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# The Cost of Reinforcement: Selection on Flower Color in Allopatric Populations of *Phlox drummondii*\*

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**ABSTRACT:** Reinforcement is the process by which increased reproductive isolation between incipient species evolves due to selection against maladaptive hybrids or costly hybrid mating. Reinforcement is predicted to create a pattern of greater prezygotic reproductive isolation in regions where the two species co-occur, sympatry, than in allopatry. Although most research on reinforcement focuses on understanding the evolutionary forces acting in sympatry, here we consider what prevents the alleles conferring greater reproductive isolation from spreading into allopatry. We investigate flower color divergence in the wildflower *Phlox drummondii*, which is caused by reinforcement in the regions sympatric with its congener *Phlox cuspidata*. Specifically, we performed common garden field experiments and pollinator observations to estimate selection acting on flower color variation in allopatry. We combine our estimates of maternal and paternal fitness using simulations and predict how flower color alleles migrating from sympatry will evolve in allopatry. Our results suggest that strong pollinator preference for the ancestral flower color in allopatry can maintain divergence between allopatric and sympatric populations.

**Keywords:** reinforcement, speciation, *Phlox drummondii*, pollinator preference, flower color.

## Introduction

Natural selection can favor the accumulation of reproductive isolation (RI) during the formation of species through a process termed reinforcement (Wallace 1889; Dobzhansky 1940; Butlin 1987; Howard 1993). Reinforcement occurs when selection against hybridization or mating between incipient species causes the evolution of increased prezygotic RI. Reinforcing selection favors the evolution of novel RI mechanisms in regions of sympatry or close parapatry, where hybridization

can occur, but not in allopatry, where the species do not have the opportunity to mate. Consequently, reinforcement can produce a pattern of greater prezygotic RI in sympatry than in allopatry (Dobzhansky 1937; Coyne 1989).

Different amounts of RI in the sympatric and allopatric areas of species' ranges has been documented in a wide variety of organisms including insects, birds, mammals, and plants (reviewed in Howard 1993; Servedio and Noor 2003; Pfennig and Pfennig 2009), suggesting that reinforcement can play an important role in speciation. For many organisms, the identification of this geographic pattern in RI has led to further research on the evolutionary processes responsible for divergence in sympatry. For example, research has aimed to understand the types of RI mechanisms that evolve (Noor 1995; Higgie et al. 2000; Lemmon 2009), the genetic architecture of RI (Geyer and Palumbi 2003; Ortiz-Barrientos et al. 2004; Sæther et al. 2007; Hopkins and Rausher 2011) and the types of selection acting on RI in sympatry (Nosil et al. 2003; Pfennig 2003; Albert and Schluter 2004; Hopkins and Rausher 2012).

A question related to the evolution of increased RI through reinforcement that has seldom been investigated is why the ancestral trait is maintained in the allopatric area of a species' range (but see Pfennig 1998, 2000; Higgie and Blows 2007). One possibility is that there has been little gene flow between regions of allopatry and sympatry and not enough time has passed since divergence for the increased RI to spread into allopatric populations. Alternatively, the traits conferring increased RI in sympatry are disadvantageous in allopatry. The mechanism underlying selection in allopatry is likely to vary depending on the type of RI that evolves in sympatry. For example, it has been shown that when the evolution of RI in sympatry involves modification of a sexually selected trait, the novel trait value conferring RI can be an unreliable indicator of mate quality and therefore selected against in allopatry (Ryan and Rand 1993; Pfennig 1998). A number of studies have documented a trade-off between the advantages of species recognition and mate quality recognition (Collins and Lueddem 2002;

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Pryke and Andersson 2008; Rosenthal and Ryan 2011) and, specifically, for reinforcement and sexual selection maintaining different trait values in sympatry and allopatry (Pfennig 2000; Higgie and Blows 2007, 2008). For cases in which reinforcement does not alter a sexually selected trait, such as in most plants systems, selection in allopatry has not been investigated.

Here we report investigations on why traits in the wildflower *Phlox drummondii* that have evolved due to reinforcement in a region of sympatry with the congener *Phlox cuspidata* have not spread into the region of allopatry. Both species have similar light blue flowers throughout allopatric areas of their ranges, but where the two species co-occur, *P. drummondii* has dark red flowers. In sympatry, the two species hybridize but their offspring are nearly sterile, creating strong selection against hybridization (Levin 1967, 1975; Ruane and Donohue 2008). Previous experiments have shown that the change in flower color is caused by substitutions at two genetic loci, with one locus determining whether flowers are light or dark and the other determining whether flowers are blue or red (Hopkins and Rausher 2011). At least one of these substitutions increases assortative mating and decreases costly hybridization between the two species (Hopkins and Rausher 2012). Patterns of neutral genetic variation across the range of *P. drummondii* suggest substantial gene flow between allopatric and sympatric populations of this species (Hopkins et al. 2012). Our work indicates that the absence of derived flower-color alleles in the allopatric region is not due to lack of gene flow between allopatry and sympatry. We therefore predict that the “dark” and “red” alleles are selected against in the region of allopatry.

The purpose of the experiments described here is to test the supposition that derived alleles are disfavored in allopatry. Specifically, we address two hypotheses about flower color evolution in allopatric populations: (1) The dark and red alleles have detrimental pleiotropic effects on survival, fruit-set and or seed production in allopatry. This is the maternal fitness hypothesis. (2) Pollinators prefer the ancestral light blue color compared to any of the other phenotypes (light red, dark blue, or dark red) that could be produced by migration of those alleles into the allopatric region. This is the paternal fitness hypothesis. We test these hypotheses by performing common garden field experiments in the native allopatric range of *P. drummondii* and pollinator observations in controlled experimental plots.

## Methods

### *Study Organism*

*Phlox drummondii* is an annual herb native to central and eastern Texas that inhabits roadsides, open fields, and pastures. Individuals germinate in late fall or early spring and

flower and set fruit from March through June or July. *Phlox drummondii* is largely self-incompatible (Lawrence and Turner 1962) and is pollinated by Lepidoptera, predominantly pipevine swallowtail butterflies (*Battus philenor*) and a variety of skipper species (family *Hesperiidae*; Hopkins and Rausher 2012).

Flower color variation within *P. drummondii* is caused by cis-regulatory mutations in two genes involved in the production of floral anthocyanin pigments (Hopkins and Rausher 2011). The hue locus (*H*), determines whether an individual produces both blue and red pigments (ancestral state) or just red pigments (derived state). The locus codes for the enzyme F3'5'h, which is highly expressed in the ancestral, dominant allele (*H*; “blue” allele) and down-regulated in the derived, recessive allele (*h*; “red” allele). The second locus, termed the intensity locus (*I*), determines the amount of anthocyanin pigments produced. At the intensity locus, there is lower expression of the ancestral allele (*i*; “light” allele), resulting in less pigment than in the dominant, derived allele (*I*; “dark” allele). The ancestral, allopatric phenotype is light blue (*iiHH*), the derived flower color is dark red (*IhHh*), and the potential intermediate flower colors are dark blue (*IIHH*) and light red (*iihh*). For clarity, we will refer to the homozygous genotypes by their corresponding flower colors as opposed to their genotype letters.

Common garden field experiments have shown that the dark allele at the intensity locus in *P. drummondii* decreases hybridization with *Phlox cuspidata* (Hopkins and Rausher 2012). Observations of pollinator movement indicate that pollinators move more frequently between the two species when they both have light flower color than when *P. drummondii* has dark flowers (Hopkins and Rausher 2012). The pattern of pollinator constancy results in assortative mating based on flower color in sympatric populations of *P. drummondii* and *P. cuspidata*. It is still unknown how and why the red allele evolved at the hue locus.

