# Speciation: The Strength of Natural Selection Driving Reinforcement

**Our understanding of how natural selection drives the speciation process remains a mystery. A recent study shows how selection for flower color differences causes the evolution of reproductive isolation between two plant species of the genus *Phlox*.**

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*Why is not all nature in confusion instead of species being, as we see them, well defined?*

– Charles Darwin (1859)

One of the most striking observations about nature is its discontinuity — organisms cluster into discrete groups, or species, which are recognizably distinct [1–3]. This is largely because species consist of groups of individuals that interbreed with each other but are reproductively isolated from other such groups [2]. The answer to Darwin's question — why are species well defined? — is that reproductive isolation evolves and facilitates the accumulation of differences between populations, keeping them distinct. Although the last 25 years have witnessed a strong revival of interest in speciation (*e.g.*, [2–5]), the role of natural selection in the origin of species remains a mystery [2,4]. In a recent paper published in *Current Biology*, Hopkins and colleagues [5] show how natural selection drives the speciation process between two hybridizing species from the plant genus *Phlox*, thus filling a major gap in our understanding of the origins of biodiversity.

Natural selection never favors the production of unfit offspring, so at first its role in speciation (through which populations lose the ability to mate successfully) was mysterious. However, once two populations are diverged enough to produce unfit hybrids, natural selection acts to prevent their formation by strengthening reproductive isolation through the process of reinforcement [4]. Speciation by reinforcement occurs when previously geographically separated populations come into secondary contact and hybridize.

Individuals that can mate with members of the same population instead of those from the divergent population enjoy higher fitness because they do not waste gametes producing sterile or inviable hybrid offspring. In these cases, natural selection is thought to directly favor the evolution of species as discrete groups [2–4].

Hybrid zones provide the ideal geographic setting to test if natural selection has played a role in the evolution of reproductive isolation in hybridizing species. The test consists of a comparison of the strength of reproductive isolation exhibited by members of a single species living within and outside of the hybrid zone. If reinforcement is acting, then individuals from the hybrid zone (sympatric) should have stronger reproductive isolation against the hybridizing species than individuals from outside the hybrid zone (allopatric).

Until recently empirical evidence for reinforcement was lacking, leading several evolutionary geneticists to deem the theoretical process improbable in nature (*e.g.*, [6,7]). One of the first convincing studies of reinforcement came from the plant genus *Phlox*, which has since developed into a major model for the study of speciation. Interbreeding species of *Phlox* were observed to produce partially sterile hybrids [8], and evidence of reinforcement in hybrid zones between *Phlox* species started to accumulate [9]. Levin focused on the study of *Phlox drummondii* and *Phlox cuspidata*, two sister species with overlapping ranges in the southern United States that hybridize in nature [10]. Throughout allopatric areas of their ranges, both species produce light blue flowers that are similar in color and hue, a trait that is characteristic of the *Phlox* genus. In

areas where these two species co-occur, *P. cuspidata* shows the characteristic blue flower color but *P. drummondii* has dark red flowers (Figure 1). This newly evolved flower color has been shown to compartmentalize pollinators that are attracted to different flower colors, increasing assortative mating, and thus minimizing the likelihood of hybridization [9,10]. Natural selection has driven the evolution of corolla color to prevent maladaptive hybridization, directly favoring the origin of two distinct *Phlox* species. The contemporary field of research on reinforcement owes quite a debt to these small blue and red prairie flowers, as they first demonstrated the feasibility that reinforcement could evolve and be detected. Now, examples of this characteristic signature of reinforcement are known from several taxonomic groups [2,4].

