal effects should therefore increase the chances that character displacement, as opposed to competitive exclusion, will transpire. However, in contrast to within-generation plasticity, maternal effects also have carry-over effects on subsequent generations (see Introduction). In fact, maternal effects may spawn an evolutionary momentum that even persists long after the environmental stimulus that originally triggered the maternal effect ceases (Kirkpatrick and Lande 1989). Thus, maternal effects provide a mechanism whereby differences that arise between populations in trait expression—such as those that might occur between populations in contrasting competitive environments—may be inherited in subsequent generations.

Our results suggest that maternal effects may indeed play a critical role in promoting character displacement. In particular, five lines of evidence support the hypothesis that a condition-dependent maternal effect mediates divergence between populations of *S. multiplicata* that have, and have not, undergone ecological character displacement (Fig. 1; see also Hypothesis and Predictions). The first such line of evidence is that females from sympatry were in poorer condition than those from allopatry. The reduced condition of sympatric females appeared to reflect, at least in part, competition from *S. bombifrons* (Fig. 2).

The second line of evidence is that, possibly because they were in poorer condition, females from sympatry invested less in offspring than did those from allopatry. Specifically, sympatric females produced smaller eggs than did allopatric females, sug-

gesting that females in different competitive environments varied in the amount of provision that they allocated to each egg.

The third line of evidence is that females in the best condition produced offspring with the highest propensity to become carnivores (Fig. 3). This tendency for better condition females to produce more carnivores may reflect the fact that females in better condition produce larger eggs, which develop into correspondingly larger tadpoles (R. A. Martin and D. W. Pfennig, unpubl. ms). Larger tadpoles, in turn, handle shrimp more efficiently and consume them faster and earlier in development than smaller tadpoles (Frankino and Pfennig 2001), thereby acquiring more of the cue—shrimp ingestion—that induces the carnivore morph.

The fourth line of evidence is that, presumably because they were in poorer condition and produced smaller eggs (see Testing Prediction 2) and tadpoles (see R. A. Martin and D. W. Pfennig, unpubl. ms), freshly captured females from sympatry produced fewer carnivores than did those from allopatry (Fig. 4A; see also Pfennig and Murphy 2000). Moreover, these population differences appeared to be inherited maternally (Fig. 4A).

Finally, a fifth line of evidence is that the reduced propensity of females from sympatry to produce carnivores disappeared when sympatric and allopatric females were equilibrated in condition (Fig. 4B).

It might be contended that some of our data (e.g., see Fig. 4A) are also consistent with the hypothesis that there is genomic imprinting with maternal expression of genes regulating morph production (e.g., Jablonka and Lamb 1995). Two observations argue against this alternative hypothesis, however. First, morph production is condition dependent: females in the best condition produced offspring with the highest propensity to become carnivores (Fig. 3). Second, the decreased propensity of sympatric females to produce carnivores disappeared once sympatric and allopatric females were equilibrated in condition (Fig. 4B). Both observations are more consistent with the hypothesis that a maternal effect, rather than genomic imprinting, influences morph production (see also R. A. Martin and D. W. Pfennig, unpubl. ms). Thus, taken together, the above five lines of evidence point to a maternal effect mediating population divergence and character displacement in spadefoot toads (Fig. 1). Generally, our results suggest that maternal effects may play an important role in character displacement.

If female condition can equilibrate in the laboratory in one year, why does female condition not equilibrate in nature and consequently balance morph production between sympatric and allopatric sites? One explanation is that females that are larger and in better condition as juveniles may achieve adult size and condition along a different growth trajectory from those that are smaller and in poorer condition. Possibly, poorer condition females can only be taken off their trajectory by long-term,

continuous, intensive feeding in the laboratory, something that they will likely not experience in the wild (where foraging opportunities are restricted to a few humid nights in the summer, when the toads emerge from their burrows; Bragg 1965). Moreover, in the laboratory, larger, better condition adults are better at acquiring food than are smaller, poorer condition adults (D. W. Pfennig, pers. obs.). Females that are larger and in better condition as juveniles (e.g., those that developed as carnivores when tadpoles) therefore likely maintain a size and condition advantage for life. Thus, differences in condition between sympatric and allopatric females may persist in the wild, thereby contributing to divergence in morph production and character displacement.

