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Ecological divergence in flowering plants: niche expansion, selection, and reproductive isolation

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ECOLOGICAL DIVERGENCE IN FLOWERING PLANTS: NICHE EXPANSION, SELECTION, AND REPRODUCTIVE ISOLATION

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by

Megan L. Peterson

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TABLE OF CONTENTS **Page**

LIST OF FIGURES AND TABLES

ABSTRACT

Ecological divergence in flowering plants: niche expansion, selection, and reproductive isolation

Megan L. Peterson

The origin and persistence of species is fundamental to the evolution of biological diversity. Species generally arise in new environments through a combination of ecological divergence and spatial isolation. Under this framework, speciation occurs through three main processes: 1) niche expansion, 2) divergent selection, and 3) the accumulation of reproductive isolating mechanisms. In this dissertation, I tested specific hypotheses about the factors governing each of these stages, using a combination of stochastic simulations and field and greenhouse experiments with life history ecotypes of common monkeyflower *Mimulus guttatus* DC (Phrymaceae). First, I tested the ecological and genetic determinants of colonization success in flowering plants. In particular, I used stochastic simulations to test the effect of mating system plasticity on the persistence and adaptation of colonizing populations in new environments. I found that a shift to a mixed mating strategy facilitated niche expansion by reducing extinction and promoting adaptation under a broad range of colonization scenarios. In my second and third chapters, I examined the causes and consequences of life history divergence among populations of *M. guttatus* in central California. In chapter two, I used comparative demography to test for local adaptation and selection on life history traits in a common garden experiment in a montane perennial environment. I found that a perennial life history strategy had the highest performance and that selection favored perennial life history traits, although the native montane perennial ecotype was not locally adapted relative to other inland perennial populations. In chapter three, I quantified multiple isolating mechanisms between a focal montane perennial population and ten other populations spanning the range of life history strategies. I found that total reproductive isolation increased linearly with life history divergence between populations. However, total isolation was achieved through a mosaic of reproductive barriers and enhancers which, individually, exhibited non-linear relationships with life history divergence. These non-linear relationships are likely due to the effects of life history and inbreeding on the evolution of specific isolating mechanisms. Taken together, this work highlights the potential for diverse evolutionary, ecological, and demographic forces to interact to determine progress during the early stages of divergence.

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STATEMENT OF CONTRIBUTION

The text of this dissertation includes reprints of the following previously published material: Chapter one: Peterson, M. L. and K. M. Kay. 2015. Mating system plasticity promotes persistence and adaptation of colonizing populations of hermaphroditic angiosperms. *The American Naturalist* 185:28-43. The co-author listed in this publication directed and supervised the research which forms the basis for the dissertation.

GENERAL INTRODUCTION

The origin and persistence of species is fundamental to the evolution of biological diversity. Under the Biological Species Concept, speciation is the evolution of barriers to gene flow between sister lineages (Mayr 1942). Comparative tests of the geography and evolutionary forces driving speciation suggest that reproductive isolation evolves primarily via divergent natural or sexual selection accompanied by some degree of spatial isolation between populations (reviewed in Coyne and Orr 2004; Fitzpatrick et al. 2009; Funk et al. 2006; Rundle and Nosil 2005). Within this framework, the process of speciation can be conceptualized as comprising three stages: niche expansion through the colonization of a new selective environment, ecological divergence, and the evolution of reproductive isolating barriers between lineages. Within species, populations in unique selective environments reflect all stages along this continuum, from maladapted sink populations to strongly isolated and locally adapted ecotypes (e.g. Anderson and Geber 2010; Berner et al. 2009; Clausen 1951; Clausen et al. 1948; Nosil and Crespi 2004). Such populations may become extinct, homogenized through gene flow, or remain partially isolated ecotypes; intraspecific divergence only rarely results in the evolution of a new, persistent biological species. Understanding the ecological and evolutionary processes that determine progress toward adaptive divergence and speciation remains a major goal of evolutionary biology (Nosil et al. 2009). In this dissertation, I tested specific hypotheses about the processes operating at each of these stages, using a combination of stochastic simulations and field and greenhouse experiments with life history ecotypes of common monkeyflower *Mimulus guttatus* DC (Phrymaceae).

Chapter one: Mating system plasticity promotes persistence and adaptation of colonizing populations of hermaphroditic angiosperms

The initial evolution and persistence of small populations is critical for understanding the process of speciation, particularly in sessile organisms such as plants. Gene flow has historically been viewed as a powerful force maintaining genetic cohesion throughout a species range, necessitating large-scale geographic isolation for speciation to occur (e.g. vicariant or geographic speciation) (Dobzhansky 1951; Jordan 1905; Mayr 1942). However, botanists have argued that gene flow is sufficiently limited in many sessile organisms to allow smaller populations to evolve more or less independently, particularly at the periphery of a species range or under strong divergent selection (Ehrlich and Raven 1969; Levin and Kerster 1974). Contemporary evidence suggests that the spatial scale of speciation is in fact related to the scale of gene flow (Kisel and Barraclough 2010), and that new plant species are often formed in small, ecologically divergent populations (Anacker and Strauss 2014; Baldwin 2005; Gottlieb 2004; Grossenbacher et al. 2014; Levin 1993). Speciation in initially small populations (e.g. quantum, peripatric, progenitor-derivative, or budding speciation) is fundamentally different from vicariant speciation in widely-distributed races because of the complex interplay of selection, genetic drift, inbreeding, and gene flow (Grant 1981; Levin 1993; Mayr 1954). Small populations face unique challenges to persist and adapt under novel environmental conditions, yet this is a frequent first step in the evolution of new plant species.

In chapter one, I used stochastic simulations to explore the ecological and genetic conditions under which a small colonizing population is able to successfully persist and adapt in a new selective environment. In particular, I focused on the role of plasticity in the self-fertilization rate of hermaphroditic angiosperms. Botanists have recognized the potential for self-fertilization to promote range and niche expansion. Baker (1967) first noted the tendency for isolated or peripheral plant populations to exhibit increased self-fertilization, and suggested that the ability to reproduce when mates are limiting (e.g. reproductive assurance) is an important predictor of colonization success. Grant (1981) later suggested that the evolution of self-fertilization in peripheral populations could promote speciation by providing reproductive isolation, and Levin (2010) highlighted the potential for novel environments to increase self-fertilization instantaneously through phenotypic plasticity. However, self-fertilization can also increase extinction risk through inbreeding depression (Darwin 1876; Goldberg et al. 2010; O'Grady et al. 2006; Stebbins 1957). I used individual-based stochastic simulations to model the joint evolution of inbreeding depression, genetic load, and local adaptation in a colonizing population as a function of the strength of selection, mate limitation, gene flow, and a plastic increase in self-fertilization. I found that a plastic shift to a mixed mating system reduced extinction risk and accelerated local adaptation, particularly when mates were limiting or gene flow from surrounding populations was high. This result suggests that partial self-fertilization is an important contributor to the persistence and adaptation of small, ecologically-peripheral plant populations. Environmental stress increases self-fertilization in diverse flowering plant species (reviewed in Levin 2010) and this response could become canalized over time, potentially explaining the frequent evolution of self-fertilizing species from outcrossing ancestors (Barrett 2002; Stebbins 1974; Wright et al. 2013b).

Chapter two: Divergence in life history traits alters the pattern and strength of phenotypic selection: comparing integrated selection among life history ecotypes of Mimulus guttatus.

A major goal of speciation research is to link natural selection in particular environments to the evolution of traits underlying reproductive barriers (Rundle and Nosil 2005; Schluter 2001; Sobel et al. 2010). Variation in the strength of selection has been suggested to explain variable progress towards speciation (Nosil et al. 2009), but testing this hypothesis requires estimates of selection that can be compared among populations and environments. Most estimates of phenotypic selection in wild populations are based on individual components of fitness, such as survival, growth, or fecundity at a given life history stage (Kingsolver et al. 2012; Kingsolver et al. 2001). Yet, adaptive divergence often involves life history traits themselves (e.g. Evans et al. 2005; Moser et al. 2012; Reznick et al. 1990; Verboom et al. 2004; Walsh and Reznick 2011); in this case, individual fitness components will differ in their importance for overall performance among populations and environments. Although there has been a growing effort to integrate selection through multiple fitness components using demographic methods (Coulson et al. 2003; Ehrlén and Münzbergová 2009; Horvitz et al. 2010; van Tienderen 2000), this approach has not yet been applied to address adaptive divergence among populations.

In chapter two, I tested the potential for life history divergence within *Mimulus guttatus* to influence the pattern and strength of phenotypic selection within a common montane environment. *Mimulus guttatus* includes populations that range from small, fast-cycling annuals in rapidly drying environments to larger short-lived perennials in more mesic habitats. In a two-year common garden experiment, I estimated mean-standardized selection gradients through individual fitness components and integrated selection based on the population growth rate λ (Hereford et al. 2004; van Tienderen 2000) for four life history traits related to ecological divergence and, potentially, reproductive isolation (Hall and Willis 2006; Lowry et al. 2008b). I found that the importance of specific fitness components for population growth varied among life history ecotypes; annual populations relied on first-year seed set whereas the performance of perennial populations also depended on vegetative growth and adult survival. Selection through individual fitness components, such as fecundity, was often similar in direction among life history ecotypes, but integrating across all fitness components altered the pattern and strength of phenotypic selection. In particular, integrated selection was weaker in perennials relative to annuals, suggesting that single fitness component measures may overestimate the strength of selection in iteroparous populations. In addition, selection on particular traits, such as flowering time, often occurred through different fitness components among life history ecotypes. This work highlights the importance of using an integrative fitness measure, such as population growth rate, to accurately compare the pattern or strength of selection among populations that differ in their overall life history strategy. Divergence in life history traits can alter the selective landscape, even within a common environment, and thus influence the trajectory of adaptive divergence.

Chapter three: Reproductive isolation accumulates through a mosaic of barriers and enhancers along a life history continuum in Mimulus guttatus.

The formalization of the biological species concept provided a framework for studying the process of speciation as the evolution of barriers to gene flow between sister lineages. Early classifications recognized that multiple isolating mechanisms, including geographic, ecological, and intrinsic barriers, contributed to speciation (Dobzhansky 1951; Grant 1963; Mayr 1942; Stebbins 1950). Geographic and ecological barriers were generally thought to operate early during race formation, whereas the evolution of intrinsic barriers marked the final stage of speciation (Dobzhansky 1940; Mayr 1947; Stebbins 1950). Despite extensive study of the magnitude and genetic basis of reproductive barriers, our understanding of the relative order in which these barriers arise remains limited, particularly during the early stages of divergence (reviewed in Coyne and Orr 2004). Comprehensive comparisons of the relative importance of multiple barriers often represent snapshots in time of the speciation process (e.g. Kay 2006; Ramsey et al. 2003; Sobel and Streisfeld 2015) whereas comparisons among taxon-pairs that differ in the degree of divergence are limited to a few easily quantified barriers (e.g. Bolnick et al. 2006; Coyne and Orr 1989; Moyle et al. 2004). Recently, Nosil (2012) suggested that selection against immigrants and hybrids should be the earliest and strongest barriers to evolve, followed by other ecological and then intrinsic barriers. Yet, in some studies, intrinsic isolation evolves early during adaptive divergence (Bomblies et al. 2007b; Lindsay and Vickery 1967) whereas in others selection against immigrants or hybrids is lacking (Gosden et al. 2015; Lowry et al. 2008b; Räsänen and Hendry 2014). Further, early-evolving barriers to gene flow may be offset by other trait interactions that serve to increase gene flow, such as mate preferences or heterosis (i.e. reproductive enhancers, Hendry 2009). Thus, in order to understand how reproductive isolation evolves, multiple potential barriers must be quantified among population-pairs that span a range of ecological divergence.

In chapter three, I quantified the strength and relative importance of five potential isolating mechanisms between ten population-pairs of *Mimulus guttatus* spanning a range of life history divergence. Using a common garden field experiment, I mimicked the dispersal of immigrant genotypes into a montane perennial focal population, and tested whether the magnitude of reproductive barriers in sympatry was correlated with phenotypic divergence in life history traits. I found that a mosaic of reproductive barriers and enhancers operated between each immigrant population and the focal population, but that total reproductive isolation in sympatry was positively correlated with phenotypic divergence. Further, I found that many individual barriers or enhancers evolved non-linearly with phenotypic divergence. This work demonstrates the importance of multiple partial barriers and enhancers in shaping total isolation during the early stages of divergence. Although a simple linear relationship between total isolation and ecological divergence appears to be a general phenomenon (Funk et al. 2006; Sexton et al. 2014), the underlying barriers generating this pattern may often evolve non-linearly and thus vary greatly in their relative importance as ecological divergence proceeds.

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Chapter 1

Mating system plasticity promotes persistence and adaptation of colonizing populations of hermaphroditic angiosperms

Megan L. Peterson and Kathleen M. Kay

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ABSTRACT

Persistence and adaptation in novel environments is limited by small population sizes, strong selection, and maladaptive gene flow. Mating system plasticity is common in angiosperms, and may provide both demographic and genetic benefits that promote niche evolution, including reproductive assurance and isolation from maladaptive gene flow. Yet, increased self-fertilization may also cause inbreeding depression, accumulation of deleterious mutations, and reduced adaptive potential. Here, we use individual-based simulations to examine the consequences of mating system plasticity for persistence and adaptation in a novel environment that imposes selection on a quantitative trait. We examine the joint evolution of local adaptation, inbreeding depression, and genetic load. We find that a plastic shift to a mixed mating system generally promotes niche evolution by decreasing the risk of extinction, providing isolation from maladaptive gene flow, and temporarily increasing genetic variance in the trait under selection, whereas obligate self-fertilization reduces adaptive potential. These effects are most pronounced under conditions of mate limitation, strong selection, or maladaptive gene flow. Our results highlight the diverse demographic and genetic consequences of self-fertilization, and support the potential role for plastic shifts in mating system to promote niche evolution in flowering plants.

INTRODUCTION

Demographic and genetic processes may limit or promote adaptation to novel environmental conditions. Such processes are fundamental to determining when and why adaptation to novel environments may fail, as at species range limits (Bridle and Vines 2007; Sexton et al. 2009), or when successful adaptation promotes the invasion of non-native species (Gilchrist and Lee 2007) or the evolution of reproductive barriers (Rundle et al. 2000; Lowry et al. 2008). Properties that influence the evolvability of species have been central to theory regarding the maintenance of sexual reproduction (Misevic et al. 2010) and outcrossing (Busch et al. 2004; Morran et al. 2011), as well as recent interest in predicting species' responses to global environmental change (Lavergne et al. 2010).

Historically, models of adaptation to novel environments focused on the genetic consequences of selection, gene flow, and genetic drift (e.g. Kirkpatrick and Barton 1997; Lenormand 2002) as well as demographic processes, such as immigration (Gomulkiewicz et al. 1999; Kawecki and Holt 2002; Holt et al. 2003; Kawecki 2003; reviewed in Kawecki 2008). However, recent work suggests that phenotypic plasticity may commonly play an important role in allowing population persistence in novel environments (Yeh and Price 2004; Chevin and Lande 2011; Reed et al. 2011). Unlike adaptive evolution, phenotypic plasticity can immediately increase local fitness following colonization of a new environment (Ghalambor et al. 2007; Lande 2009). Phenotypic plasticity may be particularly likely in harsh or stressful environments due to developmental instability or the expression of cryptic genetic variation (Badyaev 2005; Ledón-Rettig et al. 2010), and can shift a population to within the “realm of attraction” of a new fitness peak (Price et al. 2003). Phenotypic plasticity can evolve as an adaptive response to the range of environments typically encountered by an organism over space and time, or can be a passive response to stress; in either case it can be adaptive or maladaptive in a novel selective environment (Ghalambor et al. 2007).

Levin (2010) suggested that plasticity in traits affecting the mating system may have particularly important consequences for niche evolution in plants because the mating system has direct impacts on both the demographic and genetic properties of a population. Specifically, Levin (2010) suggested that niche evolution in plants may be facilitated if colonization of a novel environment is associated with increased self-fertilization via plasticity in floral traits or self-incompatibility systems. Diverse floral traits affect self-fertilization rate in plants, including the spatial and temporal separation of stigma and anthers, the proportion of cleistogamous flowers, and the expression of self-incompatibility mechanisms (Darwin 1876; Darwin 1877). Plasticity in one or more of these traits resulting in increased self-fertilization has been widely documented in response to environmental stress, including herbivory (Steets and Ashman 2004; Ivey and Carr 2005; Schutzenhofer 2007; Penet et al. 2009), pollen limitation (Travers et al. 2004), drought (Kay and Picklum 2013), heat (reviewed in Good-Avila et al. 2008), salt (Tlngdong et al. 1992), and shade (Waller 1980). Conversely, several studies have found either no plasticity in mating system or increased outcrossing in response to environmental stress (Levri and Real 1998, Kay and Picklum 2013). Thus, the magnitude, underlying traits, and environmental drivers of mating system plasticity appear to vary greatly among taxa.

From a genetic perspective, increased self-fertilization may promote adaptation by acting as a partial reproductive barrier to maladaptive gene flow or by temporarily increasing genetic variation in traits under selection. Although self-fertilization is associated with reduced genetic variation over long timescales (Glémin 2006; Leffler 2012), rapid increases in the self-fertilization rate are predicted to increase genetic variation temporarily by generating positive correlations between additive allelic effects within loci among offspring (Lande 1977). Such allelic correlations may increase the rate at which genetic variance recovers following a bottleneck (Lande 1977) and the response to selection in populations with mixed mating (Kelly 1999). Self-fertilization may further increase genetic variation in traits with more complex genetic architectures by converting epistatic or dominance variance to additive genetic variance (Carson 1990; Willis and Orr 1993; Cheverud and Routman 1996; Cheverud et al. 1999). Partial self-fertilization may also result in more rapid fixation of new, beneficial mutations (Charlesworth 1992; Glémin and Ronfort 2013) and essentially produces assortative mating for traits under selection (Lande 1977; Levin 2010).

From a demographic perspective, self-fertilization provides reproductive assurance by ensuring at least some reproduction when mates are limiting (Kalisz et al. 2004). Mate limitation may be severe in novel environments because of small population sizes and/or isolation. Plants with abiotic pollination mechanisms often exhibit density-dependent pollen limitation (Knight et al. 2005; Hesse and Pannell 2011). In animal-pollinated systems, scarce ancestral pollinators or ineffective novel pollinators may limit outcross pollen availability in new environments (Eckert et al. 2010; Rodger et al. 2013). The importance of reproductive assurance during colonization is supported by the observation that isolated or peripheral plant populations often exhibit increased self-fertilization (Baker 1967; Busch 2005; Herlihy and Eckert 2005; Moeller 2006). Correspondingly, self-fertilization is associated with invasiveness in annual weeds (Petanidou et al. 2012) and larger range sizes in *Collinsia* (Randle et al. 2009).

Despite these potential benefits of mating system plasticity for the persistence and adaptation of colonizing populations, sudden increases in self-fertilization rate are commonly associated with reduced fitness due to the expression and fixation of deleterious alleles that accumulate in previously outcrossing populations (i.e. inbreeding depression) (Charlesworth and Charlesworth 1999). If the segregating genetic load of an outcrossing population is high, a plastic increase in self-fertilization rate could decrease the efficiency of selection on quantitative traits and increase the probability of extinction following colonization (O'Grady et al. 2006). In addition, inbreeding depression is often environmentally-dependent, and may be most severe in stressful or novel environments (Heschel and Paige 1995; Armbruster and Reed 2005; but see Ronce et al. 2009). Alternatively, the process of colonization itself may result in the fixation or purging of deleterious alleles if the number of colonists is small (i.e. a bottleneck) (Lopez et al. 2009; Pujol et al. 2009; but see

Kirkpatrick and Jarne 2000). Population bottlenecks increase the frequency of rare deleterious alleles, and may result in reduced fitness regardless of self-fertilization rate. Given the diverse potential effects of mating system plasticity on the demography and evolution of colonizing populations, its overall consequences for niche evolution remain unclear.

We used individual-based simulations to examine the evolutionary and ecological contexts in which mating system plasticity may promote or inhibit niche evolution. We tested the effect of a constant plastic increase in prior self-fertilization rate on population persistence and local adaptation by examining the evolution of a quantitative trait under stabilizing selection and the probability of extinction in a novel environment. Specifically, we focus on a region of genetic and demographic parameter space in which sink populations are in a ‘race’ to become locally adapted prior to extinction. To distinguish reproductive assurance, increased genetic variation, and reproductive isolation as potential mechanisms, we tested the effects of pollen limitation, strength of selection, genetic architecture, and maladaptive gene flow on both extinction probability and local adaptation. We examined the evolution of inbreeding depression and genetic load in the colonizing population to test whether consideration of deleterious mutations alters the effects of mating system plasticity on niche evolution. Although this model includes several assumptions based on plant reproductive biology (e.g. pollen limitation and pollen dispersal), it could also be applied to hermaphroditic animal systems with mate limitation and/or gamete dispersal (e.g. broadcast spawning).

METHODS

We used stochastic simulations that track individual genotypes to model the evolution and demography of a colonizing sink population connected by gene flow with a source population. Our model is similar to that used by Holt et al. (2003) in which one-way migration occurs from a locally adapted source population to an initially maladapted sink population. However, we consider a plastic increase in self-fertilization rate following colonization of the sink habitat, the potential for pollen limitation, and the evolution of inbreeding depression and genetic load due to deleterious mutations.

Ecological assumptions

The source and sink populations are composed of diploid and hermaphroditic individuals with discrete generations. Initially, the source population contains *K* individuals (see Table 1.1 for parameter definitions and values) and the sink habitat is empty. The source population evolves for 1000 generations to reach mutation-selection equilibrium before *C* individuals are randomly selected without replacement to colonize the sink habitat. Both populations evolve for 1000 generations following colonization or until the sink population goes extinct. The order of life-history events within each generation is: fertilization, selection, density dependence, dispersal, reproduction, and death.

Prior to reproduction, individuals undergo selection. This selective period encompasses mortality at all life-history stages between fertilization and reproduction, including seed development, germination, and seedling growth. The fitness of an individual is determined by two forms of selection: optimizing selection on a quantitative trait, and purifying selection on deleterious mutations. Optimizing selection can be either directional or stabilizing, depending on the similarity of the average phenotype to the optimum. The probability of survival of an individual in habitat *i* with phenotype *z* and genetic load m is given by max{ $0,\ W_i\ (z, m)$ }, where

$$W_\mathrm{i}(z, m) = \exp\left[-\frac{(z-\theta_\mathrm{i})^2}{w^2}\right] - \ m\ , \tag{1}$$

θ_i is the optimum phenotype in habitat i, and w^2 determines the strength of optimizing selection. Following Holt et al. (2003), the source optimum is kept constant at 0, so that the magnitude of the sink optimum reflects the severity of the sink habitat. Selection against deleterious mutations is the same in source and sink habitats and is always purifying (i.e. *m* is positive and determined solely by the genotype of an individual, not its habitat).

We assume that the source and sink habitats each support a finite population size *K*. If more than *K* individuals survive viability selection, then *K* individuals are randomly sampled without replacement to become reproductive adults.

One-way pollen dispersal occurs each generation from the source population into the sink population. After colonization of the sink habitat, a proportion *I* of the outcross pollen pool in the sink population is randomly selected with replacement from individuals in the source population. Since we consider a one-time colonization event followed by pollen dispersal, extinction of the sink population is irreversible.

Each individual produces *B* ovules and contributes equally to the pool of available outcross pollen. In the source habitat, all individuals are obligately outcrossing and gametes are randomly paired between individuals in the population. In the sink habitat, all individuals self-fertilize a proportion S of unfertilized ovules. S is treated as a constant within simulations; thus there is no genetic variation for plasticity in self-fertilization rate. We focus on prior self-fertilization, in which SB ovules are self-fertilized and the remaining $(1-S)B$ ovules receive outcross pollen with probability p. Prior self-fertilization is an appropriate focus given plasticity in floral traits and results in a straightforward relationship between S and the realized self-fertilization rate (figure 1.A5). In addition, this mode of self-fertilization is considered more costly than delayed self-fertilization, because it occurs even if outcross pollen is abundant. However, we also consider a subset of simulations with delayed self-fertilization and find that the effects of the realized self-fertilization rate are similar (figure 1.A5).

Genetic assumptions

We assume that the source and sink habitats exert divergent selection on a quantitative trait that is determined additively by L loci. The phenotype of an individual, z, is the sum of the allelic values at all L loci (i.e. the genotypic value) and an environmental value drawn from a normal distribution with mean zero and variance V_e. An additional N loci contribute to the genetic load via deleterious mutations. These loci are under purifying selection for the same wild-type allele in both the source and sink habitats, so that any mutation at these loci is equally deleterious in both habitats. We assume that there is no epistasis between loci and that all loci segregate independently.

Each generation, gametes receive a single mutation at a randomly selected locus with probability $(L+N)\mu$, where μ is the per-locus mutation rate. We set μ = 0.001, which results in a per-gamete mutation rate of 0.011 – 0.080 (for $L + N = 11$ - 80), within the range of empirical estimates (Charlesworth and Charlesworth 1999). For mutations at loci contributing to the quantitative trait, mutational values are drawn from a normal distribution with mean zero and variance α^2, and added to the previous allelic value (Holt et al. 2003). Following empirical estimates, we consider values for α^2 between $1 \times 10^{-3} – 50 \times 10^{-3}$ (Lynch 1988) and values for L between 1-20. These values for L are in keeping with empirical evidence that many quantitative traits are largely determined by relatively few loci of major effect (Zeng 2005Zeng 2005). To ensure that adaptation in sink populations is difficult, we consider parameter values that result in relatively low heritability for the quantitative trait. This allows the possibility of extinction in sink populations given the high fecundity of individuals (B = 10). The heritability of traits in stressful or novel environments is an open question (Hoffman and Merilä 1999), but there is some evidence that heritability in wild populations may be reduced in unfavorable conditions, limiting the response to selection (Bennington and McGraw 1996; Charmantier and Garant 2005; Wilson et al. 2006). However, we also consider a subset of simulations with higher heritability (figures 1.A2, 1.A3).

Mutations at loci contributing to the genetic load are always deleterious (i.e. there is no back-mutation to the wild-type allele) and decrease fitness by a factor s when homozygous or sH when heterozygous, where s is the selection coefficient and

H is the dominance of the deleterious mutation. We considered three mutation classes: $s = 0$, $H = 0$; $s = 0.10$, $H = 0$; and $s = 0.01$, $H = 0.5$. These models represent selectively neutral, strongly deleterious recessive, and weakly deleterious codominant mutations, respectively, and reflect empirical observations that highly deleterious mutations tend to be more recessive than mildly deleterious mutations (Charlesworth and Charlesworth 1999). The total decrease in survival due to deleterious mutations, or the genetic load of an individual m, is determined by the sum of the effects of the single-locus genotypes across all N loci. Thus, m is always positive, resulting in purifying selection (eq. 1). We assume that deleterious mutations at separate loci contribute additively, rather than multiplicatively, to reductions in fitness to limit the total number of loci necessary to achieve high genetic load and/or inbreeding depression. Under an additive model, fitness declines more rapidly with increasing numbers of deleterious mutations, allowing a smaller total genome size per individual to improve computational efficiency. However, for the parameters we explore, these models are qualitatively similar.

We use the stochastic house-of-cards (SHC) approximation of the equilibrial genetic variance given by equation 14 in Burger and Lynch (1995) (see also Burger et al. 1989) to determine the initial genotypes at the L quantitative trait loci for the source population. K genotypes for the source population are drawn randomly from a set of five alleles per locus, generated from a normal distribution with mean zero and variance equal to the SHC (Holt et al. 2003). The source population is initially fixed for the wild-type allele at all N genetic load loci.

Simulations

In each sink population, we monitored the number of individuals and the mean and variance of the quantitative trait and genetic load. Sink populations were censused following viability selection and prior to density-dependent mortality. We measured inbreeding depression by separately simulating the production of equal numbers of outcrossed and selfed progeny, then calculating inbreeding depression as:

$$1 - \frac{W_{sf}}{W_{oc}}, \qquad (2)$$

where W_{oc} is the mean fitness of outcrossed progeny and W_{sf} is the mean fitness of selfed progeny (where fitness is given by eq. 1). These individuals were only used to measure the potential for inbreeding depression, and did not contribute to the next generation.

For each combination of parameter values (see Table 1.1), simulations were replicated 200 – 600 times and averaged to obtain general patterns (see online appendix for means and variances). We highlight patterns observed over many parameter combinations and select specific figures to illustrate these trends. All summary statistics are available in the online appendix. Raw output and code files are deposited in the Dryad Digital Repository: http://doi.org/10.5061/dryad.n5rd6. The probability of extinction was calculated as the proportion of simulations that resulted in extinction of the sink population prior to 1000 generations after colonization. All simulations were performed using R 2.13.0 (R Core Development Team 2011)

RESULTS

Niche evolution in sink populations

Immediately following colonization of the sink habitat, populations either begin to evolve towards the optimum phenotype and increase rapidly in size, or decline towards extinction (figure 1.A1). Population size is strongly associated with the average genotypic value, and extinction occurs only in maladapted populations with sizes far below K (figure 1.A1). Thus, density dependence only occurs once sink populations have begun to adapt, and the value of K does not affect colonization success. Extinction mainly occurs within the first 10 generations following colonization, and no populations become extinct after 50 generations (figure 1.A1). Given that the key demographic and genetic dynamics determining colonization success operate within the first 50 generations, we focus our results on this period. However, we also examine longer-term outcomes after 500 or 1000 generations to test whether these patterns change over time.

Genetic variation and adaptive potential

Mating system plasticity has a profound and immediate effect on the genetic variance in colonizing sink populations (figure 1.1A). In general, an increase in self-fertilization rate temporarily increases genetic variation relative to obligate outcrossing, but this effect decreases through time. Mixed mating ($0 < S < 1$) maintains higher genetic variation than obligate outcrossing for tens to hundreds of generations following colonization, whereas obligate self-fertilization results in an initial spike in genetic variation that declines rapidly to levels below obligate outcrossing populations (figure 1.1A). This general pattern was observed across a range of parameter values, though the magnitude and duration of mating system effects on genetic variance depend on the genetic architecture of the quantitative trait (figure 1.A2).