Although we know that reinforced reproductive isolation is a byproduct of selection against hybridization between species, for the most part we have very little information about how strong selection must be for reinforced traits to evolve, especially in natural systems. Three alternative approaches can be used to study reinforcement. First, hybridizing species can be exposed to each other in artificial selection experiments to observe whether enhanced reproductive isolation evolves *in vitro*. These experiments usually consist of cages containing two hybridizing sister species. Every generation, hybridization is harshly penalized by killing all produced hybrids, and starting the next generation only with pure species individuals — those whose parents did not hybridize, and who therefore have inherited traits associated with assortative mating. Reproductive isolation is measured after a few generations to detect whether this strong selection regime is enough to strengthen prezygotic isolation between the species in the experimental cages. This approach, dubbed 'kill-the-hybrid', was proposed
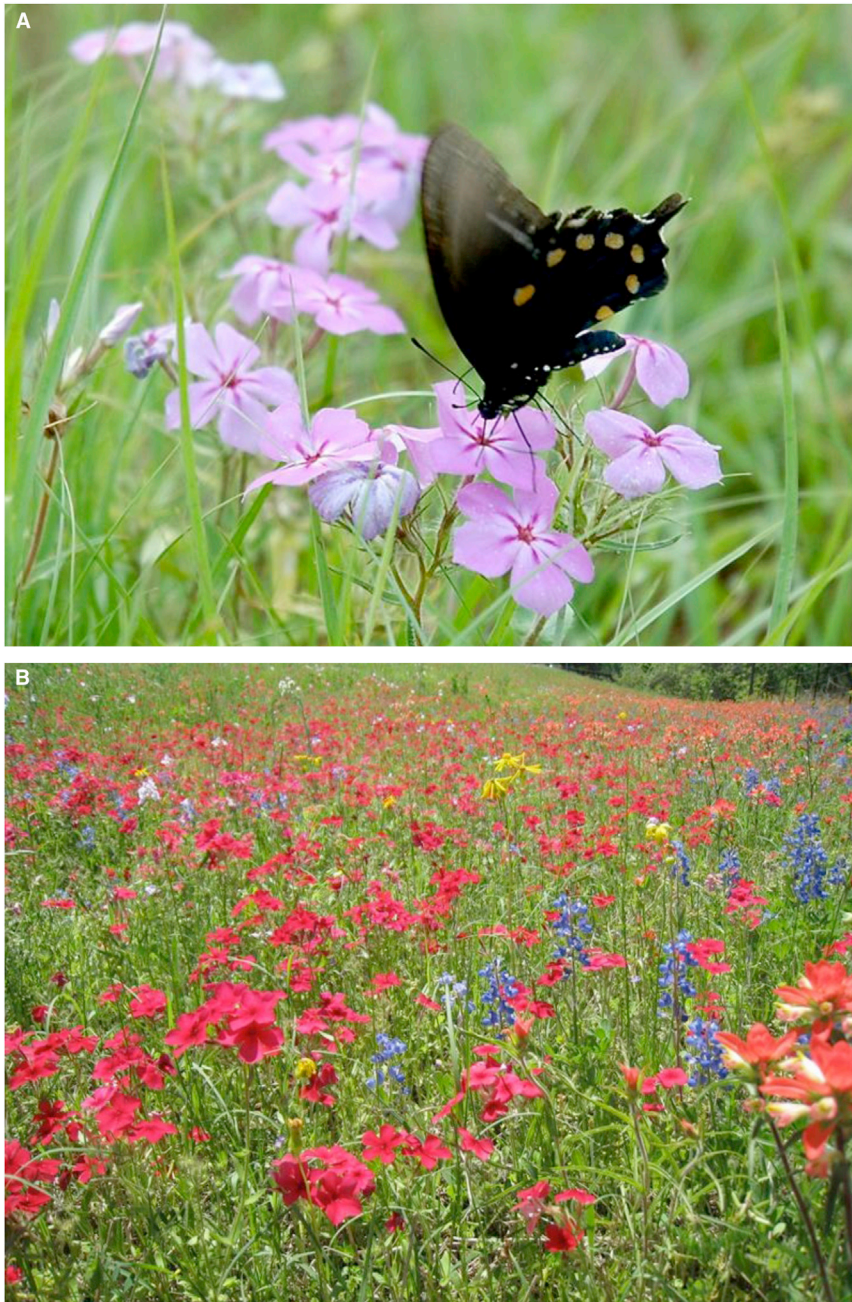
Figure 1. Evolution of flower color in *Phlox*.

(A) Wildflowers from the genus *Phlox* show a characteristic blue flower color. (B) In areas of contact with *P. cuspidata,* the Texas wildflower *P. drummondii* has evolved dark-red flowers. This newly evolved flower color causes pollinators to increase assortative mating between the plants, and thus it minimizes the likelihood of maladaptive hybridization. Photo credit (A) David L. Des Marais and (B) Robin Hopkins.

and with few exceptions (*e.g.*, [15,16]), the genetic architecture of reinforced reproductive isolation remains elusive. Once again, a notable example comes from *Phlox*. In the species pair *P. drumondii* and *P. cuspidata*, flower color variation is caused by changes in the *cis*-regulatory elements of two loci [9,17]. Changes in the expression levels of *flavonoid 3'5'-hydroxylase* alters the anthocyanin pigment composition and changes flower hue from blue to red. A change in the *cis*-regulatory elements of the second locus, *R2R3-Myb*, changes flower color intensity from light to dark. One of these alleles (*flavonoid 3'5'-hydroxylase*) seems to be evolving under the influence of strong recent selection, while the other one (*R2R3-Myb*) does not [17]. These approaches are powerful and provided the first direct measurement of the strength of reinforcing selection. Nonetheless, their scope is limited as they only reveal the influence of fast episodic selection events on particular alleles, even though natural selection ultimately acts on phenotypes and not on genes.

