Studies Investigating Evolutionary Transitions in Plant Reproduction

by

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Abstract

In this thesis I explore several topics related to the evolution of plant reproductive characters.

First, I consider mating system evolution at a single locus that simultaneously affects multiple fitness components, including pollen export, selfing rate, and viability (i.e., survival or a similar change in male and female function). I use two approaches. First, I assume frequency-independent mating, so the model characterizes prior selfing (Chapter 2). Second, I assume that selfing rates are determined by a "mass action" process, which characterizes several additional modes of selfing (Chapter 3). For both approaches, pleiotropy between increased viability and selfing rate reduces opportunities for the evolution of pure outcrossing, can favor complete selfing despite high inbreeding depression, and notably, can cause the evolution of mixed mating despite very high inbreeding depression. These results suggest that selection by non-pollinating agents may help explain mixed mating, particularly in species with very high inbreeding depression.

Second, I analyze the potential for different genome regions to harbor intra-locus sexually-antagonistic polymorphism. Such polymorphism, involving one allele that benefits fitness in males but decreases fitness in females, and a second allele with opposite effects, is believed to influence the evolution of sexual dimorphism and sex chromosome evolution; both have evolved repeatedly among plant lineages, so understanding the potential for sexually-antagonistic variation informs the evolution of dioecy. Numerical analyses confirm the previous major conclusion that sexually-

antagonistic polymorphisms are generally maintained in a larger region of parameter space if the locus is in the pseudo-autosomal region than if it is autosomal.

Finally, I consider the effect of two stressors on time to flowering to address hypotheses regarding the evolution of flowering time in heterogeneous environments. A greenhouse experiment using *Mimulus guttatus* revealed that low water and herbivory had opposite effects on time to flowering, although these effects were weak. These stressors had stronger influences on plant height and the number of flowers produced. These data, combined with previously published results, suggest that a stressor's effect on non-phenological traits may influence the evolution of flowering time through mechanisms not considered by previously published theoretical studies.

Preface

Each chapter in this thesis involved varying levels of contribution from numerous individuals.

I conceived and developed the model in Chapter 2, with guidance from Sally Otto. Sally Otto also devised the approach to analyze the model, and we shared its analysis. I wrote this chapter, with extensive editorial guidance from Sally Otto. My committee members and an anonymous reviewer provided comments that helped focus the discussion of this work. This chapter will be submitted as a co-authored paper with Sally Otto (second author).

I conceived, developed and analyzed and the project in Chapter 3. Sally Otto provided advice for the analysis and interpretation of this project, and checked the results; like the previous chapter, I wrote it with extensive editorial guidance from Sally Otto. Also like the previous chapter, my committee members and an anonymous reviewer provided comments that helped focus the discussion of this chapter. This chapter is being prepared for submission as a sole-authored manuscript.

I conceived the project in Chapter 4, performed all analyses, and wrote approximately 40% of this chapter; Deborah Charlesworth wrote the remainder. Brian Charlesworth and Sally Otto provided discussion and comments that clarified this work. This chapter has been accepted in the journal Evolution (with revisions); I am the primary author on this work, and Deborah Charlesworth is the second author. My committee members provided minor comments on the presentation of this chapter.

The experiment reported in Chapter 5 was designed with equal contri-

butions from myself, Dilara Ally and Kay Hodgins; the Otto lab group provided suggestions for experimental design. Dilara Ally, Kay Hodgins and I shared equally in the construction of the genetic lines for this experiment and in conducting the necessary pilot studies. Kay Hodgins and I shared equally in running the experiment after the pilot study stage, including tending the plants and collecting the data. Loren Rieseberg suggested that we use the data from this experiment to address the evolution of flowering time. I analyzed the data, wrote this chapter, and developed the hypotheses considered in the Discussion; Dilara Ally, Kay Hodgins and Sally Otto provided editorial comments that clarified the contents. M. Whitlock provided minor comments on the presentation of this work. This chapter is being prepared for submission for publication, with authorship in the following order: Crispin Jordan, Kay Hodgins, Dilara Ally, and Sally Otto.

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Chapter 1

Introduction

Angiosperms exhibit tremendous diversity in life history and reproductive traits. Examples include variation in the number of reproductive episodes in a plant's lifetime (monocary vs. polycarpy; Barrett et al. 1996), modes of fruit dispersal (Lorts et al. 2008), pollen vector (e.g., wind- vs. animal-pollination; Friedman and Barrett 2009), degrees of sexuality vs. asexuality (Vallejo-Marín et al. 2010), floral design (e.g., radial vs. bilateral symmetry; Sargent 2004), the degree of inbreeding (self- vs. outcross-pollination), and breeding system (e.g., hermaphrodite vs. dioecious species). Many of these traits have evolved repeatedly among plant lineages, representing evolutionary transitions with consequences for both micro- and macro-evolution.

Among these traits, reproductive traits are particularly important because they coordinate the transmission of genetic information (Barrett 2008). Consequently, variation in reproductive traits influence the amount (e.g., Ashman and Majetic 2006) and distribution of genetic variation within a species (e.g., Bakker et al. 2006). Reproductive traits (e.g., rate of self-fertilization) can also affect microevolution (Glemin et al. 2006, Wright et al. 2008), the probability of local population extinction (e.g., Morgan et al. (2005)), genome size (Wright et al. 2008), and rates of species diversification (Sargent 2004, Goldberg et al. 2010). In this thesis, I consider three aspects of plant reproduction: the evolution of self- vs. cross-pollination,

the influence of stress on the evolution of flowering time, and sexually-antagonistic variation, which theory suggests influences the evolution of sex-chromosomes, sexual dimorphism, and separate sexes (dioecy).

1.1 Models of Mating System Evolution

The evolution of selfing rate has been studied more than any other evolutionary transition in plant reproduction (Barrett 2008). This interest arises, in part, from the vast diversity of mating systems, which range from complete outcrossing to almost complete selfing (reviewed by Goodwillie et al. 2005). Indeed, great variation in selfing rate exists both among and within species (e.g., *Eichhornia paniculata*; Ness et al. 2010), and the evolution of selfing from outcrossing may be the most common evolutionary transition in flowering plants (Stebbins 1974). Such diversity both facilitates empirical studies of the forces that generate it and demands explanation.

Most research on the evolution of selfing rates has focussed on two primary hypotheses. First, self-pollination may evolve to provide reproductive assurance when pollinators are scarce or when invading a new habitat with a new pollinator fauna (Kalisz et al. 2004). This hypothesis appealed to Darwin (1876) and may apply widely because reproduction is often pollen limited in plant populations (Knight et al. 2005), although it is not the rule (see Aizen and Harder 2007 for a critique of methods that measure pollen limitation). Second, as identified by Fisher (1941), an allele that increases the rate of self-fertilization can enjoy a transmission bias relative to an allele that causes strict outcrossing, because genes in a selfer can be transmitted in two doses of a self-fertilized seed plus one dose through pollen exported to other plants, whereas genes in an outcrosser are transmitted in only two doses (Porcher and Lande 2005b).

Theoretical studies that address the evolution of selfing rate have outpaced empirical efforts and are briefly reviewed here (see Thesis Conclusion for discussion of empirical work). Early theoretical studies largely predicted the evolution of complete outcrossing or selfing, and identified inbreeding depression and pollen discounting (reduction in pollen export

due to self-pollination; Harder and Wilson 1998) as factors that balance the transmission bias and affect the outcome of mating system evolution (Nagylaki 1976, Wells 1979, Charlesworth 1980, Lande and Schemske 1985). These early results suggested that the many species that reproduce through both outcrossing and selfing in appreciable quantities (mixed mating) were cases in transition to complete selfing or outcrossing (Schemske and Lande 1985). In response, later models sought out conditions under which evolution could favour a mixed-mating strategy, exploring a variety of mechanisms (Table 1.1 summarizes many of these mechanisms; see Goodwillie et al. 2005 for a more comprehensive list of citations).

Table 1.1: Examples of processes that predict mixed mating systems; see also Goodwillie et al. (2005).

Process	**Reference**
Provide reproductive assurance	Schoen and Brown (1991) Harder and Aizen (2010)
"Excess ovule" production permits reproductive compensation with high early inbreeding depression	Harder et al. (2008)
Genetic constraints on independent evolution of pollen export and numbers of self- vs. cross-pollinated seeds	Johnston et al. (2009)
Co-evolution of pollen export and self-pollination	Lloyd (1979) Johnston (1998)
Self-pollen has a higher likelihood of reaching ovules than outcross pollen does with competing selfing	Holsinger (1991)

Continued on next page

Table 1.1 – continued

Process	Reference
Inbreeding depression differs between male- and female-function	Chang and Rausher (1999)
Biparental inbreeding reduces cost of outcrossing	Uyenoyama (1986) Yahara (1992)
Biparental inbreeding in populations with density-dependent recruitment	Ronfort and Couvet (1995)
Resource allocation	Iwasa (1990), Sakai (1995)
Overdominant inbreeding depression	Holsinger (1988) Uyenoyama and Waller (1991)
Spatial, temporal or density-dependent inbreeding depression	Holsinger (1986) Cheptou and Schoen (2002)
Fitness decreases with recurrent generations of selfing	Latta and Ritland (1993)

By and large, these efforts have been very successful, in the sense that many viable hypotheses have been raised to explain complete selfing or outcrossing or mixed mating under a wide variety of circumstances. However, few models provide adaptive explanations for mixed mating in species with very high inbreeding depression. Mixed mating could evolve in such cases to alleviate pollen limitation (e.g., Schoen and Brown 1991), but this explanation does not apply to all species (e.g., *Aquilegia canadensis*, Eckert and Herlihy 2004). Therefore, explanations for mating systems of such species that do not invoke reproductive assurance would aid future empirical studies.

Viability selection on traits that affect selfing rate may provide one explanation. Here, we define viability selection as the probability of reaching reproduction, or equivalently, fertility selection through both male and female function in a hermaphrodite. For example, numerous empirical studies suggest that smaller flowers may evolve as a byproduct of selection for faster development in unpredictable environments, which can increase selfing rates by placing anthers and stigmas closer together (e.g., Runions and Geber 2000, Mazer et al. 2004, Elle 2004). Therefore, viability selection could indirectly cause an allele promoting self-fertilization to invade purely outcrossing populations when it could not otherwise, such as with high inbreeding depression. It is not obvious, however, whether selection for increased viability would always cause the evolution of complete selfing or whether mixed mating might also evolve. In Chapters 2 and 3, I address this issue with two models that consider different modes of self-pollination.

1.2 Sexually-Antagonistic Loci Among Genomic Regions

A second major evolutionary transition in plant reproduction involves the evolution of unisexual individuals bearing only male or female parts. This transition includes approximately 10% of plant species (Barrett 2002), and can take various forms, including the co-existence of hermaphrodites with either females (gynodioecy) or males (androdioecy), or populations with only males and females (dioecy). Dioecy has evolved independently in many plant lineages and includes approximately 6% of plant species (Renner and Ricklefs 1995). For many dieocious species (including many animal species), the evolution of genetic sex determination and of sex chromosomes represent important events with implications for further evolution. For instance, sex chromosomes can play important roles in the evolution of sexual dimorphism (Rice 1984, Delph et al. 2010), which frequently occurs in dioecious plant species (Dawson and Geber 1998). In addition, differences in the effective population sizes of sex-chromosomes and autosomes

can affect the evolution of the genes linked to a sex-determining region (Vicoso and Charlesworth 2006, Vicoso and Charlesworth 2009).

Intralocus sexually-antagonistic variation (SA) occurs when one allele increases fitness in females but reduces it in males but a second allele has the opposite effect. SA is believed to play an important role in sex chromosome evolution (reviewed by Charlesworth et al. 2005, Chapter 4). Briefly, when SA polymorphism occurs at a locus in a pseudo-autosomal region ("PAR": a region of a sex chromosome where recombination occurs in the heterogametic sex), linkage disequilibrium between a male-benefit allele and a male sex-determining region can develop and favour the evolution of reduced recombination between these regions. Reduced recombination in this region will lower the effective population size and can cause genetic degeneration of this region and the evolution of heteromorphic sex chromosomes. Quantitative genetic studies suggest that notable SA variation segregates within populations (e.g., Gibson et al. 2002, Pischedda and Chippindale 2006, Harano et al. 2010, Delph et al. 2010). Thus, besides its potential importance for sexual dimorphism, sex chromosome evolution and sexual selection (Albert and Otto 2005), SA also comprises an important source of genetic variation, generally.

Given the potential influences of SA on evolution, it is useful to understand how dominance, the strength of selection, and the regions of the genome in which an SA locus may reside each affect the maintenance of SA polymorphism. Several studies have compared the potential for SA polymorphism among various regions of the genome (e.g., PAR vs. autosome, or X-chromosome vs. autosome; reviewed in Chapter 3), but they have employed disparate assumptions that hinder general conclusions. Therefore, in Chapter 4, I perform a general numerical analysis that examines the potential for SA polymorphism among autosomes, a PAR region, and a hemizygous region of the X- (or Z-) chromosome. I find that, in general, the PAR most easily harbours SA polymorphism. This result both supports hypotheses for the role of SA in reduced recombination and helps focus empirical studies that aim to find and characterize SA loci.