The effects of mating system plasticity on genetic variance have consequences for niche evolution when adaptation is limited by low genetic variation or strong selection. Under these conditions, the sustained increase in genetic variance under mixed mating allows a greater response to selection following colonization. Mixed mating populations have higher fitness than outcrossing populations for tens to hundreds of generations following colonization, suggesting a consistent early advantage to mixed mating populations in responding to selection (figure 1.1B). The magnitude of this fitness advantage each generation is slight, but it has biologically important consequences for colonization success by decreasing the time to local adaptation (figure 1.1C) and thus reducing the risk of extinction (figure 1.1D) relative to obligate outcrossing. Alternatively, the rapid erosion of genetic variation in obligately self-fertilizing populations inhibits the response to selection (figure 1.1B). When the sink habitat exerts strong selection, obligate self-fertilization results in a longer timescale for adaptation (figure 1.1C) and faster extinction than mixed mating (figure 1.1D). These general patterns are observed for a range of sink habitat severity (figure 1.1C-D) and genetic architectures (figure 1.A3).

Inbreeding depression and genetic load

In addition to effects on genetic variance, inbreeding due to mating system plasticity can increase the frequency or expression of deleterious alleles. The overall fitness effects of inbreeding may be best understood by examining inbreeding depression and genetic load together, since the former clarifies fitness differences between outcrossed and self-fertilized progeny within a population, whereas the latter encompasses the mean fitness effects of deleterious alleles for a population with a given breeding system. The consequences of mating system plasticity for inbreeding depression and genetic load depends on the mutation class considered.

Following colonization of the sink environment, individuals produced by self-fertilization exhibit both greater variance for the quantitative trait under selection (figure 1.1A) and increased expression of segregating recessive deleterious alleles. Patterns of inbreeding depression reflect the balance of these two effects. In the absence of recessive deleterious mutations, inbreeding depression in sink populations is initially negative following colonization, indicating that progeny produced by self-fertilization have on average higher fitness in the sink habitat than progeny produced by random outcrossing (figure 1.2A, B). As populations become locally adapted, inbreeding depression slowly evolves towards slightly positive values for all self-fertilization rates. This pattern reflects the role of fitness variation under stabilizing selection: greater fitness variance in self-fertilized progeny is beneficial in maladapted populations, but becomes costly as populations approach a fitness peak (Ronce et al. 2009). Weakly deleterious codominant alleles do not contribute to inbreeding depression, since their expression is not dependent on mating system, and patterns reflect those observed in the absence of deleterious mutations (figure 1.2B).

Incorporating recessive deleterious mutations alters the initial effects of self-fertilization as well as the final magnitude of inbreeding depression. Strongly deleterious recessive alleles drive a pulse of inbreeding depression following colonization that decreases with time and greater self-fertilization rates (figure 1.2C). This pulse reflects the increased expression of segregating recessive alleles in self-fertilized individuals, and decreases through time by purging. Purging occurs more rapidly with greater self-fertilization, as recessive alleles are exposed to selection.

Similarly, the evolution of genetic load also depends on the dominance and selection coefficients of deleterious mutations. Immediately following colonization, mating system plasticity has little effect on the genetic load due to codominant alleles (figure 1.3A). In contrast, the colonization bottleneck causes a spike in the genetic load due to recessive alleles that increases with greater self-fertilization (figure 1.3B). Over time, alleles under weak purifying selection become fixed by drift, slowly increasing the genetic load, whereas strongly deleterious alleles are rapidly purged. Genetic load changes most long term under obligate self-fertilization, whereas mixed mating populations maintain similar genetic load as obligate outcrossing populations. This effect is greatest for weakly deleterious alleles, which in turn contribute to a slight increase in the time to adaptation (figure 1.3C) and an increased risk of extinction (figure 1.3D) for all self-fertilization rates. These qualitative patterns are observed for a range of N (figure 1.A4). For high frequencies of strongly deleterious recessive mutations, the pulse of inbreeding depression and genetic load induced by mating system plasticity can outweigh the benefits of increased genetic variance, increasing the risk of extinction relative to obligate outcrossing (figure 1.A4).

Reproductive isolation and local adaptation

Pollen dispersal from the source population inhibits niche evolution by increasing the time to adaptation and decreasing local adaptation in obligately outcrossing sink populations (figure 1.4A,B). Self-fertilization reduces the opportunity for gene flow by decreasing the proportion of ovules that can be fertilized by immigrant gametes. For even limited pollen dispersal, mixed mating increases local adaptation and reduces the time to local adaptation relative to obligate outcrossing by acting as a partial reproductive barrier while maintaining high genetic variation and adaptive potential. When the potential for gene flow is high (pollen dispersal >10%), the benefits of obligate self-fertilization as a reproductive barrier outweigh its costs in terms of reduced adaptive potential, and any self-fertilization rate increases local adaptation relative to obligate outcrossing. Interestingly, pollen flow does not affect the extinction rate (data not shown). This is because extinction is most likely within the first 10 generations following colonization (figure 1.A1), when populations are highly maladapted and pollen flow is just as likely to increase as decrease local fitness.

The overall fitness of sink populations depends on the interaction between pollen dispersal, mutation class, and self-fertilization rate. In general, consideration of deleterious mutations decreases fitness in sink populations via genetic load relative to simulations in which fitness is determined solely by a quantitative trait. However, this fitness decrease is small relative to the effects of pollen dispersal from source populations (figure 1.4C,D). In the absence of pollen dispersal, obligately self-fertilizing populations exhibit lower fitness than populations with at least some outcrossing (figure 1.4C,D). This pattern is driven by greater fixation of deleterious alleles (figure 1.3) and decreased adaptive potential (figure 1.1). When the potential for gene flow is high (pollen dispersal > 10%), the fitness benefit of reproductive isolation exceeds the costs of decreased selection efficiency in obligately self-fertilizing populations (figure 1.4C,D). These general patterns are observed for all mutation classes.

Pollen limitation and reproductive assurance

Niche evolution requires that colonizing populations persist in the sink environment. In general, extinction risk increases with ecological or genetic factors that act to reduce sink population fitness, including strong selection (θ_{sink}) (figure 1.1D) and deleterious mutations (figure 1.3D). Incorporating pollen limitation greatly increases the risk of extinction in obligately outcrossing sink populations (figure 1.5A), but does not affect the timing or degree of local adaptation for populations that persist (data not shown). Thus, pollen limitation inhibits niche evolution demographically by preventing population persistence. Even moderate pollen limitation ($p < 0.7$) can result in high extinction risk when the sink habitat imposes strong selection ($\theta_{sink} > 2.00$) (figure 1.4B). In these cases, increased self-fertilization due to mating system plasticity decreases the risk of extinction by providing reproductive assurance. When pollen limitation and habitat severity are moderate ($0.3 < p < 0.7$ and $1.50 < \theta_{sink} < 2.25$), even limited self-fertilization or obligate self-fertilization can greatly decrease the risk of extinction relative to obligately outcrossing populations (figure 1.5). Similar patterns are observed when self-fertilization is delayed (figure 1.A5).

DISCUSSION

A plastic shift to mixed mating promotes niche evolution under a broad range of ecological conditions. Mixed mating allows populations to respond more rapidly to selection, reduces the risk of extinction, and has little effect on the accumulation of genetic load. Alternatively, a shift to obligate self-fertilization may inhibit niche evolution by slowing the response to selection, increasing the risk of extinction, and allowing the fixation of deleterious alleles. However, even obligate self-fertilization provides important reproductive assurance and isolation benefits. The interactions between mating system plasticity and pollen limitation, selection, and gene flow determine its overall consequences for niche evolution, and are discussed in greater detail below.

Extinction risk and reproductive assurance

Even moderate pollen limitation can greatly increase the extinction risk of colonizing populations, particularly when coupled with strong selection in a novel environment. Pollen limitation is common among angiosperms, occurring in some form in 62-63% of species examined (Burd 1994; Knight et al. 2005). Estimates of the magnitude of pollen limitation are subject to various methodological and publication biases (Harder and Aizen 2010), but several meta-analyses have found that fruit or seed set reductions may range from 15-75% on average (Burd 1994; Knight et al. 2005; Knight et al. 2006). Interestingly, pollen limitation and strong selection interact during colonization to greatly increase the risk of extinction and potential importance of reproductive assurance. Given this interaction, mixed mating can promote colonization of harsh environments even when the magnitude of pollen limitation is relatively low (> 10%). Previous work has emphasized the importance of immigration in allowing sink population persistence (Holt et al. 2003); here, we demonstrate a similar demographic rescue effect caused by self-fertilization. The relative benefits of immigration vs. self-fertilization for population persistence will depend on the fitness of immigrant genotypes and the strength of inbreeding depression. Although inbreeding depression decreases rapidly following colonization, selection against immigrant genotypes remains high. Thus, we find strong support for the hypothesis that self-fertilization, particularly mixed mating, will promote persistence in novel environments through reproductive assurance.

A general role for self-fertilization in range expansion is supported empirically. Baker’s Law emphasizes an association between self-compatibility and colonization success (Baker 1967), and is widely supported in native (Busch 2005; Randle et al. 2009), invasive (Van Kleunen and Johnson 2007; Kleunen et al. 2008; Hao et al. 2011) and several island floras (McMullen 1987; Anderson et al. 2001; but see Miller et al. 2008). Further, pollen limitation and self-fertilization are associated with the colonization of human-disturbed environments (Eckert et al. 2010; Harder and Aizen 2010). Previous theoretical work has examined the role of reproductive assurance during colonization in a metapopulation framework, with mixed support for Baker's Law (Pannell and Barrett 1998; Dornier et al. 2008). However, our model represents the first attempt to integrate pollen limitation and niche evolution during colonization of a novel selective environment. Our results suggest that at least partial self-fertilization may be critical for the persistence of colonizing populations under a broad set of ecological scenarios.

Gene flow and reproductive isolation

Pollen dispersal from source populations decreases fitness in sink populations by introducing maladaptive alleles and reducing local adaptation. The potential for gene flow to swamp local adaptation is well supported empirically (reviewed in Lenormand 2002), and self-fertilization is an important reproductive barrier in a variety of systems (e.g. Fishman and Wyatt, 1999; Martin and Willis 2007). Here, we show that mixed mating increases fitness in colonizing populations when gamete dispersal is moderate (>5%). Even obligate self-fertilization, which reduces fitness by limiting adaptive potential and accumulating deleterious mutations, increases fitness relative to outcrossing when gamete dispersal is high (>10%). Interestingly, these values may not be uncommon in systems with mobile gametes, such as plants or marine invertebrates. Estimates of pollen flow between plant populations range between 8-17% in *Raphanus sativus* (Ellstrand and Marshall 1985) and 8% in *Phlox drummondii* (Levin 1983). Sperm dispersal in marine invertebrates is highly variable, with estimates from 0% as close as 8 m (Yund 1990) to 20% as far as 100 m (Babcock et al. 1994). Sessile organisms may frequently experience distinct selective environments well within the spatial scale of gamete dispersal. In such cases, self-fertilization can provide an important reproductive barrier to allow local adaptation.

Self-fertilization rate and adaptive potential

In the absence of pollen limitation or gene flow, mating system plasticity can have immediate effects on the adaptive potential of colonizing sink populations. A shift from outcrossing to mixed mating confers a temporary increase in genetic variation that can accelerate adaptation and reduce the risk of extinction when adaptation is limited by low genetic variance. Although the effects of mixed mating on genetic variance and response to selection are small and transient, they occur during a critical stage in colonization and have biologically important consequences for the persistence of small populations. Obligate self-fertilization, however, limits adaptive potential by rapidly eroding genetic variation and increasing the timescale of adaptation. These effects only occur in colonizing populations if the response to selection is limited relative to the demographic risk of extinction, such as when heritability in the trait under selection is low. There is some evidence that heritability in wild populations is reduced under unfavorable conditions (Bennington and McGraw 1996; Charmantier and Garant 2005; Wilson et al. 2006; but see Husby et al. 2011), and adaptation to novel environments may further be limited by negative genetic correlations under multivariate selection (e.g. Etterson and Shaw 2001).

The effect of mating system on genetic variance and the response to selection is consistent with predictions from deterministic models. Lande (1977) found that inbreeding temporarily increases the rate of recovery of genetic variation after a bottleneck, but that this is quickly eroded to levels below random mating when inbreeding is high (> 95%). Glémin and Ronfort (2013) found a similar pattern for the time to adaptation when selection favors a partially recessive allele at a single locus. Dominance or epistatic interactions may further increase the effect of self-fertilization on genetic variance (Carson 1990; Willis and Orr 1993; Cheverud et al. 1999), relative to our model, which only considers selection on an additive trait. In an artificial selection experiment in *Mimulus*, Holeski and Kelly (2006) found that inbreeding increased the genetic variance of traits under selection. Further, when mating system had a significant positive effect on the response to selection, it was greatest for mixed mating and reduced for complete self-fertilization. Here, we show that a plastic shift to mixed mating may enhance adaptive potential in maladapted populations when selection acts on an additive, polygenic trait.

The duration of a plastic mating system shift following colonization will depend on the biology of a specific organism, including the traits, timing (prior vs. delayed), environmental drivers, and genetic variation underlying the plastic response. Enhanced self-fertilization may also occur purely due to density-dependent effects on outcross pollen availability (i.e. mate or pollinator limitation) rather than plasticity in floral traits; in these cases, self-fertilization rates would decline rapidly as the colonizing population became more abundant. Although we focus on the immediate consequences of mating system plasticity within the first 50 generations, we also examine any longer-term consequences of a sustained increase in prior self-fertilization. Even under the extreme scenario that a shift from outcrossing to obligate self-fertilization is sustained over 1000 generations, there is remarkably little long-term cost on adaptive potential. However, we consider adaptation to a single, constant environment; fluctuating environmental conditions, such as during coevolution, may further limit the adaptive potential of highly self-fertilizing populations (Busch et al. 2004; Glémin and Ronfort 2013). It is important to note that this model examines the immediate effects of mating system plasticity on the ability of populations to successfully colonize novel environments, not the evolution of mating systems themselves or the macroevolutionary consequences of self-fertilization. Over longer time scales, primarily self-fertilizing lineages may represent evolutionary dead-ends (Stebbins 1957; Holsinger 2000; Escobar et al. 2010; Goldberg et al. 2010).

Inbreeding depression, genetic load, and the cost of self-fertilization

The accumulation of deleterious mutations is thought to limit the long-term fitness and adaptive potential of self-fertilizing populations (Heller and Smith 1978). However, comparative approaches testing for higher deleterious substitution rates in selfing lineages have yielded mixed results: no effect of mating system was found in *Triticeae* or *Arabidopsis* (Wright et al. 2002; Escobar et al. 2010), though signals of reduced selection efficiency were found across angiosperms and in *Eichhornia* (Glémin et al. 2006; Ness et al. 2012). By examining the evolution of deleterious alleles during colonization and niche evolution, we find that obligate self-fertilization is associated with greater genetic load, reflecting decreased effective recombination (Muller's ratchet) (Heller and Smith 1978; Charlesworth and Wright 2001).

Interestingly, even limited outcrossing is sufficient to reduce the genetic load to outcrossing levels. This is consistent with previous theoretical work demonstrating that recombination rates are sufficient at intermediate self-fertilization rates to achieve levels of purging expected under random mating (Charlesworth et al. 1993). However, consideration of more strongly deleterious (e.g. lethal) mutations at more loci could alter the pattern of purging by generating identity disequilibrium or selective interference (Lande et al. 1994). Thus, sustained and /or stronger inbreeding depression could outweigh any demographic benefits for very high frequencies of strongly deleterious recessive alleles.

Mating system plasticity vs. evolution

By focusing on the effects of mating system plasticity induced by a novel environment, this work differs from recent investigations of the adaptive potential of mating systems (Glémin and Ronfort 2013) or the evolution of self-fertilization (e.g. Morgan and Wilson 2005; Dornier et al. 2008). Adaptation to novel environmental conditions is limited by small population sizes, strong selection, and maladaptive gene flow. Thus, previous theoretical work has emphasized the importance of population persistence and isolation for niche evolution to occur (reviewed in Kawecki 2008). Increased self-fertilization in a historically outcrossing population will have unique genetic consequences, such as the exposure of genetic variance and expression of recessive deleterious alleles, relative to a historically self-fertilizing population. Further, if a novel environment enhances the self-fertilization rate, then the benefits of reproductive assurance, isolation, and increased genetic variation will immediately act to reduce the risk of extinction and increase the response to selection following colonization.

However, it is not clear that these same benefits could be achieved through the evolution of increased self-fertilization for two reasons. First, the risk of extinction operated mainly within the first 10 generations following colonization, suggesting that the importance of reproductive assurance for niche evolution decreases rapidly with time since colonization, as populations either become extinct or begin to adapt. Second, the fate of any allele that acts to increase the self-fertilization rate will depend on the relative fitness of selfed and outcrossed progeny within a population (Lande and Schemske 1985). Colonization is associated with a spike in inbreeding depression due to the expression of recessive deleterious alleles, which may oppose the evolution of increased self-fertilization within the first 10 generations when extinction risk is greatest. Although these alleles are rapidly fixed or purged with self-fertilization, it remains unclear how the cost of inbreeding depression and the benefit of isolation and increased genetic variance would interact to drive the evolution of mating systems in novel environments.

Self-fertilization mechanisms and Levin's model

In concordance with Levin's (2010) verbal model, we test the consequences of a plastic, constant shift to prior self-fertilization in a novel environment. This could represent plasticity in the degree of self-compatibility, the timing of male and female function, the proportion of cleistogamous flowers, or any of the diverse floral traits that influence prior self-fertilization rates and respond to abiotic stress (reviewed in

Levin 2010). However, novel environments may also enhance self-fertilization via density-dependent or delayed mechanisms, in which self-fertilization increases only when the population size is small and/or outcross pollen is limiting. These alternative mechanisms would operate early in the colonization process; self-fertilization would decline quickly as populations grew and outcross pollen became available. Density-dependent effects might also be greater (i.e. obligate self-fertilization in the absence of mates) relative to plasticity in floral traits. Empirical data on the relative frequency and strength of these contrasting mechanisms would clarify whether the duration and magnitude of environmental effects on self-fertilization rate are related and, perhaps, negatively correlated. Although we do not include density-dependent self-fertilization in this model, it is likely that the short duration of this mechanism would provide the benefits described here when extinction risk is greatest, while preventing any longer-term costs of self-fertilization or benefits of reproductive isolation. However, additional work is necessary to test these predictions.

We find that mating system plasticity via prior self-fertilization promotes persistence and local adaptation of colonizing populations under a broad range of ecological scenarios and self-fertilization rates. In combination with general empirical support for plasticity in mating system traits, pollen limitation, and gene flow across selective environments, these results provide strong theoretical support for Levin's (2010) verbal model of niche evolution via environment-enhanced self-fertilization.

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Table 1.1: Description of the parameters used in the model and the ranges of values considered.

Parameter	Description	Range of values
K	Carrying capacity of the source and sink	100
C	Number of colonists to the sink habitat	10
Z	The phenotype of an individual	--------
θ_{source}	The optimum phenotype in the source	0
θ_{sink}	The optimum phenotype in the sink	1.25 – 2.50
V_e	The environmental variance	0.5
w^2	The strength of stabilizing selection	1.5
m	The genetic load of an individual	--------
I	Proportion of the outcross pollen pool drawn from the source population	0.0 – 0.5
B	The number of ovules produced by an individual	10
S	The proportion of ovules that receive self pollen	0 - 1
p	The probability of an ovule receiving outcross pollen	0.3 - 1.0
L	The number of loci underlying the quantitative trait	1 - 20
N	The number of loci underlying the genetic load	10 - 60
μ	The per-locus mutation rate	0.001
s	The selection coefficient for deleterious mutations	0.0 – 0.1
H	The dominance of deleterious mutations	0.0 – 0.5
α^2	The mutational variance for the quantitative trait	0.001 – 0.050

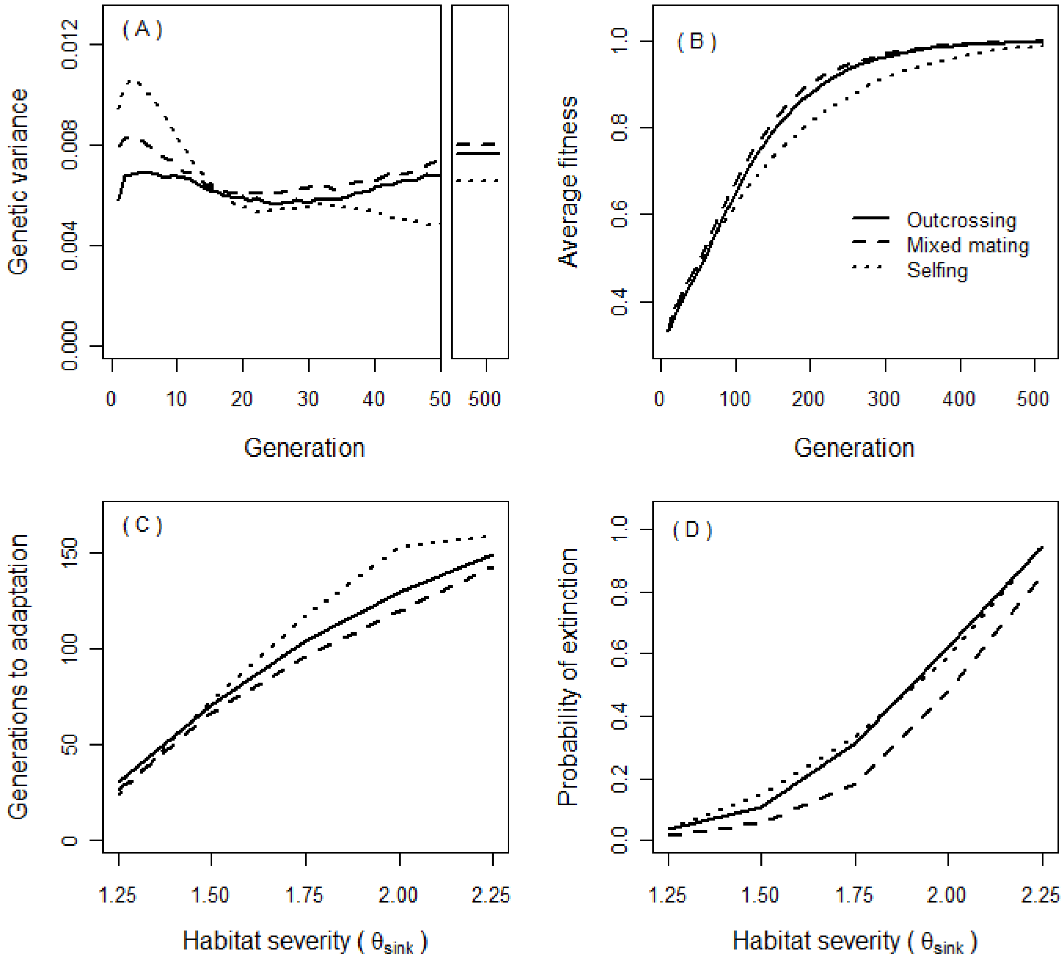

Figure 1.1: Effects of self-fertilization on niche evolution in sink populations. A) Evolution of genetic variance during the first 50 generations following colonization, and final values after 500 generations. Data are the variance among genotypic values within a population. B) Average fitness within sink populations over 500 generations following colonization. C) Mean number of generations for the population mean fitness to reach 0.5. Data are averages for those simulations that reached this fitness threshold within 1000 generations; no populations went extinct after reaching this point. D) Proportion of sink populations that went extinct within 1000 generations. For all panels, data are averages for 200 replicate simulations for obligate outcrossing (S = 0, solid line), mixed mating (S = 0.5, dashed line), and obligate self-fertilization (S = 1, dotted line). For all simulations (unless otherwise stated): $\theta_{\text{sink}} = 1.5$, $I = 0$, $p = 1$, $C = 10$, $L = 10$, $\alpha^2 = 2.5 \times 10^{-3}$.

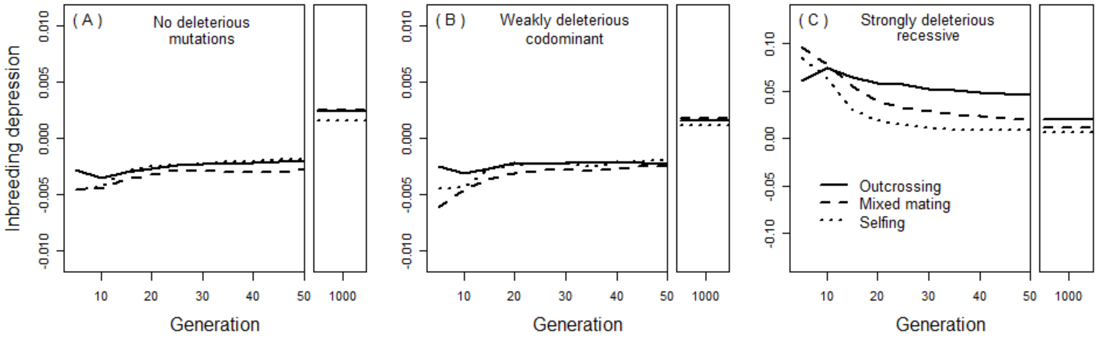

Figure 1.2: Evolution of inbreeding depression in sink populations for three mutation classes: A) No deleterious mutations, B) Weakly deleterious codominant mutations (s = 0.01, H = 0.5), and C) Strongly deleterious, recessive mutations (s = 0.10, H = 0.0). Data are inbreeding depression (eq. 2) for separately simulated outcrossed and self-fertilized progeny. Note the different y-axis scale in C and the break in the x-axis showing dynamics during the first 50 generations following colonization and final values after 1000 generations. For all panels, data are averages for 200 replicate simulations for obligate outcrossing (S = 0, solid line), mixed mating (S = 0.5, dashed line), and obligate self-fertilization (S = 1, dotted line). For all simulations (unless otherwise stated): θ_{sink} = 1.75, I = 0, p = 1, C = 10, L = 10, N = 10, $\alpha^2 = 2.5 \times 10^{-3}$.

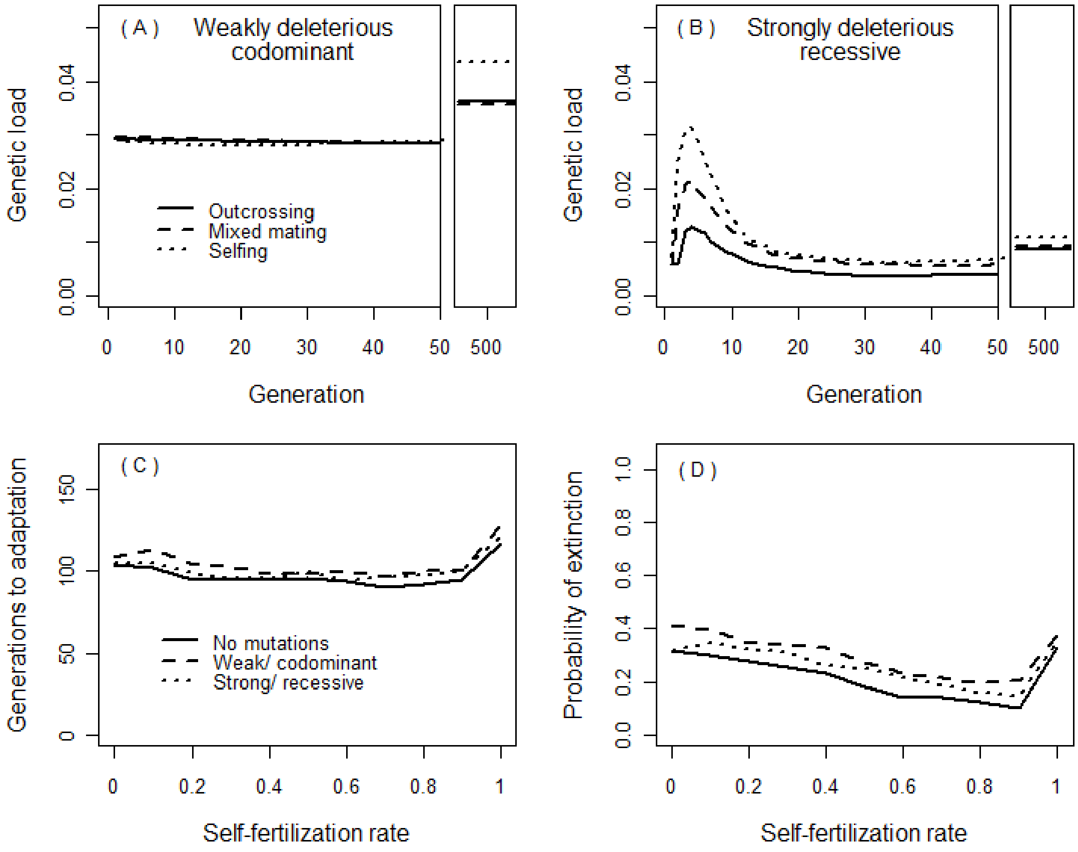

Figure 1.3: Consequences of deleterious mutations for niche evolution in sink populations. Top panels: Evolution of genetic load in sink populations due to A) weakly deleterious codominant mutations (s = 0.01, H = 0.5) and B) strongly deleterious recessive mutations (s = 0.10, H = 0.0). Data are the mean genetic load (i.e. reduction in fitness due to deleterious mutations, or *m*) for: obligate outcrossing (solid line), mixed mating (dashed line), and obligate self-fertilization (dotted line). Note the break in the x-axis showing dynamics during the first 50 generations following colonization and final values after 500 generations. Bottom panels: Effects of no deleterious mutations (solid line), weakly deleterious codominant mutations (s = 0.01, H = 0.5, dashed line), and strongly deleterious recessive mutations (s = 0.10, H = 0.0, dotted line) as a function of the self-fertilization rate. C) Mean number of generations for the population mean fitness to reach 0.5. D) Proportion of sink populations that went extinct within 1000 generations. For all panels, data are averages for 600 replicate simulations. For all simulations (unless otherwise stated): $\theta_{\text{sink}} = 1.75$, $I = 0$, $p = 1$, $C = 10$, $L = 10$, $\alpha^2 = 2.5 \times 10^{-3}$.