### *Common Garden Experiments: Test for Maternal Fitness Variation*

To test hypothesis 1, that the dark and red alleles have detrimental pleiotropic effects on maternal fitness, we compared relative fecundity and survival between the four homozygous flower color genotypes (light blue, dark red, light red and dark blue) in a common garden in the allopatric (western) part of the native range. The experiment was replicated over 2 years with very different climatic conditions. During 2010 there was a mild spring with ample precipitation, whereas in 2011 there were extreme heat and record drought.

*Crossing Design.* We performed three generations of con-

trolled crosses to create experimental seeds used in the common garden experiments. All seeds had a known homozygous genotype at both flower color loci and a randomized genetic background with respect parental flower color genotype at unlinked loci and were outcrossed in the last generation to minimize inbreeding depression. Although the specific populations and crosses differed between the two years (table B1), the general design was the same for both experiments and similar to previous field experiments performed in the sympatric range (Hopkins and Rausher 2012). First, we created  $F_2$  families by crossing a light blue individual from an allopatric population to a dark red individual from a sympatric population and self-fertilizing the  $F_1$  offspring. We genotyped the  $F_2$  individuals at the flower color loci as described by Hopkins and Rausher (2011) and identified individuals homozygous at both the intensity and hue loci. Individuals with the same homozygous genotype were crossed between  $F_2$  families to create the experimental seeds. Across the 2 years we created five experimental families (two in 2010 and three in 2011), each with four homozygous flower-color genotypes (with the exception of one family in which insufficient dark red seeds were produced).

*Planting and Growth.* Experimental plants germinated and grew the first true leaf in greenhouses at the University of Texas at Austin. To synchronize germination, we soaked seeds in 500 ppm gibberellic acid for 48 h, planted them in water-saturated Metro-Mix 360 (Sun Gro Horticulture, Bellevue, WA), and stratified them at 4°C for 7 days. Both common garden experiments occurred in the Experimental Gardens at the University of Texas Brackenridge Field Laboratory (Austin, TX). This field station is in the native range of *P. drummondii* and the surrounding natural populations contained light-blue-flowered individuals (R. Hopkins, personal observation).

For the 2010 experiment, we planted seedlings in a randomized block design on February 25. We included 100 individuals of each of the four homozygous flower color genotypes split between two blocks. We also planted 300 additional light blue individuals randomly interspersed with the focal individuals to more realistically mimic conditions under which a derived flower color allele would invade an allopatric population. In this way, we distorted the flower color frequency such that most of the plants in the common garden had the ancestral light blue flower color native to that region. We did not collect data on these additional individuals. Plants were given supplementary water twice during the first week after transplant. Transplant survival was surveyed after 10 days, at which point individuals were allowed to grow, flower, and set fruit naturally. The 55 individuals that did not survive transplanting were excluded from all analyses. Survival and

flowering time were monitored weekly and fruits were counted as they ripened. We bagged a subset of the fruits on 124 individuals using green tulle to collect and count number of seeds produced per fruit.

For the 2011 experiment, 150 seedlings of each flower color genotype were planted across two blocks on March 2. We also planted 400 nonfocal light-blue-flowered individuals randomly throughout the plots. Plants were watered twice in the first week after transplant and then surveyed for transplant survival. The 64 individuals that did not survive transplanting were excluded from analyses. To ensure some survival to flowering during the drought, we watered the plots every other week through the middle of July. Plants were monitored for survival weekly, and fruits were counted as they were produced. As in 2010, randomly chosen subsets of fruits were bagged on 305 individuals to determine number of seeds produced per fruit for each genotype.

*Statistical Analyses.* The data from the two common garden field experiments were analyzed to determine whether genotype at the flower color loci effected survival, fruit-set, and number of seeds per fruit. These are all aspects of maternal fitness.

In 2010, all individuals that survived transplanting also survived to flower; we therefore analyzed only variation in fruit-set and seeds per fruit and not survival. We used a mixed-model ANOVA in PROC MIXED (SAS 9.4, SAS Institute, Cary, NC) to determine whether variation in fruit-set was explained by genotype at the hue locus or the intensity locus or an interaction between the two. Fruit-set was log transformed before analysis. The random effects in the model included family, block, all two-way interactions between random and fixed effects, and the three-way interaction between block, intensity, and hue. Significance of random effects was determined using a likelihood ratio  $\chi^2$  statistic. First, the complete model was run, and second, a model was run without one of the random effects. The difference in the log likelihoods between the two models was calculated and used to test for the importance of the random effect in the model using a  $\chi^2$  distribution with 1 degree of freedom. The likelihood ratio test was performed for each of the random effects in succession.

On average the experimental plants in 2010 produced 2.4 seeds per fruit. We used the same ANOVA model described above to determine whether variation in number of seeds per fruit is explained by the intensity locus or hue locus.

In 2011, 17% of the individuals died before flowering, allowing us to analyze survival and fruit-set together and separately. First, we analyzed variation in fitness as estimated from combined survival and fruit-set data. We

transformed the fruit-set data by adding 1 to all values and log transforming. All individuals that did not survive to flower were assigned zero fruits. We used a mixed-model ANOVA in SAS PROC MIXED and included genotype at the intensity locus and the hue locus and an interaction as fixed effects. Family, block, and all two-, three-, and four-way interactions with the fixed effects were included as random effects in the model. We analyzed the significance of random effects as described above.

In our second model, we separated the fitness components of survival and fruit-set by excluding all individuals that did not survive to flower. We reran the same ANOVA model described above to determine whether the intensity or hue locus affected variation in fruit-set for those individuals who survived to flower. As in 2010, an average 2.4 seeds were produced per fruit, and we used the same ANOVA model to determine whether flower color affected variation in number of seeds produced per fruit.

Finally, we determined whether flower color genotype affected survival to fruit-set using a logistic regression in PROC CATMOD (SAS 9.4). In this model, we asked whether the survival was affected by intensity genotype, hue genotype, block, family, all two-way interactions, and the three-way interaction between intensity, hue, and block.

#### *Pollinator Observations: Test for Paternal Fitness Variation*

We tested hypothesis 2, that pollinators preferred the ancestral light blue flower color, by observing pollinator visits in arrays of potted plants.

*Array Design.* We performed observations on three array types, each of which included two flower-color genotypes. With the first array, we looked for pollinator preference acting on the hue locus by including light-blue- and light-red-flowered plants. Second, we investigated pollinator preference on the intensity locus by including light-blue- and dark-blue-flowered plants. Finally, we compared the ancestral light blue flower color to the derived double mutant dark red flower color. For each array type we alternated flower colors in a 4 × 6 grid. In total, each color had the same number of open flowers (ranging from 518 to 1,254 across days). Pollinator observations were performed on 3 days for each array type between 10 a.m. and 4 p.m. during May 2012. For each pollinator we recorded the color of each plant visited and the order of visits. From these data we calculated the total number of plants visited of each color by each pollinator and the number of transitions between each color. Pollinator visits were counted only if the pollinator's proboscis was seen entering a corolla tube.

*Statistical Analyses.* Pollinator preference was calculated as the ratio of light blue plant visits to total plant visits summed

across all pollinators for each array type. A value of 0.5 represents no preference, and a value greater than 0.5 represents preference for light blue flowers. We calculated 95% confidence intervals around estimates of pollinator preference using bootstrap resampling. Specifically, for each array type we resampled our data with replacement at the level of pollinator and recalculated a bootstrap estimate preference value from all the resampled pollinator visitation scores. Each resampled pool contained the same number of pollinators as the observed data. We resampled 10,000 times and determined 95% confidence interval based on the top and bottom 2.5% bootstrap resampled values.

Over 95% of the pollinators visiting the arrays were pipe-vine swallowtails or skippers. To determine whether these two pollinator types differed in behavior, we reanalyzed the observation data by pollinator type (excluding the 13 visits from other pollinators). Since each array type was observed across 3 days we also split the data by day and recalculated preference and confidence intervals for each day.