1.3 The Role of Stress In Promoting Divergence Via Phenological Shifts

Adaptive population divergence, whether it results in speciation or simply local adaptation, occurs most easily with reduced gene flow among populations (reviewed by Lenormand 2002). Factors at various stages of reproduction can act as barriers to gene flow among populations, including: geographic separation, phenological differences among populations, pollinator isolation, pollen precedence, and selection against gene-flow (e.g., migration load, or hybrid inviability) (Ramsey et al. 2003). Barriers that occur earlier in reproduction can contribute more strongly to reduced gene flow (Coyne and Orr 2004).

Differences in phenology can cause genetic isolation in time (Hendry and Day 2005), and it is widely believed that the evolution of flowering time could greatly reduce gene flow. Flowering times between members of a species that inhabit different environments frequently differ (reviewed by Levin 2009), and genetic differences in flowering time between environments can evolve in at least two ways. First, selection could act directly on time to flowering, as suggested by local adaptation of flowering time in several species (e.g., Hall and Willis 2006). Second, environmental conditions, themselves, can affect time to flowering (reviewed by Levin 2009). Theory suggests that such effects can promote assortative mating by environment-type, promoting further genetic divergence (Stam 1983, Gavilets and Vose 2007; see Levin 2009 for alternative mechanisms of divergence in flowering time).

In Chapter 5, I address the latter of these two mechanisms for divergence in flowering time with two goals. First, using a greenhouse experiment, I determine the influence of two common stressors, low water and herbivory, on flowering time and two other ecologically important traits (flower number and plant height). These data help present a more complete picture of how stress influences flowering time. Second, I use these data to present novel hypotheses for how stress may affect gene flow through its influence on ecological processes. These results speak to population diver-

gence in mating system when stress influences the evolution of selfing rate.

1.4 Ongoing Work

In addition to the work reported in this thesis, my PhD also included an empirical study that involved several of the themes addressed here. Specifically, to better understand variation in inbreeding depression among environments (e.g., stressful vs. benign) (Armbruster and Reed 2005), which theory suggests can promote the evolution of mixed mating (Holsinger 1986, Cheptou and Schoen 2002), I tested whether maternal effects influence the expression of inbreeding depression in *Mimulus guttatus* (in preparation).

1.5 Conclusions

The projects in coming chapters largely contribute to understanding evolutionary transitions in plant reproduction by proposing new hypotheses. The final chapter of this thesis briefly reviews empirical efforts that address the themes of this thesis, particularly mating system evolution. I highlight some strengths of current empirical studies, but also suggest approaches to test both the ideas in this thesis and hypotheses relating to evolutionary transitions in plant reproduction, more generally.

Chapter 2

Functional Pleiotropy and Mating System Evolution in Plants: Frequency-Independent Mating

2.1 Summary

Mutations that alter the morphology of floral displays can change multiple fitness components simultaneously, such as pollen export and selfing rate. Therefore, functional pleiotropy may influence the evolution of both mating systems and floral displays, two characters with high diversity among angiosperms. Functional pleiotropy between viability and selfing rate may also occur when the morphology of floral displays (e.g., flower size) affects survival or male and female function similarly. The influence of viability selection on mating system evolution has not been studied theoretically. We model plant mating system evolution when a single locus simultaneously affects the selfing rate, pollen export, and viability. We assume frequency-independent mating, so our model characterizes prior selfing. Functional pleiotropy between increased viability and selfing rate

reduces opportunities for the evolution of pure outcrossing, can favor complete selfing despite high inbreeding depression, and notably, can cause the evolution of mixed mating despite very high inbreeding depression. Functional pleiotropy through pollen export and viability do not have independent effects on the evolution of selfing rate. These results highlight the importance of functional pleiotropy for mating system evolution and suggest that selection by non-pollinating agents may help explain mixed mating, particularly in species with very high inbreeding depression.

2.2 Introduction

Flowering plants are famous for the diversity of their mating systems and floral displays: plant mating systems vary from almost complete selfing to complete outcrossing (reviewed by Goodwillie et al. 2005), while the diversity of floral and inflorescence form rivals the diversity of reproductive organs in any other group of organisms (Barrett 2002). The evolution of mating systems in plants frequently involves a change in floral display, implying that functional pleiotropy (by which we mean the correlation between a single morphological trait and other functional traits caused by the underlying action of genes with pleiotropic effects) may be common in mating system evolution (Ritland 1991, Kohn and Barrett 1994, Galen 1999, Fishman 2000). In particular, evolution of the proportion of self-fertilized offspring can occur via changes in either the amount or timing of self- vs. outcross-pollen deposition (Lloyd 1992), self- and outcross-pollen tube growth rates, or the abortion of fertilized ovules (Harder et al. 2008, Porcher and Lande 2005b). Changes in deposition of self and outcross pollen often derive from modifications of floral display (e.g., Kohn and Barrett 1994, Vallejo-Marín and Barrett 2009), as floral form often controls opportunities for contact between the anthers and stigma (e.g., Karron et al. 1997) as well as the exchange of pollen between its vector and the floral organs (e.g., Armbruster et al. 2004). Similarly, the number (Harder et al. 2004) and arrangement of flowers affect pollen movement among flowers within a plant (wind pollination: Friedman and Barrett 2009, animal pollination:

Hainsworth et al. 1983, Jordan and Harder 2006). Therefore, selection to alter the selfing rate may, in turn, affect other aspects of fitness such as pollen receipt or export. Conversely, direct selection on floral displays (e.g., on flower size, shape, number, or arrangement) can influence opportunities for autogamy (within-flower self-pollination) or geitonogamy (between-flower self-pollination), thereby potentially changing the mating system.

Selection on traits that affect plant viability (i.e., the probability of surviving to reproduction, generalized to include fertility to the extent that it affects male and female components equally) extend the pleiotropic effects of floral display mutations beyond the mating system and pollination. Although most studies assume a nearly universal role for pollinators in the evolution of floral diversity (Elle 2004), numerous non-pollinating agents can select for floral characters that may influence mating system, pollination, and viability (Galen 1999, Strauss and Whittall 2006). In this light, selection for faster development in unpredictable or stressful environments may be particularly important because plants with prolonged development may lose all opportunities to reproduce, and small flowers may evolve as a byproduct (Guerrant 1989, Runions and Geber 2000, Mazer et al. 2004, Elle 2004, Snell and Aarssen 2005). For example, in a field trial, small-flowered genotypes of *Mimulus guttatus* were 12 times more likely to survive to flowering than large-flowered genotypes, because the latter group matured too slowly to reproduce before a terminal drought period (Mojica and Kelly 2010). In turn, viability selection that favours smaller flowers may, itself, indirectly impose selection on the mating system through a variety of routes. For example, selfing rates could evolve due to indirect selection on herkogamy (the physical separation of anthers and stigmas), which often correlates with selfing-rate (e.g., Karron et al. 1997, Brunet and Eckert 1998, Herlihy and Eckert 2007). If smaller flowers place male and female organs closer together (e.g. Armbruster et al. 2002, but see Fenster et al. 1995), they may experience altered levels of self-pollination (reviewed in Elle 2004) and/or pollen-discounting (the use of pollen for self-pollination that might have been exported; Harder and Wilson 1998). Alternatively, selection for faster floral development can reduce the temporal separation of

pollen presentation and ovule receptivity (dichogamy) (Mazer et al. 2004), reducing the efficacy of pollen export and increasing the probability of self-pollination when male and female functions overlap in time.

Factors independent of development time may also link viability and mating system. For example, small flowers may increase viability by increasing tolerance to water stress (e.g., Galen et al. 1999); because flowers transpire notable quantities of water, plants with more or larger flowers may close stomata in response to decreased water-leaf potential, and therefore experience decreased carbon assimilation (reviewed by Lambrecht and Dawson 2007; note that, as above, faster development may also avoid water stress). Interactions with plant enemies may also play a role. For instance, smaller flowers received less damage from ants in *Polemonium viscosum* (Galen 1999). Similarly, foliar herbivory can cause plants to produce smaller flowers (Strauss 1997), and a genetic correlation links tolerance to herbivory and petal size in *Brassica rapa* (Strauss et al. 1999); therefore, tolerance to herbivory could affect viability, selfing (as described above) and interactions with pollinators. Clearly, functional pleiotropy can link viability, selfing rate and pollen export through a variety of means.

A notable fraction of models that address mating system evolution consider the role of functional pleiotropy on transitions between selfing and outcrossing, although most authors do not explicitly explain their models in these terms. Most of these studies focus on relationships between shifts in the mating system and other aspects of pollination (Lloyd 1979, Schoen et al. 1996; Harder and Wilson 1998; Johnston 1998; Johnston et al. 2009). For example, Johnston (1998) showed that mixed mating (the use of both self- and outcross-pollen) can evolve when pollen discounting increases with selfing rate, whereas Johnston et al. (2009) explored mating system evolution when functional constraints cause the number of ovules pollinated by self- or outcross-pollen to evolve non-independently. Other authors consider additional roles for functional pleiotropy. For example, Iwasa (1990) and Sakai (1995) examine the effect of resource allocation on the evolution of selfing rates, while Uyenoyama (1986) and Yahara (1992) model mating system evolution when the genetic costs of outcrossing evolve

with the mean population selfing rate. In contrast, the effect of pleiotropy between mating system modifiers and plant viability remains largely unexplored.

We present a model in which mating system, viability, and pollen export are linked through a common morphological basis. Specifically, we analyze a model in which mating is frequency-independent and seed-production is not limited by pollen-receipt, as assumed for many models of mating system evolution (e.g., Wells 1979, Charlesworth 1980, Lande and Schemske 1985). In functional terms, one can envision our model as one of the evolution of "prior selfing" (the deposition of self-pollen and stigma receptivity that occurs before the receipt of outcross pollen; Lloyd and Schoen 1992). For instance, selfing rates will be frequency independent if 1) self-pollination occurs sufficiently early that self- and outcross-pollen tubes do not compete for access to ovules, and 2) all plants subsequently receive enough outcross pollen to fertilize all the ovules that were not fertilized by self-pollen (if pollen receipt limits seed production, then selfing rates depend on the amount of pollen exported in the population). Chapter 3 explores a similar model of pleiotropy that includes selfing rates determined by "mass action" (Holsinger 1991), in which a genotype's selfing rate depends on the frequency of various phenotypes in the population (frequency-dependent mating; e.g., competing- or facilitated-selfing, Lloyd and Schoen 1992).

We then explore the impact of pleiotropy between selfing rate and viability, as well as pollen export, on the evolution of selfing and outcrossing. Given the current debate over whether mixed mating systems are evolutionarily stable versus are simply in transition to either complete selfing or outcrossing (Schemske and Lande 1985, Goodwillie et al. 2005), we pay special attention to the conditions favoring the evolution of stable mixed mating systems. Early models of mating system evolution focussed on the role of inbreeding depression and provided the foundation for this debate (particularly Lande and Schemske 1985, reviewed in Goodwillie et al. 2005). In these models selfing is typically favoured because an allele that increases selfing can be transmitted in three doses to the next generation

(two in a selfed seed plus one in exported pollen) whereas an allele for outcrossing is passed on to the next generation in only two doses (one copy in the maternal seed and one copy in exported pollen; Fisher 1941, Porcher and Lande 2005a). Inbreeding depression is crucial to mating system evolution because it can negate this transmission bias for selfing, so that many models predict the evolution of complete outcrossing when inbreeding depression is low, and the evolution of complete outcrossing when inbreeding depression is high (e.g., Lloyd 1979, Charlesworth et al. 1990). Our results reveal that functional pleiotropy has strong and important effects on mating system evolution; in particular, in contrast to other models of mating system evolution that assume no pollen limitation, our model predicts that pleiotropy between selfing and increased viability can cause mixed mating despite high inbreeding depression.

2.3 Model Description

We describe mating system evolution with a one-locus, two-allele model, where the locus in question simultaneously affects several plant traits (Table 2.1 summarizes parameters). The homozygote for the resident A allele produces a fraction of its seeds, θ, by self-pollination. It exports a fraction $(1 - d\ \theta)$ of its pollen to other plants, where d represents the loss of pollen export due to processes involved with selfing (e.g., pollen discounting, Charlesworth 1980; but see Harder and Wilson 1998). The fitness of offspring produced by selfing is reduced by inbreeding depression by a factor (1 - δ), which we assume is relatively constant over the time course of this model.