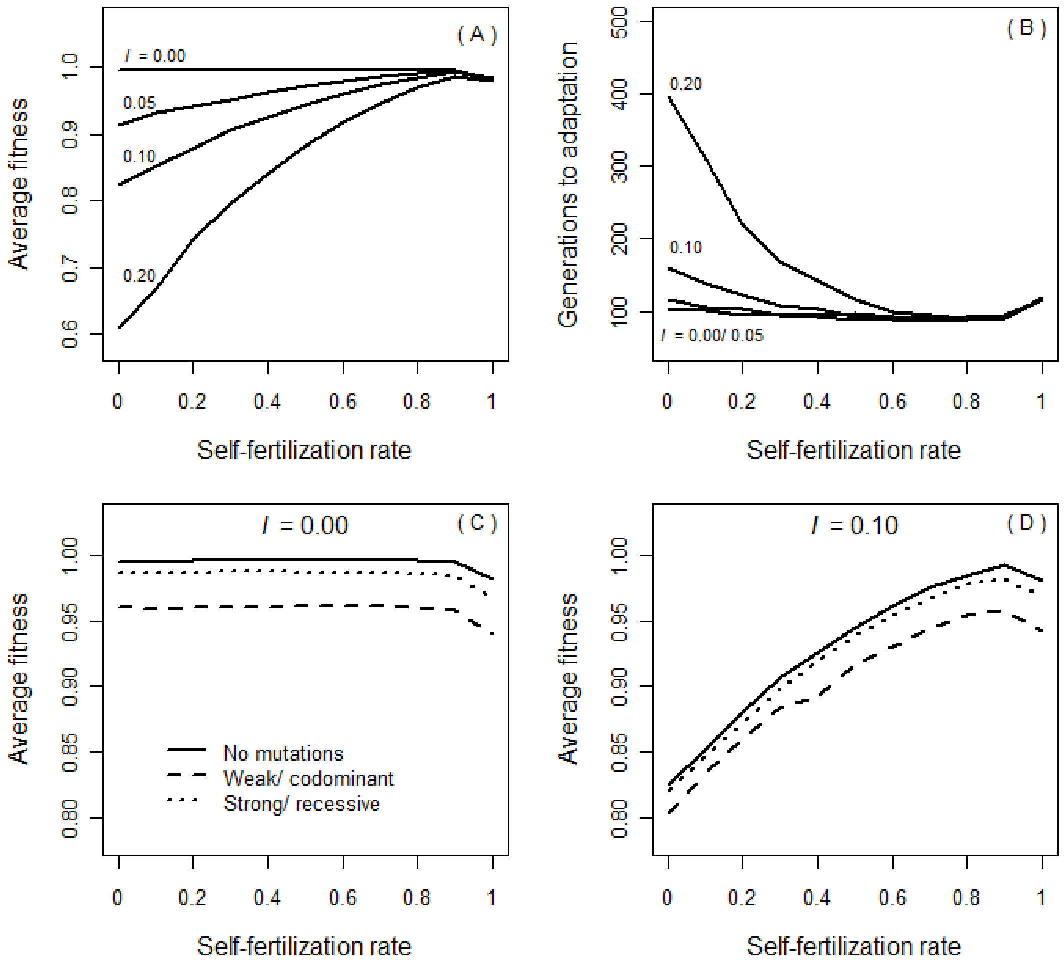

Figure 1.4: Effects of pollen flow on sink niche evolution as a function of the self-fertilization rate. A) Population mean fitness after 500 generations. B) Mean number of generations for the population mean fitness to reach 0.5. C and D) Population mean fitness after 500 generations with no pollen flow (C: *I* = 0.00) and moderate pollen flow (D: *I* = 0.10) for three mutation classes: no deleterious mutations (solid line), weakly deleterious codominant mutations (*s* = 0.10, H = 0.5; dashed line) and strongly deleterious recessive mutations (*s* = 0.10, H = 0.0; dotted line). For all panels, data are averages for 400 replicate simulations. For all simulations: θ_{sink} = 1.75, *p* = 1, *C* = 10, *L* = 10, $\alpha^2 = 2.5 \times 10^{-3}$.

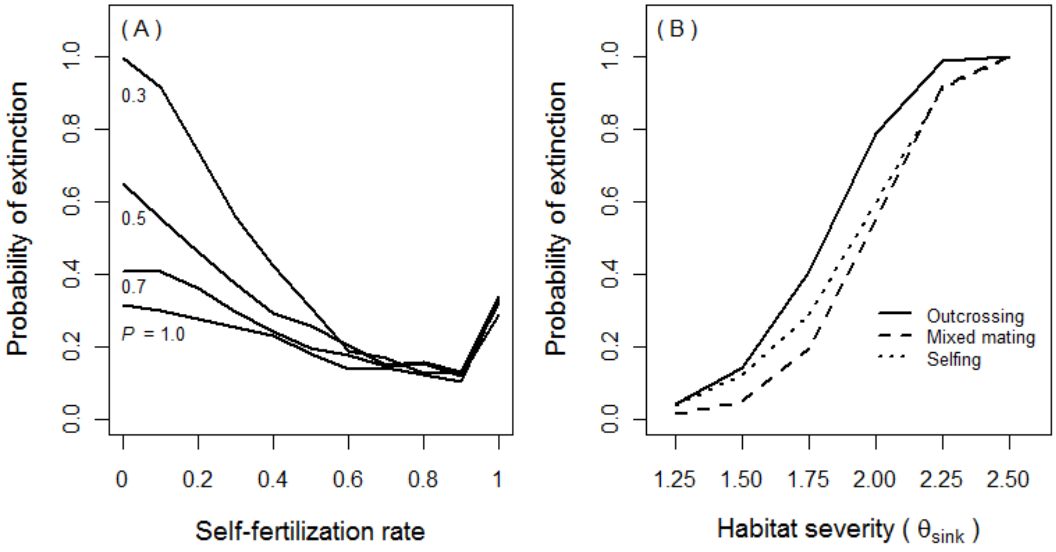

Figure 1.5: Effects of pollen limitation on the probability of extinction in sink populations. A) Effects of pollen limitation (*p*) as a function of the self-fertilization rate when $\theta_{sink} = 1.75$. B) Effects of habitat severity (θ_{sink}) when $p = 0.7$ for obligate outcrossing ($S = 0$, solid line), mixed mating ($S = 0.5$, dashed line), and obligate self-fertilization ($S = 1.0$, dotted line). Data are the proportions out of 400 replicate populations that became extinct within 1000 generations following colonization. For all simulations: $I = 0$, $C = 10$, $L = 10$, $\alpha^2 = 2.5 \times 10^{-3}$.

APPENDIX

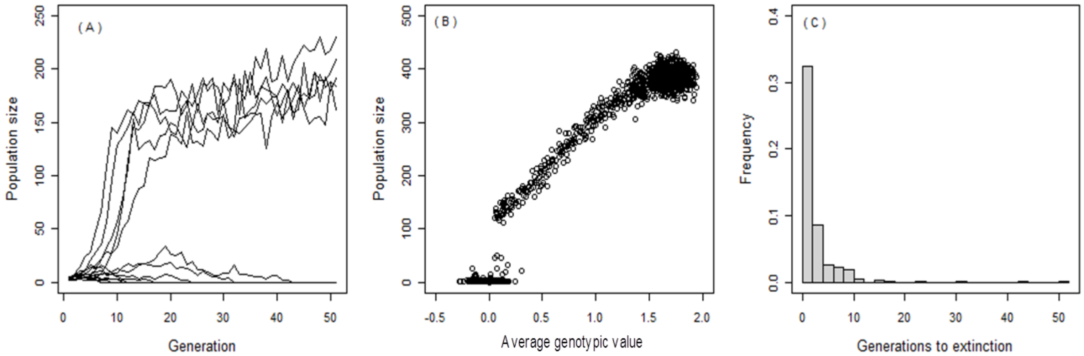

Figure 1.A1: Representative sink population dynamics following colonization. A) Population sizes either increase rapidly or decline to extinction within the first 50 generations following colonization. B) Population size reflects local fitness in the sink environment, and extinction does not occur beyond a threshold of local adaptation. C) Extinction only occurs within the first 50 generations. Data are a histogram of generations to extinction for the 163 out of 400 replicate simulations that went extinct prior to 1000 generations. Data shown here are from simulations with $\theta = 1.75$, $p = 0.7$, $S = 0$, $I = 0$, C=10, L=10, $\alpha^2 = 2.5 \times 10^{-3}$, and no deleterious mutations, but general patterns are representative of all simulations.

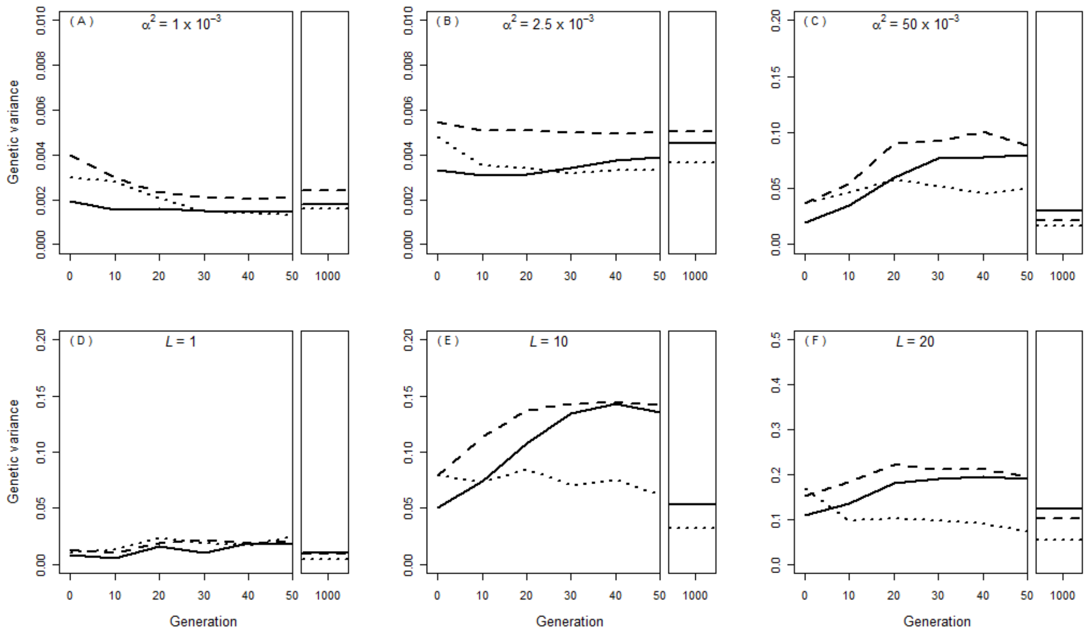

Figure 1.A2: Evolution of genetic variance depends on genetic parameters and mating system plasticity. Top panels: Effects of mutational variance (α^2). Bottom panels: Effects of number of loci (*L*) underlying the quantitative trait. Data are the variance of genotypic values within a population, averaged over 200 replicate populations, for obligate outcrossing ($S = 0$, solid line), mixed mating ($S = 0.5$, dashed line), and obligate self-fertilization ($S = 1.0$, dotted line). Note the different y-axis scales and the break in the x-axis showing dynamics during the first 50 generations following colonization and final values after 1000 generations. For all simulations (unless otherwise stated): $\theta = 2.5$, $I = 0$, $p = 1$, $C = 10$, $L = 5$, and $\alpha^2 = 5.0 \times 10^{-3}$.

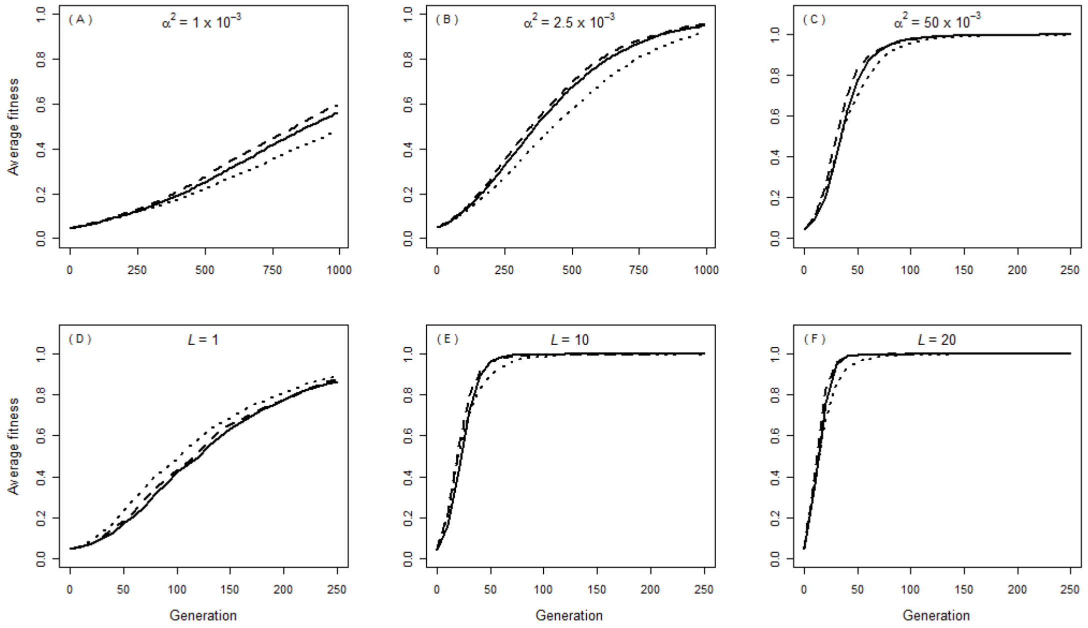

Figure 1.A3: Niche evolution in sink populations depends on genetic parameters and mating system plasticity. Top panels: Effects of mutational variance (α^2). Bottom panels: Effects of number of loci (*L*) underlying the quantitative trait. Data are the mean population fitness through time, averaged over 200 replicate populations, for obligate outcrossing (*S* = 0, solid line), mixed mating (*S* = 0.5, dashed line), and obligate self-fertilization (*S* = 1.0, dotted line). Note the different x-axis scales in A and B. For all simulations (unless otherwise stated): $\theta = 2.5$, $I = 0$, $p = 1$, $C = 10$, $L = 5$, and $\alpha^2 = 5.0 \times 10^{-3}$.

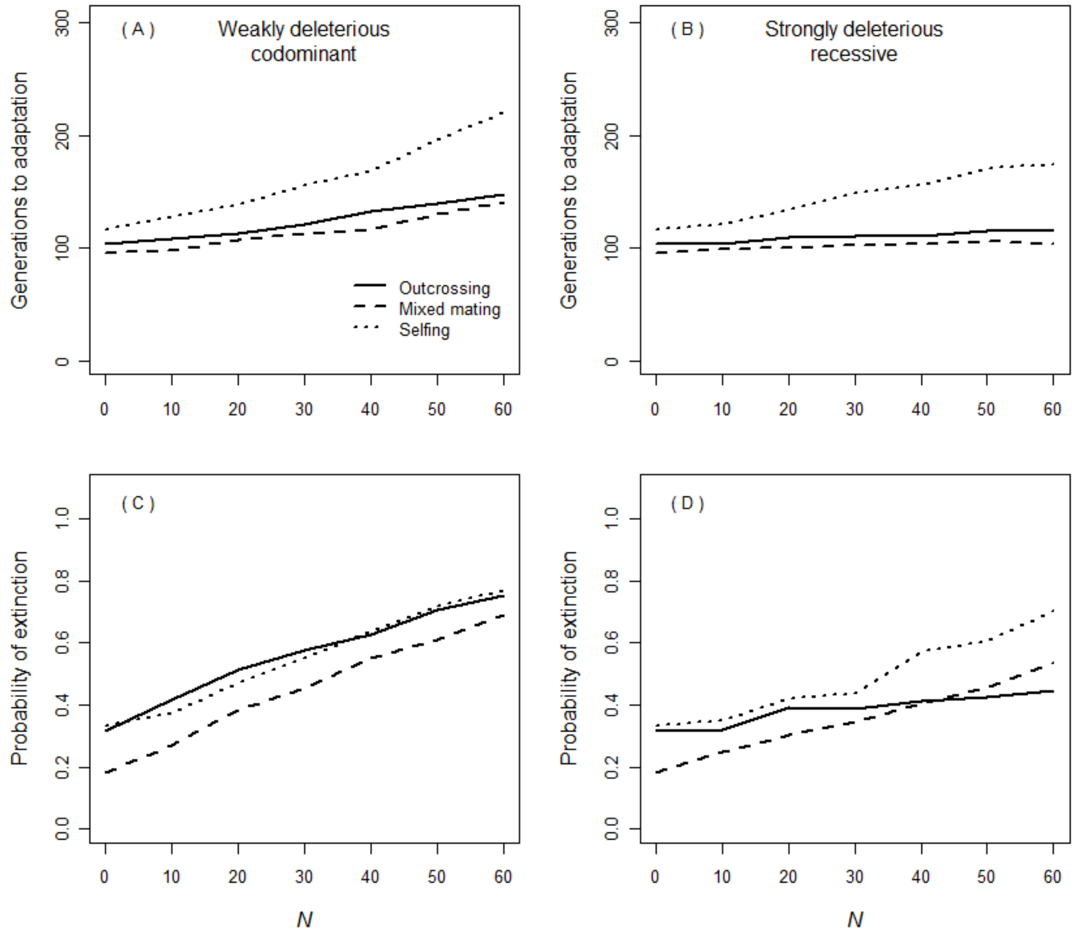

Figure 1.A4: Effects of the number of genetic load loci (*N*) for weakly deleterious codominant mutations (A and C) and strongly deleterious recessive mutations (B and D). Top panels: Average time to adaptation increases with obligate self-fertilization and is reduced under mixed mating. This pattern is observed for a range of *N* under both mutation classes. Bottom panels: The probability of extinction increases with *N* for all mating systems and both mutation classes. Extinction risk is reduced under mixed mating and similar or increased under obligate self-fertilization for a range of *N*. At high frequencies of strongly deleterious recessive alleles, extinction risk is increased under mixed mating relative to obligate outcrossing (D). This suggests that very high frequencies of strongly deleterious recessive alleles in the source population can diminish the demographic benefits of mating system plasticity during colonization. In these simulations, this trade-off occurs for $N > 40$, which results in inbreeding depression above 56% in the source population. However, this value represents inbreeding depression solely due to strongly deleterious ($s = 0.1$) and completely recessive alleles, and cannot be clearly related to empirical measures of inbreeding depression, which encompass complex mutational loads with a range of fitness effects and dominance coefficients.

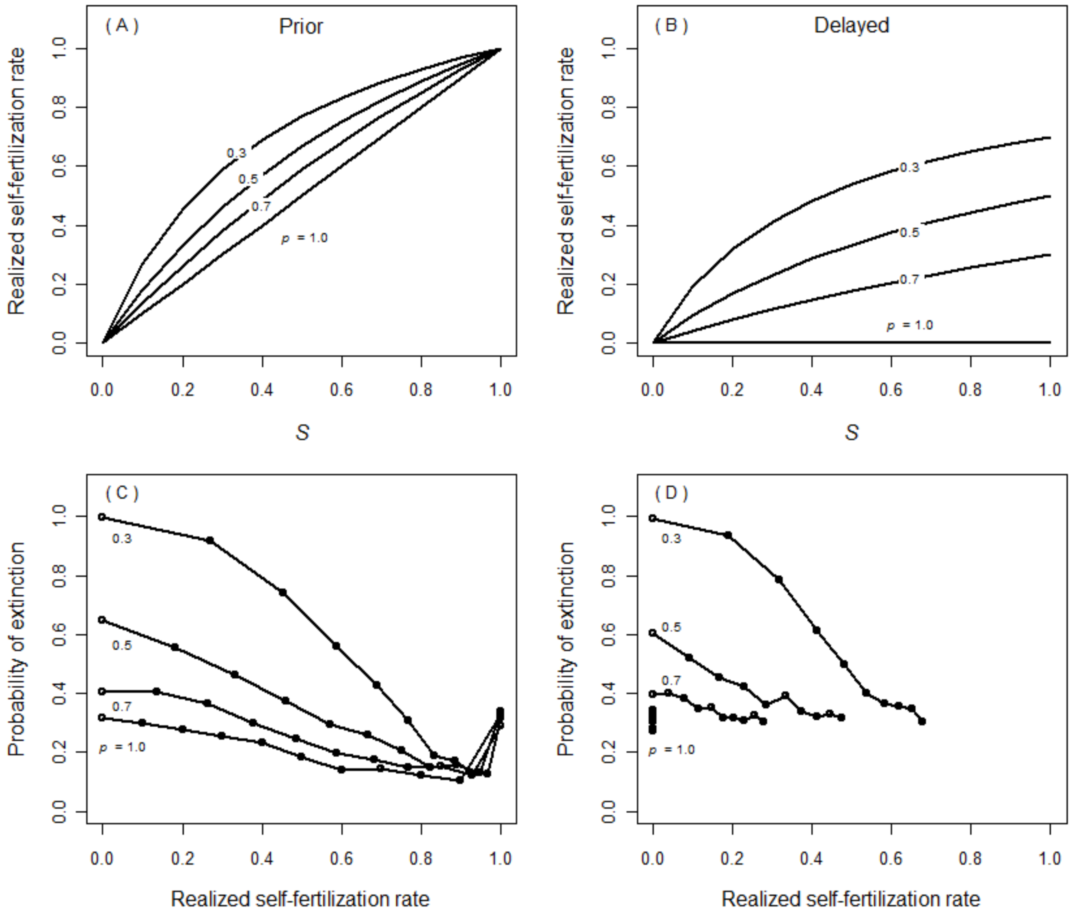

Figure 1.A5: Effects of the mode of self-fertilization on the probability of extinction. Top panels: Realized self-fertilization rate as a function of the parameter S for prior (A) and delayed (B) self-fertilization mechanisms. Realized self-fertilization rate is the average proportion of the total offspring produced by an individual that are self-fertilized, and is equal to $S\ /\ [S + (p\ (1 - S)\ )\ ]$ for prior self-fertilization and $\big(S\ (1 - p)\big)\ /\ [S + (p\ (1 - S)\ )\ ]$ for delayed self-fertilization. Bottom panels: The probability of extinction depends on the realized self-fertilization rate, and this pattern is qualitatively similar for prior (C) and delayed (D) self-fertilization mechanisms.

Chapter 2

Divergence in life history traits alters the pattern and strength of phenotypic selection: comparing integrated selection among life history ecotypes of *Mimulus guttatus*

INTRODUCTION

The concept of fitness is fundamental to the process of evolution by natural selection. Organisms must allocate finite resources to different components of fitness, including survival, growth, and reproduction, as part of an overall life history strategy. The optimal life history strategy is determined by local environmental conditions, including the timing and magnitude of extrinsic mortality and the effect of size or age on survival and fecundity (reviewed in Stearns 1992). When different populations experience contrasting selective environments, life history divergence can evolve rapidly (Lowry et al. 2008; e.g. Reznick et al. 1990; Walsh and Reznick 2011). Thus, adaptive divergence between environments may frequently involve fitness components themselves in addition to morphological or physiological traits. By altering the relative importance of survival, growth, and fecundity for overall performance, life history divergence can shape the strength and pattern of phenotypic selection and influence the trajectory of adaptive divergence.

The timing and frequency of reproduction is a major axis of life history divergence with profound impacts on the evolution of fitness components. Semelparous organisms reproduce in a single massive effort, whereas iteroparous organisms balance multiple, smaller reproductive bouts with investment in survival and growth. Iteroparity essentially trades current reproduction for potential future reproduction; this strategy can evolve when adult survival is greater or more consistent than juvenile survival (Charnov and Schaffer 1973; Cole 1954; Young 1990), or when fecundity increases disproportionately with age or size. Alternatively, semelparity is favored in organisms with large costs to initiate reproduction (e.g. migration in salmon) or in environments that impose a limited lifespan (e.g. annual plants in ephemeral habitats). These contrasting reproductive strategies differ in the relative importance of fecundity in any given reproductive period for lifetime fitness. In semelparous organisms, an increase in fecundity translates directly to increased fitness. Conversely, increased fecundity may come at the cost of reduced survival or growth in iteroparous organisms (reviewed in Reznick 1985), and could in fact decrease lifetime fitness. Correspondingly, reproductive effort is consistently greater in semelparous organisms (Browne and Russell-Hunter 1978; Hautekèete et al. 2001; Young 1990), whereas adult survival and growth have greater impacts than fecundity on population growth in iteroparous organisms (Oli and Dobson 2003; Silvertown et al. 1993).

Understanding adaptive divergence requires linking selection in particular environments to the evolution of traits underlying local adaptation. Although life history trade-offs among fitness components are well-developed theoretically and well-documented empirically (reviewed in Reznick et al. 2000; Stearns 1989; Stearns 1992), most estimates of selection in wild populations are based on single fitness components such as survival, growth, or fecundity in a given year (Kingsolver et al. 2012; Kingsolver et al. 2001; Siepielski et al. 2009). An implicit assumption of this approach is that the fitness components used to measure phenotypic selection are themselves under strong directional selection (Kawecki and Ebert 2004). When adaptive divergence involves life history traits, single fitness component estimates of selection will be biased and potentially misleading. This is especially true in iteroparous taxa, where traits that increase fecundity in a given reproductive period – a commonly used fitness measure - may not increase lifetime fitness if survival or growth is reduced. For these reasons, there has been a growing effort to develop and apply methods that integrate multiple fitness components to estimate selection.

Ultimately, the relationships between fitness components and total fitness can be best understood from a demographic perspective (Lande 1982). In stable populations, lifetime reproduction (R_0) is an appropriate fitness measure and can be estimated using the aster statistical framework (Shaw et al. 2008). In systems where the timing of reproduction is important, such as in growing populations or comparisons among life history strategies, the mean rate of increase of a population or genotype (r or λ) is more appropriate (Giske et al. 1993). Van Tienderen (2000) showed that selection through individual fitness components can be integrated by their effects on population growth using elasticities, and this approach has since been expanded to accommodate temporal and environmental stochasticity in demographic rates (Horvitz et al. 2010). In this framework, trait elasticities reflect the proportional change in mean population fitness (i.e. the population growth rate λ) given a proportional change in the trait mean, and are thus analogous to mean-standardized selection gradients (Hereford et al. 2004).

There are several reasons to expect that patterns of integrated selection will differ fundamentally from single fitness component estimates. For example, although fecundity selection is often stronger and more consistent than viability selection (Kingsolver et al. 2012; Siepielski et al. 2010), variation in adult survival has a greater proportional impact on population growth rates than variation in fecundity across a broad swath of taxa (reviewed in Crone 2001). In general, the most variable fitness components exert the smallest relative effects on population growth (Burns et al. 2010; Pfister 1998; Sæther and Bakke 2000), potentially weakening the strength of integrated selection relative to single fitness component measures. As yet, demographic approaches to measuring natural selection have only been implemented in a few systems (Coulson et al. 2003; Ehrlén and Münzbergová 2009; Gamelon et al. 2011; Horvitz et al. 2010). These studies have highlighted the complexity of integrated selection, finding that selection often acts through multiple fitness components simultaneously and that the strength and sign of selection varies among life history stages and years. Whether previous conclusions about the average strength, pattern, or temporal scale of natural selection will apply towards estimates of integrated selection is an important question that awaits empirical study (Horvitz et al. 2010).

Geographic variation in plant reproductive strategy provides an excellent opportunity for testing the consequences of life history divergence on natural selection and divergent adaptation. The evolution of annual or perennial life history strategies are frequently associated with divergent environmental conditions (Drummond 2008; Drummond et al. 2012; Evans et al. 2005; Young 1990). Further, environmental differences in juvenile vs. adult mortality (i.e. Cole's Paradox) (Charnov and Schaffer 1973; Cole 1954) are well-linked theoretically to consistent differences in the importance of specific fitness components, such as reproductive effort (Fone 1989; Primack 1979; Silvertown and Dodd 1996). Together, annual and perennial plants may commonly exhibit contrasting patterns of integrated selection, even within a common environment, due to differences in overall life history strategy.

Here, I address the potential for life history divergence to alter the strength and pattern of natural selection in common monkeyflower, *Mimulus guttatus*. Populations of this species range from diminutive and fast-cycling annuals in rapidly drying habitats to large perennials where water is abundant. Natural selection in annual habitats has been well-characterized; the early onset of drought imposes strong selection to maximize first-year seed set prior to the death of all adults (Hall and Willis 2006; Lowry et al. 2008; Mojica et al. 2012; Murren et al. 2009). However, in mesic environments (hereafter "perennial environments"), year-round water availability permits adult survival, and the relative importance of different fitness components in shaping the pattern of selection is unknown. In transplant experiments, coastal perennial populations have higher survival and seed set than annual populations in coastal habitat (Hall and Willis 2006; Lowry et al. 2008), at least in part due to differences in salt spray tolerance (Lowry et al. 2009). Yet it remains unclear how survival, growth, and reproduction contribute to overall performance in perennial environments and how individual fitness components interact to shape selection on life history traits. In a two-year common garden experiment in a montane perennial habitat, I compared demographic performance and natural selection among eleven populations of *M. guttatus* spanning four life history ecotypes, from fast-cycling annuals to montane perennials, to ask:

1. Is there evidence of local adaptation at the level of life history strategy (i.e. annual or perennial), ecotype, and population?
2. How do specific fitness components contribute to total fitness (λ) within and among ecotypes and years?
3. How does the pattern of selection through individual fitness components compare to integrated selection across the life cycle?

To address these questions, I used matrix projection models and life table response experiments (LTREs) to compare the population growth rate (λ) of four life history ecotypes of *M. guttatus* when grown in a montane perennial habitat. I compared the relative importance of fecundity, survival, and vegetative growth to λ for each ecotype through elasticity analysis. Finally, I estimated phenotypic selection on four life history traits through individual fitness components and used path analysis to estimate integrated selection through the net effects of each trait on λ.

METHODS

Study system

Common monkeyflower (*Mimulus guttatus* DC, Phrymaceae) is a highly variable herbaceous annual or short-lived perennial widely distributed throughout Western North America. Annual populations of *M. guttatus* occur in seasonally-drying habitats including seeps and meadows, whereas perennials occur in areas with year-round moisture along the coast or inland along streams or lakes. Although several major life history traits are correlated with a chromosomal inversion (*DIV1*) that separates annuals and perennials throughout much of the range (Lowry and Willis 2010; Oneal et al. 2014), it is increasingly recognized that there is widespread variation in life history traits within each of these broad strategies. Annual populations exhibit clinal variation in phenological, physiological, and vegetative traits that reflect climatic variation in the length of the growing season (Kooyers et al. 2015), whereas perennial populations from coastal, inland, and montane habitats comprise separate ecotypes with consistent phenotypic differences (e.g. Lowry et al. 2009). Coastal and inland perennials overwinter as vegetative rosettes produced from above-ground stolons (Hall and Willis 2006), whereas montane perennials primarily overwinter as below-ground rhizomes (Nesom 2012) (M. Peterson *pers obs.*). Given extensive phenotypic variation in these and other traits and incomplete reproductive barriers, taxonomists have variously recognized between 4-20 species in the *M. guttatus* complex (reviewed in Nesom 2012). Recent genomic evidence suggests extensive introgression among ecotypes, reflecting the recent origin and /or on-going gene flow among taxa in this complex (Oneal et al. 2014).

