

Evolution of Phenotypic Plasticity and Gene Expression during Character Displacement

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Character displacement – trait evolution that arises as an adaptive response to competition between species – is central to the origins, abundance and distribution of biodiversity. Yet, until recently, little was known of character displacement’s underlying mechanisms. Although character displacement is assumed to arise solely through changes in deoxyribonucleic acid (DNA) sequence, many species can also respond adaptively to competition by facultatively altering traits via phenotypic plasticity, which frequently entails changes in gene expression. New research has revealed that these two mechanisms might often act together during character displacement. In particular, character displacement might proceed through an initial phase in which trait (and gene expression) differences are environmentally induced to a latter phase in which such differences become fixed via changes in DNA sequence. This plasticity-first hypothesis has increasing support, suggesting that it may be a common pathway to character displacement.

Introduction

Competition has long been considered a key driver of evolution by natural selection. Indeed, in *The Origin of Species* (1859 (2009)), Darwin emphasised how competition can stimulate divergence both within and among species. In Darwin’s view, new species, and the evolution of trait differences between them,

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arise because divergent natural selection minimises competition among initially similar populations or species. Thus, when resources are limited and competition is intense, individuals that are the most dissimilar to their competitors will experience reduced competition and ultimately be favoured by selection. Over generations, such divergent natural selection causes competing groups to evolve greater dissimilarity. This process, Darwin argued, explains why even closely related species often differ, especially in traits involved in competition.

In the decades following these ideas, ecologists and evolutionary biologists have come to recognise that Darwin was largely correct. Species often do compete with each other. However, we now know that species compete not only for resources, such as food and habitat (Schoener, 1983), but also for opportunities to reproduce successfully, such as when species hybridise or interfere with each other’s mating signals (Pfennig and Pfennig, 2012). Regardless of whether competition occurs over resources or successful reproduction, competition is (by definition) always deleterious to all parties. In some cases, competitive interactions can even drive a species locally extinct via ‘competitive exclusion’, as first demonstrated by Gause (1934) (reproductive interactions can similarly cause a species to become locally extinct through ‘reproductive exclusion’ or ‘sexual exclusion’; Gröning and Hochkirch, 2008). We also know that Darwin was correct about how natural selection can mitigate the costs of competition. Specifically, these costs abate – and the chances of species coexistence thereby increase – when species evolve distinctive resource use or reproductive traits that enable them to obtain resources or reproduce successfully in the face of competition (**Figure 1a,b**). Such trait evolution that occurs as an adaptive response to competition between species for resources or successful reproduction – dubbed ‘ecological character displacement’ and ‘reproductive character displacement’, respectively – is now widely viewed as central in the origins, distribution and abundance of the Earth’s biodiversity, just as Darwin had first suggested (Schluter, 2000; Dayan and Simberloff, 2005; Pfennig and Pfennig, 2012). **See also: Interspecific Competition; Ecological Character Displacement; Darwin and the Idea of Natural Selection**

But what is the evidence for character displacement? To determine if character displacement has occurred, researchers often