Finally, we calculated Bateman's Constancy Index (BCI; Bateman 1951; Waser 1986), to determine whether there was assortative mating caused by pollinator movement between *P. drummondii* flower color varieties. Constancy refers to the tendency for pollinators to move more frequently between phenotypically similar plants than between different plants. The measure of constancy (BCI) is independent of the number of each type of plant visited (preference). The BCI ranges from 1, which represents pollinators transitioning only between like colored flowers, to -1, which represents pollinators transitioning only between unlike flowers. A BCI of 0 represents pollinators randomly transitioning between plants with respect to flower color. We used a contingency  $\chi^2$  test to evaluate whether the BCI represented a significant deviation from random movement.

#### *Balance of Selective Forces*

To estimate the expected net evolutionary change caused by selection on flower color, we modeled gene frequency change at the two flower-color loci. Details of the model are presented in appendix A. We summarize briefly here. For given allele frequencies at the two flower color loci, the initial genotype frequencies are calculated assuming Hardy-Weinberg equilibrium. We then calculate the genotypic frequency of males and females that contribute to mating in the populations, allow them to mate randomly, and calculate genotypic frequencies and allele frequencies in the next generation.

The frequency of females that contribute to the next generation was determined by the starting genotypic frequency multiplied by both the probability of survival and the fecundity of that genotype, all renormalized by dividing

by the sum across all genotypes. The frequency of males contributing to the next generation was calculated as the starting genotypic frequency multiplied by the probability of survival and the pollinator preference for that genotype, all renormalized by dividing by sum across all genotypes. Males and females randomly mate proportionally to their frequency in the population after selection. We allow for free recombination between the two flower color loci and calculate the genotypic frequency for the next generation. Selection and recombination was iterated until allele frequencies stabilized at equilibrium. Starting allele frequencies ranged from 0.05 to 0.95 in increments of 0.05 for each allele and each combination.

For the base model, we assume that survival and fecundity showed dominance corresponding to the dominance patterns of flower color. We also assume that all genotypes produce similar amounts of pollen regardless of their ability to produce fruits or flowers and that visits by pollinators always remove the same amount of pollen. Because reproduction in *Phlox* may not satisfy these assumptions, we examined alternative models with different assumptions. First, we allow for additive effects of survival and fecundity by assigning values to heterozygous genotypes that are the mean of the corresponding homozygotes. Second, we allow male pollen production to differ among genotypes and be proportional to fecundity. The rationale for this alternative assumption is that pollen production and fruit production may be proportional to the number of flowers, as may be the number of flowers visited by a pollinator on a single plant. See appendixes for details of these alternative models. For each year, we examined the four models representing combinations of the assumption about pollen production and the assumption about dominance.

For the 2010 model, survival was 100% for all genotypes and fecundity was assumed to be the average fruit production for each genotype in the common garden experiment. For the 2011 model, survival probability was assumed to be the average survival rate for each genotype from the common garden experiment. We assumed fecundity as the average number of fruits produced by individuals that survived to flower (method 1). We also used a second method (method 2) to calculate fecundity, which was to use the average fruit production calculated across all individuals in the experiment regardless of survival to flower and divide by the survival probability.

## Results

### *Common Garden Experiments: Test for Maternal Fitness Variation*

The common garden field experiments performed in 2010 and 2011 aimed to test the hypothesis that the ancestral

flower color genotypes were favored in the allopatric range of *Phlox drummondii*. Our data provide little evidence that genotype at the flower color loci negatively affects maternal fitness.

**2010 Experiment.** In the 2010 common garden experiment, all individuals that survived transplanting produced at least one flower. All four genotypes produced similar numbers of seeds per fruit (table B2), with differences being nonsignificant (table 1). Fruit number thus appears to be a good index of female fecundity. Moreover, because in 2010 all individuals survived to reproduce, it appears to be a good index for female fitness.

Mean fruit production was variable among flower colors, although we found no statistical difference between genotypes. The dark red individuals produced the most fruits (fig. 1A), and the average fruit-set for the dark genotypes (dark blue and dark red) was greater than for the light genotypes (light blue and light red; 190.28 and 141.11, respectively). However, these differences were not statistically significant, as judged by the hue, intensity, and hue by intensity effects in the ANOVA (table 1). None of the random factors in the model show a significant effect (table B3). Our experiment thus provides little evidence that female fitness differs among flower-color genotypes. Data from this experiment can be found in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.f710n> (Hopkins and Rausher 2014).

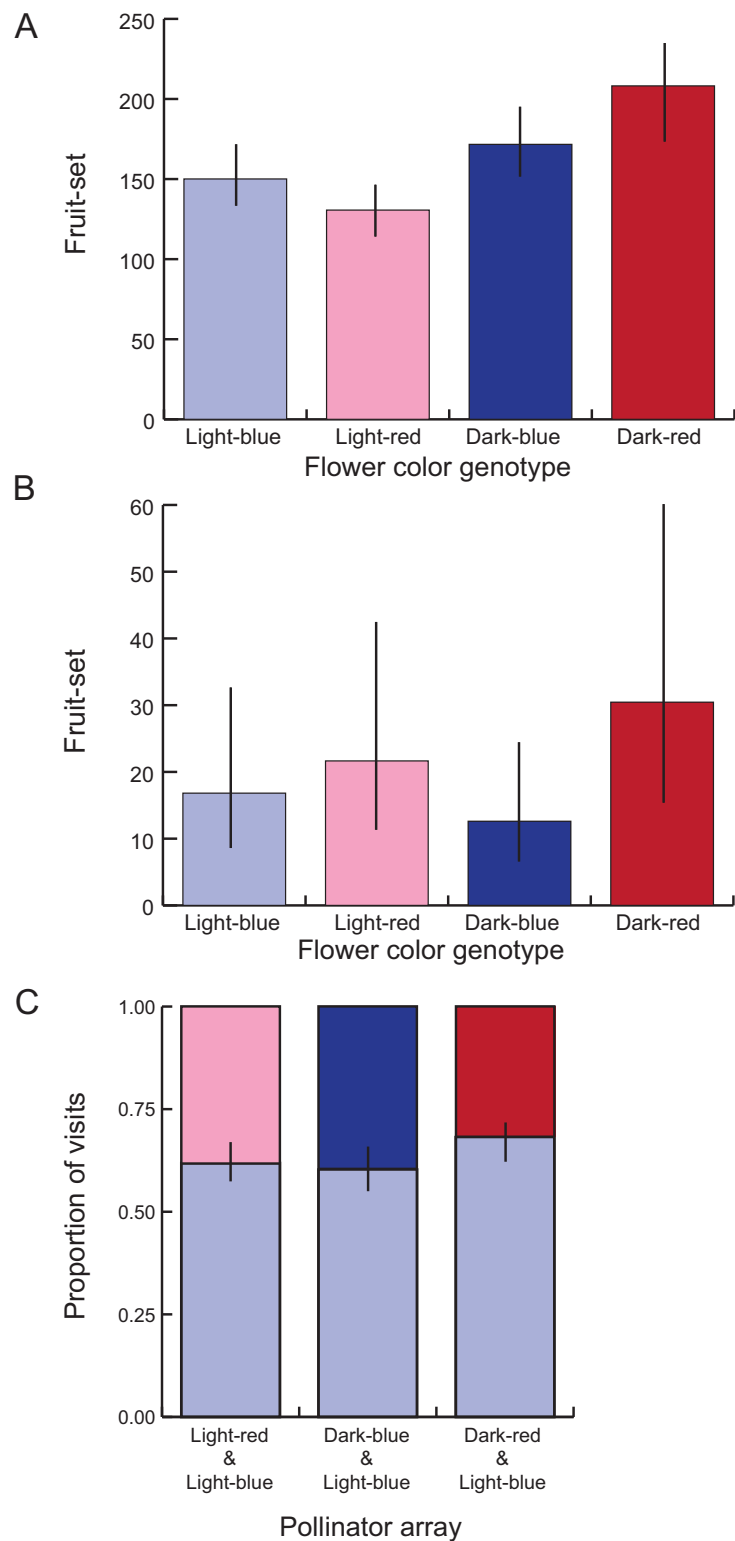
**2011 Experiment.** As in 2010, all four genotypes produced similar numbers of seeds per fruit (table B4), with differences being nonsignificant (table 2). Because some individuals did not survive to flower, we first assessed whether genotypes differed in survival probability. A logistic regression showed significant effects of hue genotype and block (table 3). Individuals with red alleles survived to flower more than individuals with blue alleles (table B7).