Seed collections

In 2010, I collected maternal seed families from eleven populations of *M. guttatus* in the central Sierra Nevada and surrounding foothills. These populations span the range of life history variation among inland populations of *M. guttatus* in California (table 2.A1). I classified each population into one of four ecotypes based on morphology and habitat characteristics (figure 2.1). Montane perennials (*M. corallinus* sensu Nesom 2012) occur above 1450 m elevation in the Sierra Nevada and produce few flowers, investing instead in extensive below-ground mats of branching rhizomes. Inland perennials (*M. guttatus* sensu Nesom 2012) are more morphologically and ecologically variable but generally occur along inland lakes and streams and produce horizontal stems (i.e. stolons) that root at nodes and give rise to clonal rosettes. Annual populations (*M. micranthus* sensu Nesom 2012) are highly variable in phenological and morphological traits, but I divided populations in this study into two groups to facilitate comparisons: fast-cycling annuals occupy rapidly-drying seeps and flower early at a small size, whereas robust annuals occupy seasonally drying meadows or creeks, experience longer growing seasons, and flower later and at larger sizes.

Common garden transplant

To compare demographic performance and natural selection within and among life history ecotypes, I transplanted cohorts of seedlings from each of the eleven populations over two years into a common garden field experiment. The common garden site was a montane meadow surrounding a small stream supporting a native population of montane perennial *M. guttatus* in Stanislaus National Forest (N 38.32107, W 119.91607, 2040 m). This native population (Eagle Meadows) was included as one of the eleven experimental populations to assess local adaptation at the population level within the montane perennial ecotype.

In 2012 and 2013, I transplanted 40 seedlings from each population into experimental plots located along the stream bank and within the distribution of native *M. guttatus* at this site. Each experimental plot contained one individual from each population in a fully randomized position, for a total of 40 plots per year. To prevent genetic contamination of the native population, I emasculated all flowers on experimental plants and transplanted all seedlings in 4” round pots buried within the local substrate (to contain below-ground rhizomes from non-native genotypes). To reduce transplant shock and mitigate any effects of pots on root development, I watered all plots to field capacity at the time of transplant and at each subsequent census. Experimental seedlings in 2012 were derived from field-collected seeds pooled from 30 maternal families within each population and germinated in the UCSC greenhouse. In 2013, seedlings were randomly sampled from among the germinants in the seed germination experiment (see below) to allow natural variation in the timing of germination following snowmelt. Transplants occurred following snowmelt on June 14, 2012 and May 26, 2013.

Trait measurement

I censused the survival and status (vegetative, flowering, fruiting, dead) of all experimental individuals at 3-10 day intervals from the time of transplant through the end of the growth season. This was the first snowfall on October 21 in 2012 and drought-induced mortality of all experimental individuals by September 27 in 2013. For each individual, I measured four traits related to life history strategy: seedling rosette size, flowering time, flower size, and flowering stem diameter. In a survey of 74 populations of *M. guttatus*, Friedman et al. (2015) found that perennial genotypes flower later and produce larger flowers, thicker primary stems, and more vegetative growth than annual genotypes. I estimated the size of each seedling at the time of transplant as the product of the rosette diameter measured at the widest point and at a 90° angle to that point. Seedling rosette size at the time of transplant reflects early above-ground growth, and may encompass variation in germination date, early growth rate, or early allocation to above- vs. below-ground biomass. I measured flowering time as the number of days from the transplant date to the first census interval with at least one open flower, and also measured the corolla width of the first flower and the diameter of the primary flowering stem at the basal node on the same day. The thickness of the primary flowering stem reflects investment in biomass allocated to sexual reproduction, whereas flower size has previously been shown to be a target of fluctuating selection in annual populations (Mojica and Kelly 2010; Mojica et al. 2012). Smaller flowers accelerate reproduction but decrease fecundity

(Mojica and Kelly 2010), suggesting that more mesic perennial habitats with longer growth seasons should favor larger flowers.

Vital rate estimation

To compare the demographic performance of life history ecotypes, I estimated vital rates for each population by tracking the survival, growth, and reproduction of each experimental individual.

Survival and growth – I censused adult overwinter survival and counted the number of vegetative rosettes each year following snowmelt (May 11, 2013 and May 1, 2014). Inland perennials overwinter as vegetative rosettes, whereas montane perennials usually regenerate from below-ground rhizomes each spring (M. Peterson, *pers. obs.*). Both stolons and rhizomes root at nodes and can readily fragment to become physiologically independent but genetically clonal rosettes (i.e. ramets) (Truscott et al. 2006).

Fecundity – In order to prevent pollen contamination of the native population, all experimental flowers were emasculated, preventing measures of seed set. Instead, I estimated two components of fecundity: flower number and ovule number per flower. Each year, I counted the total number of flowers produced by each experimental individual and estimated ovule number per flower from a subset of individuals in each population. I collected ovaries from second flowers and preserved them in ethanol, then scraped ovules from each locule onto separate microscope slides using clean forceps. Ovules were dyed with lactophenol aniline blue and counted under a dissecting microscope. I fit linear mixed models with population and year as fixed effects and plot as a random effect to estimate mean ovule number.

Seed germination experiment - I estimated seed overwinter survival and germination rates from 96 field-collected seeds per population during the winter of 2012-2013. I pooled seeds from 10 to 30 maternal families within each population and planted single seeds into separate plug tray cells filled with HP Pro Sunshine Potting Mix in a fully randomized design. Plug trays were placed in the field prior to the first snowfall on October 21, 2012, and germinants scored following snowmelt on May 11, 2013. I used generalized linear mixed models with a binomial error distribution and tray as a random effect to estimate seed germination rates for each population.

Recruitment plots – Because *Mimulus* individuals produce hundreds of ovules per fruit, raw fertility estimates and experimental germination rates would wildly inflate population growth rates and fecundity elasticities. In reality, pollen and resource limitations on seed set, safe-site limitation, and seed predation and dispersal will act to reduce the proportion of ovules that successfully recruit as seedlings. To better understand recruitment dynamics in this site, I established 5 recruitment plots within the native *M. guttatus* population. Following snowmelt in the spring of 2014, I set up plots at 1 m intervals along the stream bank in the area of peak *M. guttatus* abundance. I sampled natural *M. guttatus* recruits by collecting 10 cm x 10 cm soil cores from each plot on May 1 and again on May 16. In each core, I excavated every *M. guttatus* recruit to determine whether it was a seedling or a clonal rosette arising from a below-ground rhizome. For each rosette, I traced its network of rhizomes to determine whether it was physically connected to any other rosettes. From this data, I estimated the total proportion of *M. guttatus* recruits that were seedlings vs. clonal rosettes.

Matrix projection models

I constructed matrix projection models to compare demographic performance within and among life history ecotypes and years. I modeled the growth of each stage-classified population as **N**(t+1) = **AN**(t) where **N**(t) is a vector of stage-classified individuals at time t and **A** is a 3 x 3 matrix of transition rates. I collapsed census data collected throughout each year to estimate annual transitions based on a pre-reproductive census (i.e. at the start of the growth season right after snowmelt). At this time, individuals can exist in one of three stages: seed in the seed bank, newly germinated seedling, or vegetative rosette (figure 2.2). Either seedlings or rosettes can flower and contribute to the seed or seedling class or survive to produce vegetative rosettes in the subsequent time step, while the transition from seed to seed reflects seed dormancy (matrix 1).

Transition matrices – I assembled separate transition matrices for each population *i* and year *j* and also pooled matrices across populations within ecotypes and/or across years. Seed germination rate G^i was estimated as the mean germination rate for each population *i* from the seed germination experiment (see above). Annual seed bank survival *D* was treated as a constant across all years and populations. I estimated *D* as 0.534 based on a greenhouse seed viability study using pooled *M.*

guttatus seeds from multiple Sierra Nevada populations (Elderd and Doak 2006). Flower production (F^{ij}), overwinter survival (S^{ij}), and rosette production (R^{ij}) were estimated for each population *i* and year *j* from the observed transition frequencies in each seedling cohort. Sample sizes for estimating adult performance were small (mean N per perennial population: 6, range: 4-7) and estimates were limited to 2013 when overall performance was low (due to drought, see results). However, there was no evidence that fecundity, survival, or growth differed between rosettes and seedlings in 2013 (Inland perennial: Fruits: F = 2.44, df = 1, P = 0.12; survival and rosette production was zero for all individuals. Montane perennial: Fruits: F = 0.77, df = 1, P = 0.38; Survival: χ^2 = 0.22, df = 1, P = 0.64; Rosettes: F = 0.58, df = 1, P = 0.45). For these reasons, I assumed that rosette performance was equivalent to seedling performance within each population and year. Under this assumption, a perennial plant may produce new individuals either sexually by producing a viable seed or seedling, or clonally by producing a new vegetative rosette. In fact, rosettes may frequently represent physiologically independent clones (see results) and, given their larger sizes, this assumption is a conservative estimate of the importance of survival and growth in perennial populations.

To translate individual-level observations of flower production into estimates of viable seed set, I estimated two additional parameters: ovule number per flower, and a retention rate *A* that captures the transition from ovule to viable seed. Ovule number per flower (O^{ij}) was estimated as the mean ovule number in the second flower for each population *i* in each year *j* (see above). Ovule production per plant is certainly an overestimate of viable seed production due to pollen and resource limitations on seed development, seed predation, and safe-site limitation. *Mimulus* seeds are small and easily dispersed by wind and water (Truscott et al. 2006; Waser et al. 1982), suggesting that many seeds may be lost from the relatively narrow band of suitable stream bank habitat at this site. I examined natural recruitment dynamics in the native *M. guttatus* population to estimate the proportional recruitment success of ovules relative to rosettes, *A* (see results).

The transition matrix for population *i* in year *j* took the general form:

$$\begin{array}{lccc} & \text{Seed (t)} & \text{Seedling (t)} & \text{Rosette (t)} \\ \text{Seed (t+1)} & D\left(1-G^{i}\right) & F^{ij}O^{ij}A\left(1-G^{i}\right) & F^{ij}O^{ij}A\left(1-G^{i}\right) \\ \text{Seedling (t+1)} & DG_{i} & F^{ij}O^{ij}AG^{i} & F^{ij}O^{ij}AG^{i} \\ \text{Rosette (t+1)} & 0 & S^{ij}R^{ij} & S^{ij}R^{ij} \end{array} \qquad \text{(mat 1)}$$

Population projections and bootstrapping – I used the dominant eigenvalue of each transition matrix, λ, as a measure of overall demographic performance and assessed the contribution of each transition rate to λ through standard sensitivity and elasticity analyses (Caswell 2001; de Kroon et al. 1986). To account for uncertainty in vital rates, I used bootstrapping to estimate bias-corrected 95% confidence intervals around λ, sensitivities, elasticities, and life table response experiment (LTRE) effects (see below) (Caswell 2001). For each data set of N individuals, I randomly selected N individuals with replacement to construct a new transition matrix. As above, I estimated flower production (*F*) , survival (*S*), and rosette production (*R*) from observed seedling transition frequencies in the new data set and used these values to parameterize both seedling and rosette vital rates. To estimate total viable seed set, I drew values for O^{ij} from a normal distribution with the observed mean and standard deviation for population i and year j and values for A from a normal distribution with mean 6.7 x 10^{-4} and standard deviation 2.4 x 10^{-4} (see results), truncated to ensure $A > 0$. When populations or years were pooled for analysis, I drew a separate value of O^{ij} for each population and year in the pooled data set, and multiplied individual flower production by the appropriate value. I used a similar approach for G^i when populations were pooled. This process was replicated 10,000 times to generate a distribution of λ, sensitivities, elasticities, and LTRE effects for each dataset.

LTRE analysis

I tested the contributions of year, ecotype, and population within ecotype to variation in λ with life table response experiments (LTREs) (Caswell 1989; Caswell 2001). I modeled the effects of ecotype and year on λ as:

$$\lambda^{ij} = \lambda^{\cdot\cdot} + \alpha^{i} + \beta^{j} + \alpha\beta^{ij}$$

where $\lambda^{\cdot\cdot}$ is the population growth rate of a pooled reference matrix, α^i is the effect of ecotype i, β^j is the effect of year j, and $\alpha\beta^{ij}$ is the interaction between ecotype and year. I also tested the effects of population on λ in separate analyses within each ecotype. I constructed matrices for each treatment level ($\mathbf{A}^{i\cdot}$, $\mathbf{A}^{\cdot j}$, and $\mathbf{A}^{ij}$) by pooling across all other treatments. For example, the 2012 treatment matrix ($\mathbf{A}^{\cdot 2012}$) was constructed by pooling data across all ecotypes in 2012. All analyses were conducted with the ltre function in the popbio package (Stubben and Milligan 2007) in R v. 3.1.2 (R Core Development Team 2011).

Selection analysis

I estimated phenotypic selection on four traits related to life history strategy: seedling rosette size, flowering time, flower size, and flowering stem diameter. I estimated selection through four fitness components: total flower production, ovule number per flower, overwinter survival, and vegetative rosette production. In order to increase sample sizes for detecting selection, I pooled data from populations within each life history ecotype based on non-significant LTRE effects of population within ecotype (see results). For each trait, I compared three measures of selection. Direct selection estimates are standard selection gradients measured through individual fitness components, such as fruit production or adult survival (Lande and Arnold 1983). Integrated selection estimates incorporate individual selection gradients across multiple fitness components as well as the contributions of each fitness component to λ (figure 2.3). Finally, total integrated selection includes selection through correlated traits to estimate the total effect of a trait on λ (figures 2.3, 2.4).

Direct selection - For each ecotype, I estimated selection gradients as the partial regression coefficients in multiple regressions of relative fitness on all traits (Lande and Arnold 1983). I calculated relative fitness and estimated mean-standardized selection gradients by dividing each fitness component and trait by the mean value for that ecotype (Hereford et al. 2004). Mean-standardized selection gradients can be directly compared to integrated selection estimates, which are on a mean-standardized scale (Hereford et al. 2004; van Tienderen 2000). However, I also include variance-standardized selection gradients in the appendix to facilitate comparisons to other studies. I log-transformed seedling size values as log(seedling size + 0.01) prior to standardization. For survival, I assessed significance by fitting logistic regressions with binary survival data, then used linear models with relative survival to estimate coefficients (Coulson et al. 2003). Annual ecotypes only survive for a single growth season and all phenotypic selection is mediated through fecundity, which incorporates variation in survival to reproduction. In the montane perennial ecotype, too few individuals flowered to fit multiple regressions with all four traits. Instead, I included flowering time and stem size since these were the only traits with significant or marginally significant relationships in univariate regressions (i.e. selection differentials).

Integrated selection – I combined estimates of direct selection through individual fitness components (see above) using elasticity path analysis to estimate integrated selection on each trait (van Tienderen 2000). Integrated selection on a trait can be understood as the elasticity of a trait, or the effect of a proportional change in mean trait value on λ. The elasticity of a trait is the product of the mean-standardized selection gradient and the elasticity of a fitness component, summed across all fitness components (figure 2.3). I compared trait elasticities based only on selection gradients significant at $\alpha = 0.05$ to a separate analysis including marginally significant gradients ($\alpha = 0.10$) to test the potential for low statistical power to alter qualitative conclusions.

Total integrated selection – For each trait, I also estimated total integrated selection by incorporating the effects of phenotypic correlations between traits according to an *a priori* causal model (figure 2.4) (Morrissey 2014). I ascribed causal relationships between traits according to the timing of trait expression and prior research. For example, seedling size may affect reproductive traits later in the life cycle, whereas variation in reproductive traits is unlikely to cause variation in seedling size within a single generation. Alternatively, increased flower size is genetically correlated with later flowering and larger size at flowering in annual *M. guttatus* (Kelly and Mojica 2011; Mojica and Kelly 2010). I estimated trait correlations within each ecotype using multiple linear regressions of each trait on all other traits using mean-standardized values. I estimated total selection by summing over all significant paths, including those through correlated traits (see figure 2.2). This is analogous to correlated selection as described by Lande and Arnold (1983):

$$\beta_i = \sum_{j=i}^{n} \beta_j \times Cor(i,j)$$

where β_j is the selection gradient (or elasticity) of trait *j*, Cor(*i*,*j*) is the correlation coefficient between traits *i* and *j*, and n is the number of trait correlations significant at $\alpha = 0.05$.

All analyses were performed in R v. 3.1.2 (R Core Development Team 2011).

RESULTS

Natural recruitment dynamics in a montane meadow

The majority of new recruits in each plot were vegetative rosettes derived from below-ground rhizomes, rather than newly germinated seedlings. Most rosettes were physiologically independent from other rosettes within each core; only 3 out of 368 belonged to ramets with more than one rosette. Soil cores from each plot yielded a total of 38 seedlings and 200 vegetative rosettes on May 1 and 24 seedlings and 168 rosettes on May 16. The proportion of seedlings decreased over time, but this difference was not statistically significant ($\chi^2=1.13$, df=1, P=0.288), so I pooled all cores to estimate a mean relative recruitment rate of 0.168 seedlings per rosette with standard deviation (among cores) of 0.061. To estimate ovule retention *A*, I compared this estimate of relative recruitment to an estimate of relative production of ovules to rosettes. In 2012, experimental individuals from the Eagle Meadows population produced an estimated 392 ovules (0.64 fruits/individual · 613 ovules/flower) and 1.56 vegetative rosettes per seedling, on average. I used the formula:

$$0.168 = A\left(\frac{392}{1.56}\right)$$

to estimate the relative retention from ovules to seedlings *A* as having a mean value of 6.7×10^{-4} with standard deviation 2.4×10^{-4}. These estimates were used to parameterize a truncated normal distribution to draw random values of *A* (see *Bootstrapping* in methods).

Local adaptation among life history strategies and populations

Evidence for local adaptation to the montane site is mixed, and depends on the level of comparison. Life history ecotypes contributed significantly to overall variation in λ in LTRE analyses, whereas population within ecotype had no significant effects. Fast-cycling annuals, the most ecologically divergent ecotype, consistently had the lowest performance whereas inland perennials consistently had the highest performance (table 2.1). Although there was broad overlap in the confidence intervals around estimates of λ, these two ecotypes were the only ones to exert statistically significant effects on overall λ in the LTRE analysis (figure 2.5B). Fast-cycling annuals had a significantly negative effect on λ whereas inland perennials had a significantly positive effect on λ, providing some evidence that a perennial life history strategy is most successful in this habitat.

However, the montane perennial life history strategy, which is native to montane habitats, showed little evidence of local adaptation relative to other life history ecotypes. The montane perennial ecotype did not have higher survival or rosette production compared to the inland perennial ecotype, and had lower fruit production and seed germination rates (table 2.1). Within the montane perennial ecotype, there is some suggestion that the native Eagle Meadows population exhibits higher performance relative to other montane perennial populations, but these differences were not statistically significant.

Variation in fitness components among ecotypes and years

Year had a profound effect on the performance of all ecotypes. 2013 was a severe drought year, and estimates of λ were consistently lower in 2013 compared to 2012 (table 2.1). Although there was overlap in the confidence limits surrounding λ estimates between years, 2013 had a significantly negative effect on λ in the LTRE analysis (figure 2.5A). There were no significant interactions between year and ecotype (figure 2.5C) and the rank order of λ among ecotypes remained consistent (table 2.1).

In general, both flower number and ovule number per flower decreased in 2013 relative to 2012 (table 2.1). Drought conditions in 2013 increased mortality in August and September, truncating the growth season compared to 2012 when many individuals continued to flower into the late fall. In fact, all above-ground biomass died back by the end of September. One consequence of this drought was that inland perennials were unable to overwinter or produce clonal rosettes through above-ground stolons. In contrast, some montane perennials regenerated from below-ground rhizomes the following spring (table 2.1).

Specific transition rates contributed to variation in λ among ecotypes within each year. In 2012, differences in performance among ecotypes were determined by first-year fecundity ($\alpha_{sdl,sdl}$) and, in perennial ecotypes, second-year fecundity ($\alpha_{sdl,ros}$), growth ($\alpha_{ros,sdl}$), and survival ($\alpha_{ros,ros}$) (figure 2.5D). In 2013, differences in performance among ecotypes were largely determined by first-year fecundity ($\alpha_{sdl,sdl}$) (figure 2.5E). Transitions associated with growth ($\alpha_{ros,sdl}$) and survival ($\alpha_{ros,ros}$) had a negative effect in inland perennials in 2013, but a positive effect in montane perennials, reflecting the contrasting effects of drought on these overwintering strategies.

Phenotypic correlations among life history traits

In general, traits related to the timing of reproduction and size of reproductive structures were positively correlated across ecotypes and years (table 2.2). Flowering time, flowering stem diameter, and flower size tended to be positively correlated with each other, suggesting a trade-off between early reproduction and the biomass allocated to reproduction. Seedling size was generally negatively correlated with flowering time, indicating that seedlings with greater above-ground rosette growth flowered earlier. In robust annuals, seedling size was also positively correlated with flower size in 2012 and flowering stem diameter in 2013, suggesting that seedlings with greater above-ground growth also produced larger reproductive structures in this ecotype.

Selection on life history traits

Direct selection - The strength and pattern of direct selection varied among ecotypes, years, and fitness components (table 2.3, appendix). In general, fitness components related to fecundity showed consistently positive relationships with flowering stem diameter and flower size across ecotypes and years (table 2.3, figures 2.6, 2.7). Thicker flowering stems or larger flowers are associated with more ovules per flower and/or more flowers. Conversely, selection on flowering time via fecundity contributions varied from positive to negative among years and ecotypes, though selection through ovule number was always positive whereas selection through fruit number was always negative (table 2.3, figures 2.6, 2.7).

Within perennial ecotypes, direct selection through rosette production was only detected in 2012 (table 2.3, figure 2.6). In contrast to the pattern for fecundity selection, selection on flowering stem diameter through rosette production was negative in montane perennials, though this was only marginally significant (figure 2.6D). In inland perennials, later flowering and larger seedlings increased rosette production, though selection on seedling size was only marginally significant (figure 2.6C). In 2012, inland perennials only experienced selection through rosette production, whereas in 2013 all selection in this ecotype occurred through fecundity (figures 2.6C, 2.7C). There was no significant relationship between adult overwinter survival and any of the four traits examined in either year.

Integrated selection – Integrating selection by estimating trait elasticities altered the strength of selection relative to estimates through individual fitness components. For most traits, integrated selection was weaker than direct selection because individual fitness components are weighted by their elasticities, which are necessarily less than one. This effect was most pronounced in perennial ecotypes because fitness component elasticities were decreased in perennials relative to annuals, which exhibit high elasticities for fecundity (table 2.A2). Large selection gradients in perennial ecotypes occurred through fitness components with smaller elasticities (figure 2.6C-D, 2.7D). Correspondingly, integrated selection on flowering time was generally weaker in perennials relative to annuals (figure 2.8), even though direct selection gradients through individual fitness components were larger in perennials (table 2.3, figures 2.6, 2.7). In one case, integrated selection was stronger than direct selection because selection occurred in the same direction through multiple fitness components. Robust annuals experienced selection for larger flowering stem diameter through both fruit number and ovule number per fruit in 2013 (figure 2.7B), resulting in strong integrated selection for larger stems (figure 2.8). A similar pattern occurred in 2012 (figure 2.6B) and for larger flowers in inland perennials in 2013 (figure 2.7C), though in both cases selection through fruit number was only marginally significant.

Total integrated selection – Correlations among traits had diverse effects on the strength of selection (figure 2.8). Positive correlations among traits increased the strength of selection in some cases. In fast-cycling annuals, for example, selection for increased flowering stem diameter caused correlated selection for larger flowers and later flowering in both years (figures 2.6A, 2.7A). Similarly, positive correlations between flowering stem diameter and flowering time increased the strength of selection on both traits in robust annuals in 2012 (figure 2.6B). In other cases, correlations among traits opposed the direction of selection on individual traits, weakening the strength of selection overall. For example, selection for earlier flowering in robust annuals in 2013 was opposed by a positive phenotypic correlation between flowering time and flowering stem diameter combined with selection for larger flowering stems (figure 2.7B). Similarly, selection for larger seedlings in fast-cycling annuals in 2013 was opposed by a negative correlation between seedling size and flowering time combined with selection for later flowering (figure 2.7A). In one case, multiple trait correlations opposed each other, effectively canceling out any effect of correlated selection on stem diameter in robust annuals in 2013. In this example, a positive correlation between stem diameter and flower size combined with selection for larger flowers was opposed by a positive correlation between stem diameter and flowering time combined with selection for earlier flowering (figure 2.7B). These opposite and nearly equal effects resulted in an estimate of total integrated selection very similar to the strength of integrated selection on stem diameter alone (1.613 and 1.637, respectively; figure 2.8).

DISCUSSION

I compared demographic performance and phenotypic selection among four life history ecotypes of *M. guttatus* to test the consequences of life history divergence on the pattern and strength of selection in a montane perennial habitat. I found that selection in a perennial habitat was generally consistent with perennial phenotypes, including later flowering and larger flowering stems and flowers. However, the fitness components mediating selection as well as their importance for population growth differed among life history ecotypes, highlighting the importance of integrating selection across the life cycle to understand adaptive divergence.

Local adaptation in perennial *M. guttatus*

Although divergent life history strategies within *M. guttatus* have become a model system for ecological genomics and speciation, the selective forces maintaining life history variation have not been well characterized in perennial populations. The adaptive significance of rapidly developing genotypes in seasonally-drying habitats is intuitive, yet evidence that a perennial life history strategy is adaptive in habitats with greater water availability remains scarce. Although coastal perennials are locally adapted to coastal habitats relative to annuals, this appears to be largely mediated by increased tolerance to salt spray, and thus greater seedling survival and fruit set, rather than an advantage of perenniality per se (Lowry et al. 2009; Lowry et al. 2008). In this study, I directly compared the demographic performance of annual and perennial ecotypes within a montane perennial habitat and tested how specific transitions in the life-cycle contribute to differences in performance using LTREs. With this approach, I found mixed evidence for local adaptation. A significant effect of ecotype on overall performance, combined with non-significant effects of population within ecotype, support that there are real differences in life history strategy and performance among these *a priori* ecotypes. I found some evidence that a perennial life history strategy is adaptive in a perennial habitat, since inland perennials consistently had the greatest demographic performance whereas fast-cycling annuals had the lowest. Further, transitions associated with adult survival and vegetative growth ($\alpha_{ros,sdl}$ and $\alpha_{ros,ros}$) contributed positively to λ in the montane perennial ecotype, whereas fecundity transitions ($\alpha_{sdl,sdl}$ and $\alpha_{ros,ros}$) contributed negatively.

However, there was no evidence that the montane perennial ecotype is locally adapted since inland perennials and robust annuals outperformed montane perennials in both years. One potential explanation for the relatively poor performance of the montane perennial ecotype is that the importance of below-ground rhizomes was not fully captured during the time-frame of this study. Indeed, montane perennial genotypes invest extensively in below-ground rhizomes and may be able to persist during harsh conditions when other strategies lacking below-ground structures would become extinct. Natural recruitment plots suggest that seedling recruitment is low relative to rosette recruitment. Longer-term monitoring of the relative performance of seedlings and rosettes under a range of environmental conditions, including drought, competition, and frost, would refine estimates of demographic performance. Alternatively, the low performance of the montane perennial ecotype could be due to genetic load, given extensive clonal growth and low flower production and seedling recruitment. Within the montane perennial ecotype, I did find some evidence for local adaptation as the native Eagle Meadows population outperformed both foreign populations, although this difference was not significant.

Integrated selection on life history traits

As expected, life history ecotypes exhibit different fitness strategies even within a common montane environment. The performance of annual ecotypes is influenced most strongly by seed production, whereas perennial performance is also achieved through the growth and survival of clonal rosettes. Among perennials, montane populations produce few flowers and rely extensively on clonal growth through below-ground rhizomes. This divergence in the relative importance of specific fitness components influences the net pattern of selection. Interestingly, the direction of fecundity selection was similar across life history ecotypes, consistently favoring greater allocation to reproductive structures including larger flowers and larger reproductive stems. Conversely, selection through clonal growth, when detected, favored smaller reproductive stems and seedlings (though some of these effects were only marginally significant). These contrasting effects of individual fitness components resulted in contrasting selective landscapes for different life history ecotypes even within a common environment. In particular, integrated selection tended to be weaker in perennials relative to annuals due to the reduced elasticity of any one fitness component.

A major goal of evolutionary biology has been to document the strength of selection in wild populations. In a review of mean-standardized selection estimates, Hereford (2004) found surprisingly strong directional selection through individual fitness components and suggested that these are unlikely to represent the strength of selection in general. One potential explanation is trade-offs among fitness components in the direction of selection, yet evidence for such trade-offs is mixed and based on relatively few studies (Kingsolver and Diamond 2011; reviewed in Schluter et al. 1991). I only detected concurrent selection on the same trait through multiple fitness components for stem size and marginally for flower size. In these cases, selection through multiple fitness components increased the strength of integrated selection, because underlying selection gradients through fruit and ovule number were in the same direction. These traits are directly related to overall investment in reproductive biomass; such “size” traits may frequently be positively correlated and increase multiple components of fitness due to environmental covariance (Mitchell-Olds and Shaw 1987).

I found some evidence for conflicting selection among fitness components in comparisons across ecotypes and years. Later flowering consistently increased ovule production but decreased fruit production, whereas larger stems increased fecundity but decreased rosette production. However, I never detected these effects within the same ecotype and year, so it is unclear whether these trade-offs act simultaneously to decrease the strength of selection.

Even if selection on a given trait primarily occurs through a single fitness component, life history divergence will still affect the strength of selection. Failing to integrate multiple fitness components will bias the strength of selection, since individual fitness components contribute unequally to population growth among environments and life history strategies (e.g. Horvitz and Schemske 1995). Integrating selection incorporates this variation by multiplying selection gradients by the elasticities of their respective fitness components. Since elasticities are necessarily less than one, integrated measures of selection will always be weaker than selection gradients, unless multiple fitness components are positively correlated. Thus, previous conclusions about the average strength of selection in wild populations are likely overestimates (Hereford et al. 2004; Kingsolver et al. 2001). The strength of selection gradients depends in part on the degree of fitness variation, however demographic reviews have found that the fitness components with the largest elasticities are often the least variable within populations (Burns et al. 2010; Pfister

1998; Sæther and Bakke 2000). In this study, I found that strong selection gradients in perennials occurred through fitness components with smaller elasticities, weakening the strength of integrated selection relative to annuals. Large selection gradients may reflect less important fitness components, rather than strong phenotypic selection, and this potential bias should be greatest in long-lived iteroparous organisms where some fitness components will only contribute weakly to total fitness.

Correlated selection and life history evolution in *M. guttatus*

Incorporating phenotypic correlations among traits frequently altered the strength of selection. Although phenotypic correlations determine the strength and pattern of selection, inference about the evolutionary response to selection requires an understanding of the underlying genetic variance-covariance structure. However, there are several lines of evidence to suggest that the phenotypic correlations observed here reflect genetic correlations within or among ecotypes. First, the randomized plot design used in this experiment minimizes the potential for environmental effects to generate trait correlations (Mitchell-Olds and Shaw 1987) while the use of highly divergent ecotypes and multiple populations within each ecotype maximizes the potential for genetically based trait variation. Second, previous research has shed light on the genetic basis of many traits in this species. Below, I explore how selection through correlated traits may drive the evolution of perennial ecotypes in this species.