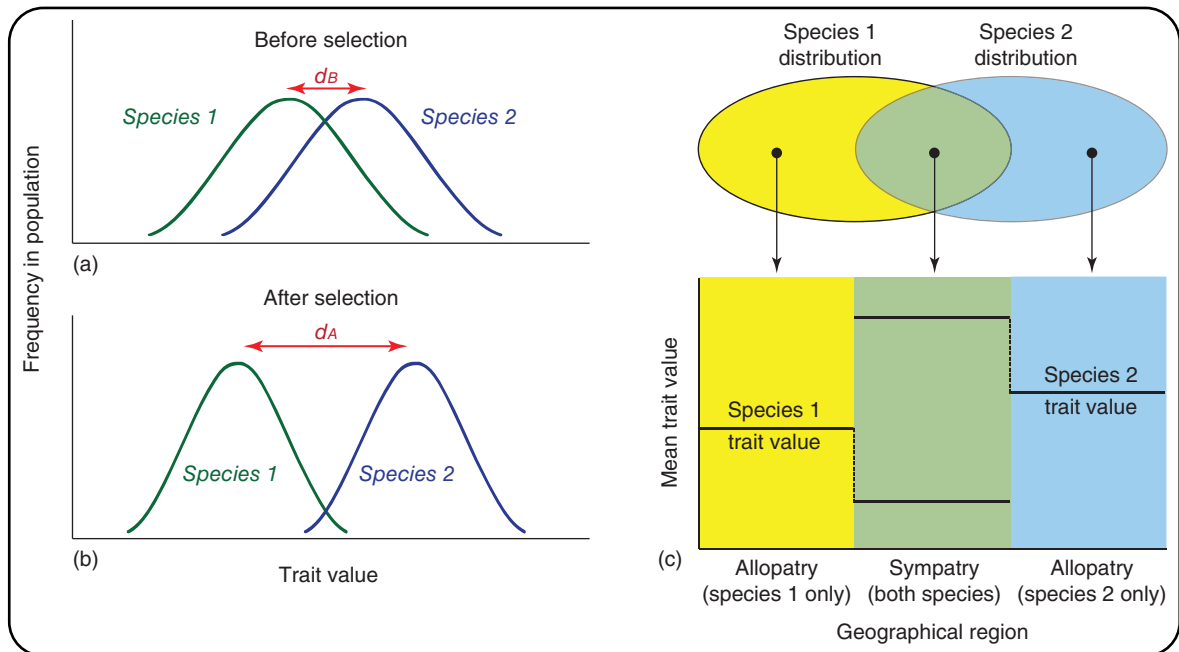


Figure 1 How character displacement unfolds and is detected. (a) Initially, two species encounter each other and overlap in traits associated with resource use or reproduction, indicated here by the two overlapping bell-shaped curves. Character displacement arises when individuals most dissimilar from the average resource-use or reproductive phenotypes of another species are more successful at acquiring resources or at reproducing than other members of their population. (b) Consequently, the most divergent individuals should experience the highest fitness, and the two species should evolve to be less similar to each other. Character displacement is indicated when the difference between species in mean trait value is greater after selection (d_A) than before selection (d_B). In practice, this process is most often detected by a distinctive pattern of divergence (c) in which the two species are more dissimilar to each other in sympatry, where there is selection for divergence, than in allopatry, where there is no such selection.

compare populations of the same species that cooccur with another species (sympatric populations) with those populations that occur in the absence of the other species (allopatric populations). From such a contrast, the signature of character displacement can be identified – greater dissimilarity between species that occur in sympatry than where those same species occur in allopatry (**Figure 1c**). A classic case in which character displacement was detected this way comes from the seed-eating finches that inhabit the Galápagos islands (i.e. Darwin’s finches). On these islands, several finch species have diverged in the morphology of their beak in order to minimise competition and to facilitate utilisation of alternative resource types (Grant, 1972). Importantly, differences in beak morphology are exaggerated in these sympatric populations compared to allopatric populations (Grant, 1972). Numerous other examples of character displacement have now been documented in diverse taxa, ranging from plants to animals and from invertebrates to vertebrates, and in diverse traits, ranging from those involved in resource use to those involved in reproduction (reviewed in Schluter, 2000; Dayan and Simberloff, 2005; Pfennig and Pfennig, 2012). **See also: Darwin’s Finches**

Some of the strongest evidence for character displacement comes from studies aiming to fulfil rigorous criteria that rule out evolutionary alternatives (Schluter, 2000). Experimental studies have also been used to confirm specific predictions of the character displacement hypothesis, such as showing that natural selection favours divergent phenotypes during competition

(Schluter, 1994). In addition, compelling support for character displacement comes from observations in which researchers have documented the process unfolding, in both the lab (Tyerman *et al.*, 2008) and the field. As an example of the latter, Grant and Grant (2006) observed on an island in the Galápagos that the medium ground finch diverged from its competitor in beak size and shape (and, hence, in diet). Another case comes from *Anolis* lizards inhabiting small islands in the southeastern United States (Stuart *et al.*, 2014). Here, an invading species (*Anolis sagrei*) caused the resident species (*Anolis carolinensis*) to move to higher perches. These *A. carolinensis* subsequently evolved larger toepads (**Figure 2**). Thus, competition can indeed promote divergent trait evolution among species.

Despite this mounting evidence for character displacement, however, the underlying proximate (i.e. genetic and developmental) mechanisms that mediate character displacement have received less attention. In the following section, we review what is known about these mechanisms.

Proximate Mechanisms of Character Displacement

As with many forms of adaptive evolution, character displacement is assumed to arise exclusively through changes in the frequencies of underlying genes, and ultimately, therefore, changes