Following our conception of functional pleiotropy, we envisage selfing rate evolution through selection on an underlying floral (or plant) trait (e.g., flower size or colour). A newly introduced allele, a, alters the underlying trait by an amount, ϕ, which simultaneously affects the selfing rate, pollen discounting, and viability, so selection on the trait (and selfing rate) depends on all these contributions to fitness. The homozygous mutant genotype self-pollinates $(\theta + \Delta\theta\ \phi)$ of its seeds, experiences pollen discounting

Table 2.1: Summary of Model's Parameters

Symbol	Description
ϕ	Magnitude of change in the focal plant trait
Δv, $\Delta\theta$, Δd	Sensitivities of viability, selfing rate, and pollen discounting, respectively, to change in the focal trait
θ	Initial selfing rate
h_v, h_θ, h_d	Dominance of resident allele (A) for changes in viability, selfing rate, and pollen discounting, respectively
δ	Inbreeding depression

by an amount $(d + \Delta d\ \phi)$, and has a relative viability of $(1 + \Delta s\ \phi)$. Here $\Delta\theta$, Δd and Δs represent the sensitivity (rate of change) of each character to changes in the underlying trait, ϕ. Without loss of generality, we choose the direction of the underlying trait axis such that increasing the trait ($\phi > 0$) increases the selfing rate ($\Delta\theta > 0$). Thus, mutants with $\phi > 0$ increase selfing rates, while mutants with $\phi < 0$ decrease selfing rates. Positive values of Δv and Δd thus imply that alleles that augment selfing also improve viability and decrease pollen export, respectively; negative values of Δv and Δd imply reduced viability and improved pollen export, respectively. We characterize the heterozygote by multiplying ϕ for the homozygous mutants traits by $(1 - h_\theta)$, $(1 - h_d)$ and $(1 - h_v)$, where h_x is the dominance coefficient of the resident allele (ranging from 0 to 1) with respect to selfing rate, pollen export, and viability, respectively.

As an aside, as presented, our model focusses on the evolution of an underlying trait due to selection from a variety of influences (i.e., pleiotropic effects), and not on the selfing-rate, per se. As an alternative approach, one could consider the selfing rate, itself, as the underlying trait, and characterize pleiotropic effects on viability and pollen export as the rate of change in these traits relative to changes in the selfing rate (in fact, we follow this perspective to transform our model using the terms f_v and f_d; see Stability Analysis). Although this alternative perspective focuses the model on the evolution of selfing rate, our focus on the evolution of an underlying trait (and the use of ϕ) holds some advantages. Foremost, this framework is con-

sistent with our definition of functional pleiotropy. Second, although selfing rates can evolve in response to changes in many types of traits, defining ϕ focusses the reader's thinking along a single trait axis (e.g., flower size). Finally, focussing on a single trait axis helps to justify our assumptions of similar dominance effects for viability, selfing rate, and pollen export (see Stability Analysis, below).

We describe the frequency of the resident homozygote and mutant genotypes using recursion equations that summarize a life cycle of selection followed by reproduction (self- and outcross-pollination).

Viability selection changes genotype frequencies (P_i) according to

$$\begin{aligned} P_{AA}^{v} &= \frac{P_{AA}}{\bar{W}} \\ P_{Aa}^{v} &= \frac{P_{Aa}\ (1 + (1 - h_v)\ \Delta v\ \phi)}{\bar{W}} \\ P_{aa}^{v} &= \frac{P_{aa}\ (1 + \Delta v\ \phi)}{\bar{W}} \end{aligned} \tag{2.1}$$

where dividing by $\bar{W}$, the average viability, ensures that the frequencies sum to one.

Mating comprises self- and outcross-pollination. We let $Self_{Total}$ and $Outcross_{Total}$ represent the relative contributions to the next generation via selfing and outcrossing, respectively. We assume that there is no pollen limitation (all ovules are fertilized), and that self-fertilizing an ovule removes it from the pool of ovules available for outcrossing (i.e. complete ovule discounting occurs, Lloyd 1992). Among the self-pollination component, genotypes are produced in proportions:

$$\begin{aligned} P_{AA}^{self} &= \frac{P_{AA}^{v}\ \theta\ (1 - \delta) + \frac{1}{4}\ P_{Aa}^{v}\ (\theta + (1 - h_\theta)\ \Delta\theta\ \phi)\ (1 - \delta)}{Self_{Total}} \\ P_{Aa}^{self} &= \frac{\frac{1}{2}\ P_{Aa}^{v}\ (\theta + (1 - h_\theta)\ \Delta\theta\ \phi)\ (1 - \delta)}{Self_{Total}} \\ P_{aa}^{self} &= \frac{\frac{1}{4}\ P_{Aa}^{v}\ (\theta + (1 - h_\theta)\ \Delta\theta\ \phi)\ (1 - \delta) + P_{aa}^{v}\ (\theta + \Delta\theta\ \phi)\ (1 - \delta)}{Self_{Total}}, \end{aligned} \tag{2.2}$$

where $Self_{Total}$ is given by the sum of the numerators.

Among all of the ovules, the frequencies that remain available for outcrossing with the A or a allele equal

$$\begin{aligned} Ovule_A &= \frac{P^v_{AA}\,(1-\theta) + \frac{1}{2}\,P^v_{Aa}\,(1-(\theta+(1-h_\theta)\,\Delta\theta\,\phi))}{Outcross_{Total}} \\ Ovule_a &= \frac{\frac{1}{2}\,P^v_{Aa}\,(1-(\theta+(1-h_\theta)\,\Delta\theta\,\phi)) + P^v_{aa}\,(1-(\theta+\Delta\theta\,\phi))}{Outcross_{Total}}, \end{aligned} \tag{2.3}$$

respectively, where $Outcross_{Total}$ equals the total fraction of ovules available for outcrossing (the sum of the numerators in (2.3)). Additionally, selfing can diminish opportunities to export pollen due to pollen discounting, such that plants disperse pollen with allele frequencies:

$$\begin{aligned} Pollen_A &= \frac{P^v_{AA}\,(1-\theta\,d)}{Pollen_{Total}} \\ &\quad + \frac{\frac{1}{2}\,P^v_{Aa}\,(1-(\theta+(1-h_\theta)\,\Delta\theta\,\phi)\,(d+(1-h_d)\,\Delta d\,\phi))}{Pollen_{Total}} \\ Pollen_a &= \frac{\frac{1}{2}\,P^v_{Aa}\,(1-(\theta+(1-h_\theta)\,\Delta\theta\,\phi)\,(d+(1-h_d)\,\Delta d\,\phi))}{Pollen_{Total}} \\ &\quad + \frac{P^v_{aa}\,(1-(\theta+\Delta\theta\,\phi)\,(d+\Delta d\,\phi))}{Pollen_{Total}} \end{aligned} \tag{2.4}$$

where $Pollen_{Total}$ is the sum of the numerators in (2.4). Outcross pollen and ovules unite randomly to produce offspring in proportions:

$$\begin{aligned} P^{out}_{AA} &= Pollen_A\,Ovule_A \\ P^{out}_{Aa} &= Pollen_A\,Ovule_a + Pollen_a\,Ovule_A \\ P^{out}_{aa} &= Pollen_a\,Ovule_a. \end{aligned} \tag{2.5}$$

Accounting for the different contributions through selfing versus outcrossing, the frequencies of each genotype at the start of the next generation

equal:

$$
\begin{aligned}
P'_{AA} &= \frac{P_{AA}^{out}\, Outcross_{Total} + P_{AA}^{self}\, Self_{Total}}{Outcross_{Total} + Self_{Total}} \\
P'_{Aa} &= \frac{P_{Aa}^{out}\, Outcross_{Total} + P_{Aa}^{self}\, Self_{Total}}{Outcross_{Total} + Self_{Total}} \\
P'_{aa} &= \frac{P_{aa}^{out}\, Outcross_{Total} + P_{aa}^{self}\, Self_{Total}}{Outcross_{Total} + Self_{Total}}
\end{aligned}
\tag{2.6}
$$

Stability Analysis - We performed a local stability analysis, asking when the a allele could invade a population fixed for the A allele. Two key assumptions simplified our analysis. First, we assumed that the invading allele had a small effect on the underlying trait. While this assumption simplifies the analysis of the model, it also reflects the view that multiple genes (many of which have a small effect) control most floral traits (Kalisz and Kramer 2007). Additionally, we assumed that the dominance of the invading allele was similar for selfing rates, pollen discounting, and viability (i.e., $h_\theta = h_d = h_v$, all of which we denote by h). The assumption of similar dominance for the three plant characters is motivated by the idea that the characters all derive from an underlying change in a single plant trait. However, this assumption may be unmet in some circumstances. For example, dominance coefficients will not be equal if a small change in flower size causes a proportionate change to viability, but a non-linear change to the selfing rate. General, but more complicated, results for arbitrary dominance coefficients are also available (upon request).

To determine when the a allele can invade a population fixed for the A allele, we constructed a stability matrix by linearizing the recursions. The a allele can invade a population fixed for A when the leading eigenvalue, λ, of this matrix exceeds one. Subtracting one from the leading eigenvalue,

invasion occurs when $\beta = \lambda - 1$ is positive, where:

$$\begin{aligned}\beta = & \frac{2(1-h)(1-\theta)+\theta(1-\delta)}{2\,(1-d\,\theta)\,(1-\delta\,\theta)\,(2-\theta-\delta\,\theta)} \\ & \cdot[(2\,\Delta v+\Delta\theta-d\,\Delta\theta-2\,\delta\,\Delta\theta-\Delta d\,\theta-2\,d\,\Delta v\,\theta-2\,\Delta v\,\delta\,\theta \\ & +2\,d\,\delta\,\Delta\theta\,\theta+\Delta d\,\theta^2+2\,d\,\Delta v\,\delta\,\theta^2)\,\phi].\end{aligned} \tag{2.7}$$

In equation (2.7), the fraction is positive so that the sign of β is determined by the term in the square brackets, which is the focus of our analysis. This expression is easier to understand if we define $f_v = \frac{\Delta v}{\Delta \theta}$ as a measure of the relative sensitivities of viability and selfing rate to changes in the underlying trait. If viability and selfing rate increase at the same rate then $f_v = 1$. Alternatively, if a change in the trait has no effect on viability then $f_v = 0$. Similarly, we define $f_d = \frac{\Delta d}{\Delta \theta}$ as a measure of the relative sensitivities of pollen discounting and selfing rate to changes in the underlying trait. When pollen discounting and selfing rate increase at the same rate with ϕ, $f_d = 1$, whereas $f_d = 0$ when altering the underlying trait does not affect pollen discounting. By dividing by $\Delta\theta$, we can present the expression in square brackets as:

$$\gamma = [(1-d)\,(1-2\,\delta)-2\,d\,\delta+2\,d\,\delta\,\theta-f_d\,\theta\,(1-\theta)+2\,f_v\,(1-d\,\theta)\,(1-\delta\,\theta)]\,\phi. \tag{2.8}$$

Thus, if we consider a mutation that increases the selfing rate ($\phi > 0$), such a mutant will spread if the term in square brackets in (2.8) is positive. Equation (2.8) involves effects due to pleiotropy between selfing rate and either pollen export (term multiplied by f_d) or viability (term multiplied by f_v), plus effects of selfing, itself (the remaining terms). Increased selfing is favoured when the terms sum such that γ is positive for a given initial selfing rate, whereas negative γ indicates selection for increased outcrossing. Figure 2.1 illustrates how each term contributes to the value of γ for a variety of parameter values.

We analyzed (2.8) to determine how the combination of viability selection and pollen discounting influences the invasion of alleles that increase

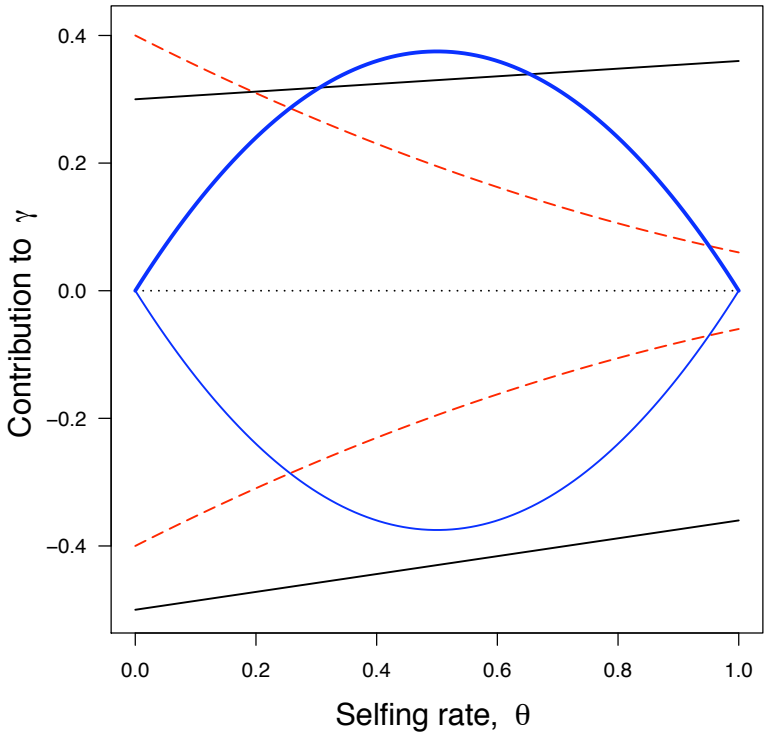

Figure 2.1: Illustration of the forms (and contributions) of the components of γ (equation 2.8) to selection for increased selfing as functions of θ; selection for higher selfing increases with more positive contributions to γ, and the dotted line marks neutrality. Blue, solid curves indicate the value of the portion of γ multiplied by f_d: top (thick) $f_d = -1.5$, bottom, $f_d = 1.5$. Red, dashed curves indicate the value of the portion of γ multiplied by f_v for $f_v > 0$ (top) and $f_v < 0$ (bottom) ($d = 0.5$, $\delta = 0.7$, $f_v = 0.2$ or -0.2). Black, solid straight lines show contributions by the remaining, selfing-related components of γ: top, $d = 0.1$, $\delta = 0.3$, bottom $d = 0.1$, $\delta = 0.7$. Importantly, for a given set of parameters, the values of the red, blue and black functions sum to produce γ, whose various forms over the range $0 \leq \theta \leq 1$ are illustrated in Figure 2.2.

self-pollination in populations with selfing rates ranging from complete selfing to complete outcrossing. Additionally, we determined when mixed mating could evolve. Specifically, we determined whether there was an intermediate selfing rate ($0 < \theta^* < 1$) that could not be invaded by alleles that either slightly increased or slightly decreased selfing rates. Our approach examines the shape of γ as a function of the initial selfing rate, θ, and how this shape is influenced by the various parameters; Figures 2.2A-F illustrate the variety of shapes of γ encountered in our analysis; stable mixed mating is possible in Figure 2.2D-F, because an allele for increased selfing can invade ($\gamma > 0$) for a low initial selfing rate but not ($\gamma < 0$) for a higher initial selfing rate.