Although maternal effects are increasingly seen as being important in adaptive evolution (Roach and Wuff 1987; Gliwicz and Guisande 1992; Fox et al. 1997; Mousseau and Fox 1998; Agrawal et al. 1999; Hendrickx et al. 2003; Bashley 2006; Plaistow et al. 2006; Räsänen and Kruuk 2007; Allen et al. 2008; Bossdorf et al. 2008), relatively little is known of their possible contributions to population divergence (Räsänen and Kruuk 2007; but see Badyaev et al. 2002). The present study contributes to filling this void. Our study illustrates how a maternal effect can mediate divergence between neighboring conspecific populations that differ in exposure to a heterospecific competitor. Specifically, in sympatric populations of *S. multiplicata*, a maternal effect appears to generate a self-reinforcing cycle that is triggered when *S. bombifrons* outcompete *S. multiplicata* for shrimp, thereby precluding them from experiencing the environmental cue (shrimp ingestion) that induces the production of a larger, carnivore-morph tadpole (Pfennig and Murphy 2000, 2002). Because *S. multiplicata* produce only the smaller omnivore-morph tadpoles in sympatry (Pfennig and Murphy 2000, 2002; Pfennig et al. 2007), this may explain why they mature into smaller, poorer condition adults (Pfennig and Pfennig 2005; this study). These smaller, poorer condition adults, in turn, produce mostly omnivores in the next generation, because of a condition-dependent maternal effect (Fig. 3). Once started, this process should be self-reinforcing and thereby persist for generations. In each generation, smaller, poorer condition females will produce omnivores, which will tend to mature as smaller, poorer condition adults that produce more omnivores in the next generation. Moreover, because this process is not expected to occur in nearby allopatric populations of *S. multiplicata*, sympatric and allopatric populations will undergo character displacement in their propensity to produce the carnivore morph. In this way, a maternal effect mediates divergence between populations.

Our results further suggest that maternal effects may mediate such divergence on a short time scale. In our system, a maternal effect may have enabled allopatric and sympatric populations of *S. multiplicata* to evolve different propensities to produce alternative resource-use morphs in less than 60 spadefoot generations:

S. multiplicata and *S. bombifrons* appear to have first come into contact in the San Simon Valley as recently as 120 years ago (Pfennig 2003; Rice and Pfennig 2008; A. Chunco, unpubl. data). By mediating rapid character displacement, maternal effects may buffer populations from extinction (via competitive exclusion) as morphological evolution proceeds. Thus, maternal effects may render character displacement, as opposed to competitive exclusion, more likely to transpire.

The recognition that maternal effects can mediate rapid character displacement helps resolve a long-standing discrepancy between theory and data regarding the speed of character displacement. Most models assume that character displacement will be a long, drawn-out process (Slatkin 1980; Taper and Case 1985). Yet, empirical data have shown that character displacement can be rapid (e.g., Fenchel 1975; Diamond et al. 1989; Yom-Tov et al. 1999; Grant and Grant 2006; Pfennig et al. 2006). This discrepancy between theory and data vanishes when one considers that condition-dependent maternal effects can mediate rapid character displacement. Indeed, when underlain by a maternal effect, even canalized character displacement (*sensu* Pfennig and Murphy 2002), where differences in trait production between sympatry and allopatry are developmentally fixed, could potentially evolve in only two generations following contact with a heterospecific competitor (Fig. 1).

Maternal effects may also facilitate the subsequent evolution of character displacement by buffering populations from extinction while genetic changes accumulate, which produce the divergent trait in the absence of the maternal effect. Different alleles or genotypes may become fixed in different populations that differ in exposure to a heterospecific competitor, for at least two reasons (Pfennig and Murphy 2002). First, as one resource-use phenotype is expressed continuously in a population, and as the alternative phenotype is never expressed, alleles that regulate expression of this "hidden" phenotype would not be exposed to selection, and thus they would be at risk of chance loss through drift or gradual mutational decay (West-Eberhard 1989). Second, if individuals in a population begin to express an environmentally induced phenotype that lessens costly interactions with heterospecifics, and if there is underlying genetic variation in the population in the magnitude and direction of the plastic response, then selection should favor those alleles or gene combinations that best stabilize, refine, and extend this trait's expression through the process of genetic accommodation (*sensu* West-Eberhard 2003). Following strong and persistent divergent selection, alternative alleles may thereby become fixed in different populations (for possible examples of genetic accommodation from natural populations, see Van Tienderen 1990; Gurevitch 1992; Day et al. 1994; Losos et al. 2000; Sword 2002; Parsons and Robinson 2006; Gomez-Mestre and Buchholz 2006; Ledón-Rettig et al. 2008). Thus, by biasing the expression of resource-use phenotypes, maternal effects

may facilitate the further evolution and elaboration of character displacement. Indeed, maternal effects may often be decisive in determining the initial direction and rate of evolution during the early stages of character displacement.