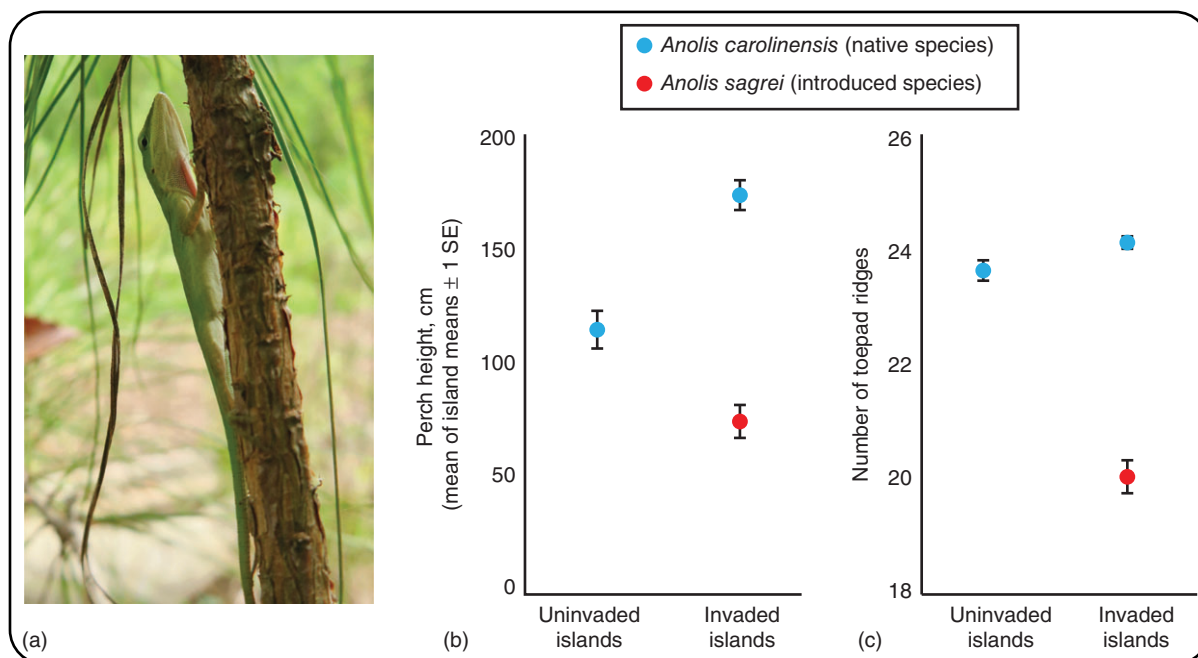


Figure 2 An example of character displacement observed in the wild. (a) On a series of small islands off the east coast of Florida, USA, (a) the native lizard, *A. carolinensis*, prefers to perch in trees (b) about a meter above ground. However, when another species, *A. sagrei*, was introduced onto some of these islands, *A. carolinensis* shifted to a higher perch site, and thereby reduced competition with this lower perching introduced species. (c) Moreover, in only 20 generations, *A. carolinensis* on the invaded islands had adapted to their higher perch sites by evolving changes to their toes, which they use to grasp trees. Based on data in Stuart *et al.* (2014). Photo by D. Pfennig.

in DNA base-pair sequence (Schluter, 2000; Dayan and Simberloff, 2005). According to this view, natural selection favours those individuals that are the most phenotypically dissimilar from their competitors. Consequently, this selection thereby also favours the genes that encode for these divergent traits. In this way, as the frequency of dissimilar individuals increases, so does the frequency of genes that contribute to that phenotypic dissimilarity. Indeed, among the widely cited criteria for demonstrating character displacement is showing that the phenotypic shift is accompanied by (and therefore, presumably, caused by) a genetic shift (Schluter and McPhail, 1992; Schluter, 2000).

A number of studies have shown that populations experiencing character displacement do indeed differ in the frequencies of genes that appear to encode the traits that are undergoing divergence. For example, genomic regions have recently been identified that vary systematically among Darwin's finch species with different beak morphologies. One such region contains a gene (*hmga2*) associated with variation in beak size (Lamichhaney *et al.*, 2016). This locus is under strong selection in populations of the medium ground finch that are experiencing character displacement (see section titled 'Introduction'), suggesting that changes in this gene have mediated competition-driven shifts in beak size in these populations (Lamichhaney *et al.*, 2016). Other studies involving various species and types of traits have similarly identified genetic loci that appear to be undergoing character displacement (reviewed in Pfennig and Pfennig, 2012).

Interestingly, although these genetic shifts sometimes appear to involve changes in DNA coding sequence (such as regions

of the genome encoding enzymes or cellular components), in many cases, genetic shifts appear to involve changes in regulatory regions of the genome (such as regions encoding transcription factors or signalling molecules that affect the expression of other genes; Pfennig and Pfennig, 2012). An example of the latter is the *hmga2* gene mentioned in the previous paragraph. This gene encodes for a protein that regulates the expression of other genes by potentiating other transcription factors (Lamichhaney *et al.*, 2016, e.g. the aforementioned two loci involved in mediating character displacement in Darwin's finch species; see also McGraw *et al.*, 2011; Hopkins and Rausher, 2011). That selection would preferentially target such regulatory regions over coding regions makes sense, given that changes in coding sequence often impact many other aspects of development (other than just the trait under selection). These findings are therefore consistent with the emerging viewpoint that changes in morphology generally result from changes in the spatiotemporal regulation of gene expression during development (Carroll, 2008). Thus, during the evolution of character displacement, selection might often favour evolutionary changes in gene regulation rather than in coding sequence.

Yet, an adaptive response to competition need not arise solely through changes in coding or regulatory sequence – shifts in traits associated with resource use or reproduction might instead reflect *environmentally induced* phenotypic change; that is 'phenotypic plasticity' See also: **Genomic Studies of Adaptation in Natural Populations; Adaptation and Natural Selection: Overview; Ecological Developmental Biology**. Indeed, such

facultative shifts might often be the first step in the process of character displacement and thereby play a crucial role in *jump-starting* subsequent genetic divergence. In the following section, we discuss these ideas in more detail.

Phenotypic Plasticity as Instigator of Character Displacement

To understand how phenotypic plasticity could initiate character displacement, consider that many species can facultatively alter their resource-use traits as an adaptive response to interspecific competition (Pfennig and Pfennig, 2012). For example, in response to low-light conditions, such as those induced by a competitor, many species of plants facultatively elongate their stems, suppress branching, alter the width of their leaves and/or accelerate flowering (Sultan, 2007). In addition, in response to low levels of food, such as what happens in the presence of a competitor, the larvae of many species of echinoids (sea urchins) facultatively allocate more tissue to their ciliated arms, which they use to acquire their food (Hart and Strathmann, 1994). Such competitively induced changes can instigate rapid, widespread and adaptive divergence between competing species (Turcotte and Levine, 2016). This speed with which phenotypic plasticity can mitigate costly competitive interactions (typically, within an individual's lifetime) is especially important, for character displacement is a time-limited process. If divergence transpires too slowly, a population risks becoming extinct owing to competitive or reproductive exclusion as noted in the Introduction.