Finally, the obvious test — the direct measurement of the magnitude of selection on the actual phenotypes involved in reproductive isolation — has never been performed. That is precisely the merit of the most recent study by Hopkins *et al.* [5]. Usually, reinforced reproductive isolation is detected only in hybrid zones, not in allopatric populations. The novel test of selection hinges on determining how suddenly reinforced reproductive isolation weakens as the population transitions from sympatry into allopatry. The prediction is simple: the more geographically abrupt the changes in reinforced phenotypes, the stronger natural selection is inferred to be acting on those phenotypes. This approach, dubbed 'cline theory', was formulated initially by Haldane in 1943 [18] to study adaptation more generally and was later co-opted to study hybrid zones [19]. A more recent and nuanced approach is to establish the natural distribution of the phenotypes involved in reproductive isolation and establish whether the geographic changes in the traits are consistent with evolutionary models that invoke natural selection or whether they can be explained by simple geographic differentiation and the vagaries of genetic drift. These are

in 1950 [11] and has been performed almost exclusively in *Drosophila* [12,13], and certainly only in animals. Under such strong selection, reproductive isolation can evolve quickly, but these experiments cannot test the biologically realistic range of selective effects that can lead to reinforcement.

A second type of study has aimed to detect the signatures of natural selection in genes involved in reinforced reproductive isolation in putatively hybridizing species (*e.g.*, [14]). Determining the genetic basis of the traits involved in reproductive isolation is the obvious logistical challenge to this approach,

the approaches that Hopkins and colleagues took to quantify the strength of reinforcing natural selection in the case of speciation in the *Phlox* system [20].

Hopkins and colleagues took painstaking measurements of the distribution of *P. drumondii*, *P. cuspidata*, and their hybrids across a large swath of their natural distribution and measured the fitness of each of the three genotypes across their geographical range. In total, the authors measured the flower color and hue of over 10,000 *P. drummondii* individuals from 32 populations across 5 transects. In all transects they found sharp changes in phenotypic frequency in *P. drummondii* flower color. The boundary can be explained by strong selection on flower color in populations of *P. drummondii* sympatric with *P. cuspidata*, but it does not provide precise estimates of the magnitude of selection. The authors next took their fine assessment of the geographic distribution of phenotypes and their fitness, and applied classical population genetics techniques to determine the most likely explanation for the distribution of the observed genotypes. For each genotype (two pure species and the hybrids), the model calculates the relative fitness in sympatry, the relative fitness in allopatry, the levels of dispersal between allopatric and sympatric populations, and the locations of the boundaries between the allopatric and sympatric zones in each of the five transects. With this population genetics model, they precisely estimated the strength of

selection. Not only do their results confirm that selection against hybridization is exceptionally strong in *Phlox*, but the model also supports previous observations that weaker, but significant, selection favors alternative alleles at both flower color loci in sympatric and allopatric *P. drummondii* populations. This could explain why the dark red flower phenotype does not spread to the whole geographic range of *P. drummondii*.

Why is this study important? First, it demonstrates the utility of studying natural variation in traits involved in reproductive isolation across the whole geographic range of a species. Second, it revitalizes a classic population genetics approach (cline theory) to understand the strength of natural selection in nature. Finally, these results provide a cutting edge quantitative analysis of reinforcement in a taxon that has been crucial for our modern understanding of the speciation process, that mystery of mysteries.

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# Neural Coding: Sparse but On Time

**To code information efficiently, sensory systems use sparse representations. In a sparse code, a specific stimulus activates only few spikes in a small number of neurons. A new study shows that the temporal pattern across sparsely activated neurons encodes information, suggesting that the sparse code extends into the time domain.**

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In a natural environment, the sensory information for all modalities is rich and highly dynamic in time. To make sense of this permanent flow of

information, brains — as well as manmade artificial systems — need strategies to encode and process the information efficiently. In a sensory nervous system, information about the outside world is represented in different types of neural code. At the

sensory periphery this is a dense population code, meaning that the information is represented in a large proportion of highly active (sensory) neurons. As the information is transferred to higher level processing stages, the neural code often changes from dense to sparse. In sparse coding [1] the information is represented by only a small fraction of all neurons (population sparseness) and each activated neuron generates only few action potentials (temporal sparseness) for a highly specific stimulus configuration (lifetime sparseness). The sparse code has been