Among individuals that survived to flower, there was variation among genotypes in fruit production (table B4), although the differences were not significant (table 2). The mean number of fruits produced by all genotypes was substantially smaller and the coefficients of variation (standard error/mean) for mean fruit production were sub-

**Table 1:** Fruit-set and seeds-per-fruit ANOVA results from 2010 common garden experiment

Fixed effect vari- ance parameter	Fruit-set			Seeds per fruit		
	df	F	P	df	F	P
Hue genotype	1	.08	.8253	1	4.37	.2841
Intensity genotype	1	9.73	.1972	1	.85	.5253
Hue × intensity	1	2.98	.3340	1	.79	.538

Note: Results for random effects included in the model are in table B3.



**Figure 1:** Male and female fitness components for each flower color genotype. *A*, Average fruit set from 2010 common garden experiment, with bars representing 1 SE. *B*, Average fruit set for all individuals from 2011 common garden experiment, with bars representing 1 SE. *C*, Proportion of pollinator visits to the two flower colors in each of the three arrays. Bars represent bootstrap 95% confidence interval.

stantially greater than in 2010, presumably due to the drought conditions (tables B2, B4). Because of the large variation among individuals, our power to detect differences was reduced.

Finally, we examined differences in overall female fitness as measured by the product of survival and fruit production. As in the previous year, there was substantial variation in the genotype means (fig. 1B) but no significant effects of hue, intensity, or their interaction (table 2). It should be noted, however, that the hue effect had a large *F* value (5.02) associated with it, suggesting that failure to detect a significant hue effect may have been due to lack of power. If such an effect really exists, the red allele would be favored, given that mean fitness for both light and dark red genotypes was greater than for the light and dark blue genotypes (table B4). Nevertheless, this experiment does not provide evidence that genotypes significantly differed in fruit production. Data from this experiment can be found in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.f710n> (Hopkins and Rausher 2014).

*Pollinator Observations: Test for Paternal Fitness Variation*

Pollinator observations were performed on three days for each of the three arrays. We observed 278 pollinators and total of 1,000 visits to plants in the arrays. Among the pollinators, 95% were either pipevine swallowtail butterflies or skipper butterflies, and the remaining 13 pollinators were various Lepidoptera including *Junonia coenia* (common buckeye) and *Vanessa virginiensis* (American painted lady).

*Preference.* In the array experiments, we compared pollinator visitation rates to plants with light blue flowers to visitation rates to the other three possible derived flower colors (light red, dark blue, or dark red). Each array type involved one of these derived colors. In all three arrays we found significant preference for the light blue genotype. Data from this experiment can be found in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.f710n> (Hopkins and Rausher 2014).

Overall, pollinators visited flowers of the light blue ge-

**Table 3:** Survival to flowering logistic regression results from 2011 common garden experiment

Effect parameter	df	$\chi^2$	<i>P</i>
Intercept:	1	134.07	<.0001
Intensity genotype	1	.05	.8155
Hue genotype:	1	10.31	.0013
Family	2	.56	.7542
Block:	1	9.07	.0026
Intensity × hue	1	.1	.3996
Intensity × family	2	2.48	.2895
Intensity × block	1	.52	.4687
Hue × family	2	3.79	.1503
Hue × block	1	.31	.5766
Family × block:	2	7.74	.0209
Intensity × hue × block	1	.09	.7689
Likelihood ratio	8	15.99	.0426

Note: Maximum likelihood estimates by each effect parameter are in table B6.

notype 50 percent more often than plants with either light red or the dark blue flowers. The difference was even more extreme for individuals with dark red flowers: pollinators visited plants with light blue flowers twice as often as plants with dark red flowers. All of these preferences were highly statistically significant, as judged by the 95% confidence intervals for the proportion of visits to light blue plants (fig. 1C, table 4).

When visits are analyzed separately by pollinator type, we found, in general, that the preferences of pipevine swallowtails were similar to those of the skippers (table 4). Both pollinator types preferred light-blue-flowered genotypes to each of the alternative genotypes, and these preferences were all statistically significant (*P* < .05) except for the light blue versus light red for the skippers. Furthermore, when we analyzed pollinator visits by day of visit, we found significant preference for the light blue genotype on each of the 3 days for each of the three array types (table 4). Our conclusion that pollinators prefer plants with light blue flowers thus seems robust.

*Constancy.* Pollinators did not move more frequently between plants that had the same flower color than between

**Table 2:** Fruit-set for all individuals, seeds per fruit, and fruit-set for flowered individuals ANOVA results from 2011 common garden field experiment

Fixed effect variance parameter	Fruit-set (all individuals)			Seeds per fruit			Fruit-set (flowered individuals)		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Hue genotype	1	5.02	.2673	1	1.03	.4952	1	.5	.607
Intensity genotype	1	.01	.9317	1	.02	.9	1	.19	.7394
Hue × intensity	1	1.64	.4221	1	.58	.5858	1	1.2	.4714

Note: Results for random effects included in the model are in table B5.



**Table 4:** Pollinator preference estimates and 95% bootstrap confidence intervals (CIs) for the three arrays

Data	N	Estimate	95% CI
Light red array:			
Total	84	.619	.571–.666
Swallowtail	38	.627	.570–.687
Skippers	41	.570	.473–.659
Day 1	33	.609	.535–.683
Day 2	32	.636	.563–.712
Day 3	19	.619	.500–.723
Dark blue array:			
Total	118	.603	.553–.653
Swallowtail	53	.567	.508–.629
Skippers	63	.680	.615–.748
Day 1	70	.580	.523–.642
Day 2	35	.615	.514–.707
Day 3	13	.752	.672–.870
Dark red array:			
Total	74	.682	.634–.732
Swallowtail	37	.635	.582–.687
Skippers	30	.909	.818–.979
Day 1	37	.630	.571–.694
Day 2	10	.646	.521–.800
Day 3	27	.794	.714–.880

plants with different flower colors (table 5). This apparent lack of constancy suggests that pollinator behavior does not lead to positive assortative mating within *P. drummondii* flower colors. In fact, the BCI for all the array types was negative, indicating that the pollinators moved more frequently than expected between plants with different flower colors. The disassortative movement is likely a consequence of the design of the arrays: nearest neighbors have different flower colors. Given this design, we suspect that the negative BCI reflects a tendency for pollinators to move to the closest neighbor plant.

#### *Balance of Selective Forces*

To better understand the balance between our field-based estimates of maternal fitness and paternal fitness, we modeled evolutionary trajectories starting from various combinations of allele frequencies at the two flower-color loci (fig. 2). When data from 2010 is used in the base model (equal pollen production, dominance of fecundity and survival), there is one stable equilibrium corresponding to fixation of the light and blue alleles. With data from 2011, we used two methods to calculate fecundity. In our base model, both estimates of fecundity resulted in a stable equilibrium corresponding to fixation of the light allele and the blue allele. With the second method of calculating fecundity (starting with the average fruit-set across all in-

dividuals regardless of survival), the model shows a second stable equilibrium with the fixation of the dark and the red alleles. Nevertheless, using either estimate of fecundity across both years, there is a basin of attraction for the light blue genotype. Our results indicate that when the red and dark alleles are rare, as would be the case when migration introduces these alleles from sympatric populations, selection will act to eliminate both of these alleles.