But how can phenotypic plasticity promote *evolution*? After all, evolution is defined as genetic change, and phenotypic plasticity has long been considered a 'nongenetic' (phenotypic) response.

The key to understanding how phenotypic plasticity can promote evolution is the concept of the 'reaction norm' (Schlichting and Pigliucci, 1998). Individuals within the same population often harbour genetic variation in the degree to which they respond to environmental cues (Scheiner, 1993). In other words, different genotypes typically express different environmentally contingent phenotypic responses; that is reaction norms. Thus, when selection favours the evolution of plastic responses that mitigate competition, it also favours certain reaction norms (genotypes) over others, which can ultimately lead to competition-driven evolution; that is character displacement (Figure 3).

Eventually, trait divergence that started out as an environmentally induced shift might become 'fixed' in a population, such that these divergent traits are produced constitutively (Wilson, 1992). According to the 'plasticity-first' hypothesis for the evolution of character displacement (Pfennig and Pfennig, 2012), character displacement might evolve from an initial phase in which phenotypic divergence is environmentally induced to a later phase in which this divergence becomes genetically fixed. This sequence might occur if (1) there is underlying genetic variation in how individuals plastically respond to interspecific competition (i.e. if reaction norms differ among genotypes); (2) selection acts on this variation to refine (via quantitative genetic changes) these induced phenotypes over time (i.e. 'genetic accommodation' occurs; *sensu* West-Eberhard, 2003) and (3) these environmentally induced traits evolve to the point of

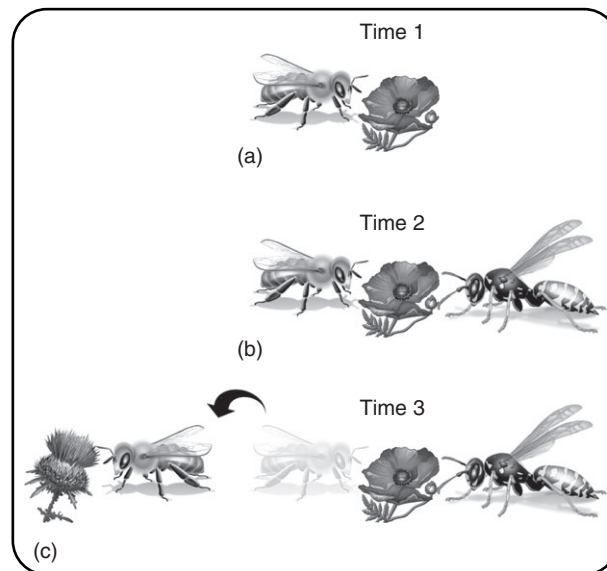


Figure 3 An example of how phenotypic plasticity can initiate character displacement. In this example, (a) a bee feeding on flower is (b) joined by a superior competitor, a wasp, whose presence, in turn, (c) causes the bee to shift to an alternative type of flower. Such a sequence of events would constitute ecological character displacement if bees with different genotypes differ in their propensity to respond to wasps, and if those that leave a flower in response to wasps experience less competition and thereby come to predominate in the population. Moreover, as described in the main text, these induced responses might even promote fixed (i.e. constitutively expressed) differences between species in foraging behaviour. Redrawn from Pfennig, D. W., and K. S. Pfennig, 2012. *Evolution's wedge: competition and the origins of diversity*. University of California Press, Berkeley, CA.

fixation as a result of recurrent selection to minimise interspecific competition.

This loss of plasticity during the evolution of character displacement might occur when maintenance or expression of plasticity is costly (Murren *et al.*, 2015). In such situations, selection can actively eliminate plasticity and cause fixation of the favoured phenotype (this process, in which selection causes a formerly environmentally induced phenotype to become fixed, is known as ‘genetic assimilation’; sensu Waddington, 1953). Alternatively, when nonfavoured phenotypes are expressed infrequently and thereby experience relaxed selection (Van Dyken and Wade, 2010), plasticity could be lost through accumulation of mutations and/or random genetic drift (Masel *et al.*, 2007). Through either route, character displacement could evolve from an initial phase in which trait divergence is environmentally induced to a later phase in which this divergence is genetically fixed.

Given that plasticity can be lost and thereby lead to plasticity-first character displacement, what are the underlying mechanisms by which this process could take place? One such plausible mechanism is via maternal effects, which arise when a female’s phenotype influences its offspring’s phenotype, independent of the direct effects of the female’s coding sequences on its offspring’s phenotype (Mousseau and Fox, 1998). To see how this mechanism works, consider that as a result of ecological character displacement, interacting species will come to utilise different resources (Schluter, 2000). The quality of these resources is often asymmetric, such that one species utilises a more profitable resource than the other (Pfennig and Pfennig, 2005; Grant and Grant, 2006). As a consequence of this asymmetry, females utilising the lower quality resource may mature at a smaller body size and/or in poorer condition and, therefore, produce offspring that are also smaller and in poorer condition. If this shift towards smaller body size makes these individuals more dissimilar to their competitors, then these individuals will be favoured by selection. Furthermore, because maternal effects can be reliably transmitted between generations (Plaistow *et al.*, 2006; Allen *et al.*, 2008), a maternal effect might facilitate the self-reinforcing loss of plasticity in sympatric populations.