Total integrated selection consistently favored larger flowers in this perennial habitat, potentially explaining the observation that perennial populations have larger flowers than annuals throughout the species range (Friedman et al. 2015). Flower size has been shown to be a target of selection in several annual populations of *M. guttatus* (Mojica and Kelly 2010; Murren et al. 2009). However, the direction of selection on flower size is both spatially and temporally variable due to positive genetic correlations between flower size, flower time, and ovule and pollen production per flower (Kelly and Mojica 2011; Mojica and Kelly 2010). Thus, in an annual habitat, large flowers decrease survival to reproduction by delaying flowering but increase seed set per flower, and the net pattern of selection on flower size depends on the timing of drought-induced mortality (Mojica and Kelly 2010; Mojica et al. 2012). Although I did not detect correlations between flower size and flowering time directly, flower size was frequently positively correlated with flowering stem diameter, which in turn was often correlated with later flowering. Further, selection on flower size only occurred through components of fecundity. This suggests that the longer growth season in perennial habitats ameliorates viability selection on flower size, resulting in strong fecundity selection for larger flowers. Selection also favors larger flowers in coastal perennial habitats, further supporting this hypothesis (Hall and Willis 2006).

Selection on flowering time was variable between years and ecotypes. Flowering time and plant size appear to be widely correlated among populations of *M. guttatus*. In a survey of annual populations, Kooyers et al. (2015) found that plant height and stem diameter are positively correlated with flowering time, suggesting that these traits have evolved together as part of a drought-escape strategy. Similarly, in an F2 mapping population between annual and coastal perennial genotypes, flowering time and stolon production map to several shared QTL, suggesting that vegetative growth through stolons is pleiotropically linked to later flowering (Friedman et al. 2015). These broad patterns are reflected in this study and influence the strength and pattern of selection. In both annual ecotypes, later flowering is correlated with larger flowering stems across both years. Given that direct selection consistently favored larger flowering stems, this correlation either increased selection for later flowering or weakened selection for earlier flowering. Further, inland perennials experienced selection for later flowering through increased rosette production in 2012, supporting a link between delayed flowering and vegetative growth. Thus, in a perennial habitat, selection may generally favor later flowering either through fecundity selection for larger flowering stems or selection through rosette production. This is in contrast to annual habitats, which impose strong fecundity selection for early flowering before the onset of drought (Hall and Willis 2006).

Temporal variation in phenotypic selection

The strength and direction of phenotypic selection is highly variable over time (Siepielski et al. 2009), providing one potential explanation for the paradox of strong selection within populations and evolutionary stasis. Given the apparent rarity of stabilizing selection in wild populations, shifting patterns of directional selection may be evidence of a population "wobbling" around a fitness peak (Kingsolver and Pfennig 2007; Siepielski et al. 2009). Alternatively, selection may not be detected at all if the breadth of a fitness peak exceeds the phenotypic variance, so that the fitness landscape appears flat. In the native montane perennial ecotype, I only detected selection on flowering time and marginal selection for smaller flowering stems in 2012. Interestingly, selection on flowering time was weak and changed directions between years. Additional temporal replication could test whether this native montane perennial ecotype is near a fitness peak for later flowering.

Throughout the Sierra Nevada, 2013 was a severe drought year due to low snowpack in the previous winter. Across all ecotypes, fecundity and survival was reduced in 2013 and LTREs confirm that 2013 contributed negatively to overall population growth. The earlier onset of drought-induced mortality in 2013 increased the strength of selection for larger flowers and flowering stems and caused selection to favor earlier flowering in robust annuals. In inland perennials, the death of all above-ground biomass due to drought imposed an annual life-cycle, changing the pattern of selection from traits that maximized rosette production in 2012 to traits that maximized fecundity in 2013. This resulted in a more “annual-like” pattern of selection for larger flowers and flowering stems. Although the time-scale of this study is limited, this suggests that dry years can exert selection for traits that maximize first-year fecundity even within a perennial habitat, supporting a potential path for the evolution of an annual life history strategy from a perennial ancestor.

Estimating phenotypic selection throughout the life-cycle

By estimating integrated selection, this study highlights the complexity of fitness landscapes once multiple fitness components are considered over several years. Recent reviews have identified the use of single fitness components and lack of temporal replication as major limitations toward a general understanding of selection in natural populations (Kingsolver et al. 2012; Siepielski et al. 2010). The few studies that have examined integrated selection have found that the strength and pattern of selection varies among fitness components and years, often altering qualitative conclusions (Coulson et al. 2003; Ehrlén and Münzbergová 2009; Horvitz et al. 2010). This study further supports the limited utility of selection estimates based on single fitness components, particularly for iteroparous taxa. For example, the relative importance of individual fitness components in inland perennials changed dramatically over two years. This growing appreciation of the complex interactions among fitness components and traits has motivated the development of methods to decompose the effects of specific environmental conditions and fitness components on long-term selection (Horvitz et al. 2010). Clearly, this is a promising approach for linking selection to specific environments and forecasting evolutionary responses to environmental change. Although previous empirical estimates of integrated selection have relied on long-term demographic data sets (Coulson et al. 2003; Horvitz et al. 2010), this study demonstrates that integrated selection can be estimated even within an experimental framework (e.g. Ehrlén 2003) or over a relatively short time period . By collecting data on multiple fitness components and estimating a few additional demographic parameters, researchers stand to gain a much richer understanding of natural selection.

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Table 2.1: Demographic parameters for each ecotype and year. The mean and bias-corrected 95% confidence limits (in parentheses) of λ for each ecotype of *M. guttatus* and year over 10,000 bootstrap replicates. The estimated mean vital rate and standard error (in parentheses) for each ecotype and year. Note that the germination rate *G* was estimated in 2012 and did not vary between years in matrix models.

Year	**Ecotype**	**λ**	***G***	***F***	***O***	***S***	***R***
2012	Fast-cycling annual	0.87 (0.40,1.66)	0.711 (0.056)	7.02 (1.10)	229.2 (48.7)	0	0
	Robust annual	2.00 (0.73, 3.55)	0.741 (0.048)	9.40 (0.89)	420.8 (29.0)	0	0
	Inland perennial	3.06 (1.55, 5.05)	0.705 (0.067)	6.88 (1.09)	615.6 (43.4)	0.163 (0.081)	5.38 (1.36)
	Montane perennial	1.18 (0.67, 1.93)	0.479 (0.063)	0.49 (0.97)	686.4 (77.9)	0.145 (0.064)	6.00 (1.29)
2013	Fast-cycling annual	0.27 (0.19, 0.43)	---	3.08 (0.56)	83.7 (23.9)	0	0
	Robust annual	0.68 (0.32, 1.14)	---	5.83 (0.41)	189.5 (15.8)	0	0
	Inland perennial	1.06 (0.47, 1.86)	---	4.09 (0.57)	478.7 (23.1)	0	0
	Montane perennial	0.47 (0.33, 0.82)	---	0.30 (0.46)	472.0 (55.2)	0.082 (0.049)	3.20 (1.55)

Table 2.2: Trait means and correlations for each ecotype of *M. guttatus* and year. Values along the diagonal are the trait mean and standard deviation in parentheses in units days, mm, mm, and log(mm^2) for flower time, flower size, stem diameter, and seedling size, respectively. Otherwise, values are the correlation coefficients from multiple regressions of the mean-standardized trait given by the column name on each of the mean-standardized traits specified in the Trait column. Due to the limited number of flowering individuals in the montane perennial ecotype, I only tested correlations between flower time and stem diameter. Significant values ($P < 0.05$) are in bold. * denotes marginally significant values ($P < 0.10$)

Year	**Ecotype**	**Trait**	**Flower time**	**Flower size**	**Stem diam.**	**Seedling size**
2012	Fast-cycling annual	Flower time	27.5 (11.88)	-0.164	**0.576**	**-0.362**
		Flower size	-0.250	16.25 (3.30)	**0.812**	0.060
		Stem diam.	**0.304**	**0.281**	1.75 (0.63)	0.079
		Seedling size	**-0.944**	0.102	0.392	5.54 (1.04)
	Robust annual	Flower time	37.47 (15.34)	-0.058	**0.223**	-0.025
		Flower size	-0.150	16.46 (4.05)	0.078	**0.106**
		Stem diam.	**0.319**	0.043	2.41 (0.80)	0.036
		Seedling size	-0.367	**0.590**	0.366	6.29 (0.64)
	Inland perennial	Flower time	47.49 (11.47)	-0.200	-0.257	**-0.322**
		Flower size	-0.129	16.99 (3.13)	0.243	-0.118
		Stem diam.	-0.093	0.136	2.87 (0.75)	-0.112*
		Seedling size	**-0.978**	-0.552	-0.939*	5.74 (0.61)
	Montane perennial	Flower time	53 (14.37)	-----	0.037	-----
		Flower size	-----	19.37 (5.56)	-----	-----
		Stem diam.	0.017	-----	1.76 (0.45)	-----
		Seedling size	-----	-----	-----	4.99 (0.84)

2013	Fast-cycling annual	Flower time	19.97 (10.79)	0.028	**0.330**	**-0.353**
		Flower size	0.084	11.69 (3.24)	**0.507**	0.219
		Stem diam.	**0.419**	**0.216**	0.75 (0.33)	0.235*
		Seedling size	**-0.778**	0.162	0.409*	2.56 (2.26)
	Robust annual	Flower time	34.37 (13.88)	-0.078	**0.376**	**-0.351**
		Flower size	-0.163	12.72 (3.16)	**0.673**	0.043
		Stem diam.	**0.335**	**0.287**	1.17 (0.52)	**0.204**
		Seedling size	**-0.451**	0.027	**0.295**	3.40 (1.18)
	Inland perennial	Flower time	55.67 (11.07)	0.319	0.237	-0.641*
		Flower size	0.201	16.59 (4.56)	0.487	0.395
		Stem diam.	0.061	0.201	2.43 (0.89)	0.113
		Seedling size	-0.188*	0.184	0.128	2.96 (1.09)
	Montane perennial	Flower time	51.15 (18.27)	-----	0.374	-----
		Flower size	-----	15.99 (6.40)	-----	-----
		Stem diam.	0.268	-----	1.69 (0.71)	-----
		Seedling size	-----	-----	-----	2.49 (1.37)

Table 2.3: Significant or marginally significant mean-standardized selection gradients for each ecotype of *M. guttatus* and year. Only partial regression coefficients that were significant or marginally significant ($P \leq 0.10$) are included. All coefficients, including variance-standardized selection gradients, are available in the Appendix. Trait measurements are as described in the methods.

Year	**Ecotype**	**Trait**	**Fitness component**	**β_μ (SE)**	**P-value**
2012	Fast-cycling annual	Stem diam.	Fruits	0.845 (0.380)	0.03
	Robust annual	Flower time	Ovules	0.504 (0.202)	0.02
		Flower size	Ovules	1.31 (0.330)	< 0.01
		Stem diam.	Fruits	0.458 (0.279)	0.10
		-----	Ovules	0.592 (0.219)	0.01
	Inland perennial	Flower time	Rosettes	1.106 (0.517)	0.04
		Seedling size	Rosettes	1.607 (0.900)	0.09
	Montane perennial	Flower time	Fruits	-1.92 (0.685)	0.01
		Stem diam.	Rosettes	-0.66 (0.377)	0.10
2013	Fast-cycling annual	Flower time	Ovules	0.603 (0.240)	0.02
		Flower size	Fruits	0.928 (0.557)	0.10
		Stem diam.	Ovules	0.987 (0.255)	< 0.01
		Seedling size	Fruits	1.282 (0.479)	0.01
	Robust annual	Flower time	Fruits	-0.957 (0.346)	< 0.01
		Flower size	Ovules	1.011 (0.204)	< 0.01
		Stem diam.	Fruits	1.745 (0.327)	< 0.01
		-----	Ovules	0.304 (0.131)	0.02
	Inland perennial	Flower size	Fruits	1.943 (1.069)	0.08
		-----	Ovules	1.242 (0.348)	< 0.01
		Stem diam..	Ovules	0.465 (0.194)	0.03
	Montane perennial	Flower time	Ovules	1.113 (0.342)	0.03

APPENDIX

Table 2.A1: Locality and life history information for all populations of *M. guttatus* in this study.

Population	Elevation (masl)	Latitude	Longitude	Habitat	Life-history Ecotype
Red Hills	313	37.857	-120.457	Serpentine seep	Fast-cycling annual
Bald Mountain	1693	38.137	-120.094	Gravelly seep	Fast-cycling annual
Peoria Basin	293	37.933	-120.520	Creek in grassland	Robust annual
Traverse Creek	681	38.873	-120.818	Serpentine creek	Robust annual
Sprague Rd	842	37.819	-120.152	Creek in mixed woodland	Robust annual
Big Oak Flat	1225	37.829	-119.958	Marshy meadow	Robust annual
Kyburz	1371	38.768	-120.290	Stream in pine forest	Inland perennial
Girard Creek	1515	38.731	-120.240	Stream in pine forest	Inland perennial
Silver Fork	1959	38.664	-120.219	Stream banks and surrounding meadow	Montane perennial
Eagle Meadows	2046	38.320	-119.920	Small stream	Montane perennial
Silver Creek	2066	38.588	-119.786	Marshy meadow	Montane perennial

Table 2.A2: Transition rate elasticities for each ecotype of *M. guttatus* and year. Fecundity elasticity (e_{FEC}) is the elasticity of fruit number or ovule number through the sum of all transitions involving fruit number F^{ij} or ovule number O^{ij}. Growth elasticity (e_{GRO}) is the elasticity of survival or rosette number through the sum of all transitions involving survival S^{ij} or rosette production R^{ij}. Subscripts for elasticities follow the format *e* [stage (t+1), stage (t)]. Note: values are the median across 10,000 bootstrap replicates. Due to rounding and sampling errors, elasticities may not sum to exactly one.

Year	Ecotype	e_{11}	e_{21}	e_{31}	e_{12}	e_{22}	e_{32}	e_{13}	e_{23}	e_{33}	e_{FEC}	e_{GRO}
2012	Fast-cycling annual	.034	.151	0	.151	.663	0	0	0	0	.814	0
	Robust annual	.005	.064	0	.064	.867	0	0	0	0	.931	0
	Inland perennial	.002	.036	0	.023	.394	.213	.012	.200	.111	.633	.327
	Montane perennial	.010	.031	0	.004	.013	.111	.027	.082	.718	.128	.830
2013	Fast-cycling annual	.342	.223	0	.223	.196	0	0	0	0	.426	0
	Robust annual	.041	.159	0	.159	.640	0	0	0	0	.800	0
	Inland perennial	.032	.145	0	.145	.677	0	0	0	0	.823	0
	Montane perennial	.177	.108	0	.031	.017	.117	.072	.045	.352	.172	.497

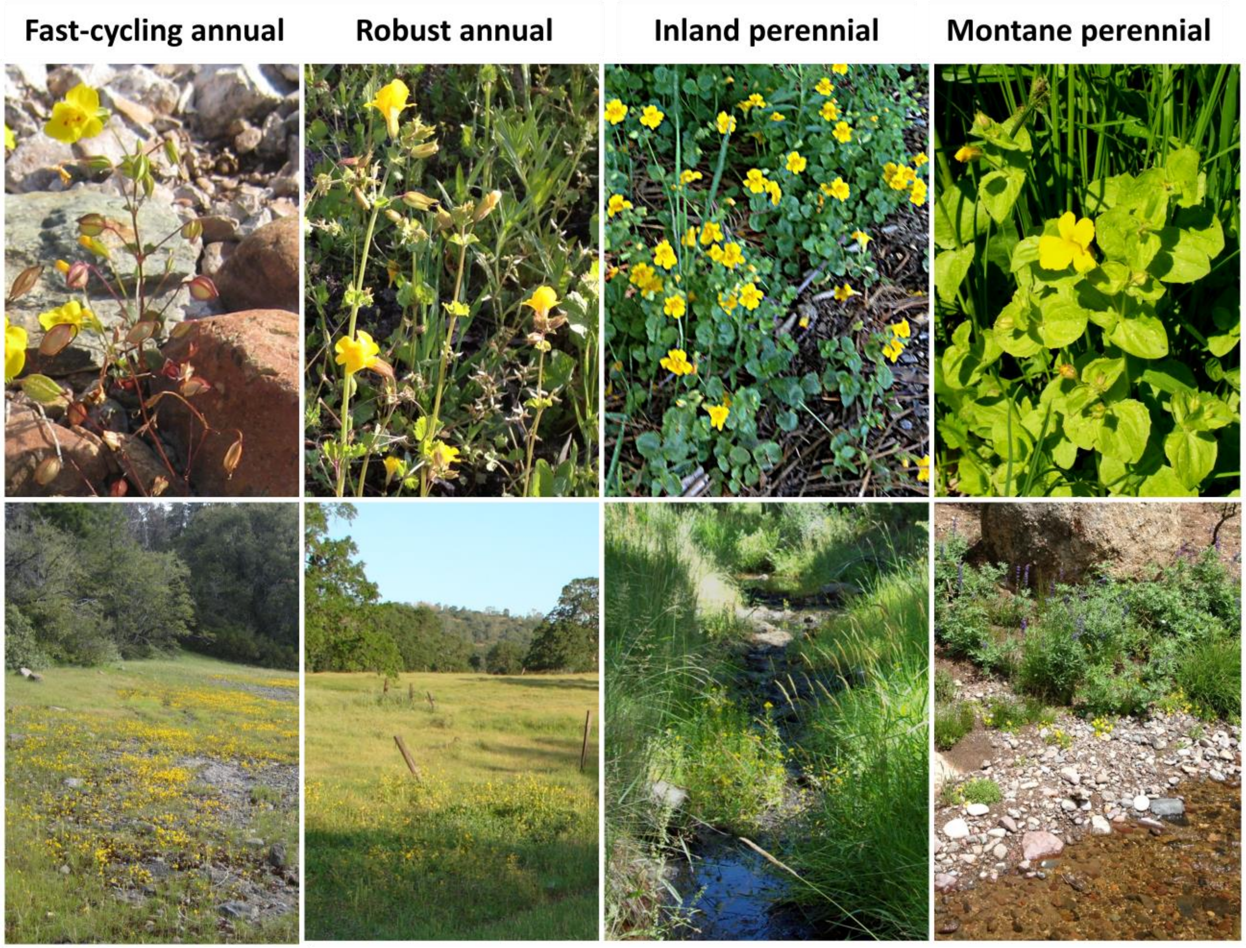

Figure 2.1: Representative growth form (top) and habitat (bottom) of each life history ecotype of *M. guttatus*. Fast-cycling annuals occur in fast-drying seeps and flower early. Robust annuals occur in seasonal streams or meadows and flower later at a larger size. Inland perennials occur along streams or lakes and produce vegetative rosettes through stolons. Montane perennials occur above 1450 m in the Sierra Nevada and produce rosettes through rhizomes.

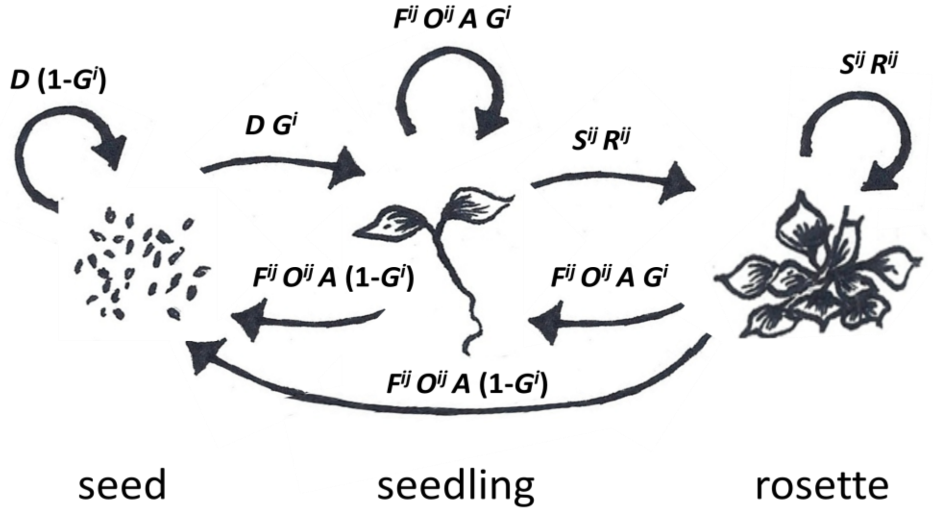

Figure 2.2: Life cycle graph of *M. guttatus* based on a census at the start of the growth season. Individuals can exist as seeds in the seed bank, newly germinated seedlings, or rosettes derived from above-ground stolons or below-ground rhizomes.

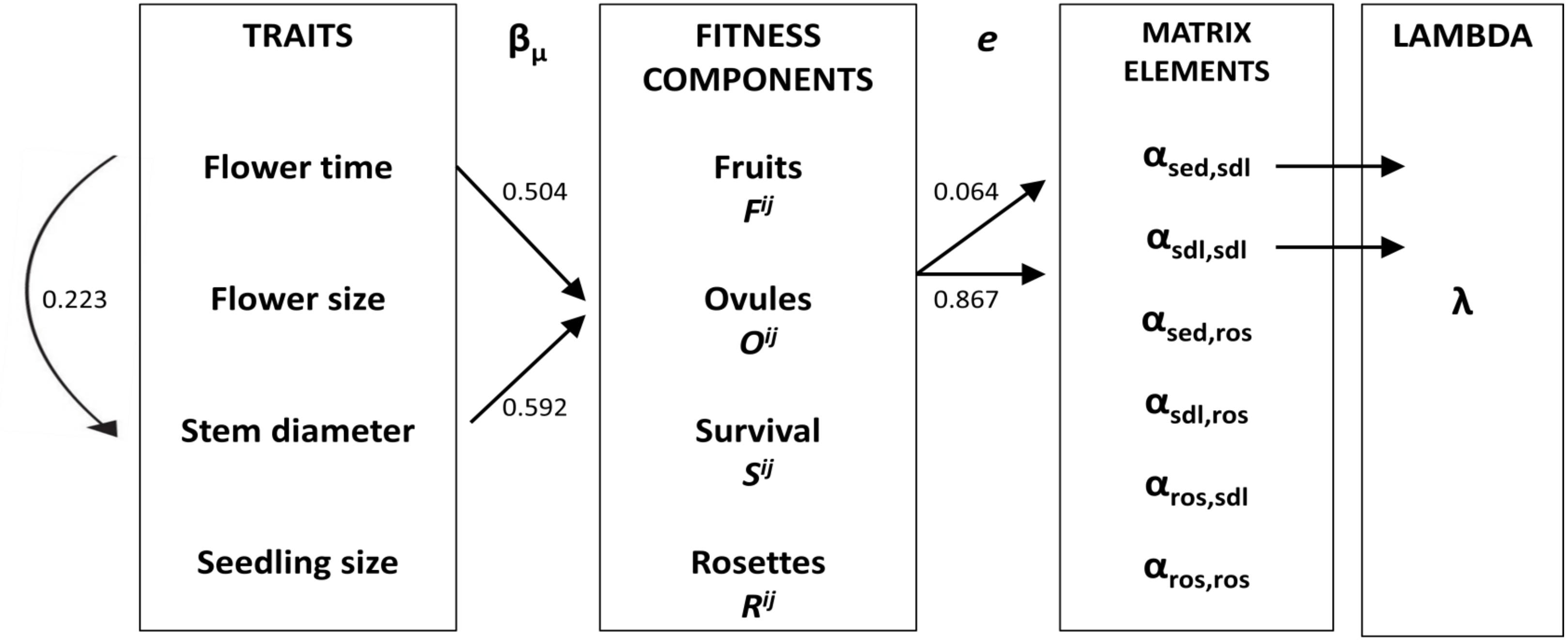

Figure 2.3: Diagram illustrating the elasticity path analysis used to estimate integrated and total integrated selection in *M. guttatus*. Arrows and values represent integrated and total integrated selection on flower time in the robust annual ecotype in 2012 (see Tables 2.2, 2.3). Integrated selection on a trait is calculated by summing over all significant paths connecting that trait to fitness components (i.e. mean standardized selection gradients, β_μ) through the effects of those fitness components on lambda (i.e. vital rate elasticities, *e*). In this example, integrated selection on flower time is 0.504·(0.064+0.867) = 0.469. Total integrated selection includes paths through correlated traits weighted by the phenotypic correlation. In this example, total integrated selection on flower time is [0.504+(0.223·0.592)]·(0.064+0.867) = 0.592. A positive phenotypic correlation between stem diameter and flowering time increases selection for later flowering in this example.

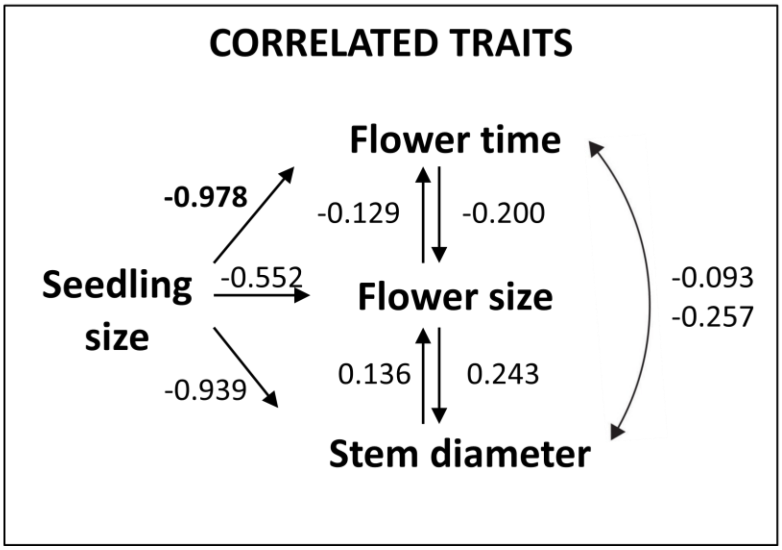

Figure 2.4: Causal model for correlated selection among traits in *M. guttatus*. Arrows represent potential causal relationships between traits. Seedling size may influence flower time, flower size, or stem diameter. Flower time, stem diameter, or flower size may influence each other. Values represent correlation coefficients in the inland perennial ecotype in 2012 (see Table 3). Only the path from seedling size to flower time is significant (in bold) and could contribute to correlated selection in this example.

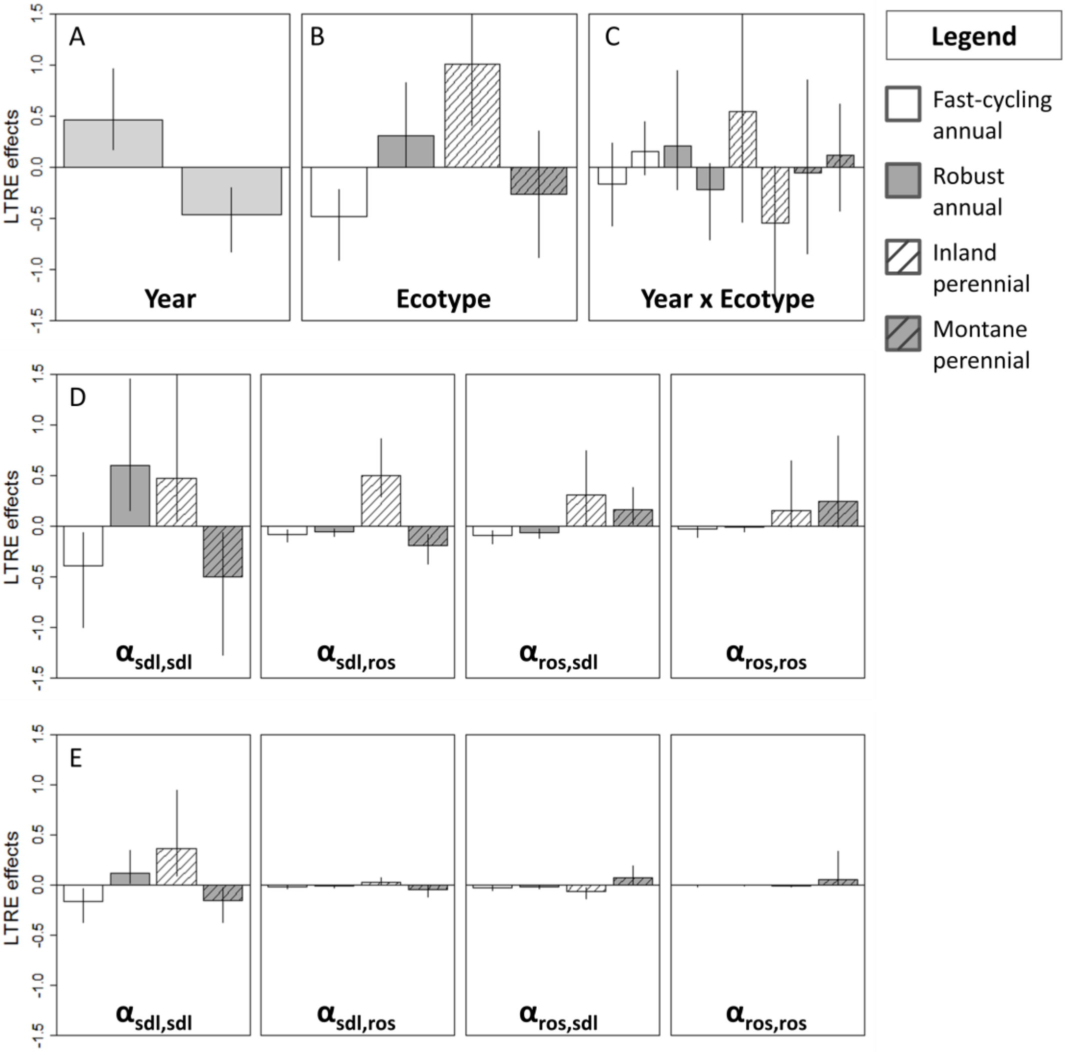

Figure 2.5: Contributions of year, ecotype, and vital rates to overall variation in λ through life table response experiments. LTRE effects and bias-corrected 95% confidence intervals over 10,000 bootstrap replicates. A) Main effect of year (left to right: 2012 and 2013). B) Main effect of ecotype. C) Interactions between the effects of ecotype and year (left: 2012, right: 2013). D, E) The contributions of specific transition rates α for each ecotype in 2012 (D) and 2013 (E). Subscripts for transition rates follow the format α [stage (t+1), stage (t)], where sdl = seedling and ros = rosette. Other vital rates had consistently small effects and are not shown.