We begin our analyses by considering the invasion of selfing and outcrossing into purely outcrossing and selfing populations, respectively. We then use these results to partition "maps" of parameter space (plots of pollen discounting, d, versus inbreeding depression, δ) into regions that support different evolutionary outcomes when selfing is rare or outcrossing is rare. We then extend our analysis to consider how the primary regions may be partitioned further to support additional mating systems to produce Figures 2.3 and 2.4. This graphical approach allows us to summarize our analytical findings with figures that present evolutionary outcomes for a variety of cases.

2.4 Results

We build our analysis from the simplest to most complete models to better understand the processes that influence the evolution of selfing rates. We first begin by considering mating system evolution with no pleiotropy. We turn second to cases where the underlying trait does not affect viability, and third to cases where the trait does not affect pollen discounting. Finally, we consider the complete model.

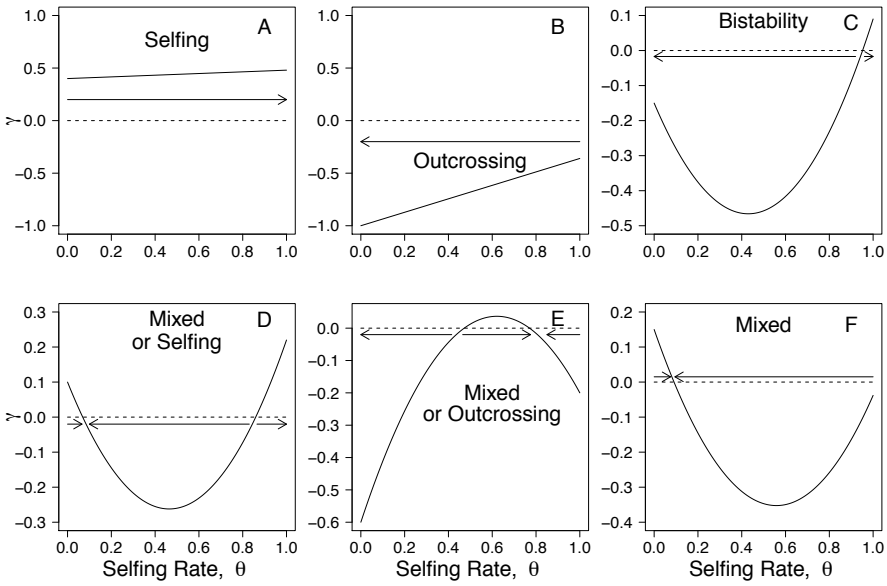

Figure 2.2: Examples of γ (equation 2.8) that illustrate outcomes described in the text; selection favours either increased selfing or outcrossing when $\gamma > 0$ or $\gamma < 0$, respectively, for a given initial selfing rate, θ. Arrows indicate the direction of evolution. Illustrates the evolution of: (A) Complete selfing (increased selfing always favoured, as $\gamma > 0$ for $0 \leq \theta \leq 1$): $d = 0.2$, $\delta = 0.2$, $f_v = f_d = 0$; (B) Complete outcrossing: $d = 0.4$, $\delta = 0.8$, $f_v = f_d = 0$; (C) Bistability (outcrossing or selfing favoured for low or high θ, respectively): $d = 0.55$, $\delta = 0.5$, $f_v = 0.2$, $f_d = 1.6$; (D) Either mixed mating or complete selfing (see text for explanation): $d = 0.5$, $\delta = 0.4$, $f_v = 0.2$, $f_d = 1.6$; (E) Either mixed mating or complete outcrossing; $d = 0.5$, $\delta = 0.75$, $f_v = 0.2$, $f_d = -1.8$; (F) Mixed mating (selfing favoured for low but not high θ): $d = 0.05$, $\delta = 0.6$, $f_v = 0.2$, $f_d = 1.6$. The general properties displayed here correctly illustrate the evolutionary outcomes of the model, although the exact shapes of the curves vary according to the parameters (quantitatively, not qualitatively).

2.4.1 Mating System Evolution Without Pleiotropy

This case, in which no pleiotropy with viability or pollen discounting occurs, is essentially the same model as Charlesworth's (1980) model of selfing rate evolution in a hermaphrodite, and provides results that inform later cases that include pleiotropy.

To examine mating system evolution without pleiotropy, we set $f_d = f_v = 0$ in (2.8):

$$\gamma_{Simple} = [(1-d)\ (1-2\ \delta) - 2\ d\ \delta + 2\ d\ \delta\ \theta]\ \phi. \tag{2.9}$$

Note that γ_{Simple} increases linearly with θ. If inbreeding depression is sufficiently low, $\delta < \frac{1-d}{2}$, γ_{Simple} is positive regardless of the current level of selfing and increased selfing evolves until $\theta = 1$ (complete selfing; e.g., Figure 2.2A). The diagonal line in Figure 2.3A displays the condition $\delta = \frac{1-d}{2}$, below which complete selfing evolves when $f_d = f_v = 0$. In contrast, selection always favours increased outcrossing rates (so complete outcrossing evolves) when inbreeding depression is sufficiently high ($\delta > \frac{1}{2}$; e.g., Figure 2.2B), given by the horizontal line in Figure 2.3A. For reference to later analyses, note that the diagonal and horizontal lines in Figures 2.3 and 2.4 mark the conditions for the invasion of selfing and outcrossing into completely outcrossing and selfing populations, respectively.

It can be shown that for intermediate inbreeding depression, $\frac{1-d}{2} < \delta < \frac{1}{2}$, and $f_d = f_v = 0$, γ_{Simple} has the form shown in Figure 2.2C. In this case the direction of evolution depends on the initial selfing rate: for initial selfing rates below $\theta^* = \frac{2\ \delta - 1 + d}{2\ d\ \delta}$ increased outcrossing is favoured, and above this point selfing is favoured. Therefore, either complete outcrossing or complete selfing can evolve, forming a region of "Bistability" between the straight lines in Figure 2.3A (see also Johnston 1998, Porcher and Lande 2005a,b).

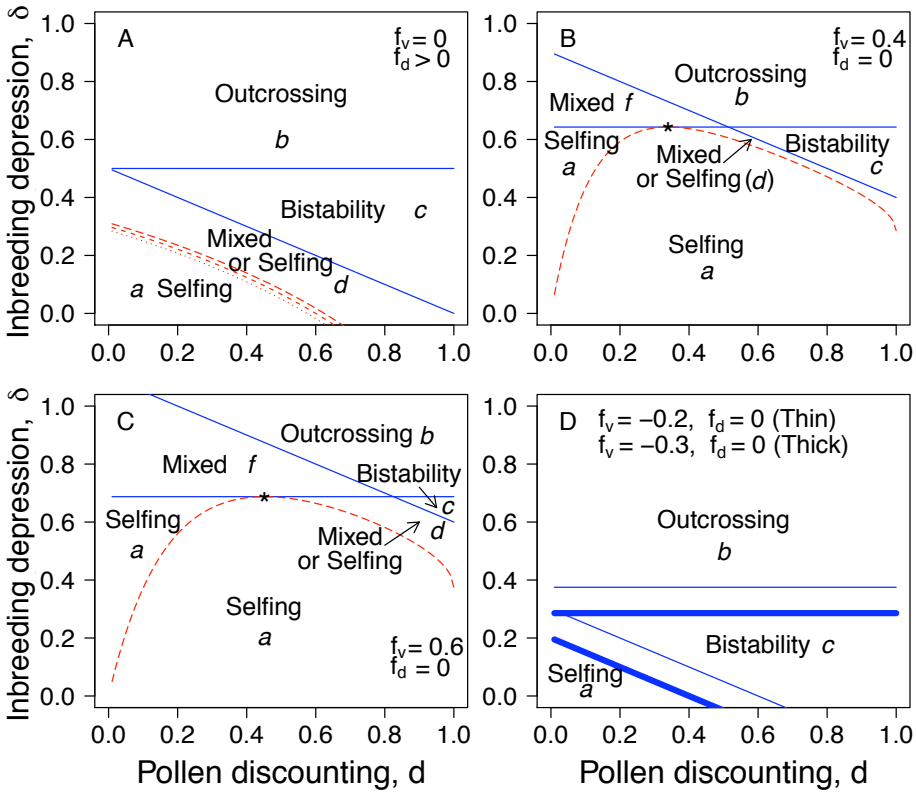

Figure 2.3: Parameter values yielding complete outcrossing, bistability, complete selfing, or mixed mating for sub-models. Increased selfing can invade a purely outcrossing population in regions beneath the diagonal line, whereas increased outcrossing can invade purely selfing populations in regions above the horizontal line. Dashed curves mark transitions between zero and two roots for θ between 0 and 1 (see text and Figure A.1 for details). Italicized letters indicate the panel in Figure 2.2 that illustrates the selection scenario applicable to the given region of each plot. (A) $f_v = 0$, $f_d = 1.5, 1.6, 1.7$, with shorter dashing indicating more extreme values of f_d. (B) and (C) show two examples where $f_d = 0$ and $f_v > 0$; the "*" indicates where the dashed curve touches the horizontal line. (D) Two examples where $f_d = 0$ and $f_v < 0$.

2.4.2 Pleiotropy Between Pollen Discounting and Mating System

To build upon our first case, we studied the influence of pleiotropy between the selfing rate and pollen discounting (f_d) on mating system evolution, which is similar to analyses in other models (particularly Johnston 1998). Figure 2.3A summarizes typical results for this case when increased selfing causes a reduction in pollen export ($f_d > 0$).

To determine the effect of pleiotropy between only pollen discounting and the selfing rate, we set $f_v = 0$ in (2.8):

$$\gamma_{f_d} = [(1-d)\ (1-2\,\delta) - 2\,d\,\delta + 2\,d\,\delta\,\theta - f_d\,\theta\ (1-\theta)]\ \phi, \qquad (2.10)$$

which is a similar condition to (2.9), except for the addition of a term multiplied by f_d.

If the underlying trait affects pollen export ($f_d \neq 0$), how does this influence mating system evolution? Equation (2.10) reveals that, because f_d is multiplied by $\theta\ (1-\theta)$, pleiotropic effects on pollen discounting do not affect mating system evolution in completely selfing ($\theta = 1$) or outcrossing ($\theta = 0$) populations. Instead, f_d affects the evolution of selfing most at intermediate selfing rates by adding a quadratic curve to the linear relationship between γ_{f_d} and the initial selfing rate, θ, seen when $f_d = 0$ (Figure 2.1). f_d has weak effects on mating system evolution in highly selfing or highly outcrossing populations for different reasons. In populations with low selfing rates, pleiotropic effects on pollen discounting do not greatly affect invading alleles because individuals must self to experience pollen discounting (see 2.4), but most reproduction occurs via outcrossing. In contrast, in highly selfing populations pollen discounting involves small costs because few outcrossing opportunities exist (Kohn and Barrett 1994). This latter result arises because our selfing rates are specified as parameters and are not functions of the phenotypes in a population (mass-action models; e.g., Holsinger 1991, Harder and Wilson 1998).