Another plausible mechanism for the plasticity-first scenario of character displacement (which may or may not be mutually exclusive with maternal effects) is via evolutionary changes in the degree of plasticity in gene expression – specifically, in the amount of gene product. Indeed, gene expression is often environment specific (Aubin-Horth and Renn, 2009), and an evolutionary shift in gene expression is increasingly viewed as important in mediating population divergence (Pavey *et al.*, 2010). If each individual encounters the same selective environment (i.e. selection is coarse grained), then formerly induced differences in gene expression might ultimately become fixed. Thus, shifts in gene expression plasticity might parallel and facilitate shifts in resource-use phenotypes during character displacement. **See also: Diet, Changes in Gene Expression and Human Evolution**

In the following section, we illustrate how these three proximate mechanisms are potentially intertwined by describing recent research into the mechanisms of character displacement in spadefoot toads (genus *Spea*). In these toads, genetic changes, maternal

effects and shifts in gene expression have all been shown to play an important role in mediating character displacement.

Plasticity-first Evolution and Character Displacement in Spadefoot Toads

Spadefoot toads have become models for studying competition and phenotypic plasticity. Members of the genus *Spea* are especially relevant for these studies because they have evolved a unique resource polymorphism as an adaptive response to minimise resource competition: in this case, competition within species (Martin and Pfennig, 2009). In particular, *Spea* tadpoles produce alternative, environmentally induced morphs: an ‘omnivore’ morph, which feeds on detritus and microorganisms and which possesses small jaw muscles, smooth keratinised mouthparts, numerous denticle rows and a long gut, or an alternative ‘carnivore’ morph that is characterised by large jaw muscles, notched and keratinised mouthparts, few denticle rows and a short gut (**Figure 4a,b**). This carnivore morph is specialised for eating fairy shrimp and other tadpoles, and it can be induced by the consumption of shrimp or tadpoles (Levis *et al.*, 2015). However, the frequency with which carnivores are produced – and how extreme they are – varies among species, populations and sibships, suggesting underlying genetic variation in propensity to produce and express the carnivore phenotype.

In southeastern Arizona and southwestern New Mexico, USA, two species (*Spea multiplicata* and *Spea bombifrons*) have undergone character displacement with each other (**Figure 4c**). In ponds containing only a single species (i.e. allopatry), both *Sp. multiplicata* and *Sp. bombifrons* produce similar frequencies of both morphs. By contrast, in nearby ponds where both species occur (i.e. sympatry), each species becomes nearly monomorphic, with *Sp. multiplicata* producing mostly omnivores and *Sp. bombifrons* producing mostly carnivores. Such character displacement in morph production lessens competition between these species for food and thereby enables them to coexist: this is because *Sp. multiplicata* is the superior competitor for detritus, and *Sp. bombifrons* is the superior competitor for shrimp (Pfennig and Murphy, 2000, 2002; Pfennig *et al.*, 2007).

Importantly, these species differ in the proximate mechanism by which they are diverging from each other. While a condition-dependent maternal effect mediates production of only omnivores in sympatric *Sp. multiplicata* (Pfennig and Martin, 2009), in *Sp. bombifrons*, divergence in morph production between allopatry and sympatry reflects genetically fixed differences (Pfennig and Martin, 2010). In particular, because *Sp. multiplicata* produce both morphs in allopatry, but only omnivores in sympatry, females from sympatry mature smaller and in poorer condition than females from allopatry (carnivores metamorphose at a larger size than omnivores, because they monopolise the more nutritious shrimp resource). Consequently, the two populations diverge in maternal investment (Pfennig and Pfennig, 2005), such that sympatric females invest less into offspring by producing smaller eggs, whereas allopatric females invest more into offspring by producing larger eggs. Smaller eggs