Results for the alternative models for 2010 are qualitatively similar to the base model (fig. B1). Fixation of the light and blue allele is a stable equilibrium when fecundity and survival are additive and when male success is proportional to fecundity. In 2011, the results depend on the method of estimating fecundity. When the first method is used, all alternative models find a stable equilibrium with the fixation of the light and blue allele. When the second method is used, there is not a stable equilibrium of fixation of the light and blue alleles.

#### Discussion

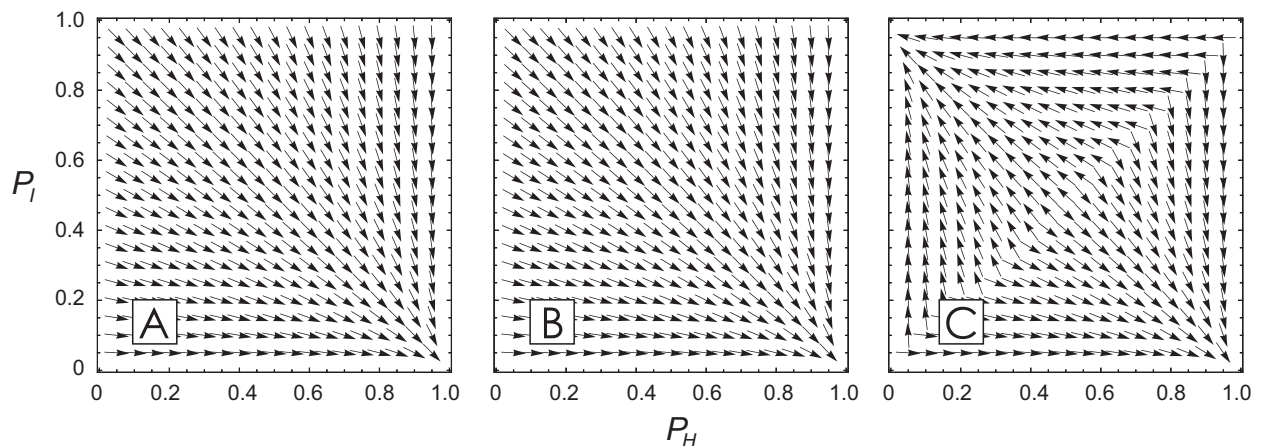
When Dobzhansky first formalized the hypothesis that costly hybridization could create selection for increased prezygotic reproductive isolation, he predicted that this process would result in greater prezygotic reproductive isolation in sympatric populations than in allopatric populations (Dobzhansky 1937, 1940). Numerous studies have documented this pattern in a wide variety of organisms, and much research has attempted to understand the evolutionary forces responsible for divergence in sympatric populations (reviewed in Howard 1993; Servedio and Noor 2003; Ortiz-Barriontos et al. 2009; Pfennig and Pfennig 2009).

While reinforcing selection may explain increased prezygotic isolation in sympatry, little is understood about why the signature of reinforcement persists, that is, why alleles conferring increased reproductive isolation in sympatry do not spread to regions of allopatry (but see Pfennig 2000; Higgie and Blows 2007). Two non-mutually exclusive hypotheses can account for the persistence of the ancestral allele: either gene flow has not introduced these alleles into allopatric populations or there is selection against these alleles in allopatric populations. In the case of *Phlox drum-*

**Table 5:** Bateman's constancy index (BCI) from pollinator observation arrays

Array	BCI	$\chi^2$	df	P
Light red	-.36	34.88	1	<.0001
Dark blue	-.22	7.062	1	.0078
Dark red	-.22	9.53	1	.002

Note: Each array contained the genotype indicated in the "Array" column and the light blue genotype.



**Figure 2:** Evolutionary trajectories at the hue and intensity loci. A, The results from model of 2010 data. B, Results from model of 2011 data using method 1 to estimate fecundity. C, Results from model of 2011 data using method 2 to estimate fecundity.  $P_I$  is frequency of the dark allele at the intensity locus.  $P_H$  is the frequency of the blue allele at the hue locus. Each arrow corresponds to a different pair of starting frequencies and points in the direction of the equilibrium to which gene frequencies converge. The lower right corner of each graph represents fixation of the light and blue alleles and is a stable equilibrium in both years. In 2011, using method 2 to estimate fecundity, there is a second stable equilibrium corresponding to fixation of the  $I$  (dark) allele and the  $H$  (red) allele.

*mondii*, the former explanation seems unlikely because there is evidence for extensive gene flow and little isolation by distance over much greater distances than separate light blue and dark red populations (Hopkins et al. 2012). Instead, it seems likely that the light and blue alleles, characteristic of allopatric populations, are maintained by natural selection.

In this investigation we attempted to determine whether there is net selection favoring the maintenance of the light and blue alleles in allopatric populations. We found that pollinators visit plants with light blue flowers more frequently than they visit genotypes with other flower colors. Although we have not directly quantified male fitness, previous work in *Phlox* indicates that patterns of pollinator movement correspond to patterns of paternity (Hopkins and Rausher 2012), and investigations in other systems indicate that male fitness is frequently correlated with visitation rates (Broyles and Wyatt 1990; Galen 1992; Ashman 1998; Jones and Reithel 2001). It is therefore likely that the higher visitation rate to light blue plants generates a higher male success to these individuals compared to the other genotypes.

Counteracting pollinator preference is a difference in survival, detected in the 2011 experiment, which favors the red allele over the blue allele. In addition, the estimate of fruit production for at least one alternative genotype was greater than that for the light blue genotype in both years. While differences in fruit production were not significant in either year, our statistical power to detect differences may be low, especially in 2011. In 2010, point estimates indicate that individuals carrying the dark allele

produced more seeds than individuals carrying the light allele. These data suggest that selection acting through maternal fitness (survival to fruit and fruit production) may act counter to selection acting through paternal fitness.

To assess the balance among these selection forces, we simulated the effects of both components of fitness on evolutionary trajectories at the two flower-color loci. Using the data from 2010, all variations of the model predict the allopatric populations will remain light blue. The net effect of selection under the 2010 model is to prevent the red and dark alleles from spreading into the region of allopatry. Using the data from 2011, our base model also finds that selection would disfavor dark and red alleles migrating into an allopatric population. The results of our alternative models, which investigate assumptions about dominance of fecundity and survival, and male success, differ depending on which estimate of fecundity was used. When fecundity is estimated from the number of fruits produced by individuals in the common garden that survived to flower, the red and the dark allele cannot invade a light blue population. But, when the alternative method of estimating fecundity is used, relaxing the assumptions of dominance or male success results in a loss of the light blue stable state. Despite the strong selection acting through pollinator preference (selection coefficients between 0.5 and 1 favoring the light blue flowers), in one of these alternative models, the dark allele is able to invade, and in all three, the red allele is able to invade a light blue population. In other words, the survival and seed-set advantage of the other genotypes outweighs the pollinator

preference for light blue. Although it is likely that pollinator preference will maintain the light and blue alleles in allopatry, future work investigating dominance of fitness effects, and male fitness variation, is needed to confirm these findings.

As discussed above, our calculations are based on the point estimates of fruit production and survival from the common-garden field experiments, which may suffer from low statistical power, and from the pollinator observations, which we are assuming are proportional to siring success. These calculations make two additional assumptions: (1) pollinator movement between plants of different colors is random, producing no assortative mating (i.e., mating is random); and (2) there is no pollen competition among pollen genotypes at the hue and intensity loci.