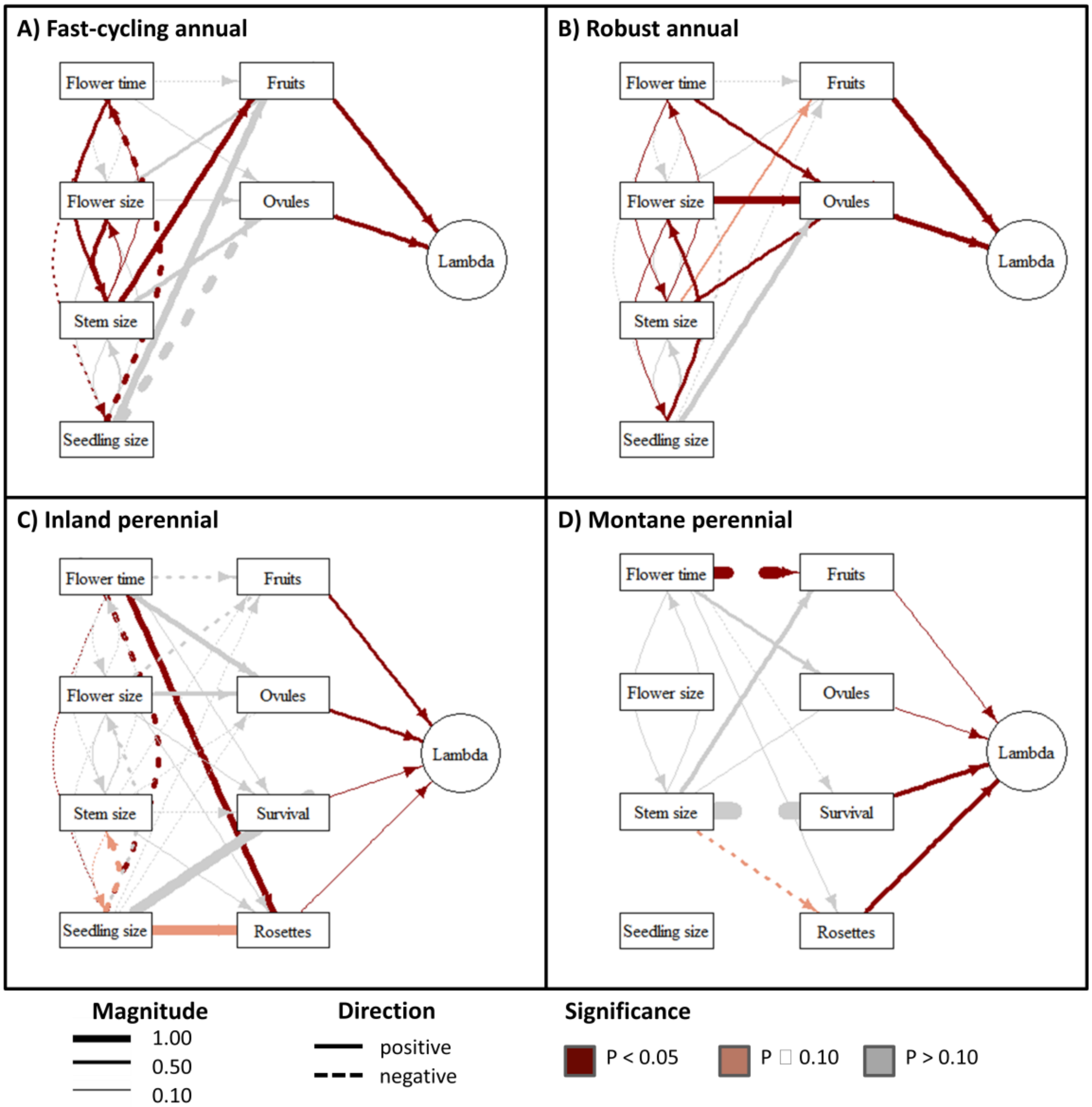

Figure 2.6: Path diagrams illustrating integrated and total integrated selection for each ecotype of *M. guttatus* in 2012. Arrows reflect positive (solid) or negative (dashed) relationships, where the arrow width is proportional to the magnitude of the coefficient. Statistical significance is indicated by red ($p < 0.05$), salmon ($p \leq 0.10$), and light gray ($p > 0.10$) arrows. Following figure 2.3, arrows between traits represent phenotypic correlation coefficients (Table 2.3), arrows between traits and fitness components are mean-standardized regression coefficients (Table 2.A2), and arrows between fitness components and lambda are elasticities (Table 2.A2).

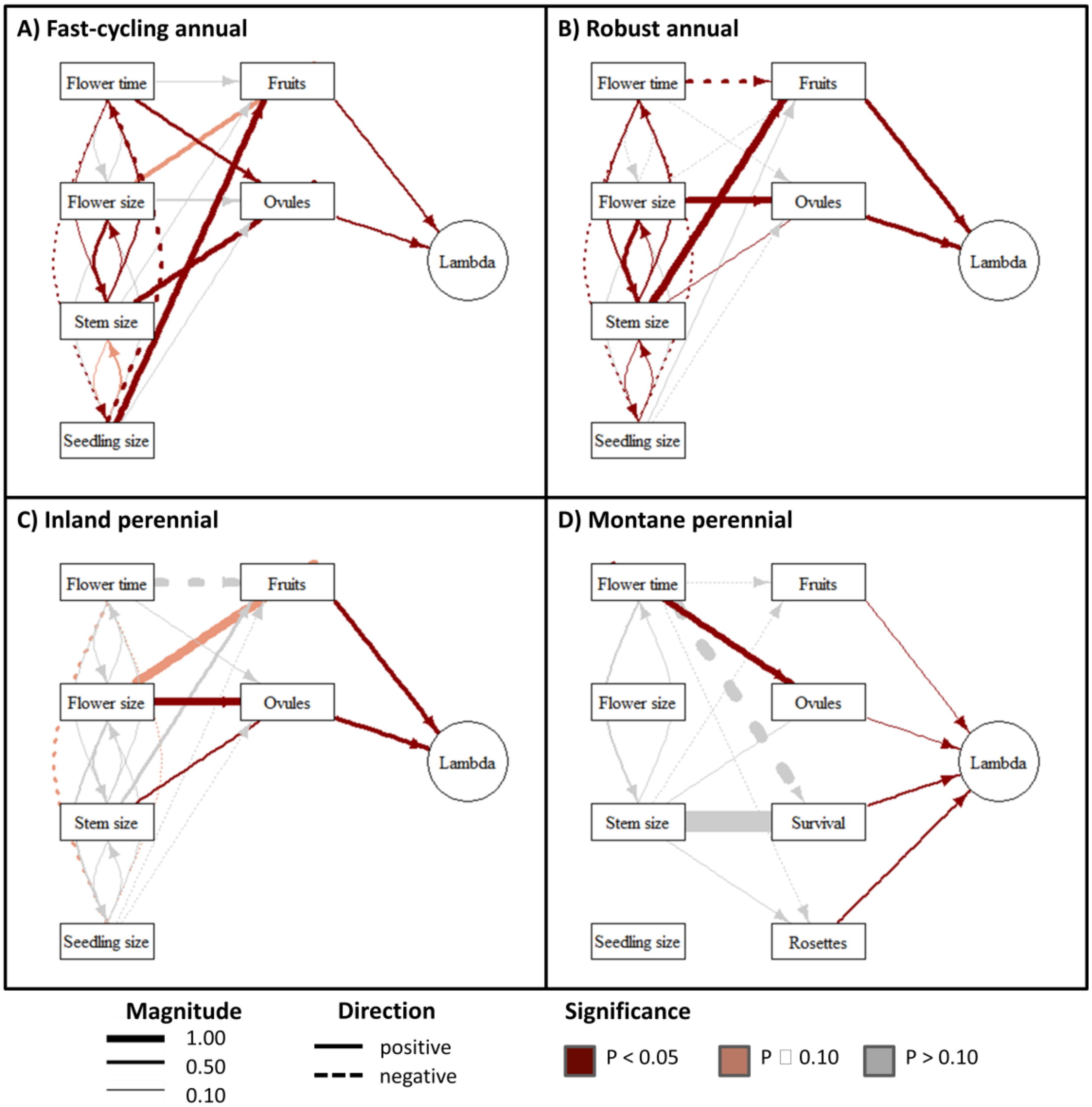

Figure 2.7: Path diagrams illustrating integrated and total integrated selection for each ecotype of *M. guttatu* in 2013. Arrows reflect positive (solid) or negative (dashed) relationships, where the arrow width is proportional to the magnitude of the coefficient. Statistical significance is indicated by red ($p < 0.05$), salmon ($p \leq 0.10$), and light gray ($p > 0.10$) arrows. Following figure 2.3, arrows between traits represent phenotypic correlation coefficients (Table 2.3), arrows between traits and fitness components are mean-standardized regression coefficients (Table 2.2), and arrows between fitness components and lambda are elasticities (Table 2.A2).

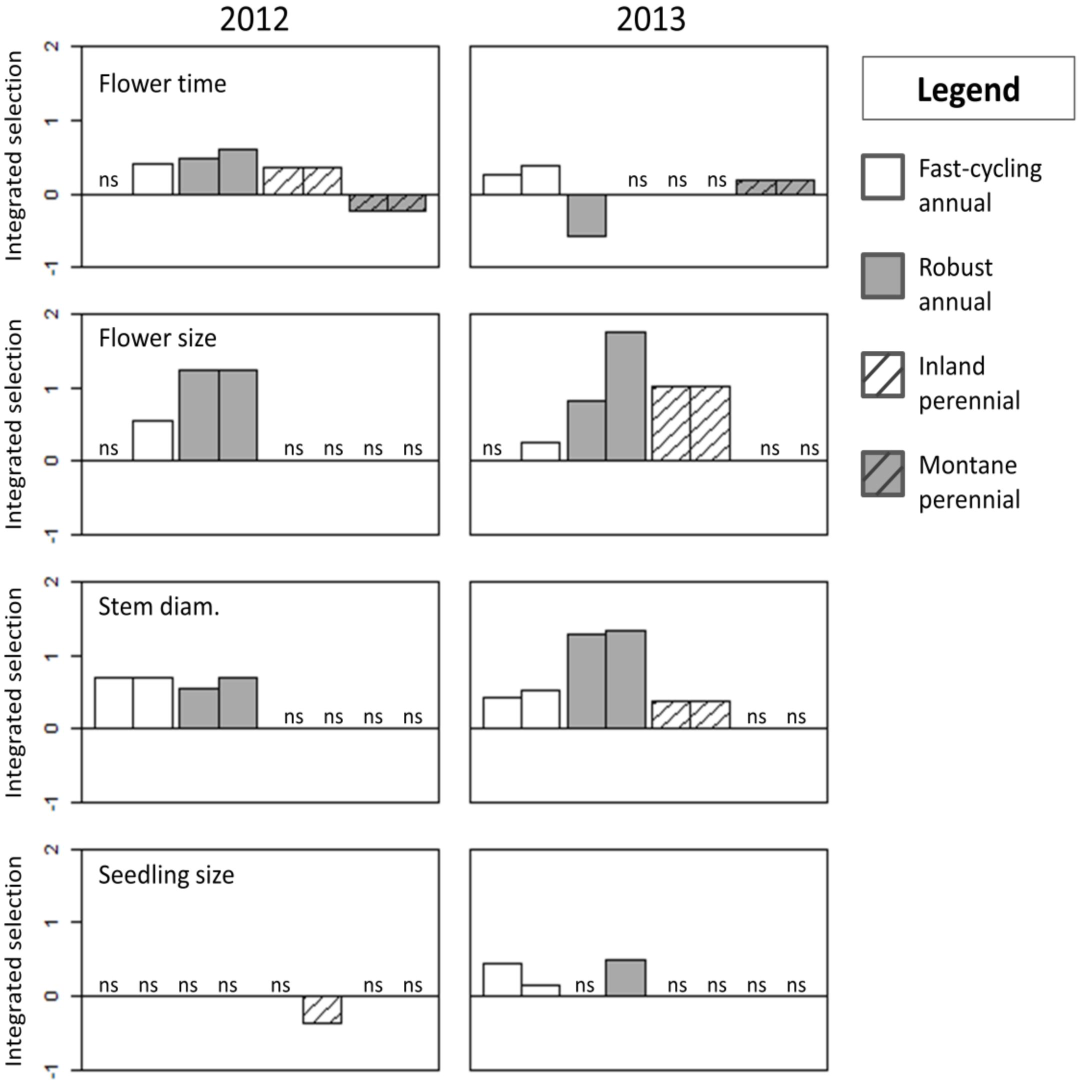

Figure 2.8: Integrated and total integrated selection on four life history traits for each ecotype of *M. guttatus* and year. Selection in 2012 (left panels) and 2013 (right panels). Within each ecotype, bars represent integrated (left) and total integrated (right) selection estimates. Estimates are the sum of all significant paths (i.e. red paths in figures 2.6, 2.7) from a trait to fitness (integrated selection, left bars) and all significant paths including those through correlated traits (total integrated selection, right bars). Missing bars (ns) reflect traits that lacked any significant paths to λ.

Chapter 3

Reproductive isolation accumulates through a mosaic of barriers and enhancers along a life history continuum in *Mimulus guttatus*

INTRODUCTION

Species encounter divergent selective environments throughout their range and local adaptation to these environments is the primary mechanism driving speciation (reviewed in Mayr 1947; Rundle and Nosil 2005; Schluter 2009). Adaptive divergence can generate reproductive isolation directly by decreasing the fitness of immigrant or hybrid genotypes and indirectly by shaping traits related to mate choice or reproductive compatibility. Yet, ecological speciation is not a necessary consequence of adaptive divergence, and speciation is best viewed as a continuum of increasingly strong and irreversible reproductive isolation in sexually reproducing species (Clausen 1951; Dobzhansky 1951; Grant 1981; Mallet 2008; Nosil et al. 2009; Wu 2001). Understanding the factors that promote or constrain progress towards speciation requires documenting the order in which specific reproductive barriers evolve as well as the mechanisms driving their evolution (Faria et al. 2014).

Comparative approaches have found strong support for increasing genetic isolation with greater ecological divergence (Berner et al. 2009; Funk et al. 2011; Rosenblum and Harmon 2011; Shafer and Wolf 2013). Yet, ascribing causality with molecular signatures of gene flow is difficult because reduced gene flow may in fact promote adaptive divergence, rather than vice versa (Moore et al. 2007; Räsänen and

Hendry 2008). A stronger test is to quantify how reproductive barriers themselves evolve with adaptive divergence (Hendry 2009). A few studies have implemented this comparative approach by regressing reproductive barriers onto adaptive divergence among taxon-pairs. For example, mating isolation increases with divergence in color pattern in cichlids (Seehausen et al. 2008) while intrinsic post-zygotic isolation increases with body size divergence in fish (Bolnick et al. 2006). Across a broad swath of taxa, reproductive isolation is positively correlated with ecological divergence between taxon-pairs (Funk et al. 2006). However, to my knowledge, no study has quantified the strength of multiple reproductive barriers among population-pairs that vary in their degree of adaptive divergence within a species (i.e. the speciation continuum). Thus, it is unknown how multiple barriers evolve with adaptive divergence within a system or whether the relative importance of different barriers changes as population pairs become more ecologically divergent. In a recent symposium, these questions were identified as "perhaps the most important contribution" towards advancing the field of ecological speciation (Faria et al. 2014).

Comparing the evolution of multiple reproductive barriers along the speciation continuum is necessary to understand how total reproductive isolation evolves during adaptive divergence. Ecological reproductive barriers may be offset by other trait interactions that act as reproductive enhancers (i.e. increasing gene flow, Hendry 2009), particularly in the early stages of divergence (e.g. Gosden et al. 2015; Räsänen and Hendry 2014). In *Colias* butterflies, for example, males from high-elevation populations prefer the lighter wing patterns of low-elevation females over the more thermally efficient dark patterns of high elevation females (Ellers and Boggs 2003). Selection against immigrants and hybrids have been predicted to be the earliest and strongest reproductive barriers to evolve during adaptive divergence, whereas other ecological or intrinsic barriers are predicted to evolve later or more sporadically (Nosil 2012). Although most speciation studies find strong selection against immigrants (reviewed in Nosil et al. 2005), the evidence for ecological selection against hybrids is mixed, with many closely related taxa exhibiting heterosis (reviewed in Arnold and Hodges 1995; Edmands 2002; Lowry et al. 2008a). Further, intrinsic post-zygotic barriers can evolve as a direct by-product of divergent selection and could be important in the early stages of divergence (e.g. Bomblies et al. 2007a; Rogers and Bernatchez 2007; Wright et al. 2013a). Finally, the relative importance of multiple barriers will likely vary throughout a species range, depending on the spatial distribution, opportunities for gene flow, strength of selection, and adaptive potential for a given population. Quantification of these barriers among closely-related population pairs that span a range of adaptive divergence is necessary to test these predictions (Faria et al. 2014; Hendry 2009; Nosil 2012).

To clarify the evolution of reproductive barriers along a speciation continuum, I compared five reproductive barriers among 10 population pairs spanning a range of life history divergence in common monkeyflower, *Mimulus guttatus* DC (Phrymaceae). Western North American monkeyflowers are a model system for dissecting the evolution of ecological reproductive barriers (Lowry et al. 2008b; Ramsey et al. 2003; e.g. Schemske and Bradshaw 1999; Sobel and Streisfeld 2015).

Speciation in this group is characterized by a budding process, in which small ecologically divergent populations give rise to new species (Grossenbacher et al. 2014), and this ecogeographic divergence is an important component of reproductive isolation (Sobel 2014). The *M. guttatus* species complex is particularly diverse, comprising annual and perennial taxa that occupy a wide range of edaphic, coastal, and montane habitats (Wu et al. 2008). Yet despite this extensive adaptive divergence, genomic variation in *M. guttatus* is partitioned geographically rather than among habitats or life history strategies, reflecting recent divergence and on-going gene flow (Oneal et al. 2014; Puzey and Vallejo-Marín 2014). Thus, ecotypic divergence within *M. guttatus* provides an ideal system for testing the evolution of early reproductive barriers in an actively diverging complex.

I used a common garden experiment to mimic the dispersal of immigrant genotypes from ten populations in central California into a focal population and quantified the ecological and intrinsic barriers to gene flow that would operate in sympatry. I used a regression approach to test whether the strength of individual barriers and total isolation were related to adaptive phenotypic divergence in life history traits. Finally, I compared the contributions of immigrant and hybrid fitness, flowering and pollinator isolation, and intrinsic crossing barriers to total reproductive isolation for each population and ecotype. Taken together, this work provides a comprehensive evaluation of the reproductive barriers (and enhancers) operating along a continuum of adaptive divergence for this species.

METHODS

Study system

Mimulus guttatus (DC, Phrymaceae) is an herbaceous annual or short-lived perennial plant that grows in mesic habitats in western North America. Life history divergence in this system is driven by variation in the length of the growth season. Annual populations occur in seasonally-drying seeps or meadows, whereas perennial populations occur in habitats with year-round moisture such as lakes, streams, and coastal seeps. Annual and perennial genotypes differ in several life history traits that are linked to a chromosomal inversion region (*DIV1*) that occurs throughout the species range (Lowry and Willis 2010). In general, perennials flower later at a larger size, produce larger flowers and stems, and reproduce vegetatively through clonal rosettes (Friedman et al. 2015). Yet within each of these broad life history strategies, populations exhibit extensive adaptive variation in morphological, phenological, and life history traits. Among annual populations, clinal variation in the length of the growth season is correlated with variation in drought escape traits including flowering time and size at first flower (Kooyers et al. 2015). Among perennial populations, coastal, inland, and montane habitats are associated with distinct ecotypes. Coastal perennials have evolved tolerance to salt spray (Lowry et al. 2009), whereas montane perennials invest extensively in below-ground rhizomes and produce few flowers (see Ch. 2).

Despite this extensive adaptive divergence, taxa within the *M. guttatus* species complex are at least partially interfertile and taxonomists have divided this complex into as few as 4 and as many as 20 species in different treatments (reviewed in Nesom 2012). Although the most recent treatment by Nesom (2012) splits *M. guttatus* into multiple morphological species, recent genomic evidence has challenged this view (Oneal et al. 2014). Thus, to be consistent with genomic evidence as well as previous work in this complex, I treat life history strategies in this study as ecotypes within *M. guttatus* sensu lato (Grant 1924).

Seed collections

In 2010, I surveyed the central Sierra Nevada mountains and surrounding foothills and collected maternal seed families from eleven populations of *M. guttatus* spanning the range of life history variation in this region (table 3.1, figure 2.1 & 3.1A). I characterized each population as one of four ecotypes based on local habitat characteristics and plant morphology in the field (figure 2.1). Montane perennials (*M. corallinus* sensu Nesom 2012) occur in the Sierra Nevada above 1450 m elevation, invest in below-ground rhizomes which give rise to clonal rosettes, and produce few flowers and seedlings (see Ch. 2). Inland perennials (*M. guttatus* sensu Nesom 2012) occur along inland lakes and streams at mid- to low elevations and produce clonal rosettes through above-ground stems (i.e. stolons). Annual populations (*M. micranthus* sensu Nesom 2012) lack overwintering structures, invest extensively in seed production, and senesce after a single year. I divided annual populations in this study into two ecotypes to reflect variation in the length of the growth season: fast-cycling annuals grow in rapidly-drying seeps and flower early at a small size, whereas robust annuals occur in meadows or creeks with longer growing seasons and flower later at larger sizes (figure 2.1).

Common garden transplant

I quantified the pattern and strength of reproductive isolation along a life history continuum by estimating reproductive barriers operating between a focal montane perennial population (Eagle Meadows) and ten other populations sampled across four life history ecotypes (table 3.1). Ecological reproductive isolation in annual habitats for M guttatus is well characterized: late-flowering perennial genotypes fail to flower before the onset of drought, imposing strong habitat isolation (Hall and Willis 2006; Lowry et al. 2008a). However, the barriers (if any) operating within a mesic montane perennial habitat, with an extended growth season, are unknown. I used a common garden approach to mimic seed dispersal of an immigrant genotype into a montane perennial population to test for barriers to gene flow as a function of phenotypic divergence. The common garden site was a small stream in a montane meadow supporting a native montane perennial population (Eagle Meadows) in Stanislaus National Forest (N 38.32107, W 119.91607, 2046 m).

In 2012 and 2013, I transplanted cohorts of seedlings from each of the ten immigrant populations and the native Eagle Meadows population into experimental plots located along the stream bank. Each plot contained one individual per population in a fully randomized design with a total of 40 plots per year. I emasculated all experimental flowers to prevent genetic contamination of the native population. I also transplanted all seedlings into 4” round pots, buried within the substrate of the stream bank, to contain below-ground rhizomes. Plots were approximately 1.0 x 0.5 m with 10-20 cm between pots. I watered all plots to field capacity at each census to prevent transplant shock and ameliorate any effects of pots on root growth. Experimental seedlings in 2012 were germinated in the UCSC greenhouse from field-collected seeds pooled from 30 maternal families per population. In 2013, I left field-collected seeds to overwinter in plug trays at the common garden site to allow natural variation in the timing of germination following snowmelt. Experimental seedlings were randomly sampled from among those germinants. I transplanted seedlings following snowmelt on June 14, 2012 and May 26, 2013, when native seedlings and ramets were actively growing in the area of the experimental plots.

Quantifying ecological divergence

I tested whether reproductive barriers were correlated with ecological divergence by quantifying phenotypic distance in four life history traits in the common garden experiment. For each individual, I measured time to first flower, flower size, flowering stem diameter, and rosette production. In general, inland and coastal perennials exhibit larger values for each of these traits compared to annual genotypes (Friedman et al. 2015). Further, there is some evidence that these traits are under divergent selection in annual and perennial habitats. Drier habitats or years are associated with selection for earlier flowering and smaller flowers, whereas later flowering and larger flowers and flowering stems are favored in wetter habitats or years (Hall and Willis 2006; Mojica et al. 2012; see Ch. 2). Investment in rosette production is an important component of overall fitness in perennial populations (see Ch. 2), whereas seasonal drought prevents the survival of adults or vegetative clones in annual habitats (Hall and Willis 2006; Lowry et al. 2008b).

I censused all experimental individuals at 3-10 d intervals from the date of transplant to the end of the growth season (first snowfall on October 21, 2012 and drought-induced mortality of all individuals by September 27, 2013). I measured flowering time as the number of days from transplant to the first census interval with an open flower and also measured the corolla width of the first flower and the diameter of the primary flowering stem at the basal node on the same day. I measured rosette production as the number of vegetative rosettes following snowmelt the next year (May 11, 2013 and May 1, 2014). I used principal components analysis (PCA) to collapse among-population variation in each of these four traits. I transformed trait values in each year to z-scores and used the mean values for each population to estimate principal components using the princomp function in R. v. 3.1.2 (R Core Development Team 2011). I quantified divergence in these putatively adaptive traits by calculating the Euclidean distance between each immigrant population and the focal population in a phenotypic space defined by the first two principal components. The geographic distance between each immigrant population and the focal population was calculated using the earth.dist function in the fossil package in R (Vavrek 2011).

Immigrant fitness

I compared the fitness of different life-history strategies by estimating of the population growth rate, λ, of each population . This fitness measure integrated differences in survival, fecundity, vegetative growth, and the timing of reproduction to compare the mean rate of increase of different life history strategies. I used matrix projection models to estimate λ for each population and year (see Ch. 2). For annual genotypes, λ combines germination rates, seedling survival to reproduction, flower production, and ovule production per flower. For perennial genotypes, λ also incorporates adult overwinter survival and vegetative rosette production. I used bootstrapping to construct percentile confidence intervals around λ estimates.

Flowering time overlap

At each census, I recorded the number of open flowers for each individual. I estimated flowering time distributions for each population and year as the proportion of total flowers produced by that population that were open on a given census date. I estimated flowering time the overlap in flowering time between each immigrant population I and the focal population F as:

$$\sum_{j=1}^{n} \sqrt{I_j \times F_j}$$

where n is the number of census dates, I_j is the proportion of flowers produced by an immigrant population that are open on the jth census date, and F_j is the proportion of flowers produced by the focal population that are open on the jth census date. This measure ranges from zero (no overlap) to one (complete overlap).

Pollinator visitation

Annual and perennial ecotypes of *M. guttatus* are pollinated by large-bodied bees, including honey bees (*Apis mellifera*), bumblebees (*Bombus* sp.), and large-bodied solitary bees (e.g. *Osmia*, *Megachile*) (Bodbyl Roels and Kelly 2011; Martin 2004; Peterson et al. 2015; Rae and Vamosi 2012). I estimated the potential for these shared pollinators to discriminate against immigrant flowers in sympatry on the basis of UV nectar guide patterns. Although I initially selected the populations in this study without any *a priori* knowledge of UV floral variation, Peterson et al. (2015) recently found that UV floral pattern is polymorphic among populations of *M. guttatus* in central California. Further, UV nectar guide pattern is correlated with life history strategy and influences pollinator visitation. Choice trials in natural populations of *M. guttatus* and controlled experimental arrays have demonstrated that wild bees and captive *Bombus impatiens* prefer the learned, local UV pattern and discriminate against flowers with the unfamiliar UV pattern (Peterson et al. 2015). I took advantage of these results to estimate pollinator discrimination against immigrant genotypes based on UV floral patterns. I assumed that wild bees foraging in the focal population will be less likely to visit the flowers of an immigrant genotype if they have a different UV pattern than the focal population, but will not discriminate against immigrant genotypes with the same UV pattern. Although this approach ignores other floral traits that could influence pollinator choice, Peterson et al. (2015) found no effect of flower size or maternal line on pollinator visitation in choice trials. I estimated the pollinator discrimination rate from controlled choice trials using

Bombus impatiens trained on the same UV phenotype as the focal population from Peterson et al. (2015).

F1 seed production

I tested for intrinsic post-mating barriers between each population and the focal population by performing controlled crosses. In November 2013, I grew individuals from 18 maternal families from the focal population and five maternal families from each of the ten immigrant populations in the UCSC greenhouse. Maternal families had been randomly outcrossed within populations in a previous greenhouse generation. Due to differences in the timing of flowering among populations, I planted 4-6 individuals from each maternal family in two cohorts on November 8 and November 22, 2013 to increase flowering overlap. Seedlings were grown in 3.5” square pots filled with Premier Pro Mix HP potting soil and transplanted to 6” round pots as required. All individuals were grown under a 14 hr day/ 10 hr night schedule and fertilized weekly with Peters General Purpose 20-20-20 fertilizer.

I performed reciprocal crosses between families within each population and also between the focal population and each immigrant population. For each reciprocal cross, families were randomly paired and individuals within families selected each day based on flower availability. For each pollination, I collected pollen from a sire by tapping dehiscent anthers against a clean microscope slide, then used clean forceps to deposit pollen onto the receptive stigma of a freshly emasculated maternal flower. I performed 10-33 pollinations for each population pair and crossing direction and recorded the order of pollinations on a given maternal plant as a covariate. Following fruit maturation, I photographed seeds from each fruit on a white background and used ImageJ (U. S. National Institutes of Health, v1.48) to count seed set using the particle analysis function.

To test seed viability, I scored germination rates for up to 36 seeds from each fruit according to availability (mean: 18.4, sd: 2.2). For each fruit, I planted 2-4 seeds into each of 6-10 plug tray cells filled with Premier Pro Mix HP potting soil in a randomized design . Plug trays were kept in the UCSC greenhouse under a 14 hr day/ 10 hr night schedule and bottom watered to maintain saturated soil. Most germinants (> 96%) occurred within 30 d following planting, but I scored germination for a total of 60 d. I estimated total viable seed set for each fruit as the product of seed number and germination rate.

Each maternal population differed in the average number of seeds produced per fruit according to variation in ovule number per flower. Given these different distributions, I fit separate mixed models for each maternal population to estimate mean viable seed set from within and between population crosses using the nlme package (Pinheiro et al. 2007). For each maternal population, I included sire population and the pollination order as fixed effects and maternal family as a random effect. I tested the significance of fixed effects with type 3 F-tests and used planned linear contrasts to test for pairwise differences in within and between population seed set with the multcomp package (Hothorn et al. 2008). Finally, I estimated mean viable seed set given the first pollination on a maternal plant for each cross type using the lsmeans package (Lenth and Herva 2015).

Hybrid fitness

I estimated extrinsic hybrid fitness in the focal montane perennial habitat for a subset of six immigrant populations spanning all four ecotypes. Relative hybrid performance, particularly among ecologically similar populations, may depend on the history of inbreeding. Hybridization can increase relative fitness between ecologically similar or inbred populations but decrease relative fitness between ecologically divergent or outbred populations (Edmands 2002; reviewed in Waser 1993). Because *M. guttatus* has a mixed mating system, I manipulated the history of inbreeding to test whether this influenced relative hybrid performance as a function of adaptive divergence.