In conclusion, our study suggests that a maternal effect can promote rapid, adaptive divergence between populations that differ in exposure to a competitor. Because populations that undergo such divergence might eventually become reproductively isolated from one another, maternal effects may therefore fuel speciation and adaptive radiation. Future studies are needed to evaluate more fully the possible contributions of maternal effects to evolutionary diversification.

ACKNOWLEDGMENTS

We thank K. Pfennig, A. Rice, and M. McGee for laboratory and field assistance, K. Pfennig, C. Ledón-Rettig, J. Wolf, and two anonymous referees for helpful comments on the manuscript, and the American Museum of Natural History's (AMNH) Southwestern Research Station (SWRS) for housing and logistical support. This work was funded by NSF grants DEB-0234714 and DEB-0640026 and conducted in compliance with the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill under application #06-047.0-A. Field collections were conducted under Arizona collecting permit SP719496 and New Mexico collecting permit 1857.

LITERATURE CITED

- Agrawal, A. A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science* 294:321–326.
- Agrawal, A. A., C. Laforsch, and R. Tollrian. 1999. Transgenerational induction of defences in animals and plants. *Nature* 401:60–63.
- Allen, R. M., Y. M. Buckley, and D. J. Marshall. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. *Am. Nat.* 171:225–237.
- Arthur, W. 1982. The evolutionary consequences of interspecific competition. *Adv. Ecol. Res.* 12:127–187.
- Badyaev, A. V., and Oh, K. P. 2008. Environmental induction and phenotypic retention of adaptive maternal effects. *BMC Evol. Biol.* 8:3.
- Badyaev, A. V., G. E. Hill, M. L. Beck, A. A. Derven, R. A. Duckworth, K. J. McGraw, P. M. Nolan, and L. A. Whittingham. 2002. Sex biased hatching order and adaptive population divergence in a passerine bird. *Science* 295:316–318.
- Bashley, F. 2006. Cross-generational environmental effects and the evolution of offspring size in the Trinidadian guppy *Poecilia reticulata*. *Evolution* 60:348–361.
- Bernardo, J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *Am. Zool.* 36:216–236.
- Bossdorf, O., C. L. Richards, and M. Pigliucci. 2008. Epigenetics for ecologists. *Ecol. Lett.* 11:106–115.
- Bragg, A. N. 1965. Gnomes of the night: the spadefoot toads. Univ. of Pennsylvania Press, Philadelphia, PA.
- Brown, W. L., and E. O. Wilson. 1956. Character displacement. *Syst. Zool.* 5:49–64.
- Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer, Sunderland, MA.
- Day, T., and K. A. Young. 2004. Competitive and facilitative evolutionary diversification. *BioScience* 54:101–109.
- Day, T., J. Pritchard, and D. Schluter. 1994. A comparison of two sticklebacks. *Evolution* 48:1723–1734.
- Dayan, T., and D. Simberloff. 2005. Ecological and community-wide character displacement: the next generation. *Ecol. Lett.* 8:875–894.
- DeWitt, T. J., and S. M. Scheiner. 2004. Phenotypic plasticity: functional and conceptual approaches. Oxford Univ. Press, New York.