Our results indicate these assumptions are at least partially justified. In particular, we found no evidence for pollinator constancy (table 5), which would result in positive assortative mating. Instead, we found an apparent excess of movements between plants of different genotypes, which would generate negative assortative mating. We believe, however, that this pattern reflects a tendency to move to the closest plant in our arrays, which were always a different genotype. The tendency to move to a nearest neighbor could mask a low degree of pollinator constancy that might cause small deviations from random mating and Hardy-Weinberg genotype frequencies, but we suspect these deviations would not greatly affect the shape of the adaptive landscape. We have no evidence indicating flower color hue or intensity causes variation in pollen competitive ability. Variation in competitive ability would affect estimates of paternal fitness but would have to be large to counteract the substantial pollinator preference and therefore alter the expected evolutionary trajectories.

Our model is deterministic and based on the point-estimate means (not variances) of maternal and paternal fitness. Future work should explore more thoroughly how different strengths of selection will alter the expected pattern of flower-color variation across the allopatric and sympatric range of *P. drummondii*.

This study does not address why pollinators exhibit a preference for light blue flowers. This preference may be predominantly innate. The strong preference for blue flowers by the pipevine swallowtails is consistent with previous experiments showing innate preference for blue and purple paper flowers over red paper flowers (Weiss 1997). However, it is possible that preference for light blue is at least partially learned. Pipevine swallowtail butterflies have been shown to alter their color preference after experience to optimize reward (Weiss 1997; Weiss and Papaj 2003). If preference is partially learned, the strength of preference may increase with frequency of flower color phenotype leading to frequency-dependent dynamics. In regions of

allopatry, previous foraging experience would likely reinforce the strong preference for the light blue color. In sympatric areas, where divergence in flower color is favored due to reinforcement, pollinators may prefer, or at least not disfavor, the dark red flower color because it is at high frequency in this region. This would imply that, once reinforcement evolved in *P. drummondii*, there is no longer a cost associated with the increase in reproductive isolation in sympatry. Understanding the dynamics of pollinator preference is an interesting area for future research.

Our work demonstrates how multiple aspects of pollinator behavior can affect the evolution of flower color. Our previous work in this system shows that flower-color divergence in sympatric populations causes pollinator constancy within *Phlox* species. In other words, when both *P. drummondii* and *P. cuspidata* have the same light blue flower color pollinators move equally among and between species, but when *P. drummondii* has dark red flowers, pollinators move more frequently within plants of the same species than between plants of different species. Traits that increase pollinator constancy are favored because constancy decreases costly hybridization between the *Phlox* species. Here we find that a different aspect of pollinator behavior, preference, causes selection for the light and blue alleles in allopatric populations. Of note, we find no evidence of constancy based on flower color within the species *P. drummondii*. The research in this system reveals how, even when the pollinator community is the same across environments, the direction of selection on flower morphology caused by pollinators can change depending on the environment.

In conclusion, our results suggest that the spread of alleles fixed by reinforcement selection in the region of sympatry between *P. drummondii* and its congener *P. cuspidata* are prevented from spreading to *P. drummondii*'s region of allopatry by selection favoring the ancestral alleles. While the derived alleles favored in sympatry may also confer some survival or fecundity advantage in allopatry, this advantage is likely more than offset by disadvantage in pollen transmission due to pollinator preference for the ancestral phenotype. Our work suggests that pollinators play a major role in generating selection on flower color that both leads to reinforcement in sympatry and maintains the ancestral flower color in allopatry.

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APPENDIX A

Model of Flower Color Evolution

In this appendix we describe the models of allele frequency change at the two flower color loci. The hue locus had two alleles, *h*, corresponding to red, and *H*, corresponding to blue. The intensity locus has two loci, *i*, corresponding to light, and *I*, corresponding to dark.

Let the initial genotype frequency be

$$G = [G_{IIHH}, G_{IiHH}, G_{iiHH}, G_{IIHh}, G_{IiHh}, G_{iiHh}, G_{IIhh}, G_{Iihh}, G_{iihh}],$$

The initial allele frequencies are

$$p_I = \left[ 1, 1, 1, \frac{1}{2}, \frac{1}{2}, \frac{1}{2}, 0, 0, 0 \right] \cdot G,$$

$$p_H = \left[ 1, \frac{1}{2}, 0, 1, \frac{1}{2}, 0, 1, \frac{1}{2}, 0 \right] \cdot G,$$

where  $\cdot$  is the inner product operator:  $[abc] \cdot [def] = [ad + be + cf]$ .

The proportional contribution of each genotype to ovules (female contribution) is

$$G_f = \frac{G \times \mathbf{1} \times \mathbf{m}}{\sum G \times \mathbf{1} \times \mathbf{m}},$$

where the times sign indicates multiplication of corresponding elements, and the denominator indicates the sum of the elemental products. Vector  $\mathbf{1}$  is the vector of survival probabilities corresponding to each of the nine genotypes, and  $\mathbf{m}$  is the vector of fecundity estimates corresponding to each of the nine genotypes.

The proportional contribution of each genotype to pollen (male contribution) is

$$G_m = \frac{G \times \mathbf{1} \times \mathbf{p}}{\sum G \times \mathbf{1} \times \mathbf{p}},$$

where  $\mathbf{p}$  is the vector of pollinator preferences relative to the light blue genotype for each of the nine genotypes.

There are only four possible haploid gamete genotypes: IH, iH, Ih, ih. The frequencies of these gamete types in the ovules after selection are

$$g_f = G_f \begin{bmatrix} 1 & 0 & 0 & 0 \\ \frac{1}{2} & \frac{1}{2} & 0 & 0 \\ 0 & 1 & 0 & 0 \\ \frac{1}{2} & 0 & \frac{1}{2} & 0 \\ \frac{1}{4} & \frac{1}{4} & \frac{1}{4} & \frac{1}{4} \\ 0 & \frac{1}{2} & 0 & \frac{1}{2} \\ 0 & 0 & 1 & 0 \\ 0 & 0 & \frac{1}{2} & \frac{1}{2} \\ 0 & 0 & 0 & 1 \end{bmatrix} = [g_{IH}^f, g_{iH}^f, g_{Ih}^f, g_{ih}^f].$$

The matrix in brackets is the segregation matrix. The columns represent the four gametes, and each row represents the proportion of gametes that are produced by the corresponding genotype in  $G_r$ .

Similarly, the male gametes have haplotype frequencies of

$$g_m = G_m \begin{bmatrix} 1 & 0 & 0 & 0 \\ \frac{1}{2} & \frac{1}{2} & 0 & 0 \\ 0 & 1 & 0 & 0 \\ \frac{1}{2} & 0 & \frac{1}{2} & 0 \\ \frac{1}{4} & \frac{1}{4} & \frac{1}{4} & \frac{1}{4} \\ 0 & \frac{1}{2} & 0 & \frac{1}{2} \\ 0 & 0 & 1 & 0 \\ 0 & 0 & \frac{1}{2} & \frac{1}{2} \\ 0 & 0 & 0 & 1 \end{bmatrix} = [gm_{IH}, gm_{iH}, gm_{Ih}, gm_{ih}].$$

Male and female gametes combine randomly:

$$\begin{aligned} Z &= g_m \circ g_f = \begin{bmatrix} gm_{IH} \\ gm_{iH} \\ gm_{Ih} \\ gm_{ih} \end{bmatrix} \circ [gf_{IH}, gf_{iH}, gf_{Ih}, gf_{ih}], \\ &= \begin{bmatrix} gm_{IH}gf_{IH} & gm_{IH}gf_{iH} & gm_{IH}gf_{Ih} & gm_{IH}gf_{ih} \\ gm_{iH}gf_{IH} & gm_{iH}gf_{iH} & gm_{iH}gf_{Ih} & gm_{iH}gf_{ih} \\ gm_{Ih}gf_{IH} & gm_{Ih}gf_{iH} & gm_{Ih}gf_{Ih} & gm_{Ih}gf_{ih} \\ gm_{ih}gf_{IH} & gm_{ih}gf_{iH} & gm_{ih}gf_{Ih} & gm_{ih}gf_{ih} \end{bmatrix}, \end{aligned}$$

where  $\circ$  designates the outer product operator.