To manipulate the history of inbreeding, I generated outcrossed and self-fertilized lines within each of 12 families from the focal population over two greenhouse generations (Figure 3.2). In October 2011, I applied two pollination treatments to 12 individuals from separate outcrossed families: self pollen and outcross pollen from an additional, unique outcrossed family. In March 2012, I grew self and outcrossed lines from within each of these 12 families to serve as dams in crosses with each of the six immigrant populations. For each immigrant population, I grew five individuals from separate field-collected maternal families to serve as sires. For each family in the focal population, both outcrossed and selfed lines were cross-pollinated with each of the six immigrant populations to generate F1 hybrids. I pooled pollen for each pollination from 2-5 individuals from a given immigrant population by tapping dehiscent anthers on a shared microscope slide. I applied these mixed pollen loads onto receptive stigmas of freshly emasculated flowers. Montane perennial populations produce few flowers but many ovules per flower. Pooling pollen across sire individuals allowed me to generate F1 hybrid families that incorporated genetic variation in the sire population.

On May 27, 2013, I transplanted F1 hybrids and outcrossed and selfed maternal lines into the focal montane perennial habitat to assess hybrid relative fitness. Due to space constraints with the existing common garden experiment (described above), I transplanted seedlings for this experiment along two small streams also supporting montane perennial populations of *M. guttatus* within 1 mi of the common garden site. Seven families yielded the full complement of crosses in both selfed and outcrossed lines (6 F1 hybrids x 2 lines). I germinated seeds from each hybrid (N=12) and maternal line (N=2) for each of these seven families in the UCSC greenhouse and planted one seedling per treatment in a randomized position into experimental plots along the streambank (Figure 3.2). I replicated eight plots per family (8 plots x 7 families = 56 replicates per treatment). As in the common garden experiment, seedlings were transplanted into 4" round pots, watered at each census, and all flowers were emasculated to prevent genetic contamination of the native population. This breeding design allowed me to compare the fitness of each F1 hybrid with its respective selfed or outcrossed maternal line, to test whether relative hybrid fitness depends on the history of inbreeding within seven families.

I tracked the survival and flower production of experimental individuals over two growth seasons (May 26, 2013 – September 6, 2014). I collected the ovaries from second flowers in 2013 and counted ovules stained with lactophenol aniline blue under a dissecting microscope to estimate ovule number per flower. In November 2012, I left seeds from each F1 hybrid and maternal line to overwinter in plug trays and scored germination following snowmelt on May 11, 2013. Several plots were destroyed by disturbances over the course of the experiment, introducing missing data into fitness estimates and preventing the use of a single model (e.g. aster models, Shaw et al. 2008) to estimate lifetime fecundity. Instead, I fit separate mixed models to each fitness component and estimated lifetime fecundity with a bootstrapping approach. In each model, I included sire population, maternal line (outcrossed or selfed), and their interaction as fixed effects, and plot nested within family as random effects. I fit a binomial mixed model to estimate germination rates using the lme4 package (Bates et al. 2011) and linear mixed models to estimate total fruit production in each year and ovule number per flower using the nlme package (Pinheiro et al. 2007). I tested the significance of fixed effects with likelihood ratio tests for binomial models and type 3 F-tests for linear models, and constructed planned linear contrasts to test pairwise differences between each F1 hybrid and its respective maternal line using the multcomp package (Hothorn et al. 2008).

To estimate total lifetime fecundity for each F1 hybrid and maternal line, I used bootstrapping to re-sample with replacement the dataset for each fitness component while preserving the family structure. I estimated lifetime fecundity as the product of the mean germination rate, mean ovule number per flower, and the sum of the mean fruit production in 2013 and 2014 for each bootstrapped data set, and estimated the median and 95% confidence intervals over 1000 replicates. I used these values to estimate the performance of each F1 hybrid (*H*) relative to the respective maternal line from the focal population (*C*) for all reproductive isolation calculations (see eq. 1-2 below).

Estimating reproductive isolation

Following Sobel and Chen (2014), I estimated the strength of individual reproductive barriers as:

$$RI = 1 - 2\left(\frac{H}{H+C}\right) \qquad \text{eq. 1}$$

where *H* is the performance of the immigrant population and *C* is the performance of the focal population in the focal habitat. This measure is bounded by -1 and 1 to allow direct comparisons between reproductive enhancers (RI < 0) and reproductive barriers (RI > 0) (Sobel and Chen 2014). I estimated total reproductive isolation by assuming that a dispersing immigrant seed faces the following sequence of potential barriers or enhancers to gene flow: relative fitness in the focal habitat, flowering time overlap with the focal population, pollinator discrimination according to UV floral pattern, relative seed production by focal pollen, and, for the subset of populations in which it was measured, relative fitness of hybrids from outcrossed lines. I combined these barriers according to:

$$RI_{total} = 1 - 2\left(\frac{\prod H}{\prod H + \prod C}\right) \qquad \text{eq. 2}$$

where $\prod H$ and $\prod C$ are the products of each individual measure of performance of the immigrant population and focal population, respectively, through the sequence of potential barriers or enhancers (Sobel and Chen 2014). Finally, I estimated the absolute contribution of each barrier to total reproductive isolation as:

$$AC_i = RI_i - RI_{i-1} \qquad \text{eq. 3}$$

where AC_i is the absolute contribution of the ith barrier in the sequence of reproductive barriers, RI_i is the total reproductive isolation due to all barriers in the sequence up to and including the ith barrier, and RI_{i-1} is the total reproductive isolation up to but excluding the ith barrier (Sobel and Chen 2014). Positive values reflect reproductive barriers that increase total isolation, whereas negative values indicate reproductive enhancers that weaken total isolation.

Testing the effect of ecological divergence

I used linear models to test for associations between the strength of each individual reproductive barrier with phenotypic and geographic distance. I compared the AIC of nested models to identify the best-fit models for each reproductive barrier using the stepAIC function. For each barrier, I considered the effects of phenotypic and geographic distance as well as a quadratic term for phenotypic distance. I considered year (2012 and 2013) in models for immigrant fitness and flowering isolation, cross direction (immigrant population as dam or sire) in models for seed production, and maternal line (outcrossed or selfed) in models for hybrid fitness. In all cases, geographic distance did not improve model fit whereas either linear or quadratic terms for phenotypic distance were retained in the best fit models (see results). Finally, I tested whether total reproductive isolation increases with phenotypic distance using two estimates of RI_{total}: estimates through seed production for all ten immigrant populations and through hybrid fitness for a subset of six immigrant populations.

RESULTS

Phenotypic divergence

Populations in this study group together in phenotypic space according to *a priori* ecotypes (figure 3.1A). Annual and perennial populations are separated along the first principal component axis. This life-history axis explained 52% of the phenotypic variance among populations and more positive values indicate later flowering, larger flowers, and greater overwinter survival and growth in both years and larger flowering stems in 2013. The second principal component axis separates populations within ecotype based on investment in reproductive structures and explains 24% of the overall phenotypic variance. More positive values along this second axis reflect smaller flowering stems but larger flowers. Phenotypic distance is not correlated with geographic distance from the focal population ($t = 0.32$, $df = 8$, $P = 0.76$; figure 3.1B, table 3.1).

Immigrant fitness

The relative fitness of immigrant populations in the montane perennial common garden differed among ecotypes and years (table 3.2). Although the confidence intervals around estimates of λ are broadly overlapping, life table response experiments (LTREs) found significant effects of year and ecotype on overall variation in λ in a previous study (see Ch. 2). In this study, the fast-cycling annual ecotype and the year 2013 exerted significantly negative effects on λ whereas the inland perennial ecotype and the year 2012 had significantly positive effects on λ. Relative performance in the montane perennial habitat acted as either a barrier or enhancer to gene flow, depending on the immigrant population and year, and this effect was significantly related to phenotypic distance (Figure 3.3A). RI due to immigrant fitness showed a significantly quadratic relationship with phenotypic distance, indicating that ecologically intermediate populations had the highest relative fitness in the focal habitat ($\beta = 0.28$, $P < .01$, N = 20). Indeed, relative immigrant fitness acted as a strong reproductive enhancer with the inland perennial ecotype in all population-year comparisons, but a reproductive barrier with the fast-cycling annual ecotype in 3 out of 4 population-year comparisons. The performance of the focal Eagle Meadows population was reduced in 2013 (table 3.2), decreasing RI due to immigrant fitness in this drought year ($\beta = -0.23$, $P = .04$, N = 20). RI due to immigrant fitness was not related to geographic distance in the full model ($\beta = 0.001$, t = 0.49, $P = 0.63$, N = 20; Figure 3.3B)

Flowering time overlap

The overlap in flowering time between immigrant populations and the focal population was influenced by the time to initiate flowering and the duration of flowering. In general, annual populations initiated flowering earlier in the season relative to perennial populations (table 3.2). In 2013, drought conditions advanced the flowering distributions of annuals relative to the focal population and significantly decreased flowering time overlap overall (table 3.2). However, all immigrant populations had some overlap in flowering with the focal population, and flowering time acted as a relatively weak reproductive barrier. The strength of isolation due to flowering time had a slight but statistically significant quadratic relationship with phenotypic distance ($\beta = 0.05$, $t = 3.05$, $P < .01$, $N = 20$; Figure 3.3C), indicating that flowering time isolation was greatest for the most ecologically similar montane perennial population and the most ecologically divergent fast-cycling annual populations. This pattern is driven by differences in the timing as well as overall production of flowers among life history ecotypes. Montane perennial populations produce few flowers; although the flowering time distributions of these populations were entirely nested within that of the focal population (table 3.2), the low total number of flowers significantly reduced the probability of flowering overlap on any given census. Conversely, fast-cycling annuals were isolated by their earlier and shorter flowering periods relative to the focal population (table 3.2). Drought conditions in 2013 increased the strength of flowering time isolation overall ($\beta = 0.06$, $t = 3.46$, $P < .01$, $N = 20$). RI due to flowering overlap was not related to geographic distance in the full model ($\beta = 0.0005$, $t = 0.97$, $P = 0.35$, $N = 20$; Figure 3.3D).

Pollinator visitation

Among the populations in this study, UV floral pattern was perfectly correlated with life history. Although I selected these populations with no *a priori* knowledge of UV floral patterning, I found that all six fast-cycling and robust annual populations exhibited a runway pattern in which the entire lower petal absorbed UV light whereas all four inland and montane perennial populations exhibited a bulls-eye pattern in which UV absorption was limited to the corolla throat (Peterson et al. 2015). In a previous experiment, *Bombus impatiens* bees that had been trained to forage on the bulls-eye UV pattern chose to visit bulls-eye flowers over runway flowers in 69% of choice trials, regardless of plant maternal line or relative flower size (Peterson et al., 2015). Since the native focal population had a bulls-eye UV pattern, I used this value to estimate that a rare immigrant flower with the runway UV pattern would have a 0.31 probability of being visited by a pollinator and receiving focal pollen relative to an immigrant flower with the native bulls-eye UV pattern. This results in a moderate reproductive barrier of 0.38 between the focal population and each fast-cycling or robust annual population.

F1 seed production

When an immigrant population received pollen from the focal population, the effect on viable seed set differed among ecotypes. Viable seed set decreased in crosses with all annual and inland perennial populations, and this reduction was statistically significant in 7 out of 8 populations (table 3.3). Alternatively, viable seed set increased significantly in crosses with both montane perennial populations (table 3.3). I found a similar, but weaker, pattern when the focal population received pollen from each immigrant population. Viable seed set was reduced in crosses between the focal population and all annual and inland perennial populations, although this reduction was only statistically significant for 5 out of 8 populations (table 3.4).

There was no significant difference in seed set in crosses between the focal and other montane perennial populations (table 3.4). Relative viable seed set acted as a reproductive barrier between the focal population and all other ecotypes, but a reproductive enhancer or weak barrier with other montane perennial populations (Figure 3.3E). The strength of reproductive isolation due to seed set had a significantly quadratic relationship with phenotypic distance ($\beta = -0.20$, $t = -2.19$, $P = 0.04$, $N = 20$), indicating that this barrier was strongest with ecologically intermediate populations (Figure 3.3E). There was no significant effect of crossing direction ($\beta = -0.07$, $t = -0.66$, $P = 0.52$, $N = 20$) or geographic distance in the full model ($\beta = -0.001$, $t = -0.32$, $P = 0.75$, $N = 20$; Figure 3.3F).

Hybrid fitness

F1 hybrids from all crosses outperformed focal maternal lines in a montane perennial habitat, indicating heterosis (Figure 3.3G). However, the effects of sire population and maternal line breeding history varied among individual fitness components. Fruit production in both years and ovule number per flower depended on sire population (Fruit 2013: $F = 7.23$, df1=7, df2 = 605, $P < 0.01$, $N = 674$; Fruit 2014: $F = 3.24$, df1 = 7, df2 = 354, $P < 0.01$, $N = 408$; Ovule: $F = 37.53$, df1 = 7, df2 = 137, $P < 0.01$, $N = 194$), whereas maternal line breeding history did not have an overall effect on these fitness components (Fruit 2013: $F = 0.06$, df1 = 1, df2 = 605, $P = 0.81$, $N = 674$; Fruit 2014: $F = 1.60$, df1 = 1, df2 = 354, $P = 0.21$, $N = 408$; Ovule: $F = 0.11$, df1 = 1, df2 = 137, $P = 0.74$, $N = 194$). Seed overwinter survival and germination was determined by maternal line breeding history and its interaction with sire population (Maternal: $\chi^2 = 3.79$, df = 1, $P < 0.05$, N = 843; Sire: $\chi^2 = 7.92$, df = 6, $P = 0.24$, N = 843; Interaction: $\chi^2 = 19.7$, df = 6, $P < 0.01$, N = 843). Selfed maternal lines had marginally lower germination rates than outcrossed maternal lines ($z = 2.63$, $P = .09$), altering the relative performance of hybrids. F1 hybrids from outcrossed maternal lines had lower germination rates whereas hybrids from selfed maternal lines had higher germination rates compared to their respective parents (although this difference was only significant in 2 out of 12 pairwise comparisons; table 3.5). A similar pattern was found for ovule production per flower. Selfed maternal lines produced marginally fewer ovules per flower compared to outcrossed maternal lines ($z = 2.47$, $P = 0.10$), causing relative ovule production to decrease in hybrids from outcrossed lines and increase in hybrids from selfed lines (although only 1 out of 12 pairwise comparisons was significant, table 3.5).

Combining these various fitness components through bootstrapping clarified the net effect of maternal line and sire population on relative hybrid lifetime fitness. In all crosses, relative hybrid fitness acted as a reproductive enhancer and the magnitude of this effect was marginally greater for crosses with ecologically intermediate immigrant populations ($\beta = 0.14$, t = 2.21, $P = 0.06$, N = 12; Figure 3.3G). Further, reproductive isolation due to hybrid fitness was decreased in maternal lines that had been previously self-fertilized ($\beta = -0.24$, t = -3.13, $P = 0.01$, N = 12), indicating that a history of inbreeding increased heterosis. Reproductive isolation due to hybrid fitness was not correlated with geographic distance in the full model ($\beta = 0.001$, t = 0.63, $P = 0.55$, N = 12; Figure 3.3H).

Reproductive isolation and ecological divergence

Total reproductive isolation across a range of phenotypic divergence reflected a mix of reproductive barriers and enhancers, some of which differed between years or inbreeding history (Figure 3.3). Reproductive enhancers tended to be fitness-related and were strongest in ecologically intermediate populations (Figure 3.4). Conversely, reproductive barriers included flowering time and pollinator isolation as well as relative seed set. In general, individual barriers tended to contribute similarly to total isolation within ecotypes (Figure 3.4).

Total reproductive isolation increased with phenotypic distance (Figure 3.5). Total reproductive isolation up to viable seed set increased linearly with phenotypic distance ($\beta = 0.28$, $t = 4.56$, $P < 0.01$, Figure 3.5A). Similarly, when hybrid fitness was incorporated for a subset of populations, total isolation was reduced overall but still increased with phenotypic distance ($\beta = 0.42$, $t = 5.06$, $P < 0.01$, Figure 3.5B). Year had no significant effect in either model (All populations: $\beta = -0.09$, $t = -1.01$, $P = 0.33$; Subset: $\beta = -0.11$, $t = -0.88$, $P = 0.40$)

DISCUSSION

I found a strong correlation between total reproductive isolation and phenotypic divergence across a life-history continuum in *M. guttatus*. This pattern provides strong support for the role of ecological divergence in the initial evolution of reproductive isolation. Most individual reproductive barriers did not accumulate linearly with phenotypic divergence, and total isolation was achieved through different reproductive barriers and enhancers among ecotypes. This study supports the principal prediction of ecological speciation theory that increasing ecological divergence generates increasing reproductive isolation. However, it also highlights the complexity of the mosaic of reproductive barriers and enhancers operating during the early stages of divergence.

How do individual barriers evolve with ecological divergence?

The speciation process is best viewed as a continuum (Clausen 1951; Dobzhansky 1951; Grant 1981; Hendry et al. 2009; Mallet 2008; Nosil et al. 2009). During the early stages of ecological divergence, populations may be characterized by a mosaic of weak and reversible reproductive barriers and enhancers (Hendry 2009). The evolution of good biological species requires strong and irreversible barriers to gene flow. Various empirical systems have been suggested as examples of stages along the speciation continuum (Hendry 2009), and a gradual decrease in gene flow has been documented among taxa within pea aphids (Peccoud et al. 2009), *Heliconius* butterflies (Mallet et al. 2007), and lake-stream stickleback (Berner et al. 2009). Yet few studies have addressed how multiple barriers to gene flow evolve with increasing ecological divergence within a single species.

I found strong support for the speciation continuum conceptual framework. Using a montane perennial focal population, I found that ecologically intermediate inland perennial populations are characterized by a mosaic of reproductive barriers and enhancers that, together, result in weak or absent reproductive isolation. With increasing ecological divergence, annual populations exhibit fewer and weaker reproductive enhancers and stronger reproductive barriers, resulting in moderate isolation. It is important to note that this study measured reproductive barriers that would operate in sympatry following immigrant dispersal. Ecogeographic isolation among these ecotypes is likely an important barrier preventing such dispersal events (e.g. Sobel 2014), and was likely crucial in allowing the initial evolution of ecological divergence.

Interestingly, the strength of most individual barriers did not increase linearly with ecological divergence. Rather, weak isolation with ecologically intermediate inland perennials was the net result of strong barriers through seed set and strong enhancers through immigrant and hybrid fitness advantages. To the extent that these barriers are genetically based, this suggests that selection could rapidly increase or decrease net isolation in ecologically intermediate populations by altering these individual components. For example, the fitness advantage of hybrids could result in the purging of alleles underlying crossing barriers, given secondary contact. Alternatively, this partial crossing barrier could become stronger over time in allopatry, completely preventing the production of hybrids.

Fitness trade-offs and ecological speciation

Ecological speciation is often defined as the by-product of reciprocal local adaptation to divergent selective environments, and fitness-related reproductive barriers (e.g. selection against immigrants and hybrids) are an integral component of the ecological speciation framework (Nosil et al. 2005; Rundle and Nosil 2005). These barriers are predicted to evolve early in the speciation continuum and to be highly correlated with adaptive divergence, whereas other ecological or intrinsic barriers are predicted to evolve later or more sporadically (Nosil 2012). However, reviews of the local adaptation literature have found that reciprocal fitness trade-offs are relatively rare (Hereford 2009; Leimu and Fischer 2008). Immigrant genotypes may enjoy a fitness advantage due to maternal effects (Stanton and Galen 1997), sexual selection (Ellers and Boggs 2003), inbreeding depression (Leimu and Fischer 2008), or local adaptation of pathogens or antagonists (Crémieux et al. 2008; Summers et al. 2003). Although these factors would swamp adaptive divergence if gene flow is high (Anderson and Geber 2010; e.g. Stanton and Galen 1997), divergent selective environments are often at least partially isolated geographically (Mayr 1947). Whether strong ecological isolation can evolve in the absence of reciprocal fitness trade-offs, particularly early in the divergence process, is an intriguing question. Indeed, ecology may play a much more general role in the speciation process than those conditions usually considered within the ecological speciation framework (Sobel et al. 2010).

In this study, I found little evidence for immigrant inviability relative to the focal population. The montane perennial ecotype produces few flowers and relies on overwinter survival and vegetative growth. Inland perennial populations had high overwinter survival and growth while also producing more flowers, resulting in a net fitness advantage. Similarly, some robust annual populations were able to produce enough flowers over the extended growth season in this mesic habitat to outperform the focal population. Conversely, fast-cycling annuals appeared developmentally unable to exploit the longer growth season in this habitat, flowering earlier at a small size and producing few flowers (see Ch. 2). The relatively low performance of the montane perennial ecotype could reflect inbreeding depression and/or a bet-hedging strategy that prioritizes adult persistence through below-ground rhizomes at the expense of reproductive output in any given year (see Ch. 2). Together, the relative performance of both immigrants and hybrids was quadratic with phenotypic divergence, suggesting that ecologically intermediate genotypes enjoy a fitness advantage whereas increasing phenotypic divergence results in immigrant inviability or, potentially, reduced hybrid performance. This pattern may be common during the early stages of ecological divergence due interactions between inbreeding depression and divergent adaptation (reviewed in Waser 1993). I found strong support for this hypothesis by manipulating the inbreeding history prior to hybridization, suggesting that, in many systems, selection against immigrants and hybrids may not be the earliest or strongest barriers to evolve. Indeed, several studies have failed to find selection against immigrants (Forister 2004; Gosden et al. 2015; Räsänen and Hendry 2014) or hybrids (Lowry et al. 2008b) even when other ecological barriers are present.

Linking reproductive barriers to divergent selection in *Mimulus*

Several lines of evidence suggest that the traits underlying reproductive isolation in this study are the result of divergent selection between annual and perennial habitats. The trade-off between rapid flowering and vegetative growth is a major axis of phenotypic divergence in this species complex that is determined by multiple pleiotropic QTL (Friedman 2014; Friedman et al. 2015). Early flowering is under strong selection in fast-drying annual habitats, whereas later flowering and/or greater vegetative growth are favored in coastal and montane perennial habitats (Hall and Willis 2006; see Ch. 2). Correspondingly, flowering time isolation in immigrant populations was greater for annuals than inland perennials, whereas immigrant and hybrid fitness was greatest for inland perennials that invest in vegetative growth.

Crossing barriers were strongest with ecologically intermediate populations. Reduced viable seed set could be due to pollen-pistil incompatibilities preventing fertilization or early-acting genetic incompatibilities in developing hybrid seeds. Although the traits driving these barriers are as yet unknown, the relationship between crossing barrier strength and phenotypic divergence suggests a role for natural selection. Crossing barriers with the focal population were stronger in inland perennial populations than annual populations, and this pattern could reflect differences in life history strategy. Fecundity selection is strong in annual populations but weaker in perennial populations, where other components of fitness including survival and growth also shape the pattern of selection (see Ch. 2). Relaxed fecundity selection in perennial populations could allow more rapid genetic divergence in loci related to pollen-pistil interactions or seed development, potentially increasing the likelihood of incompatibilities. Future research should identify the mechanisms underlying crossing barriers to test this hypothesis.

Progress along the speciation continuum

What determines whether partially isolated taxa will evolve into good species, remain partially isolated ecotypes, or collapse into a single population?

Parallel ecological contrasts have yielded variable progress toward genetic isolation among White Sands lizards (Rosenblum and Harmon 2011), lake-stream stickleback (Berner et al. 2009; Berner et al. 2010), insect host-races (Peccoud et al. 2009; Stireman et al. 2005), and mimetic butterflies (Merrill et al. 2011). Several hypotheses have been developed to explain variation in progress toward speciation, including the strength and consistency of selection, the number of traits or loci mediating adaptation or isolation, and opportunities for gene flow and reinforcement (e.g. Coyne and Orr 1989; Hendry 2009; Kondrashov and Kondrashov 1999; Nosil et al. 2009). I found no evidence that reproductive isolation was related to geographic distance, a proxy for opportunities for gene flow. Alternatively, individual barriers and total reproductive isolation with a focal montane perennial population were correlated with phenotypic divergence. Yet, the strength of total isolation was still incomplete and dependent on environmental variation between years as well as the history of inbreeding. Reproductive isolation with a montane perennial population is weaker than reproductive isolation between annual and coastal perennial ecotypes in this species (Lowry et al. 2008b). In annual populations, late-flowering coastal perennial genotypes face nearly complete immigrant inviability due to their inability to reproduce prior to the onset of drought. In coastal habitats, annual genotypes have reduced fitness due to salt spray and flower earlier than the native coastal perennial genotypes, while F1 hybrids exhibit strong heterosis. Together, reproductive isolation in these habitats ranges from 0.93 – 1.00 (from eq. 2 based on values in Lowry et al.

2008b), exceeding estimates from this study which ranged from 0.60 – 0.76 for annual populations including hybrid fitness.

The differences in reproductive isolation among these life history ecotypes suggests that strong viability selection early in the life-cycle, due to drought or salt spray, is an important factor in the rapid evolution of strong reproductive isolation. Drought avoidance traits are highly correlated among populations of *M. guttatus* (Friedman et al. 2015; Kooyers et al. 2015), providing support for the view that strong selection on few traits, such as rapid development or salt tolerance, promotes speciation (Nosil et al. 2009). However, quantitative genetic studies have demonstrated a polygenic basis for both flowering time and salt tolerance in this species (Friedman 2014; Hall et al. 2010; Lowry et al. 2009), such that selection on individual loci may be weaker. Negative pleiotropy between early flowering and vegetative rosette production could generate fitness trade-offs between annual and perennial habitats (Friedman et al. 2015; Hall et al. 2010), whereas Lowry et al. (2009) found no fitness costs for salt tolerance QTL in an annual habitat. Thus, although conceptually useful, distinguishing between the stronger selection and multifarious selection hypotheses may be difficult in practice.

Summary

Ecotypic divergence in *M. guttatus* meets the expected conditions for ecological speciation. Different habitats support phenotypically discrete ecotypes and exert divergent selection on traits, such as flowering time or life history strategy, related to adaptation and reproduction. As predicted by ecological speciation theory, total reproductive isolation increases linearly with phenotypic divergence. Yet, total isolation is achieved by a mix of reproductive barriers and enhancers that do not themselves generally increase with phenotypic divergence. Rather, each barrier exhibits a unique, often quadratic, relationship with adaptive divergence. Surprisingly, immigrant and hybrid fitness is most often a reproductive enhancer and there is little evidence for fitness trade-offs despite extensive phenotypic and life history divergence. This study suggests that partial reproductive isolation can evolve under divergent selection even in the absence of strong fitness trade-offs, particularly when selective environments are geographically separated. Further, this study supports the view that early stages of divergence will be characterized by a mosaic of partial barriers and enhancers which interact with environmental, genetic, and demographic factors to either strengthen or ameliorate total reproductive isolation. Interestingly, despite these diverse relationships between individual barriers and ecological divergence, total reproductive isolation showed the predicted positive correlation. The underlying mechanisms may be diverse within and among species, but the overall pattern of reproductive isolation may be general, as suggested by meta-analyses using molecular signatures of gene flow (Sexton et al. 2014Sexton et al. 2014; Shafer and Wolf 2013).

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Table 3.1: Locality and life history information for all populations of *M. guttatus* in this study. Phenotypic and geographic (km) distances between each of the 10 immigrant populations and the Eagle Meadows focal population.

Ecotype	Population	Elevation (masl)	Latitude	Longitude	Habitat	Phenotypic distance	Geographic distance
Fast-cycling annual	Red Hills	313	37.857	-120.457	Serpentine seep	4.27	69.73
	Bald Mountain	1693	38.137	-120.094	Gravelly seep	4.34	25.41
Robust annual	Peoria Basin	293	37.933	-120.520	Creek in grassland	3.83	67.89
	Traverse Creek	681	38.873	-120.818	Serpentine creek	3.37	99.38
	Sprague Rd	842	37.819	-120.152	Creek in woodland	3.23	59.31
	Big Oak Flat	1225	37.829	-119.958	Marshy meadow	3.22	54.71
Inland perennial	Kyburz	1371	38.768	-120.290	Stream in pine forest	3.03	53.53
	Girard Creek	1515	38.731	-120.240	Stream in pine forest	2.69	59.32
Montane perennial	Silver Fork	1959	38.664	-120.219	Stream and meadow	1.93	46.28
	Silver Creek	2066	38.588	-119.786	Marshy meadow	2.56	32.01
	Eagle Meadows	2046	38.320	-119.920	Stream and meadow	----	----

Table 3.2: Fitness and flowering distributions of immigrant and focal populations of *M. guttatus* in the focal montane perennial habitat over two years. Fitness (λ) estimates and 95% confidence intervals in parentheses from bootstrapped datasets within each year (see ch. 2 for details). Flowering distributions for each population: the days to peak flower abundance and the first and last open flower (i.e. flowering range) in parentheses. Note: flowering time is given in days from transplant date – values of 1 indicate at least one rapidly developing seedling with an open flower at the time of transplant for that population. Overlap between the flowering distributions of the immigrant population and the focal Eagle Meadows population, from 0 (no overlap) to 1 (complete overlap).

		2012			**2013**		
Ecotype	**Population**	**λ (95% CI)**	**Peak flower (range)**	**Flower overlap**	**λ (95% CI)**	**Peak flower (range)**	**Flower overlap**
Fast-cycling annual	Red Hills	0.80 (0.38, 1.51)	34 (13, 76)	0.64	0.27 (0.22, 0.35)	13 (1, 64)	0.66
Robust annual	Bald Mountain	0.28 (0.13, 0.55)	34 (13, 60)	0.63	0.84 (0.35, 1.58)	46 (1, 74)	0.58
	Peoria Basin	2.76 (0.93, 5.02)	53 (13, 76)	0.80	0.58 (0.26, 1.04)	46 (1, 74)	0.68
	Traverse Creek	1.49 (0.53, 2.94)	34 (20, 76)	0.70	0.45 (0.23, 0.81)	55 (13, 74)	0.66
	Sprague Rd	1.34 (0.44, 2.88)	66 (25, 105)	0.82	0.77 (0.32, 1.44)	46 (13, 74)	0.68
Inland perennial	Big Oak Flat	2.15 (0.70,4.29)	53 (25, 76)	0.85	0.85 (0.33, 1.63)	46 (13, 74)	0.57
	Kyburz	2.58 (1.21, 4.50)	53 (39, 76)	0.79	0.94 (0.42, 1.65)	55 (33, 74)	0.73
	Girard Creek	3.38 (1.41, 6.01)	53 (25, 66)	0.82	1.12 (0.43, 2.19)	55 (23, 74)	0.76

Montane perennial	Silver Fork	0.95 (0.36, 1.87)	44 (44, 53)	0.75	0.36 (0.24, 0.75)	55 (33, 64)	0.57
	Silver Creek	0.82 (0.40, 1.62)	53 (39, 53)	0.80	0.55 (0.33, 1.41)	64 (23, 74)	0.78
Focal	Eagle Meadows	1.64 (0.50, 3.46)	53 (39, 105)	1.00	0.45 (0.32, 0.77)	55 (13, 74)	1.00

Table 3.3: Mean viable seed set for each immigrant population of *M. guttatus*, when receiving within-population pollen or pollen from the focal Eagle Meadows population. Values are the LS means and standard errors for viable seed set estimated for the first pollination on a maternal plant. *F* statistics, df, and *P*-values for the main effect of pollen source (within or focal) in separate models fit for each immigrant population (see methods).