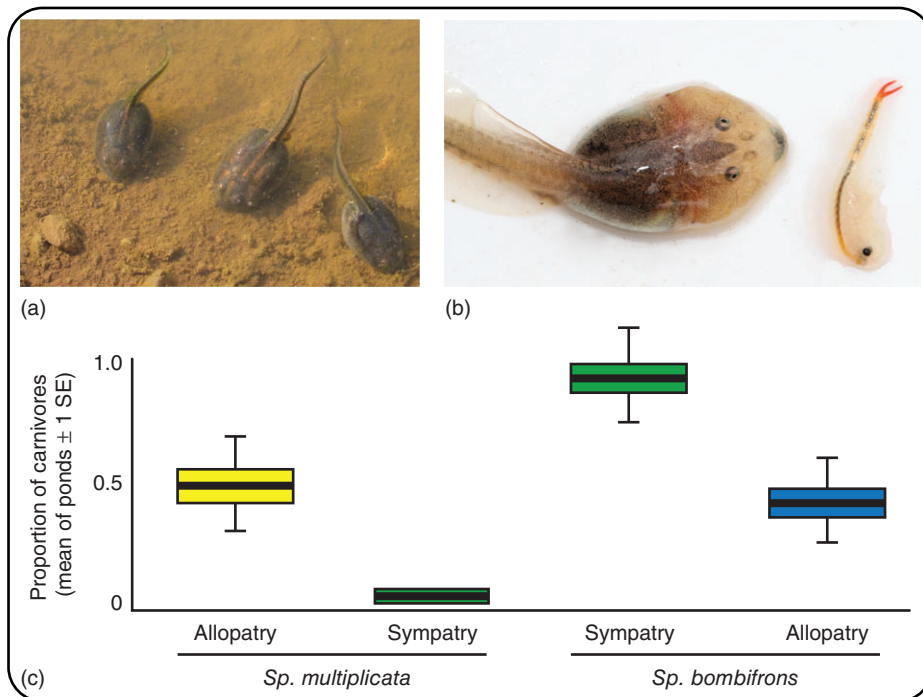


Figure 4 Character displacement in spadefoot toads, *Sp. multiplicata* and *Sp. bombifrons*. Each of these two species produces alternative tadpole resource-use phenotypes: (a) a small-headed omnivore morph, which feeds mostly on plants and detritus and (b) a large-headed carnivore morph, which feeds mostly on large animal prey, such as anostracan fairy shrimp (shown here). (c) Where each species occurs in allopatry, they produce similar, intermediate frequencies of omnivores and carnivores. However, where they occur in sympatry, *Sp. multiplicata* shifts to producing mostly omnivores, whereas *Sp. bombifrons* shifts to producing mostly carnivores. By diverging in resource-use phenotypes, these two species reduce competition for food. Photos by D. Pfennig.

hatch into smaller tadpoles, which tend to become omnivores. By contrast, larger eggs hatch into larger tadpoles, which tend to become carnivores (Martin and Pfennig, 2010a,b). In other words, variation in egg size, tadpole size and (ultimately) ecomorph production are all transmitted via a maternal effect across generations, thereby mediating divergence in these characters between allopatric and sympatric populations.

In contrast to this situation in *Sp. multiplicata*, the analogous divergence in resource-use phenotypes between allopatric and sympatric populations of *Sp. bombifrons* is not influenced by either maternal size or condition. Instead, such divergence appears to reflect genetically fixed differences (Pfennig and Martin, 2010).

This difference between these two species in the proximate mechanism of character displacement might reflect a difference between these two species in how recently each encountered the other in southeastern Arizona, where the aforementioned character displacement has been studied. Specifically, because *Sp. bombifrons* has been expanding its range out of the central United States and invading habitat in the southwestern United States formerly occupied by *Sp. multiplicata* (Pierce *et al.*, 2017), *Sp. bombifrons* on the front edge of this expansion in southeastern Arizona have had long evolutionary contact with *Sp. multiplicata* (Pierce *et al.*, 2017) and, thus, more time to accrue genetic differences. By contrast, populations of *Sp. multiplicata* in southeastern

Arizona have only recently encountered *Sp. bombifrons* and, thus, had less time to accrue such differences.

Furthermore, laboratory studies have shown that plasticity-mediated shifts in allopatric (ancestral) populations mirror the more highly canalised trait differences observed in sympatric (derived) populations that have undergone character displacement (because these two species have come into secondary contact following a range expansion, allopatry represents the ancestral condition and sympatry the derived condition; Rice *et al.*, 2009; Pierce *et al.*, 2017). In these experiments, allopatric *Sp. multiplicata* that were reared with *Sp. bombifrons* facultatively produced mostly omnivores, which mirrors the canalised morph production in sympatric *Sp. multiplicata*. Conversely, allopatric *Sp. bombifrons* that were experimentally exposed to *Sp. multiplicata* facultatively produced mostly carnivores, which mirrors the canalised morph production in sympatric *Sp. bombifrons* (*Sp. bombifrons* produce more carnivores in the presence of *Sp. multiplicata* because they are more effective at consuming shrimp (Pfennig and Murphy, 2000), the diet that induces carnivores).

Thus, different lineages of *Sp. multiplicata* appear to represent different stages in the evolution of character displacement, from its possible initial stages where phenotypic plasticity enables *Sp. bombifrons* and *Sp. multiplicata* to each respond adaptively – and immediately – to competition imposed by the other species (as

shown in the experiments described in the previous paragraph); to a subsequent stage where divergence between allopatric and sympatric populations in resource-use phenotypes is stabilised by a condition-dependent maternal effect (as shown in present-day sympatric populations of *Sp. multiplicata*) and to a later stage where this divergence becomes even more permanent via genetically fixed differences (as in present-day *Sp. bombifrons*). In other words, these species appear to have undergone plasticity-first character displacement.

Shifts in Gene Expression during Character Displacement

In the previous section, we described phenotypic shifts as a result of character displacement in spadefoot toads. Recent work has revealed parallel shifts in gene expression accompanying these phenotypic shifts in one of the species undergoing character displacement – *Sp. bombifrons*. Genes that are expressed more highly in one morph than in the other (i.e. morph-biased

genes) were identified by rearing *Sp. bombifrons* tadpoles under common conditions on a diet of shrimp (to ensure that any differences in gene expression were associated with different trophic morphologies *per se* and not dietary differences) (Leichty *et al.*, 2012). These genes were classified either as ‘carnivore biased’ (if they had significantly higher expression in carnivores than in omnivores) or ‘omnivore biased’ (if they had significantly higher expression in omnivores than in carnivores; **Figure 5a**). On the basis of what is known about the functions of these genes (Leichty *et al.*, 2012), most (if not all) are likely crucial in ecomorph functionality. Therefore, their expression levels represent molecular phenotypes. Thus, just as selection can target higher order phenotypes (**Figure 4c**), these genes can also be targeted by selection and undergo adaptive refinement (i.e. genetic accommodation) during character displacement.