The new genotype frequencies are

$$g_i = [Z[1, 1], (Z[1, 2] + Z[2, 1]), Z[2, 2], (Z[3, 1] + Z[1, 3]), (Z[4, 1] + Z[2, 3] + Z[3, 2] + Z[1, 4]), \\ Z[3, 3], (Z[3, 4] + Z[4, 3]), Z[4, 4]],$$

and the new allele frequencies are calculated as above.

#### Data

In 2010, the  $\mathbf{m}$  vector is

$$\mathbf{m} = [172.88, 172.88, 151.21, 172.88, 172.88, 151.21, 209.45, 209.45, 131.69].$$

In 2010, the  $\mathbf{l}$  vector is

$$\mathbf{l} = [1, 1, 1, 1, 1, 1, 1, 1, 1].$$

In 2011, the  $\mathbf{m}$  matrix was calculated in two ways. Method 1 used the estimate of fruit set from the individuals that survived:

$$\mathbf{m} = [28.99, 28.99, 31.24, 28.99, 28.99, 31.24, 36.30, 36.30, 30.30],$$

and method 2 used the estimate of fruit set from all experimental individuals and dividing by survival probability:

$$\mathbf{m} = [16.90, 16.90, 20.49, 16.90, 16.90, 20.49, 33.91, 33.91, 24.97].$$

The survival probabilities for 2011 are

$$\mathbf{l} = [.746, .746, .821, .746, .746, .821, .898, .898, .867].$$

The preference for each genotype is

$$\mathbf{p} = [0.658, 0.658, 1, 0.658, 0.685, 1, 0.466, 0.466, 0.615].$$

#### *Modifications of the Model*

Variation 1. We altered the above standard model by making male contribution proportional to female fecundity:

$$G_m = \frac{G \times \mathbf{l} \times \mathbf{p} \times \mathbf{m}}{\sum G \times \mathbf{l} \times \mathbf{p} \times \mathbf{m}}.$$

Variation 2. We altered the model by making survival and fecundity additive and not dominant. For 2010,

$$\mathbf{m} = [172.88, 162.04, 151.21, 191.16, 166.31, 141.45, 209.45, 170.57, 131.69].$$

For 2011, method 1:

$$\mathbf{m} = [28.99, 30.11, 31.24, 32.65, 31.71, 30.77, 36.30, 33.30, 30.30],$$

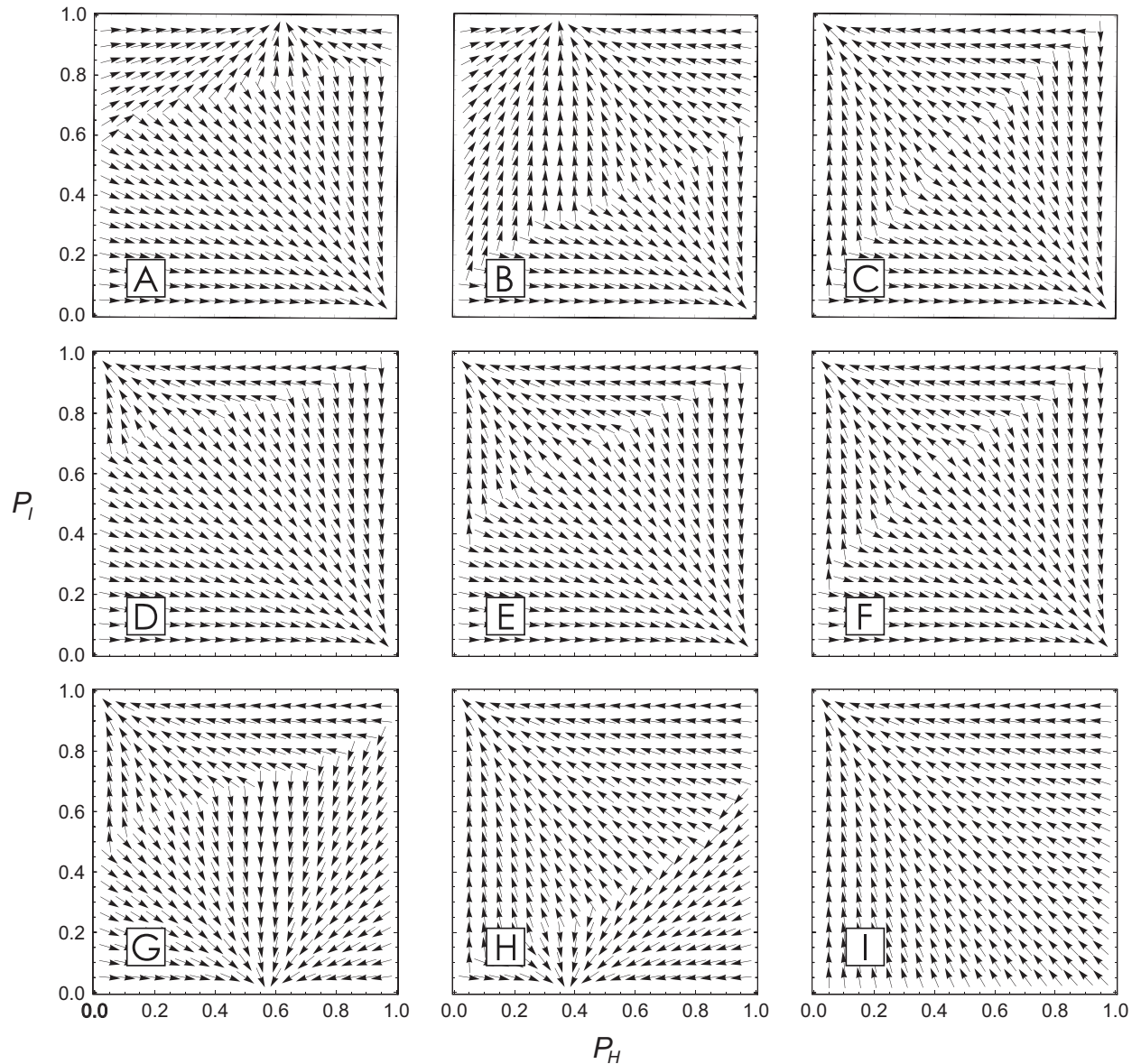
and method 2:

$$\mathbf{m} = [16.9, 18.69, 20.487, 25.41, 24.07, 22.73, 33.91, 29.44, 24.97],$$

and additive survival is

$$\mathbf{l} = [.746, .783, .821, .822, .833, .844, .898, .882, .867].$$

APPENDIX B  
Supplementary Figure and Tables



**Figure B1:** Evolutionary trajectories of the alternative models. *A–C* are results from models of data from 2010, *D–F* are results from models of 2011 using method 1 to estimate fecundity, and *G–I* are results from models of 2011 using method 2 to estimate fecundity. Results assume fecundity and survival have additive effects are shown in *A*, *D*, and *G*. Results assuming both additive effects and pollen success in proportional to fecundity are shown in *B*, *E*, and *H*. Results assuming pollen success is proportional to fecundity but fecundity and survival are dominant are shown in *C*, *F*, and *I*.