- Diamond, J., S. L. Pimm, M. E. Gilpin, and M. LeCroy. 1989. Rapid evolution of character displacement in Myzomelid honeyeaters. *Am. Nat.* 134:675–708.
- Doebeli, M. 1996. An explicit genetic model for ecological character displacement. *Ecology* 77:510–520.
- Fenchel, T. 1975. Character displacement and coexistence in mud snails (Hydrobiidae). *Oecologia* 20:19–32.
- Fordyce, J. A. 2006. The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *J. Exp. Biol.* 209: 2377–2383.
- Fox, C. W., M. S. Thakar, and T. A. Mousseau. 1997. Egg size plasticity in a seed beetle: an adaptive maternal effect. *Am. Nat.* 149:149–163.
- Frankino, W. A., and D. W. Pfennig. 2001. Condition-dependent expression of trophic polyphenism: effects of individual size and competitive ability. *Evol. Ecol. Res.* 3:939–951.
- Gliwicz, Z. M., and C. Guisande. 1992. Family planning in *Daphnia*: resistance to starvation in offspring born to mothers grown at different food levels. *Oecologia* 91:463–467.
- Gomez-Mestre, I., and D. R. Buchholz. 2006. Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *Proc. Natl. Acad. Sci. USA* 103:19021–19026.
- Gorbushin, A. M. 1996. The enigma of mud snail shell growth: asymmetrical competition or character displacement. *Oikos* 77:85–92.
- Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
- Grant, P. R. 1972. Convergent and divergent character displacement. *Biol. J. Linn. Soc. Lond.* 4:39–68.
- Grant, P. R., and B. R. Grant. 2006. Evolution of character displacement in Darwin's finches. *Science* 313:224–226.
- Gurevitch, J. 1992. Sources of variation in leaf shape among two populations of *Achillea lanulosa*. *Genetics* 130:385–394.
- Hendrickx, F., J. P. Maelfait, M. Speelmans, and N. M. Van Straalen. 2003. Adaptive reproductive variation along a pollution gradient in a wolf spider. *Oecologia* 134:189–194.
- Hoskin, C. J., M. Higgie, K. R. McDonald, and C. Moritz. 2005. Reinforcement drives rapid allopatric speciation. *Nature* 437:1353–1356.
- Jablonka, E., and M. J. Lamb. 1995. Epigenetic inheritance and evolution: the Lamarckian dimension. Oxford Univ. Press, New York.
- Kirkpatrick, M., and R. Lande. 1989. The evolution of maternal characters. *Evolution* 43:485–503.
- Ledón-Rettig, C., D. W. Pfennig, and N. Nascone-Yoder. 2008. Ancestral variation and the potential for genetic accommodation in larval amphibians: implications for the evolution of novel feeding strategies. *Evol. Dev.* 10:316–325.
- Losos, J. B., D. A. Creer, D. Glossip, R. Goellner, A. Hampton, G. Roberts, N. Haskell, P. Taylor, and J. Ettling. 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. *Evolution* 54:301–305.
- Maynard Smith, J. 1998. Evolutionary genetics. 2nd edn. Oxford Univ. Press, New York.
- McAdam, A. G., and S. Boutin. 2004. Maternal effects and the response to selection in red squirrels. *Proc. R. Soc. Lond. B.* 271:75–79.
- Mousseau, T. A., and C. W. Fox. 1998. Maternal effects as adaptations. Oxford Univ. Press, New York.
- Parsons, K. J., and B. W. Robinson. 2006. Replicated evolution of integrated plastic responses during early adaptive divergence. *Evolution* 60:801–813.