Levis *et al.* (2017) investigated if these morph-biased genes have experienced a concomitant shift in their expression plasticity that matches the evolved shifts in morph production shown in **Figure 4c**. To do so, they reared early-stage *Sp. bombifrons*

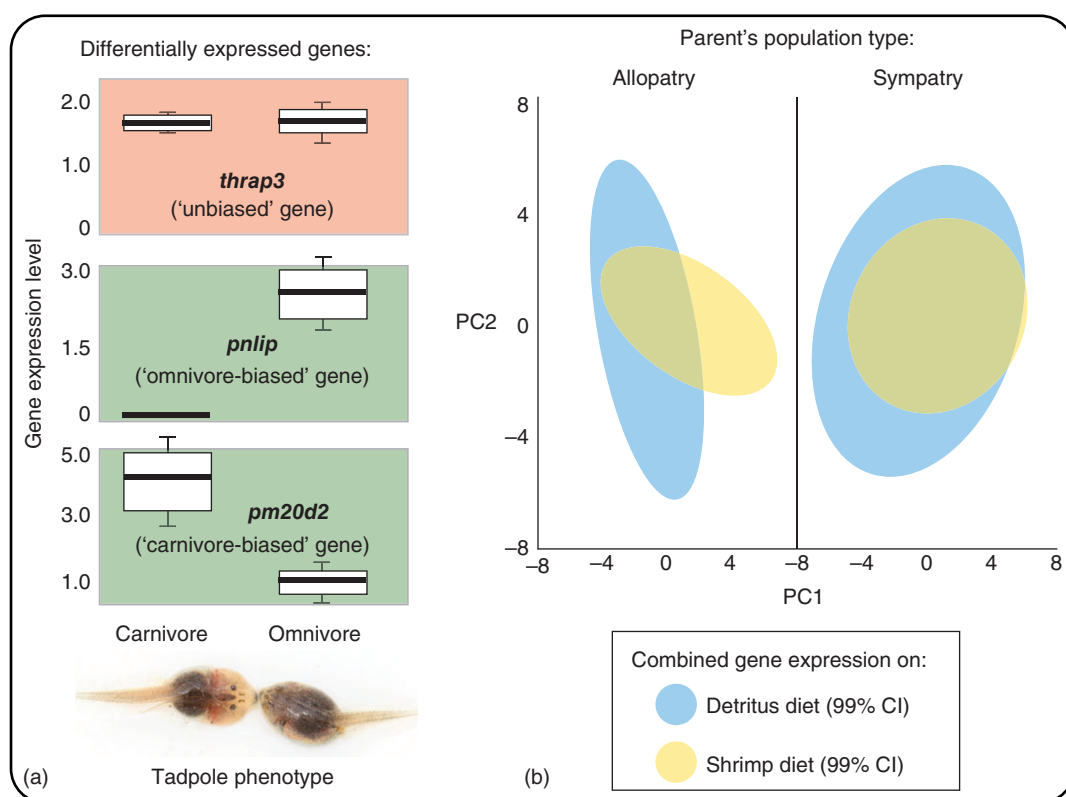


Figure 5 Evidence of gene expression evolution during character displacement. (a) Examples of genes for which levels of expression were measured in alternative carnivore and omnivore morph tadpoles of spadefoot toads when all were reared under common conditions. Although most genes display no morph-specific differences in expression level (such as the *thrap3* gene shown here), some genes are expressed more highly in omnivores than in carnivores (e.g. *pnlip*), whereas other genes are expressed more highly in carnivores than in omnivores (e.g. *pm20d2*). Data from Leichty *et al.* (2012). (b) Although different genes displayed different patterns of gene regulatory evolution in spadefoots, the combined gene expression profile of nine differentially expressed (biased) genes reveals that individuals derived from sympatry (that have undergone character displacement) have lost the diet-induced gene expression plasticity present in individuals derived from allopatry (that have not undergone character displacement). Thus, during the evolution of character displacement, gene expression has evolved in parallel with changes in morphology. Data from Levis *et al.* (2017). Photo by D. Pfennig.

tadpoles whose parents were derived from sympatry or allopatry on a diet of detritus or shrimp and measured the reaction norms of nine such biased genes. The expectation was that the expression of carnivore-biased genes should have reduced plasticity in tadpoles derived from sympatry relative to tadpoles from allopatry. This expectation was based on the observation that *Sp. bombifrons* has experienced a genetically determined shift in phenotype production between allopatric and sympatric populations such that the carnivore morph is nearly fixed in sympatry (Pfennig and Murphy, 2000, 2002).