We can prove that pleiotropic effects on pollen export cannot qualitatively affect the outcome of mating system evolution in a region of "Bista-

bility" by considering the form of the function for γ. In this region, selection favours complete outcrossing and complete selfing in populations with very low and high selfing rates, respectively. Because f_d does not influence mating system evolution in pure outcrossing or selfing populations, it cannot affect the stability of complete outcrossing or complete selfing in a region of Bistability. Therefore, the only way f_d could affect mating system evolution in this region is to introduce an additional root for θ at an intermediate selfing rate. However, to both introduce roots and maintain bistability (i.e., maintain the form of selection at the boundaries $\theta = 0$ and $\theta = 1$) requires an odd number of three or more roots for θ between 0 and 1. But, because γ is a quadratic in θ (see 2.8) it cannot yield three (or more) roots of θ, so pleiotropic effects on pollen export cannot qualitatively affect mating system evolution in the region of Bistability (see Figure A.2 for illustration of this explanation).

Thus, when $f_d \neq 0$ stable mixed mating can only arise in regions that otherwise favour either complete selfing or complete outcrossing when $f_d = 0$ (i.e., the parameter space outside "Bistability" in Figure 2.3A). Because selection favours increased selfing (or outrossing) at both high or low selfing rates in these two regions (Figures 2.2A, B), and because f_d cannot affect selection at very high or low selfing rates, there must always be an even number of roots for θ between $\theta = 0$ and $\theta = 1$, which includes only zero or two roots as possibilities for our model (see 2.8). We know that when $f_d = 0$ there are always zero roots in these regions. As f_d moves away from zero, it cannot be the case that only one root for θ appears between 0 and 1 or else the stability of complete selfing or complete outcrossing would be affected. Thus, we sought out transitions between 0 and 2 roots, i.e., where the solutions to (2.10) for θ went from complex to real. Figure A.1 illustrates this transition and provides further explanation.

We find that mixed mating systems can arise due to pleiotropy in either the region that favours complete selfing or complete outcrossing in Figure 2.3A, depending on whether the trait that increases selfing also increases or decreases pollen discounting ($f_d > 0$ or $f_d < 0$), respectively. Mixed mating arises because f_d changes the form of γ_{f_d} from either that in Figure 2.2A

to a form like that in Figure 2.2D ($f_d > 0$), or from that in Figure 2.2B to a form like that in Figure 2.2E ($f_d < 0$; note the two roots for θ in these examples). As expected, if pollen discounting grows as selfing rates rise ($f_d > 0$) the evolution of selfing is inhibited (γ_{f_d} becomes more negative), but this influences mating system evolution only for intermediate selfing rates. This causes mixed mating to evolve under conditions that would otherwise favour complete selfing had changes in the underlying trait not caused a change in pollen discounting ($f_d = 0$; the area beneath the diagonal line in Figure 2.3A). However, Figure 2.2D also illustrates that whether mixed mating or complete selfing evolves depends on the initial selfing rate. When evolution begins in a population with a sufficiently low level of selfing (e.g., $\theta = 0.05$ in Figure 2.2D), low inbreeding depression favours the evolution of increased selfing rates, but pleiotropic costs of pollen discounting can stop the evolution of further selfing and thereby produce a mixed mating system. If evolution begins in a highly selfing population (where pollen discounting has sufficiently little effect) low inbreeding depression favours the eventual evolution of complete selfing. Overall then, either mixed mating or complete selfing can evolve when $f_d > 0$ and when inbreeding depression, δ, and pollen discounting, d, lie in the region labelled "Mixed Mating or Selfing" in Figure 2.3A (the curves being defined by the transition from 0 to 2 roots for θ). Also as expected, greater sensitivity of pollen discounting relative to the selfing rate (higher values of f_d) increases the region in which mixed mating can evolve and decreases the parameter space in which only selfing evolves (Figure 2.3A).

Pleiotropy that causes plants with higher selfing rates to export more pollen ($f_d < 0$) can also produce mixed mating. As above, the outcome of selection depends on the initial selfing rate. We find that mixed mating can only evolve when inbreeding depression is high (i.e, the region above the horizontal line in Figure 2.3A; results not shown), and it is always simultaneously stable with complete outcrossing (i.e., γ_{f_d} has a shape like Figure 2.2E). To illustrate, when evolution begins in a highly selfing population, selection favours the invasion of higher outcrossing because of the high inbreeding depression (right end of Figure 2.2E). The evolution

of increased outcrossing can be stopped at an intermediate level, however, because higher outcrossing entails lower pollen export, resulting in an evolutionary stable mixed mating system. In contrast, when evolution begins in populations with a sufficiently high outcrossing rate, pollen discounting is inconsequential (see above) and high inbreeding depression favours the evolution of complete outcrossing. Analogous to the situation with $f_d > 0$, increasing the sensitivity of pollen export to selfing rate (i.e., more negative values of f_d) increases the opportunity for mixed mating to evolve and decreases the parameter space with only outcrossing (results not shown).

We confirmed that the two roots for θ always lie between 0 and 1 except when the dashed curve hits either of the solid lines (at $\delta = \frac{1-d}{2}$ or $\delta = \frac{1}{2}$); however, the dashed curve only touches these lines for relatively small values of f_d (results not shown). Specifically, when $0 < f_d < \frac{1}{4}$, the dashed curve touches the diagonal line at two points within the range $0 < d < 1$, and mixed mating is no longer supported between them. Furthermore, when $-1 < f_d < 0$, the dashed curve touches the horizontal line and mixed mating is not supported to the right of this contact point (*Mathematica* file available on request).

2.4.3 Pleiotropy Between Viability and Mating System

We next examined mating system evolution when pleiotropy occurs only between viability and selfing rate ($f_d = 0$). Figures 2.3B-D summarize the results we discuss in this section. In this case, γ becomes

$$\gamma_{f_v} = [(1-d)\,(1-2\,\delta) - 2\,d\,\delta\,(1-\theta) + 2\,f_v\,(1-d\,\theta)\,(1-\delta\,\theta)]\,\phi. \quad (2.11)$$

The last term demonstrates that increased viability associated with a higher selfing rate ($f_v > 0$) always benefits the evolution of selfing; it is strongest in populations with low selfing rates and weakens as the selfing rate rises. The viability benefit decreases for higher selfing rates because inbreeding depression and pollen discounting reduce the fitness of selfers as the selfing rate grows, counteracting the viability benefit. Similarly, a viability decrease with an increase to the underlying trait ($f_v < 0$) favours outcrossing,

especially when selfing is rare and inbreeding depression and pollen discounting are low.

We begin our analysis by determining when selfing and outcrossing can invade populations that only outcross or self, respectively, and these conditions divide parameter space into regions that support different evolutionary outcomes (as in the previous case). Viability selection (f_v) affects the invasion of mutant selfing alleles in highly outcrossing and selfing populations differently than pollen discounting (f_d) did because viability has its strongest effect when selfing is rare. In particular, invasion into completely selfing or completely outcrossing populations become functions of f_v. Increased selfing can invade a completely outcrossing population when $\delta < \frac{1}{2}(1 - d + 2\ f_v)$, which corresponds to the area below the diagonal line in Figures 2.3B-D. In contrast, increased outcrossing can invade completely selfing populations when $\delta > \frac{1+2\ f_v}{2\ (1+f_v)}$, or the area above the horizontal line in Figures 2.3B-D. These boundary conditions create up to four regions that support different evolutionary outcomes: complete outcrossing, bistability, complete selfing and mixed mating.

Considering the boundary conditions and the possibility of multiple roots for θ, mixed mating can arise in two regions, illustrated by Figures 2.3B, C. The first region lies beneath the diagonal line (selfing can invade a completely outcrossing population because of the viability benefits) yet above the horizontal line (outcrossing can invade a completely selfing population because of the cost of high inbreeding). Figure 2.2F exemplifies the general shape of γ_{f_v} as a function of θ in this region. In this region only one root for θ occurs and mixed mating is the only evolutionarily stable strategy. This region is widest when pollen discounting (d) is low. Importantly, mixed mating here requires high inbreeding depression ($\delta > \frac{1}{2}$).

Mixed mating can also arise in the region beneath both straight lines (Figure 2.3B, C) when viability benefits accompany increased selfing ($f_v > 0$). Because γ_{f_v} is a quadratic function of the initial selfing rate, a transition from zero to two roots for θ is possible in the area beneath the two straight lines, just as in the previous analysis ($f_v = 0$). Therefore, as above, we solved for when θ becomes complex, which marks the border of the pa-

rameter space that supports mixed mating (dashed curves). Again, when the two roots for θ become real, we asked whether they fall between 0 and 1 (permitting a mixed mating system) or not. As in the previous analysis, either mixed mating or complete selfing can evolve in the region between the dashed curve and the straight lines, depending on the initial selfing rate; the shape of γ_{f_v} as a function of θ in this region thus resembles the example in Figure 2.2D.

As in the previous case, when the dashed curve contacts either of the straight lines some regions of parameter space between the curve and the straight lines do not support mixed mating because the roots for θ fall outside of 0 and 1. In particular, the curve always contacts the horizontal line at the point $d = \frac{f_v\ (1+2\ f_v)}{1+2\ f_v\ (1+f_v)}$ (Figure 2.3B, C; note point marked by *), and it can be shown that mixed mating only occurs to the right of this point; only complete selfing evolves to its left within this region. Similarly, if the curve contacts the diagonal (which happens only when $f_v < 0.262$) it does so at two points, and the region between these two points of contact do not yield mixed mating (*Mathematica* file available on request).

Pleiotropy that increases viability with selfing also has important effects for the evolution of complete selfing and complete outcrossing. With more positive f_v, viability selection facilitates invasion of selfing in outcrossing populations to a greater extent than it retards the invasion of outcrossing in purely selfing populations, as shown by the greater rise in the diagonal boundary than the horizontal one (compare Figures 2.3B, C). Therefore, higher viability benefits (greater f_v) decreases the parameter space that produces exclusive outcrossing and bistability and increases opportunities for the evolution of complete selfing and mixed mating. Of particular interest, complete selfing can evolve due to viability benefits even in the face of high inbreeding depression ($\delta > \frac{1}{2}$).

In contrast, reduced viability with increased selfing ($f_v < 0$) reduces the parameter space favouring selfing, and it never produces mixed mating. Decreasing viability with selfing weakens the potential of selfers to invade purely outcrossing populations (Figure 2.3D; compare solid and dashed diagonal lines) and aids the invasion of outcrossing in selfing populations

(Figure 2.3D, lowering the horizontal line). Because pleiotropic effects on viability affect evolution most when selfing is rare, making f_v more negative favours outcrossing in purely outcrossing populations to a greater extent than it does in selfing populations. Graphically, this lowers the diagonal boundary faster than the horizontal one, which decreases opportunities for the evolution of selfing and expands regions with bistability (Figure 2.3D; compare solid and dashed lines). Thus, there are no conditions under which the lines cross, which would allow mixed mating. It can also be shown that when viability decreases with increased selfing, there are never two roots for θ that lie between 0 and 1 (see Table 2.2 substituting $f_d = 0$), so that the dashed curves seen in Figures 2.3B and 2.3C do not arise when $f_v < 0$. Finally, note that when $f_v = -0.5$, the horizontal line lies at $\delta = 0$; therefore selection exclusively favours the evolution of complete outcrossing for $f_v < -0.5$.

2.4.4 Complete Model

Pleiotropy among viability selection, pollen discounting and mating system can combine the results for the simpler models, above, as illustrated by Figure 2.4, although the conditions that determine the zone in which mixed mating arises are now more complex. Because pleiotropy between pollen discounting and the selfing rate cannot affect mating system evolution in completely outcrossing or selfing populations (recall that f_d is multiplied by $\theta\ (1-\theta)$ in equation 2.8), allowing the underlying trait to affect the level of pollen discounting never alters the position of the diagonal and horizontal lines that delineate invasion into completely outcrossing or completely selfing populations (Figure 2.4). However including f_d can generally provide opportunities for mixed mating in the same qualitative manner as it did in the model lacking viability selection (eqn 2.10), but the region in which this effect occurs depends on f_v (Table 2.2). By solving for the conditions under which there are two real roots for θ that lie between 0 and 1, one can show that for $f_d > 0$, mixed mating can occur below the two straight lines (in the "selfing" zone) and above the curve that marks the

Table 2.2: Conditions for the evolution of a stable (at least locally) mixed mating system in the complete model. Note that $(f_d + 2\ d\ \delta\ f_v)$ is the coefficient of θ^2 in equation (2.8), and therefore indicates whether γ has upward ($f_d + 2\ d\ \delta\ f_v > 0$) or downward curvature. Figure references exemplify the shapes of this equation for γ. $\zeta = -2\ d\ \delta + f_d + 2\ f_v\ (d + \delta)$. Note that ζ equals the negative value of the slope of equation (2.8) at $\theta = 0$.