- Pfennig, D. W. 1990. The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. *Oecologia* 85:101–107.
- Pfennig, D. W. 1992. Proximate and functional causes of polyphenism in an anuran tadpole. *Funct. Ecol.* 6:167–174.
- Pfennig, K. S. 2003. A test of alternative hypotheses for the evolution of reproductive isolation between spadefoot toads: support for the reinforcement hypothesis. *Evolution* 57:2842–2851.
- . 2007. Facultative mate choice drives adaptive hybridization. *Science* 318:965–967.
- Pfennig, D. W., and W. A. Frankino. 1997. Kin-mediated morphogenesis in facultatively cannibalistic tadpoles. *Evolution* 51:1993–1999.
- Pfennig, D. W., and P. J. Murphy. 2000. Character displacement in polyphenic tadpoles. *Evolution* 54:1738–1749.
- . 2002. How fluctuating competition and phenotypic plasticity mediate species divergence. *Evolution* 56:1217–1228.
- . 2003. A test of alternative hypotheses for character divergence between coexisting species. *Ecology* 84:1288–1297.
- Pfennig, K. S., and D. W. Pfennig. 2005. Character displacement as the ‘best of a bad situation’: fitness trade-offs resulting from selection to minimize resource and mate competition. *Evolution* 59:2200–2208.
- Pfennig, D. W., and A. M. Rice. 2007. An experimental test of character displacement’s role in promoting postmating isolation between conspecific populations in contrasting competitive environments. *Evolution* 61:2433–2443.
- Pfennig, D. W., A. M. Rice, and R. A. Martin. 2006. Ecological opportunity and phenotypic plasticity interact to promote character displacement and species coexistence. *Ecology* 87:769–779.
- . 2007. Field and experimental evidence for competition’s role in phenotypic divergence. *Evolution* 61:257–271.
- Pfennig, K. S., and M. J. Ryan. 2006. Reproductive character displacement generates reproductive isolation among conspecific populations: an artificial neural network study. *Proc. R. Soc. Lond. B.* 273:1361–1368.
- Plaistow, S. J., C. T. Lapeley, and T. G. Benton. 2006. Context-dependent intergenerational effects: the interaction between past and present environments and its effect on population dynamics. *Am. Nat.* 167:206–215.
- Pomeroy, L. V. 1981. Developmental polymorphism in the tadpoles of the spadefoot toad *Scaphiopus multiplicatus*. Ph.D. thesis. Univ. of California, Riverside, CA.
- Räsänen, K., and E. B. Kruuk. 2007. Maternal effects and evolution at ecological time scales. *Funct. Ecol.* 21:408–421.
- Rasband, W. S. 1996–2007. Image J. Bethesda, MD: <http://rsb.info.nih.gov/ij/>.
- Rice, A. M., and D. W. Pfennig. 2007. Character displacement: in situ evolution of novel phenotypes or sorting of pre-existing variation? *J. Evol. Biol.* 20:448–459.
- . 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.
- Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. *Annu. Rev. Ecol. Syst.* 18:209–236.
- Robinson, B. W., and D. S. Wilson. 1994. Character release and displacement in fish: a neglected literature. *Am. Nat.* 144:596–627.
- Rossiter, M. C. 1996. Incidence and consequences of inherited environmental effects. *Annu. Rev. Ecol. Syst.* 27:451–176.
- Rundle, H. D., and P. Nosil. 2005. Ecological speciation. *Ecol. Lett.* 8:336–352.
- Schlichting, C. D., and M. Pigliucci. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer, Sunderland, MA.
- Schluter, D. 2000. The ecology of adaptive radiation. Oxford Univ. Press, Oxford, U. K.
- Servedio, M. R., and M. A. F. Noor. 2003. The role of reinforcement in speciation: theory and data. *Annu. Rev. Ecol. Evol. Syst.* 34:339–364.
- Siegal, M. L., and A. Bergman. 2006. Canalization. Pp. 235–251 in C. W. Fox and J. B. Wolf, eds. *Evolutionary genetics: concepts and case studies*. Oxford Univ. Press, New York.
- Slatkin, M. 1980. Ecological character displacement. *Ecology* 61:163–177.
- Storz, B. L. 2004. Reassessment of the environmental mechanisms controlling developmental polyphenism in spadefoot toad tadpoles. *Oecologia* 141:402–410.
- Sword, G. A. 2002. A role for phenotypic plasticity in the evolution of aposematism. *Proc. R. Soc. Lond. B.* 269:1639–1644.
- Taper, M. L., and T. J. Case. 1985. Quantitative genetic models for the coevolution of character displacement. *Ecology* 66:355–371.
- . 1992. Coevolution among competitors. *Oxford Surv. Evol. Biol.* 8:63–109.
- Van Tienderen, P. H. 1990. Morphological variation in *Plantago lanceolata*: limits of plasticity. *Evol. Trend. Plant.* 4:35–43.
- Werner, E. E., and D. S. Hall. 1976. Niche shifts in sunfishes: experimental evidence and significance. *Science* 191: 404–406.
- West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20:249–278.
- . 2003. Developmental plasticity and evolution. Oxford Univ. Press, New York.
- Wilson, E. O. 1992. The diversity of life. Harvard Univ. Press, Cambridge, MA.
- Yom-Tov, Y., S. Yom-Tov, and H. Moller. 1999. Competition, coexistence, and adaptation amongst rodent invaders to Pacific and New Zealand islands. *J. Biogeogr.* 26:947–958.

Associate Editor: J. Wolf