Two of the genes examined by Levis *et al.* (2017) showed evidence of evolved shifts in their reaction norm between allopatric and sympatric populations of *Sp. bombifrons* (i.e. two genes showed evidence of genetic accommodation). The first gene (*Pm20d2*) is a carnivore-biased gene with a peptidase domain (i.e. it breaks down other proteins) that, as expected, had higher expression on a shrimp diet than a detritus diet (shrimp are a more proteinaceous resource than detritus). Interestingly, this gene maintained this plastic expression pattern in sympatry, but the overall expression level was lower. The authors speculate that this might be the result of selection improving the efficiency with which this enzyme breaks down peptides, but further genetic and enzymatic analyses are needed to confirm this possibility. *Btf3* (also a carnivore-biased gene that encodes a transcription factor) had higher expression on a shrimp diet than a detritus diet in individuals derived from allopatry, but had no diet-dependent expression in sympatry. In this case, the authors speculate that *Btf3* might play a role in switching between carnivore and omnivore phenotypes because of its function in activating downstream genes. However, this gene and *Pm20d2* both need to be investigated in the context of gene regulatory networks to facilitate better understanding of why these particular expression patterns were observed.

In addition to the observed shifts in expression of specific genes, Levis *et al.* (2017) also found that character displacement has led to an overall pattern of genetic accommodation (and potentially genetic assimilation) for the combined gene expression in sympatric (derived) populations. Specifically, when the expression levels of all nine biased genes were combined into a composite metric (the gene expression profile), there was evidence of diet dependence in gene expression profile in allopatry and loss of this diet dependence in sympatry (Figure 5b). Thus, individuals from the derived, sympatric populations have lost the diet-induced gene expression plasticity present in ancestral allopatric populations – a pattern that mirrors the aforementioned evolutionary loss of morphological plasticity in sympatric populations. These data therefore indicate that *Sp. bombifrons* have undergone plasticity-first character displacement.

Final Remarks

Although character displacement is central to the origins, abundance and distribution of biodiversity (Schluter, 2000; Dayan and Simberloff, 2005; Pfennig and Pfennig, 2012), until recently, little was known of its underlying mechanisms. Identifying these mechanisms is important, because different mechanisms can impact the speed of character displacement, and therefore,

whether or not it – as opposed to the alternative outcomes of competitive or reproductive exclusion – unfolds to completion (Pfennig and Pfennig, 2012).

A mechanism that might be crucial in promoting rapid character displacement is phenotypic plasticity. Indeed, phenotypic plasticity can mediate rapid niche shifts (within the lifetime of individual organisms) and thereby buffer populations from competitive or reproductive exclusion until more permanent changes (i.e. changes to DNA sequence) can evolve. Thus, character displacement might undergo plasticity-first evolution (Pfennig and Pfennig, 2012; Levis and Pfennig, 2016), in which it proceeds through an initial phase where trait differences are environmentally induced to a later phase in which these differences become genetically fixed (Wilson, 1992).

Ultimately, plasticity-first evolution likely entails the evolution of gene expression, and studies examining the evolution of gene expression in spadefoot toads offer compelling support for plasticity-first character displacement (Levis *et al.*, 2017). Recent efforts to uncover the mechanisms of character displacement in other organisms, including *Anolis* lizards (Losos, 2009), sticklebacks (Wund *et al.*, 2008), Darwin's finches (Lamichhaney *et al.*, 2016) and African cichlids (Parsons *et al.*, 2016), will illuminate whether the plasticity-first scenario (and, hence, the evolution of gene expression) is indeed a common pathway to character displacement.

Glossary

Canalisation or canalised Production of a particular phenotype irrespective of environmental conditions; a trait exhibits no phenotypic plasticity; fixation.

Character displacement A process by which divergent natural selection to minimise competitive interactions between initially similar populations causes evolutionary shifts in phenotype production.

Genetic accommodation A mechanism of evolution wherein a phenotype, generated by either a mutation or environmental change, is refined into an adaptive phenotype through selection driving quantitative genetic changes; can also promote either increased or decreased environmental sensitivity of the focal phenotype; when environmentally induced phenotypes lose environmental sensitivity, they undergo 'genetic assimilation'.

Genetic assimilation An extreme form of 'genetic accommodation' that occurs when selection causes initially environmentally induced (i.e. plastic) phenotypes to lose their environmental sensitivity over evolutionary time.

Maternal effects When a female's phenotype influences its offspring's phenotype, independent of the direct effects of the female's coding sequences on its offspring's phenotype; often takes the form of resource partitioning to eggs.

Phenotypic plasticity The ability of an organism to alter its behaviour, morphology and/or physiology in response to changes in environmental conditions; sometimes used synonymously with developmental plasticity.

Plasticity-first evolution A mechanism of adaptive evolution in which environmental perturbation leads, via phenotypic

plasticity, to developmental reorganisation (via, e.g. altered gene expression) and uncovers ‘cryptic genetic variation’ for, and ultimately production of, a novel developmental variant (i.e. trait) that immediately undergoes ‘phenotypic accommodation’ and is subsequently refined through ‘genetic accommodation’; some definitions include cases in which mutation initiates trait origin.

Reaction norm A graphical representation of the set of phenotypes that a single genotype produces in response to some specific environmental variable(s); individuals show plasticity if their reaction norm is nonhorizontal.

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