Mixed mating simultaneously stable with selfing - Figure 2.2D
$f_d > 0, f_d < 0$
$(f_d + 2\ d\ \delta\ f_v) > 0$
$0 < \zeta < 2\ (f_d + 2\ d\ \delta\ f_v)$
$\delta < min[\frac{1}{2}\ (1 - d + 2\ f_v), \frac{1+2\ f_v}{2\ (1+f_v)}]$
Mixed mating simultaneously stable with outcrossing - Figure 2.2E
$f_d < 0$
$(f_d + 2\ d\ \delta\ f_v) < 0$
$2\ (f_d + 2\ d\ \delta\ f_v) < \zeta < 0$
$\delta > max[\frac{1}{2}\ (1 - d + 2\ f_v), \frac{1+2\ f_v}{2\ (1+f_v)}]$
Mixed mating is the only stable state - Figure 2.2F
$\frac{1+2\ f_v}{2\ (1+f_v)} < \delta < \frac{1}{2}\ (1 - d + 2\ f_v)$

transition between 0 and 2 roots for θ. In addition, $f_d > 0$ expands parameter space that supports mixed mating in this region compared to when $f_d = 0$ (compare Figures 2.3B and 2.4A). However, for $f_d < 0$ the story is more complex, and mixed mating can arise in regions either above or below both straight lines. Table 2.2 summarizes the conditions under which mixed mating arises in each zone, and Figures 2.4C and 2.4D presents results for $f_d < 0$ that cause mixed mating in the "outcrossing" zone. Note that for $f_v < -0.5$ (at which point the horizontal line lies at $\delta = 0$), mixed mating can only evolve for $f_d < 0$.

In other respects, the complete model generally behaves as expected from combining the two simpler cases. For example, whenever pleiotropy with pollen discounting creates a zone in which the evolution of mixed mating is possible, either complete selfing or complete outcrossing exist

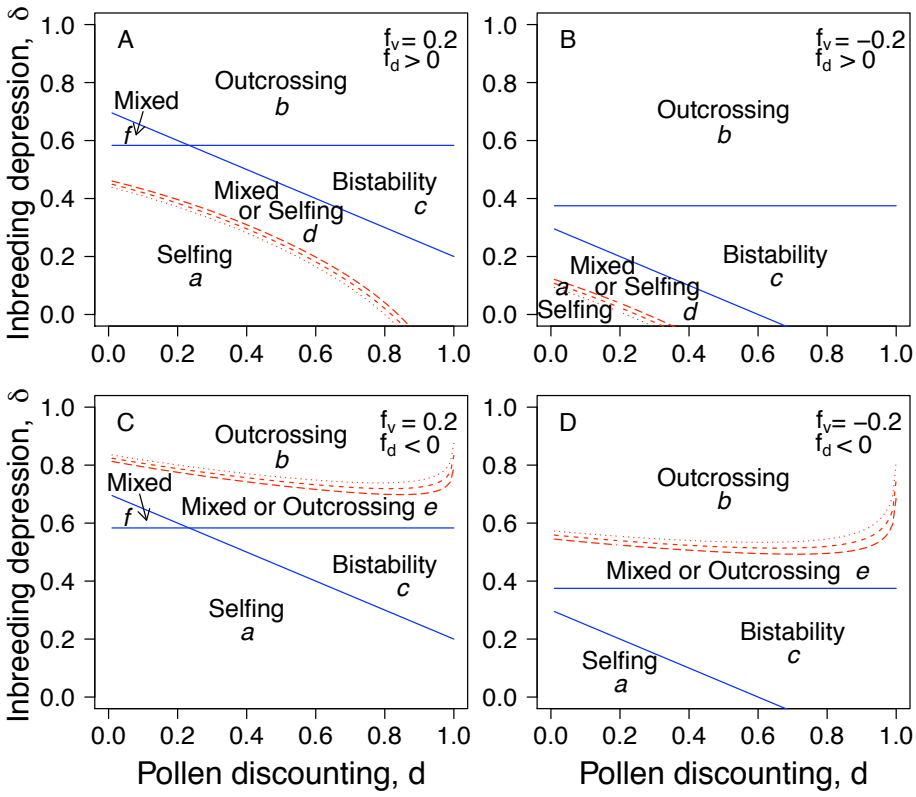

Figure 2.4: Parameter values yielding complete outcrossing, bistability, complete selfing, or mixed mating for the complete model, for combinations of negative or positive values of f_v and f_d. As in Figure 2.3, increased selfing can invade a purely outcrossing population in regions beneath the diagonal line, whereas increased outcrossing can invade purely selfing populations in regions above the horizontal line. Dashed curves mark transitions between zero and two roots for θ between 0 and 1. Italicized letters indicate the panel in Figure 2.2 that illustrates the selection scenario applicable to the given region of each plot. Shorter dashing indicates more extreme values of f_d (absolute values: 1.5, 1.6, 1.7).

as an alternate stable state (see references to Figures 2.2D and Figure 2.2E in Table 2.2). In addition, as above, increasing the absolute magnitude of f_d increases opportunities for mixed mating (Figure 2.4), except for cases where mixed mating occurs below the straight lines (in the "selfing" zone) and $f_d < 0$ (not illustrated). Additionally, f_d does not affect evolution in the parameter space between the straight lines, where either Mixed Mating or Bistability occur (Figure 2.4). Finally, as above, some regions no longer support mixed mating when the dashed curve contacts a straight line.

The predictions generated by this model (e.g., Figure 2.4) were all confirmed by numerical analysis of the eigenvalues, exploring all combinations of $0.05 \leq \Delta\theta, \delta, d \leq 0.95$, $-0.95 \leq \Delta d \leq 0.95$, and $-0.96 \leq \Delta v \leq 0.94$ in increments of 0.1; recall $f_v = \frac{\Delta v}{\Delta \theta}$ and $f_d = \frac{\Delta d}{\Delta \theta}$. Values of Δv were offset slightly to avoid fractions with a denominator equal to zero.

2.5 Discussion

2.5.1 General Implications

Given that correlations between selfing rates and other plant characters may be common, this work was motivated by the desire to understand how pleiotropy of genes shaping the evolution of selfing would affect mating system evolution. Indeed, our model of pleiotropy between the selfing rate and pollen export and / or viability has several implications for mating system evolution and yields some unique predictions. We discuss five of these below.

First, when increases in selfing rates also increase viability, mixed mating can evolve even with high inbreeding depression, even though there is no pollen-limitation in our model. Most models that predict the evolution of mixed mating in the face of high inbreeding depression involve reproductive assurance (Lloyd 1979, Schoen and Brown 1991, Lloyd 1992, Morgan and Wilson 2005, Morgan et al. 2005, some of which model prior selfing; note that models that allow the total fraction of ovules fertilized to evolve (e.g., Johnston et al. 2009) implicitly involve pollen-limitation),

although a handful do not (Iwasa 1990, Sakai 1995, Harder et al. 2008). However, to our knowledge, our model is unique in that it predicts evolutionarily stable mixed mating even with very high inbreeding depression (e.g., $\delta \approx 1$, Figure 2.3C) and without pollen-limitation. Consistent with this result, Wolf (unpubl. ms.; described in Steets et al. 2007) modeled mating system evolution in the face of sexually-transmitted diseases, where increased selfing decreased the chance of infection (like our $\Delta v > 0$) and found that mixed mating evolved when inbreeding depression was high ($\delta > \frac{1}{2}$). This result may help explain mixed mating in species with high inbreeding depression (such as those depicted in Figure 2 of Husband and Schemske 1996). For example, viability selection may help explain high selfing rates in *Aquilegia canadensis* (selfing rate = 76%, reviewed by Eckert and Herlihy 2004). This species exhibits very high inbreeding depression ($\delta > 0.95$) and low geitonogamy (and therefore likely low d, Lloyd 1992), which favours the evolution of mixed mating when $f_v > 0$ in our model. No previous theory could explain adaptive mixed mating in this species (Eckert and Herlihy 2004). An alternative explanation, an historical loss of its major pollinators (hummingbirds and native bumblebees), seems an unlikely reason for this species' high selfing rate (C. Herlihy, pers. comm.). Interestingly, *A. coerulea*, *A. formosa*, and *A. pubescens* also exhibit mixed mating (selfing rates equal 0.59, 0.21 and 0.31, respectively; Brunet and Sweet 2006, Yang and Hodges 2010), and their average inbreeding depression does not differ significantly from 1.0 (Brunet and Sweet 2006, Yang and Hodges 2010). The finding of several *Aquilegia* species with mixed mating and high inbreeding depression supports the notion that this association is not due to an historical artifact; instead functional pleiotropy common to these species may have facilitated the evolution or maintenance of mixed mating.

Second, our model supports previous verbal models that viability selection for rapid development that increases selfing could favor the evolution of complete selfing (e.g., *Collinsia parviflora*, Elle 2004; see Introduction). In addition, we show that complete selfing can evolve in this context despite high inbreeding depression ($\delta > \frac{1}{2}$, e.g., Figure 2.3C) without invok-

ing reproductive assurance benefits of autonomous selfing, which, to our knowledge, has not been shown in analyses, like ours, that assume modifiers of small effect. Some theory (e.g., Charlesworth et al. 1990, Porcher and Lande 2005a) predicts the invasion of increased selfing despite high average inbreeding depression; however, this requires alleles with a large effect on the selfing rate, and QTL studies suggest that such modifiers are rare (reviewed in Fishman et al. 2002, but see Foxe et al. 2009).

Third, our model reveals contrasting effects of $f_v > 0$ vs. $f_v < 0$: mixed mating can evolve when selfing involves a viability advantage, but it cannot if selfing decreases viability ($f_v < 0$) unless selfing also increases pollen export ($f_d < 0$). Therefore, mutations that cause $f_v < 0$ (e.g., when resource acquisition is the underlying trait, where increased resource accumulation both increases viability and flower size), do not promote mixed mating unless selfing also increases pollen export sufficiently.

Fourth, our model supports Johnston's (1998) result that mixed mating can evolve when decreased pollen export accompanies increased selfing rates, but we also show that mixed mating can evolve in the opposite case, too, when higher selfing promotes pollen export. However, this latter result ($f_d < 0$) requires that a population's initial selfing rate, θ, be relatively high (here, γ as a function of θ resembles Figure 2.2E). Therefore, this latter result cannot explain mixed mating in systems where both outcrossing is believed to be ancestral (which is thought to be typical; Stebbins 1957), and where the selfing rate evolves in small steps from $\theta = 0$ (i.e., when the initial selfing rate is close to $\theta = 0$). However, outcrossing rates could be low at the earliest stages of mating system evolution (e.g., *Leptosiphon jepsonii*, Goodwillie and Ness 2005). For example, if a self-incompatible species receives large amounts of self-pollen, then the initial rate of self-fertilization, θ, could be substantial if self-incompatibility breaks down.

Finally, our model suggests that pleiotropic effects of viability and pollen export cannot be considered independently, because viability selection establishes the boundaries (i.e., the horizontal and diagonal lines in Figure 2.4) within which pleiotropy involving pollen export affects mating system evolution. Collectively, these findings imply broad and important implica-

tions of pleiotropy for mating system evolution.

2.5.2 Evolution of Model Parameters

We expect that some of the parameters in our model could, themselves, evolve and change the dynamics of our model. In particular, purging of inbreeding depression (Byers and Waller 1999, Crnokrak and Barrett 2002) could alter the longer-term predictions. For example, consider that inbreeding depression contributes to mixed mating by diluting the benefits of increased viability at higher selfing rates (equations 2.8, 2.11). If inbreeding depression can be purged significantly, then initial increases in the selfing rate brought about through beneficial pleiotropic effects on viability could decrease inbreeding depression and facilitate the evolution of even higher selfing rates. This process could snow-ball until inbreeding depression decreases sufficiently, after which selection favours complete selfing (Figures 2.3, 2.4). Therefore, purging of inbreeding depression should diminish opportunities for the evolution of mixed mating. That said, increased selfing rates may not purge inbreeding depression enough to change the evolutionarily stable strategy. For instance, theory suggests that high inbreeding depression can be maintained despite some selfing (Lande et al. 1994, Ronfort and Couvet 1995, Morgan 2001), and simulations suggest that purging of inbreeding depression due to mildly deleterious alleles occurs very slowly, on the order of 100's to 1000's of generations (reviewed in Charlesworth and Willis 2009). Moreover, numerous species with mixed mating harbour high inbreeding depression (e.g., Eckert and Herlihy 2004, see also Figure 2 in Husband and Schemske 1996), suggesting that purging may not always be appreciable.

In addition to inbreeding depression, the nature of pleiotropy, itself, may evolve. In our model, selection that acts on a focal trait causes changes in the selfing rate, viability, and pollen export in an amount proportional to their sensitivities to the change in the focal trait, and we assume the sensitivities remain constant for any given effect on the focal trait (ϕ) and any initial selfing rate, θ. However, functional relationships between via-

bility or pollen export and the underlying trait could change for a variety of reasons, including non-linear relationships between the traits of interest (e.g., between the magnitude of herkogamy and selfing rate), the appearance of new alleles modifying these functional relationships, or a correlated response by some other trait to selection on the focal trait (see example below). Furthermore, conflicts between two co-evolving traits (e.g., a negative correlation between pollen export and viability) could be resolved either by the evolution of new traits (e.g., dichogamy, Harder et al. 2000) or recombination breaking down a correlation caused by linkage disequilibrium (Conner 2006).