Ecotype	**Population**	**Within (SE)**	**Focal (SE)**	***F*-value (df_1, df_2)**	***P*-value**
Fast-cycling annual	Red Hills	129.2 (43.1)	74.3 (42.3)	6.21 (2, 25)	.007
	Bald Mountain	195.1 (41.6)	115.7 (40.9)	9.51 (2, 23)	.001
Robust annual	Peoria Basin	932.3 (87.2)	654.8 (86.0)	40.58 (2, 23)	<.001
	Traverse Creek	300.6 (56.3)	146.1 (46.8)	10.90 (2, 27)	<.001
	Sprague Rd	223.2 (71.0)	51.4 (65.6)	2.26 (2, 25)	.126
	Big Oak Flat	215.1 (30.5)	73.7 (28.3)	21.31 (2, 40)	<.001
Inland perennial	Kyburz	358.4 (58.7)	72.3 (56.2)	27.42 (2, 43)	<.001
	Girard Creek	449.2 (50.2)	65.4 (56.3)	54.87 (2, 34)	<.001
Montane perennial	Silver Fork	326.5 (149.9)	596.3 (107.2)	7.86 (2, 21)	.003
	Silver Creek	466.5 (128.9)	568.5 (108.7)	12.56 (2, 47)	<.001

Table 3.4: Mean viable seed set for the focal population of *M. guttatus* when receiving pollen from each of the immigrant populations or in within-population crosses. Values are the LS means and standard errors for viable seed set estimated for the first pollination on a maternal plant. *Z* statistics and *P*-values from planned contrasts of seed set from crosses with each immigrant population relative to seed set from within-population crosses.

Ecotype	**Population**	**Viable seed (SE)**	***z*-value**	***P*-value**
Fast-cycling annual	Red Hills	187.2 (49.5)	-3.45	.005
	Bald Mountain	206.8 (52.7)	-3.05	.019
Robust annual	Peoria Basin	314.1 (49.1)	-1.36	.728
	Traverse Creek	295.2 (52.1)	-1.64	.509
	Sprague Rd	343.6 (48.9)	-0.88	.971
	Big Oak Flat	169.9 (47.4)	-3.94	<.001
Inland perennial	Kyburz	176.8 (45.1)	-3.84	.001
	Girard Creek	100.7 (45.6)	-5.24	<.001
Montane perennial	Silver Fork	419.6 (49.8)	0.41	1.000
	Silver Creek	307.6 (41.8)	-1.60	.538
	Within-focal	394.8 (42.5)	---	---

Table 3.5: Hybrid fitness components in a montane perennial habitat between six immigrant populations and either outcrossed or self-fertilized maternal lines from the focal population of *M. guttatus*. For each component of fitness, means and standard errors (in parentheses) for each hybrid and maternal line. *Z*- statistics and *P*-values from planned linear contrasts between each hybrid and its respective maternal line and between the two maternal lines. Significant contrasts (*P* < .05) are in bold.

			Outcrossed			**Self-fertilized**		
Ecotype	**Population**	**Fitness**	**Mean**	***Z***	***P***	**Mean**	***Z***	***P***
Fast-cycling annual	Red Hills	Germination	0.19 (0.06)	-2.54	.11	0.40 (0.10)	1.70	.60
Robust annual	Traverse Creek		0.25 (0.05)	-2.22	.24	0.30 (0.07)	0.96	.98
Inland perennial	Kyburz		0.23 (0.05)	-2.46	.14	0.34 (0.07)	1.57	.70
	Girard Creek		0.33 (0.06)	-1.07	.96	0.41 (0.09)	2.06	.33
Montane perennial	Silver Fork		0.24 (0.06)	-2.16	.27	**0.51 (0.08)**	**3.21**	**.02**
	Silver Creek		0.34 (0.06)	-0.96	.98	**0.46 (0.07)**	**2.86**	**<.05**
	Maternal line		0.42 (0.06)	---	---	0.22 (0.05)	2.63	.09
Fast-cycling annual	Red Hills	2013 Fruits	**1.92 (0.42)**	**2.85**	**.04**	2.14 (0.44)	2.34	.17
Robust annual	Traverse Creek		**1.97 (0.42)**	**2.95**	**.03**	**2.82 (0.42)**	**3.71**	**<.01**
Inland perennial	Kyburz		1.73 (0.41)	2.53	.10	1.71 (0.43)	1.56	.66
	Girard Creek		1.73 (0.41)	2.55	.10	2.10 (0.42)	2.35	.17
Montane perennial	Silver Fork		0.56 (0.42)	0.27	1.00	0.61 (0.42)	-0.55	1.00

	Silver Creek		0.46 (0.42)	0.07	1.00	0.55 (0.41)	-0.68	1.00
	Maternal line		0.42 (0.44)	---	---	0.89 (0.43)	-0.89	.99
Fast-cycling annual	Red Hills	2014 Fruits	2.19 (0.74)	1.96	.37	0.78 (0.83)	0.16	1.00
Robust annual	Traverse Creek		0.53 (0.72)	.39	1.00	0.62 (0.76)	0	1.00
Inland perennial	Kyburz		1.74 (0.72)	1.56	.66	2.09 (0.76)	1.46	.74
	Girard Creek		2.21 (0.72)	2.02	.33	2.07 (0.77)	1.42	.76
Montane perennial	Silver Fork		1.14 (0.74	0.96	.98	0.30 (0.80)	-0.31	1.00
	Silver Creek		1.55 (0.73)	1.36	.81	1.10 (0.74)	.48	1.00
	Maternal line		0.13 (0.81)	---	---	0.62 (0.74)	-0.46	1.00
Fast-cycling annual	Red Hills	Ovules	**454 (48)**	**-3.61**	**<.01**	498 (53)	0.04	1.00
Robust annual	Traverse Creek		550 (50)	-2.57	.07	584 (53)	0.94	.93
Inland perennial	Kyburz		529 (53)	-2.69	.05	518 (55)	0.24	1.00
	Girard Creek		581 (62)	-2.08	.23	556 (50)	0.66	.99
Montane perennial	Silver Fork		645 (66)	-1.39	.66	691 (69)	1.83	.36
	Silver Creek		521 (89)	-2.23	.17	569 (62)	0.74	.98
	Maternal line		789 (90)	---	---	494 (90)	2.47	.10

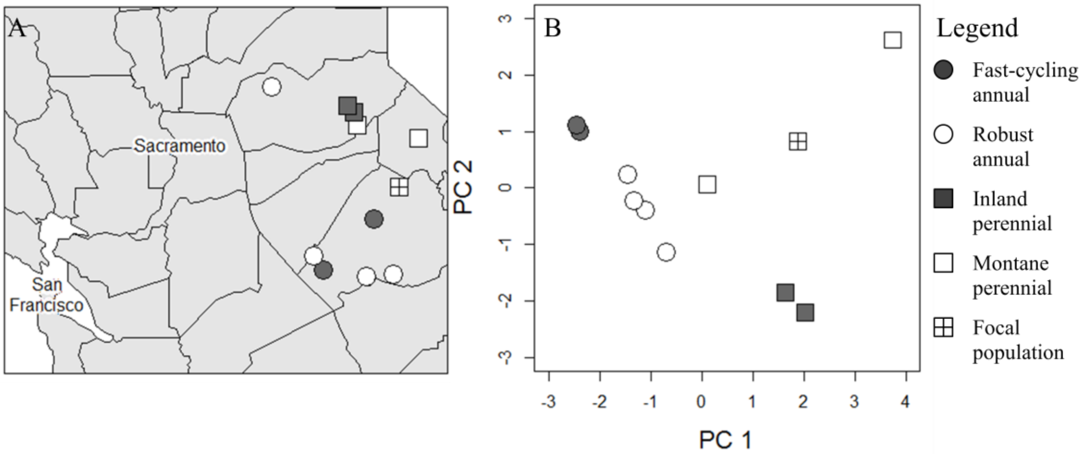


Figure 3.1: Geographic and phenotypic locations of ten immigrant populations relative to the focal montane perennial population of *M. guttatus*. A) Map of central California illustrating the geographic locations of all immigrant populations and the site of the common garden experiment within the focal population. B) Populations in phenotypic space defined by the first two axes of a principle component analysis. PC1 reflects a life-history axis from early flowering, small flowered annual plants (negative scores) to late flowering, large flowered perennial plants with increased rosette production (positive scores). PC 2 reflects a reproductive allocation axis from plants with large flowering stems and smaller flowers (negative scores) to plants with small flowering stems and larger flowers (positive scores).

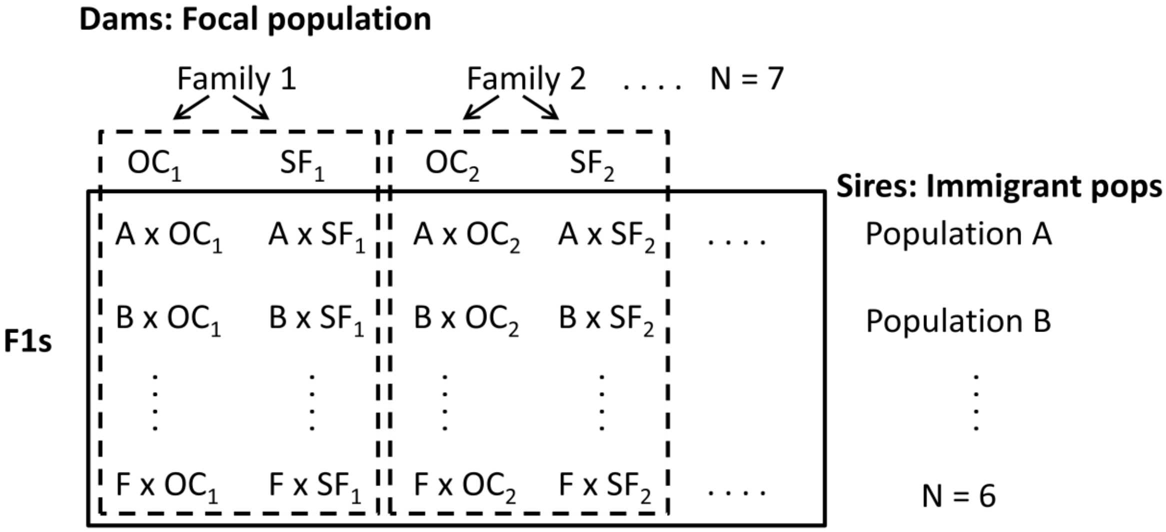
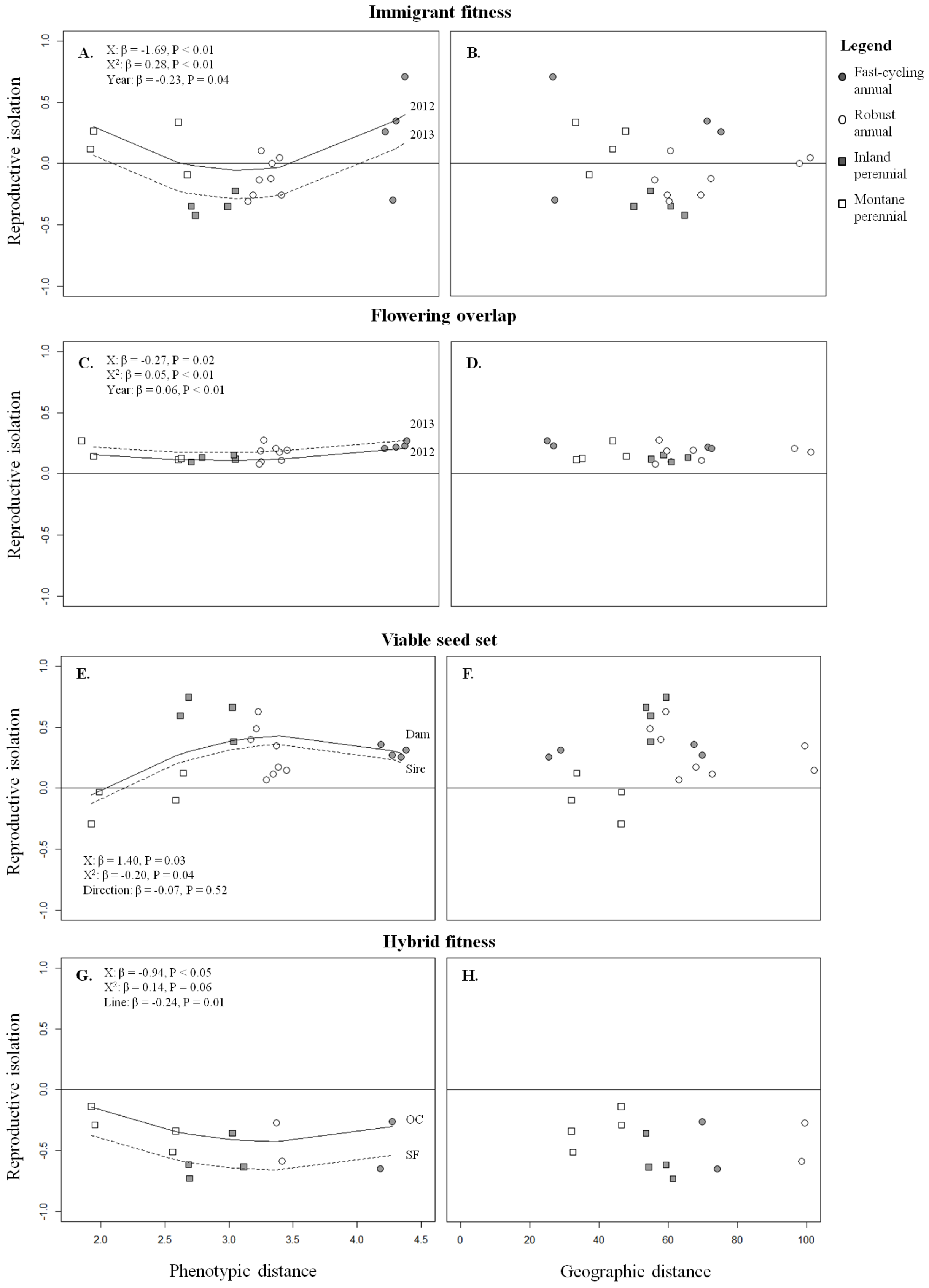


Figure 3.2: Breeding design used to manipulate inbreeding history and generate F1 hybrids between populations of *M. guttatus*. Maternal families from the focal population (top) were outcrossed and self-fertilized to create OC and SF lines within each family. These lines served as dams in crosses with each of 6 immigrant populations, which served as sires (right). Resulting F1s are given in the solid-lined center box. Dashed boxes delimit the blocking design used in the field to compare the F1 hybrids and maternal lines derived from within a focal family. All treatments for each family (dashed box) were replicated in 8 blocks in the common garden field experiment (see methods).


Immigrant fitness

Flowering overlap

Viable seed set

Hybrid fitness

Figure 3.3: Individual reproductive barriers are associated with phenotypic distance but not geographic distance. Data are the strengths of individual reproductive barriers for each immigrant population with the focal population according to eq. 1. Negative values of RI indicate reproductive enhancers whereas positive values indicate reproductive barriers. The estimates and P-values are given for the coefficients (β) in the regression $RI = \beta_0 + \beta_1 X + \beta_2 X^2 + \beta_3 Z$ where X is the phenotypic distance and Z is a categorical variable, such as year or crossing direction. Geographic distance was not a significant predictor of RI in any model. Phenotypic distance is the Euclidean distance between each immigrant population and the focal population in PC1 and PC2 coordinate space. Geographic distance is shortest distance in km between each immigrant population and the focal population. Note: points are jittered along the x-axis by up to 0.1 units phenotypic distance or 5 units geographic distance for clarity.

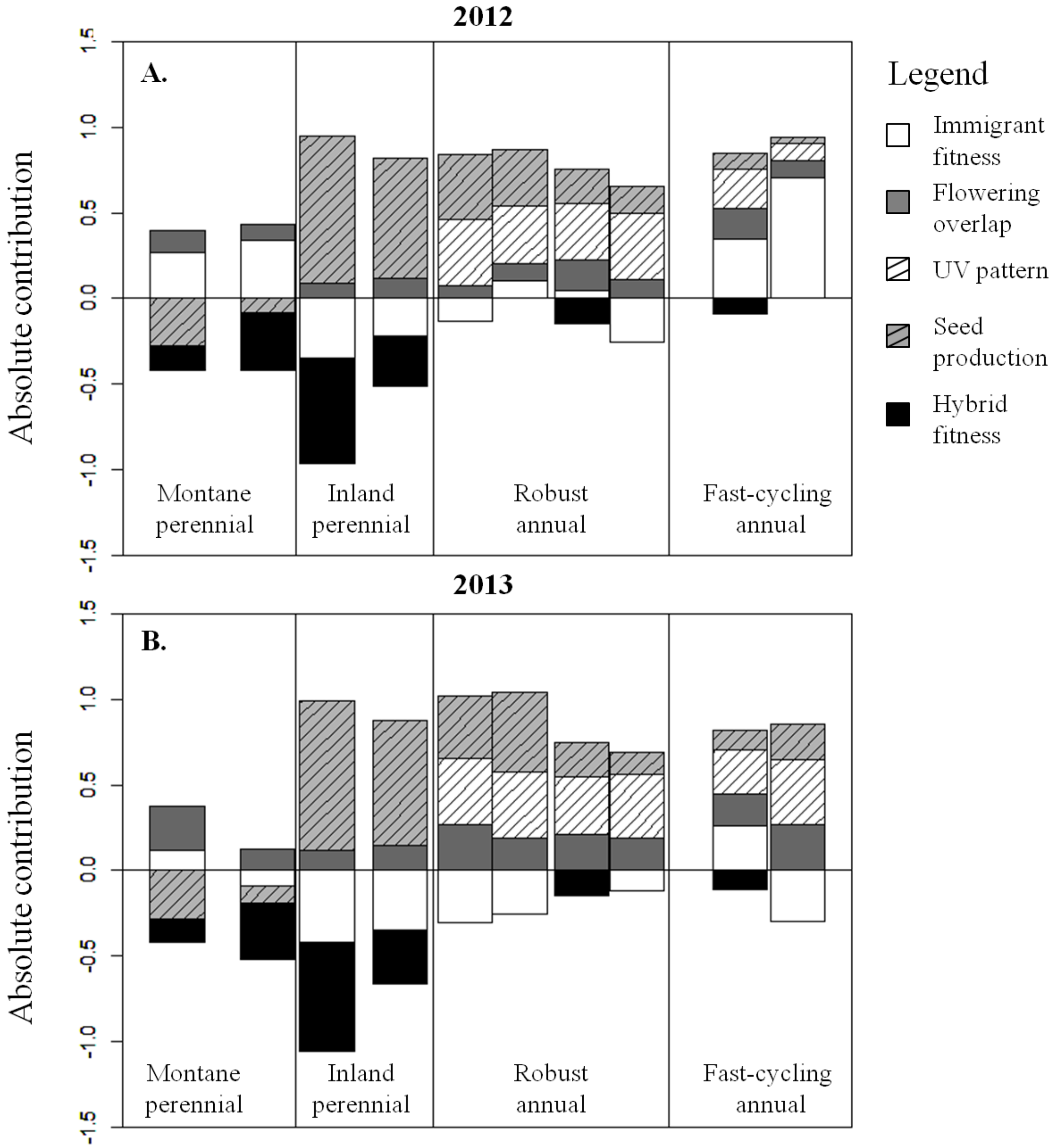


Figure 3.4: Absolute contributions of each reproductive barrier to total reproductive isolation in (A) 2012 and (B) 2013. Bars represent each immigrant population of *M. guttatus* where position along the x-axis (from left to right) is proportional to phenotypic distance from the focal population. Positive contributions indicate reproductive barriers, negative contributions indicate reproductive enhancers. Note: hybrid fitness (black) was only measured for six of the populations, and values shown here are based on outcrossed maternal lines (see methods).

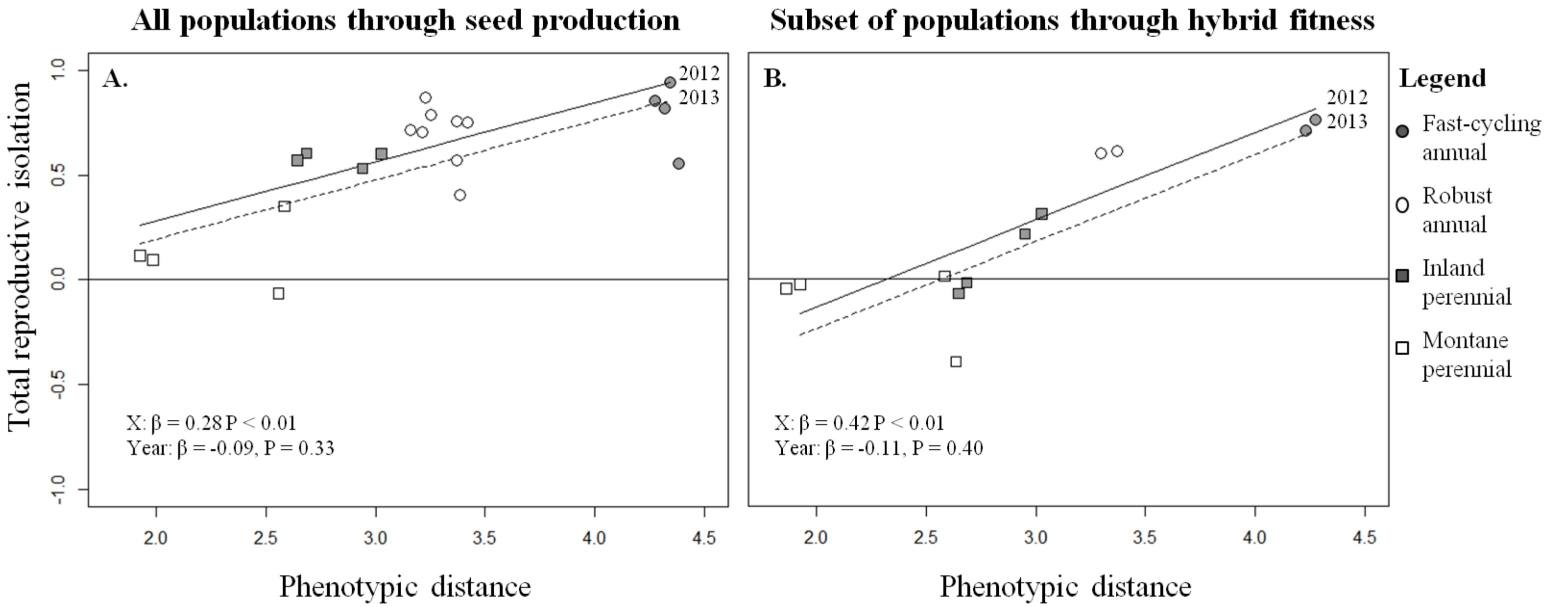


Figure 3.5: Total estimated reproductive isolation increases with phenotypic distance in *M. guttatus*. Data are the estimates of total reproductive isolation according to eq. 2 for each immigrant population in each year for A) all barriers through F1 seed production, estimated for all 10 immigrant populations, and B) all barriers through F1 hybrid fitness, estimated for six of the immigrant populations. The estimates and P-values are given for the coefficients (β) in the regression $RI_{total} = \beta_0 + \beta_1 X + \beta_2 Z$ where X is the phenotypic distance and Z is year. Note: points are jittered along the x-axis by up to 0.1 units for clarity.

SYNTHESIS

In this dissertation, I addressed several processes influencing the evolution of adaptive divergence and reproductive isolation between populations in different selective environments. Many new plant species arise as initially small, ecologically divergent populations (Anacker and Strauss 2014; Gottlieb 2004; Grossenbacher et al. 2014). In this context, the evolution of plant biodiversity depends on the ability of a species to colonize and persist in a new selective environment, respond to local selection pressures through adaptive evolution, and maintain genetic isolation from closely related lineages. Previous research has emphasized spatial isolation, time, and strong selection as critical components of diversification (reviewed in Coyne and Orr 2004; Nosil 2012). In this dissertation, I tested specific hypotheses regarding previously underappreciated mechanisms that may also determine progress at each of these stages. In chapter one, I tested the potential for plasticity in self-fertilization rate to promote persistence and initial adaptation in plant populations colonizing new selective environments. In chapter two, I examined how life history divergence, a frequent component of adaptive divergence within species (e.g. Moser et al. 2012; Vergeer and Kunin 2011; Walsh and Reznick 2011), can shape the selective landscape. Finally, in chapter three, I tested how multiple reproductive barriers and enhancers interact to determine reproductive isolation as a function of phenotypic divergence.

Taken together, this work highlights the potential for diverse evolutionary, ecological, and demographic forces to influence adaptive divergence and speciation. In chapter one, I found that if a novel environment increased self-fertilization, colonizing populations were more likely to persist and become locally adapted. Further, I found that this effect was most important in promoting colonization success when mates were limited, when adaptive genetic variation was low, or when gene flow from surrounding populations was high. Thus, this may be an important mechanism for allowing niche expansion, either through long-distance dispersal events or colonization of adjacent but strongly divergent selective environments, such as extreme soils. In chapter two, I demonstrated the potential for life history divergence to alter the importance of specific fitness components for overall performance, thus shaping the strength and pattern of phenotypic selection. In particular, I found that phenotypic selection in perennial populations of *M. guttatus* was weaker overall and driven by multiple components of fitness that varied between years, whereas annual populations experienced strong fecundity selection. Finally, in chapter three, I found that reproductive isolation between a focal montane perennial population and immigrant populations of *M. guttatus* increased with life history divergence, suggesting a role for ecological divergence in the initial evolution of reproductive isolation. However, I also found that reproductive isolation was achieved through a mosaic of reproductive barriers and enhancers, suggesting complex effects of life history on the evolution of specific traits.

One important theme that has emerged from this work is the influence of plant mating system and life history strategy on adaptive divergence and reproductive isolation. Botanists have long appreciated the potential for inbreeding depression to counter natural selection in driving patterns of local adaptation and hybrid fitness within plant species (Darwin 1876; Fenster and Galloway 2000; Price and Waser 1979; Stebbins 1957). Yet, this understanding has not been well integrated into the speciation literature. In this dissertation, I found that while partial autonomous reproduction can promote niche expansion (chapter one), the subsequent evolution of inbreeding depression within plant populations can potentially limit diversification by generating reproductive enhancers through immigrant and hybrid fitness advantages (chapter three). In perennial *M. guttatus*, vegetative reproduction is a key component of dispersal and population establishment (Lindsay and Vickery 1967; Truscott et al. 2006). Further, I found that the ability of perennials to reproduce vegetatively decreased the strength of fecundity selection (chapter two) and was associated with the evolution of stronger crossing barriers between populations (chapter three). Autonomous reproduction, either through self-fertilization or vegetative reproduction, could have general consequences for the evolution of different reproductive barriers (Brandvain and Haig 2005; Wright et al. 2013). For example, relaxed fecundity selection could accelerate the evolution of crossing barriers whereas inbreeding depression will weaken selection against immigrants and hybrids. Comparative tests of these barriers in closely related taxa that differ in mating system would clarify the role of autonomous reproduction in plant diversification, especially given its effects in promoting niche expansion.

The architects of the Modern Synthesis envisioned diverse mechanisms by which two lineages could become reproductively isolated, incorporating not only geographic isolation and natural selection but also genetic drift, inbreeding depression, sexual selection, hybridization, and chromosomal evolution (Dobzhansky 1951; Grant 1981; Mayr 1942; Stebbins 1950). Later, the delimitation of ecological speciation as a field motivated a renewed research effort while simultaneously narrowing the role of ecology to that of divergent selection between environments resulting in reduced immigrant and hybrid fitness (Nosil 2012; Rundle and Nosil 2005; Schluter 2001; Schluter 2009). This dissertation provides support for a more general role for ecology during speciation, as argued by Sobel et al. (2010), by highlighting the importance of initial niche expansion and the potential for reproductive isolation to evolve in the absence of strong fitness trade-offs. In chapter two, I found that although ecotypes of *M. guttatus* exhibit genetically based phenotypic and life history differences associated with local water availability, the montane perennial ecotype did not have a clear home-site fitness advantage over other ecotypes. Rather, a combination of divergent evolution and inbreeding depression has likely shaped the relative fitness of life history ecotypes in this environment. In chapter three, I demonstrated that reproductive isolation was positively associated with life history divergence, despite the fact that immigrant and hybrid genotypes often exhibited a fitness advantage relative to the focal montane perennial population. Several other studies have also failed to find fitness trade-offs or selection against hybrids between strongly isolated and ecologically divergent taxa (e.g. Gosden et al. 2015; Lowry et al. 2008; Räsänen and Hendry 2014). Thus, there is renewed support for the view that ecology shapes reproductive isolation through diverse mechanisms that can include divergent and uniform natural selection, sexual conflict, chromosomal evolution, genetic drift, and inbreeding (reviewed in Sobel et al. 2010; Templeton 2008).

Finally, this dissertation focused on the processes operating during the early stages of niche expansion and subsequent diversification within species. Yet an equally important determinant of biodiversity is the persistence of species through time. A budding speciation model posits that new plant species will arise as geographically-restricted and ecologically specialized populations (Crawford 2010; Gottlieb 2004). These conditions increase the risk of extinction, and many newly-diversified plant species in the California Floristic Province are listed as rare (Anacker and Strauss 2014). The very factors that promote speciation, including geographic isolation, ecological divergence, and autonomous reproduction, may also increase extinction risk. Alternatively, extrinsic factors such as the topographic complexity or climatic stability of a region may be stronger determinants of extinction risk, and thus diversification rates, over time (Lancaster and Kay 2013). To date, the biology of speciation and extinction have been largely separate research programs, yet these processes must be integrated to advance our understanding of the evolution of biological diversity. Eco-evolutionary demographic models allow the joint analysis of population growth, adaptation, and gene flow (e.g. Kinnison and Hairston 2007), and represent a promising avenue for predicting the persistence of actively diverging, partially isolated lineages.

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