**Table B1:** Population locations and crossing design for each of the five families used in the common garden field experiment

Year	Cross	F <sub>2</sub> family 1				F <sub>2</sub> family 2			
		Light blue		Dark red		Light blue		Dark red	
		Name	GPS	Name	GPS	Name	GPS	Name	GPS
2010	A	466_E7	N29.505, W-97.7292	POC2104	N30.0814, W-97.0872	DOG95	N30.2927, W-97.3423	POC2104	N30.0814, W-97.0872
2010	B	466_E7	N29.505, W-97.7292	POC2104	N30.0814, W-97.0872	DOG95	N30.2927, W-97.3423	80S1	N29.6404, W-97.6613
2011	C	696N1	N30.3236, W-97.2902	304N1	N29.6866, W-97.4177	466_E7	N29.505, W-97.7292	80S1	N29.6404, W-97.6613
2011	D	696N1	N30.3236, W-97.2902	304N1	N29.6866, W-97.4177	466_E7	N29.505, W-97.7293	POC2104	N30.0814, W-97.0872
2011	E	466_E7	N29.505, W-97.7292	80S1	N29.6404, W-97.6613	466_E7	N29.505, W-97.7294	POC2104	N30.0814, W-97.0872

**Table B2:** Fruit-set and seeds-per-fruit least squares (LS) means and standard errors (SE) for each homozygous genotype from 2010 common garden field experiment

Genotype	Fruit-set			Seeds per fruit	
	LS mean	LS mean - SE	LS mean + SE	LS mean	SE
Light blue	151.21	132.87	172.08	2.23	.17
Dark blue	172.88	152.59	195.86	2.25	.16
Light red	131.69	115.75	149.84	2.43	.16
Dark red	209.45	184.75	237.46	2.43	.17

**Table B3:** Fruit-set and seeds per fruit ANOVA results from 2010 common garden experiment

	Fruit-set			Seeds per fruit		
	df	F	P	df	F	P
Fixed effect variance parameter:						
Hue genotype	1	.08	.8253	1	4.37	.2841
Intensity genotype	1	9.73	.1972	1	.85	.5253
Hue × intensity	1	2.98	.3340	1	.79	.538
	Estimate	χ <sup>2</sup>	P	Estimate	χ <sup>2</sup>	P
Random effect covariance parameter:						
Family	0	0	1	0	0	1
Block	.0141	.6	.4385	.02789	.9	.3427
Family × block	0	0	1	0	0	1
Family × intensity	0	0	1	0	0	1
Family × hue	0	0	1	.01131	.8	.371
Block × intensity	0	0	1	0	0	1
Block × hue	0	0	1	0	0	1
Block × intensity × hue	.005814	.3	.5838	0	0	1
Residual	.4807			.1437		
-2 residual log likelihood	668.8			126.6		

Note: Both fixed and random effects reported.

**Table B4:** Fruit-set from all individuals, seeds per fruit, and fruit-set for flowered individuals least squares (LS) means and standard errors (SE) for each homozygous genotype from 2011 common-garden field experiment

Genotype	Fruit-set (all individuals)			Seeds per fruit		Fruit-set (flowered individuals)		
	LS mean	LS mean - SE	LS mean + SE	LS mean	SE	LS mean	LS mean - SE	LS mean + SE
Light blue	16.82	32.81	8.62	2.48	.12	31.24	55.30	17.65
Dark blue	12.61	24.62	6.46	2.40	.12	28.99	51.39	16.35
Light red	21.65	42.45	11.05	2.36	.12	30.30	53.64	17.11
Dark red	30.45	60.20	15.40	2.38	.12	36.30	64.42	20.45



**Table B5:** Fruit-set for all individuals, seeds per fruit, and fruit-set for flowered individuals ANOVA results from 2011 common garden field experiment, including both fixed effects and random effects

	Fruit-set (all individuals)			Seeds per fruit			Fruit-set (flowered individuals)		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Fixed effect variance parameter:									
Hue genotype	1	5.02	.2673	1	1.03	.4952	1	.5	.607
Intensity genotype	1	.01	.9317	1	.02	.9	1	.19	.7394
Hue × intensity	1	1.64	.4221	1	.58	.5858	1	1.2	.4714
	Estimate	$\chi^2$	<i>P</i>	Estimate	$\chi^2$	<i>P</i>	Estimate	$\chi^2$	<i>P</i>
Random effect covariance parameter:									
Family	.0523	.2	.6547	0	0	1	.1484	4.3	.0381*
Block	.7316	3.4	.0651	0	0	1	.5223	2.6	.1068
Family × block	.0457	.5	.4795	0	0	1	0	0	1
Family × intensity	0	0	1	0	0	1	0	0	1
Family × hue	0	0	1	0	0	1	0	0	1
Block × intensity	0	0	1	.01206	2.6	.1068	0	0	1
Block × hue	0	0	1	0	0	1	0	0	1
Family × hue × intensity	.1053	1	.3173	0	0	1	0	0	1
Family × intensity × block	0	0	1	0	0	1	0	0	1
Family × hue × block	.0157	.1	.7518	0	0	1	0	0	1
Block × intensity × hue	0	0	1	0	0	1	0	0	1
Family × hue × intensity × block	0	0	1	0	0	1	0	0	1
Residual	2.4638			.3126			1.2104		
−2 residual log likelihood	2,010.9			527.7			1,360		

**Table B6:** Maximum likelihood estimates from survival analyzes

Effect	Estimate	SE	$\chi^2$	<i>P</i>
Intercept	−1.8628	.1609	134.07	<.0001
Intensity	Dark	.0399	.1709	.05
Hue	Blue	.504	.157	10.31
Block	A	−4.091	.1358	9.07
Family	D	−.1776	.2459	.52
Family	C	.0687	.1724	.12
Family × block	D × A	.2863	.194	2.18
Family × block	C × A	−.5878	.2113	7.74
Intensity × hue	Dark × blue	.1452	.1724	.71
Intensity × block	Dark × A	.1083	.1495	.52
Intensity × family	Dark × D	.2305	.1984	1.35
Intensity × family	Dark × C	.0687	.2007	.12
Hue × block	Blue × A	−.0785	.1406	.31
Hue × family	Blue × D	.4518	.2463	3.36
Hue × family	Blue × C	−.1279	.2296	.31
Intensity × hue × block	Dark × blue × A	−.1326	.1431	.86

Note: Positive estimates indicated more individuals did not survive to flower.

**Table B7:** Survival probabilities for each genotype in 2011

Genotype	N	Proportion survived
Light blue	140	.821
Dark blue	130	.746
Light red	135	.867
Dark red	127	.898

**Table B8:** Number of transitions observed between for each of the three array types

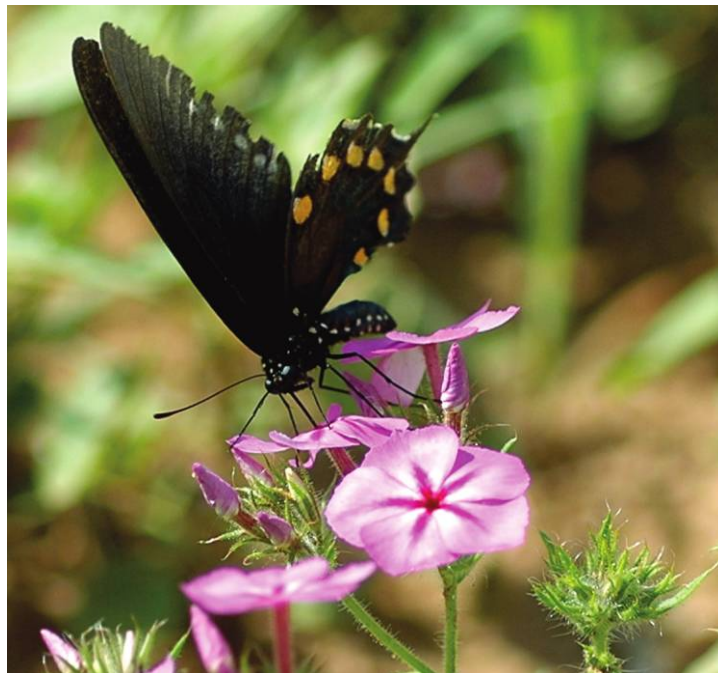
From	To	
	Light blue	Dark blue
Light blue	75	99
Dark blue	94	27
From	Light blue	Light red
	Light blue	69
Light red	69	25
From	Light blue	Dark red
	Light blue	77
Dark red	57	16

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*Phlox drummondii* with light blue flower color being pollinated by *Battus philenor*. Photo credit: David L. Des Marais.