An evolved change in a trait's sensitivity with the evolving focal trait could, in itself, create conditions that produce mixed mating. For example, consider results of artificial selection on flower size that produced a correlated response on ovule number (*Silene latifolia*, Delph et al. 2004; see also Mojica and Kelly 2010) and ovule volume (*Eichhornia paniculata*, Worley and Barrett 2000). If, initially, plants with smaller flowers experienced improved viability ($f_v > 0$), selection for small flowers could reduce the number or size of ovules, reducing fitness in a way that would counteract the viability advantage (making f_v smaller). Therefore, the correlated response to selection by ovules can decrease the fitness / viability benefit of selfing in a manner similar to inbreeding depression and cause mixed mating to evolve. Furthermore, we expect that if correlated selection can cause f_v to decrease sufficiently (e.g., to become negative), mixed mating could evolve even in a population with low inbreeding depression. If the functional relationships between f_d, f_v, and θ were known, equation (2.8) could be applied to predict the regions in which mixed mating, bistability, complete outcrossing and complete selfing are favoured, as we have done here.

2.5.3 Evidence For Pleiotropy and Possible Tests

Data suggest widespread functional pleiotropy as envisioned in our model. For instance, biometric studies commonly report correlations between flo-

ral traits associated with changes in mating system (*Mimulus*: Macnair and Cumbes 1989, Fenster and Ritland 1994; *Arenaria uniflora*: Fishman and Stratton 2004). Indeed, Ashman and Majetic (2006) report that genetic correlations among floral traits are common and (on average) positive, suggesting either widespread pleiotropy or linkage disequilibrium; these results are required for viability selection on floral traits to influence selfing rate. Ashman and Majestic (2006)'s review also reports, at most, a weak correlation between vegetative and floral traits, suggesting restricted pleiotropy between these traits and less potential for viability selection on vegetative traits to influence mating system evolution through correlated responses by floral characters; however, their sample size for this latter result was small. QTL studies also report evidence for pleiotropy between floral traits (*Mimulus*: Lin and Ritland 1997, Fishman et al. 2002; *Leptosiphon*: Goodwillie et al. 2006). Fishman et al. (2002) found pleiotropic associations between stigma-anther distance and other floral traits (but see Fenster and Ritland 1994, Lin and Ritland 1997, Fenster and Barrett 1994, Kohn and Barrett 1994), so that viability selection on floral size (e.g., corolla length) could also affect stigma-anther distance, which is known to influence selfing rate (e.g., Karron et al. 1997). Therefore, the primary requisite for our model, that pleiotropy between floral (or vegetative) traits occurs in plant populations, appears to be easily met. Additional studies that examine how correlations between plant traits (e.g., flower size and stigma-anther separation) translate into functional associations (e.g., viability and selfing rate; Elle 2004) will prove invaluable.

Our graphical analysis provides a (conceptually) straightforward approach to test our model in populations with sufficient genetic variation in selfing rate: with estimates of f_v, f_d, δ, and d, one can produce a "map" specific to a focal population and determine the population's position within it. Our model requires one to first confirm that frequency-independent processes determine selfing rates (e.g., see Schoen and Lloyd (1992) for methods to determine whether prior selfing dominates; see Aizen and Harder (2007) for methods to determine pollen-limitation). One can estimate f_v as $cov_A(\text{viability}, \theta)/var_A(\theta)$ and f_d from $cov_A(\text{discounting}, \theta)/var_A(\theta)$ and

measures of the average selfing and pollen discounting rates, even if the underlying trait under selection is uncertain (see Appendix A.1). $cov_A(x, \theta)$ refers to the genetic covariance between trait x and selfing rate, which can be determined by various methods, including use of clones (measure the mean viability and selfing rate for each clone and determine the covariance and variance among clones) and parent-offspring regression (see Lande and Price 1989 to account for natural selection and maternal effects). Due to the need for variation in the selfing rate to estimate f_d and f_v, the model can only be tested for populations that already have mixed mating systems.

2.5.4 Conclusion

Our study suggests two major conclusions relevant to understanding the diversity of mating systems among angiosperms. First, functional pleiotropy likely holds important consequences for mating system evolution. For instance, if functional pleiotropy between selfing rate and pollen export is common, then models that assume that the processes underlying pollen export remain constant as the selfing rate evolves (e.g., Wells 1979, Lande and Schemske 1985) lack key features pertinent to mating system evolution (Fishman et al. 2002, Fishman and Stratton 2004). Considerations of pleiotropy may also help explain the diversity of selfing rates in animals (Jarne and Auld 2006). Second, as a corollary of this study, we expect that changes in mating system will pleiotropically affect the evolution of floral (and plant) traits. This observation supports other theoretical (Harder and Wilson 1998) and empirical (Galen 1999) studies with this perspective. Future work that integrates the study of mating systems with floral and plant traits will likely help explain the vast diversity of these traits exhibited by flowering plants.

Chapter 3

Functional Pleiotropy and Mating System Evolution in Plants: Mass-Action Models

3.1 Summary

Floral displays are integrated structures, both functionally and genetically, so modifications to display characteristics will likely affect multiple aspects of fitness ("functional pleiotropy"), including pollen export and self-pollination, and therefore selfing rate. As a consequence, much of the great diversity of floral displays and mating systems found among angiosperms has likely not evolved independently. I extend previous mass-action models of mating system evolution to determine how functional pleiotropy that links viability (e.g., probability of survival to reproduction) and the allocation of pollen for export and selfing affects the evolution of selfing, outcrossing, and in particular, mixed mating. I show that the evolutionary outcome depends on how pollen shifts from being exported, unused, or used for selfing. The results indicate that functional pleiotropy that affects viability can explain observations not addressed by previous theory, including the evolution of stable mixed mating despite high inbreeding depression.

Consequently, pleiotropy may play a key role in explaining selfing rates for such species that exhibit otherwise enigmatic mating systems.

3.2 Introduction

Floral displays are functionally integrated structures (Armbruster et al. 2004, Harder 2009, Bissell and Diggle 2010), so mutations that affect the morphology of a floral display will affect multiple functions. For example, the relative positions of floral parts determine both how a pollen vector (e.g., wind, insect) removes pollen from anthers and its deposition onto stigmas (e.g., *Pontederia cordata*, Harder and Barrett 1993, Harder 2000). In addition to this functional integration, floral displays are genetically integrated, as indicated by common genetic correlations among display traits (Armbruster et al. 2004, Ashman and Majetic 2006, Bissell and Diggle 2010). Integration at both functional and genetic levels likely imposes constraints that affect the evolutionary divergence of populations (Schluter 1996), so that functional pleiotropy (i.e., the influence of variation in a single morphological trait on more than one functional trait, Chapter 2) has important consequences for the diversity of floral displays.

Effects of functional pleiotropy hold important consequences for the evolution of selfing rates (Ritland 1991, Kohn and Barrett 1994, Galen 1999, Fishman 2000), which vary from complete outcrossing to almost complete selfing in plants (Goodwillie et al. 2005). For example, pleiotropy that affects pollen export can reduce the transmission bias for an allele that increases selfing (Johnston 1998). A transmission bias arises because an allele that increases selfing can be passed to the next generation in three doses (two doses in a selfed seed and one in pollen exported to other plants) whereas an allele that causes strict outcrossing is transmitted in only two doses (one in the maternal seed and one in seeds sired on other plants; Fisher 1941, Porcher and Lande 2005a). This transmission bias favours the evolution of increased selfing, but it can be reduced by either pollen discounting (the reduction in pollen export due to its use in self-pollination, Harder and Wilson 1998) or inbreeding depression (δ). Classic models

of mating system evolution based on the transmission bias tend to predict the evolution of either complete selfing or outcrossing when inbreeding depression is low ($\delta < \frac{1}{2}$) or high ($\delta > \frac{1}{2}$), respectively (reviewed in Johnston et al. 2009). However, mixed mating (the use of both self- and outcross-pollen for mating) can evolve when functional pleiotropy causes the rate of pollen discounting to increase with the selfing rate (e.g., Johnston 1998). On a more general level, numerous models have shown that functional pleiotropy between aspects of pollination (e.g., attraction: Lloyd 1979; modes of selfing: Schoen et al. 1996, Harder and Wilson 1998; male and female function: Johnston et al. 2009) and selfing rate affect the conditions under which complete selfing, complete outcrossing, or mixed mating evolve.

Variation in floral and inflorescence characteristics can also be subject to viability selection (i.e., the probability of survival to reproduction, or differences in fertility that are not dependent on sex, reviewed by Strauss and Whittall 2006), so that selection by non-pollinating agents can affect mating system evolution via functional pleiotropy (Galen 1999). Consider three brief examples. First, functional pleiotropy can link viability and selfing rate because floral parts serve more than one function, including pollination functions (e.g., attraction, guiding the pollinator for effective contact with anthers and stigmas) and ovule protection (see Galen 1999 for examples). Second, because herbivory may reduce a plants' attractiveness to pollinators and increase pollen left in anthers for autogamous selfing (Penet et al. 2009), tolerance to herbivory may affect mating system evolution. Third, transgenic virus resistance in cultivated squash (*Cucurbita pepo*) affected the number of flowers visited by bees (Prendeville and Pilson 2009), suggesting that mechanisms that influence resistance to viruses could alter selfing rates by changing the incidence of geitonogamy (between-flower self-pollination). Clearly, pleiotropic interactions between viability and mating system can arise from a diversity of mechanisms, but the effects of these interactions for mating system evolution remain largely unexplored.

Only one other paper (Chapter 2) has modeled the effect of viability selection on mating system evolution through functional pleiotropy, and I

extend that work here. Chapter 2 considered the joint effects of pleiotropy between pollen discounting and viability selection on mating system evolution when the population composition does not affect selfing rates (i.e., mating is frequency-independent); that is, this work characterized the evolution of "prior selfing" (anthers dehisce, stigmas become receptive and self-pollination occurs before flowers open; Lloyd and Schoen 1992). These results revealed diverse effects of viability selection for mating system evolution, including the unique result that mixed mating can evolve despite extremely high inbreeding depression. In the current analysis, I model mating system evolution when selfing rates are functions of the relative amounts of self- and outcross-pollen received; Holsinger (1991) termed this formulation a "mass-action" model. Under mass-action, a genotype's selfing rate depends on both the amount of self-pollen it deposits and the amount of outcross pollen received from the other genotypes in the population. Therefore, selfing rates can depend on the composition of the population if genotypes export different quantities of pollen. This fundamental difference warrants a separate analysis of the effects of pleiotropy on mating system evolution. Furthermore, the mass-action models considered here can describe the evolution of "competing selfing" (autonomous selfing that occurs at the same time as outcross pollen may arrive; Lloyd and Schoen 1992), "facilitated selfing" (within-flower self-pollination due to a pollinator's actions; Lloyd and Schoen 1992), or selfing due to geitonogamy. These modes of selfing all involve the simultaneous deposition of self- and outcross-pollen, so that they can be modeled similarly (but see Lloyd 1992). Compared to prior-selfing, facilitated selfing and geitonogamy do not require special mechanisms for selfing (Holsinger 1991, Lloyd and Schoen 1992) and so may be more common. Together, this analysis and that of Chapter 2 assess the impacts of functional pleiotropy between viability and selfing rate for most modes of selfing described by Lloyd and Schoen (1992).

Under mass-action models, female selfing rates can rise due to increases in the amount of self-pollen deposited, reductions in outcross-pollen receipt, or both. Hence, I consider all three of these scenarios for a complete

analysis of mating system evolution. Due to the current debate whether mixed mating systems are evolutionarily stable strategies vs. transitions to complete selfing or outcrossing (Goodwillie et al. 2005), my analysis focuses on the influence of viability selection on the evolution of mixed mating. Like Chapter 2, this analysis reveals that consideration of pleiotropic effects on viability greatly affects predictions for mating system evolution.

3.3 Model Description

3.3.1 Characterizing Pollen Use and Pleiotropy

The model considers a single, diallelic (A, a) locus that affects both viability and pollen use; Table 3.1 summarizes the model's parameters. I assume pollen from genotype ij experiences one of three fates (c.f. Harder et al. 2008): deposition on stigmas as self-pollen, S_{ij}, removal by pollinators with the opportunity to be exported to other plants, X_{ij}, or remaining in anthers or otherwise not used in the pollination process (e.g., removed from the flower by a non-pollinating agent), N_{ij}, such that $S_{ij} + X_{ij} + N_{ij} = 1$. Note that not all pollen in the pools S_{ij} and X_{ij} successfully fertilizes ovules, and N_{ij} does not include these unsuccessful pollen grains. In the following analyses, I consider shifts in pollen use among all three possible combinations of these fates.

Following the conception of functional pleiotropy used here, evolution occurs through selection on an underlying floral (or plant) trait (e.g., flower size or colour, resistance to a virus) that simultaneously affects viability and the fraction of pollen used for self-pollination (S_{ij}) and/or export (X_{ij}). When a mutant allele, a, invades a population fixed for the A allele, it alters the underlying trait by an amount, ϕ, which can change both viability and pollen use.

Fitness is measured relative to that of the resident genotype, AA. With respect to viability (or non-sex-specific fertility), the homozygous mutant genotype has a relative viability of $W_{aa} = 1 + \Delta v\ \phi$. Here Δv represents the sensitivity (i.e., rate of change) of viability to changes in the underly-

Table 3.1: Summary of model's parameters

Symbol	Description
S_{ij}, X_{ij}, N_{ij}	Fractions of pollen allocated to either self-pollen deposition, export, or pollen unused in pollination (see text) by genotype ij, respectively
ϕ	Magnitude of change in the focal plant trait
Δv, Δp	Sensitivities of viability and allocation to pollen (self-pollination and/or export), respectively, to a change in the focal trait
h_v, h_p	Dominance of mutant allele (a) for changes in viability and pollen allocation, respectively
T	Number of pollen grains produced (equal for all genotypes)
π	Compound per-grain probability that pollen removed from a plant (1) reaches another stigma and (2) that a deposited pollen grain produces a pollen-tube that reaches an ovule
ϵ	Compound per-grain probability that pollen in the selfing pool (1) successfully adheres to a stigma and (2) that a self pollen-tube reaches an ovule
δ	Inbreeding depression

ing trait, ϕ. A change in the underlying trait also alters pollen fates. For example, consider pleiotropy that increases the pool of pollen available for selfing: the resident homozygote allocates the fraction $S_{AA} = S$ of its pollen to self-pollination, and the homozygous mutant allocates $S_{aa} = S + \Delta p\ \phi$, where Δp equals the sensitivity of self-pollen deposition to changes in the underlying trait. Without loss of generality, I choose the direction of the underlying trait axis such that increasing ϕ increases the pool of pollen available for selfing ($\Delta p > 0$). Thus, mutants with $\phi > 0$ increase self-pollen deposition, while mutants with $\phi < 0$ decrease allocation to self-pollen. A positive value of Δv thus implies that alleles that augment allocation to selfing also improve viability, whereas a negative value of Δv implies reduced viability. When considering a shift between unused pollen (N_{ij}) and exportable pollen (X_{ij}), I similarly define the trait axis such that increas-

ing ϕ increases the pool of exportable pollen. Heterozygotes have viability equal to $W_{Aa} = 1 + h_v\ \Delta v\ \phi$ and allocate the fraction $S_{Aa} = S + h_p\ \Delta p\ \phi$ of their pollen to self-pollination, where h_v and h_p equal the dominance coefficients of the mutant allele (ranging from 0 to 1) for its effects on viability and allocation to selfing, respectively.

3.3.2 Recursion Equations

The genotypes AA, Aa, and aa begin at frequencies P_{AA}, P_{Aa}, and P_{aa}, respectively. Viability selection changes genotype frequencies according to

$$\begin{aligned} P^v_{AA}\ \bar{W} &= P_{AA} W_{AA} \\ P^v_{Aa}\ \bar{W} &= P_{Aa} W_{Aa} \\ P^v_{aa}\ \bar{W} &= P_{aa} W_{aa} \end{aligned} \tag{3.1}$$

where W_{ij} is the fitness of the genotype ij and $\bar{W}$ equals the population mean fitness (the sum of the right-hand values).

Rates of self-fertilization and outcrossing depend on allocation to self-pollination and export by each genotype, as well the probabilities that self- and outcross-pollen successfully fertilize ovules. Consider a population in which all plants produce T pollen grains. For each genotype, a fraction S_{ij} of these T pollen grains has the opportunity to self-fertilize ovules, of which a fraction ϵ reaches the ovules. The fraction $(1 - \epsilon)$ does not reach ovules either because it failed to adhere to the stigma (pre-pollination failure) and/or because pollen-tubes did not reach ovules (perhaps due to a self-incompatibility reaction; post-pollination failure). Pollinators remove a fraction X_{ij} of the T pollen grains from genotype ij, and a fraction π of this pollen is deposited on other plants and successfully reaches ovules; the fraction $(1 - \pi)$ includes pollen that is either lost during the pollination process (e.g., due to pollinator grooming; pre-pollination failure) or does not fertilize ovules due to pollen-tube failure (post-pollinaton failure). Hence, ϵ and π represent compound probabilities of success for self and outcross pollen for pre- and post-pollination processes.

To calculate selfing rates (following Holsinger 1991), genotype ij, con-

sisting of n_{ij} individuals, will contribute $n_{ij}\ T\ \pi\ X_{ij}$ pollen grains to a pool of outcross pollen that is divided among all individuals in the population, N_{Pop}. Therefore, each individual receives $\frac{n_{ij}}{N_{Pop}}\ T\ \pi\ X_{ij}$, or $P^v_{ij}\ T\ \pi\ X_{ij}$, pollen grains that are able to reach ovules from genotype ij following viability selection. Individuals of genotype ij then self-fertilize the fraction

$$SRate_{ij} = \frac{T\ \epsilon\ S_{ij}}{T\ \epsilon\ S_{ij} + \pi\ (P^v_{AA}\ T\ X_{AA} + P^v_{Aa}\ T\ X_{Aa} + P^v_{aa}\ T\ X_{aa})}, \tag{3.2}$$

of its ovules (i.e., its female selfing rate), such that the number of pollen grains produced, T, cancels from the expression. Note that under this formulation, all genotypes receive equal quantities of outcross pollen, implying that genotypes are equally attractive. Furthermore, I assume that all genotypes receive enough pollen to fertilize all ovules.

To determine the frequencies of ovules fertilized by each genotype via outcrossing, first note that the female outcrossing rate for genotype ij simply equals $ORate_{ij} = 1 - SRate_{ij}$. Pollen from genotype kl fertilizes the fraction

$$FracO_{kl} = \frac{P^v_{kl}\ X_{kl}}{P^v_{AA}\ X_{AA} + P^v_{Aa}\ X_{Aa} + P^v_{aa}\ X_{aa}} \tag{3.3}$$

of outcrossed ovules (π has cancelled out of the expression above).

We can now determine the frequency of each genotype in the next generation. Accounting for the reduction in fitness of selfed offspring relative to outcrossed offspring, δ (inbreeding depression, assumed to be fixed), selfing produces genotypes with frequencies

$$\begin{aligned}
P^{self}_{AA}\ Ovule_{Tot} &= (P^v_{AA}\ SRate_{AA} + \frac{1}{4}\ P^v_{Aa}\ SRate_{Aa})\ (1-\delta) \\
P^{self}_{Aa}\ Ovule_{Tot} &= \frac{1}{2}\ P^v_{Aa}\ SRate_{Aa}\ (1-\delta) \\
P^{self}_{aa}\ Ovule_{Tot} &= (P^v_{aa}\ SRate_{aa} + \frac{1}{4}\ P^v_{Aa}\ SRate_{Aa})\ (1-\delta)
\end{aligned} \tag{3.4}$$

and outcrossing produces the frequencies

$$\begin{aligned}
P_{AA}^{out}\, Ovule_{Tot} =& P_{AA}^{v}\, ORate_{AA}\, FracO_{AA} + \frac{1}{2}\, P_{AA}^{v}\, ORate_{AA}\, FracO_{Aa} + \\
& \frac{1}{2}\, P_{Aa}^{v}\, ORate_{Aa}\, FracO_{AA} + \frac{1}{4}\, P_{Aa}^{v}\, ORate_{Aa}\, FracO_{Aa} \\
P_{Aa}^{out}\, Ovule_{Tot} =& P_{AA}^{v}\, ORate_{AA}\, FracO_{aa} + P_{aa}^{v}\, ORate_{aa}\, FracO_{AA} + \\
& \frac{1}{2}\, P_{AA}^{v}\, ORate_{AA}\, FracO_{Aa} + \frac{1}{2}\, P_{Aa}^{v}\, ORate_{Aa}\, FracO_{AA} + \\
& \frac{1}{2}\, P_{aa}^{v}\, ORate_{aa}\, FracO_{Aa} + \frac{1}{2}\, P_{Aa}^{v}\, ORate_{Aa}\, FracO_{aa} \\
& + \frac{1}{2}\, P_{Aa}^{v}\, ORate_{Aa}\, FracO_{Aa} \\
P_{aa}^{out}\, Ovule_{Tot} =& P_{aa}^{v}\, ORate_{aa}\, FracO_{aa} + \frac{1}{2}\, P_{aa}^{v}\, ORate_{aa}\, FracO_{Aa} + \\
& \frac{1}{2}\, P_{Aa}^{v}\, ORate_{Aa}\, FracO_{aa} + \frac{1}{4}\, P_{Aa}^{v}\, ORate_{Aa}\, FracO_{Aa}
\end{aligned} \tag{3.5}$$

where $Ovule_{Tot}$ equals the sum of the right hand-sides of all the expressions in (3.4) and (3.5). The frequencies of genotype ij equal the sum of the contributions through selfing and outcrossing in equations (3.4) and (3.5).

3.3.3 Stability Analysis

I performed a local stability analysis by constructing a stability matrix of the recursions and asked when the a allele could invade a population fixed for the A allele. Two key assumptions simplified this analysis (see also Chapter 2). First, I assumed that the invading allele had a small effect on the floral trait, ϕ, by using a Taylor series to linearize the recursion equations (this both simplifies the analysis and reflects the common finding that multiple genes of small effect underlie floral traits; Kalisz and Kramer 2007). Additionally, I assumed that the dominance of the invading allele was similar for viability and allocation to selfing (i.e., $h_v = h_p$, both of which I denote by h). The assumption of similar dominance for these plant characters may be reasonable because the changes in viability and pollen fates derive from changes in a common underlying trait. Equation (B.1) (Appendix B.1)

presents the invasion condition for a general model, in which pollen can simultaneously shift between all three pollen fates (S_{ij}, X_{ij}, N_{ij}). Below I present results from specific cases of this general model.

3.4 Results

3.4.1 Shifting Pollen from being Unused to Exportable Pollen

In this first case, I consider evolution that increases the pool of exportable pollen by reducing the amount of unused pollen; here, mating depends on population composition because pollen export varies among genotypes. Although one may not typically expect pollen to remain in anthers at the end of a flower's life because natural selection should not favour pollen wastage, several arguments warrant this (and the next) analysis. First, empirical measures demonstrate that pollen remains unused in anthers for numerous species (reviewed by Harder 2000), which may arise particularly when a population is not adapted to its current environment (e.g., when a plant species invades a new habitat). In addition, "unused pollen" (N_{ij}) includes pollen dislodged from flowers without the opportunity for transport, which may be a considerable cost in some species. For instance, Harder and Thomson (1989) showed that, of the pollen removed by bumblebees (*Bombus* spp.) during visits to *Erythronium americanum*, an average of $14.0\% \pm 8.3\%$ of pollen removed from flowers fell to the ground. Similarly, floral visitors that remove pollen without dispersing it to conspecifics (e.g., "pollen thieves", such as honey bees, *Apis mellifera*, which can collect pollen and disperse little; Hargreaves et al. 2010) cause pollen loss and selection to reduce N_{ij}. Second, including this analysis provides generality to this model of pollen fates. Third, we see below that selection does not universally favour the use of all pollen in the presence of functional pleiotropy.

As a specific example, many plants restrict pollen presentation to individual pollinators to maximize pollen export (reviewed by Harder and Thomson 1989). However, a reduction in pollinator availability would leave pollen unused in anthers (Harder 2000). Selection may then reduce

pollen restriction and floral longevity (because male function is satisfied sooner; Ashman and Schoen 1994), which could free resources for additional flower production and effectively increase fitness.

In this scenario allocation to self-pollen does not evolve, so $S_{ij} = S$. Genotypes ij export pollen according to $X_{AA} = X$, $X_{Aa} = X + h_p\ \Delta p\ \phi$, and $X_{aa} = X + \Delta p\ \phi$. Unused pollen is set to $N_{ij} = 1 - X_{ij} - S$.

The a allele can invade a population fixed for A when the leading eigenvalue of the stability matrix, λ, exceeds one. Subtracting one from the leading eigenvalue, invasion thus occurs when $\beta_{NtoX} = \lambda - 1$ is positive, where:

$$\begin{aligned}\beta_{NtoX} = &\frac{(S\ \epsilon\ (1-\delta) + 2\ h\ X\ \pi)}{2\ (S\ \epsilon\ (1-\delta) + 2\ X\ \pi)\ (S\ \epsilon\ (1-\delta) + X\ \pi)} \\ &\cdot [\phi\ (\Delta p\ \pi + \Delta v\ 2\ (S\ \epsilon\ (1-\delta) + X\ \pi))].\end{aligned} \tag{3.6}$$

Because the fraction is always positive, increased allocation to pollen export ($\phi > 0$) will invade if the sign of the term in square brackets is positive.

When change in the underlying trait does not affect viability ($\Delta v = 0$), selection always favours reallocating unused pollen to pollen export ($\Delta p\ \pi > 0$; see also Harder and Wilson 1998). With viability selection ($\Delta v \neq 0$), a shift of pollen from the unused pool to exportable pollen can evolve when

$$-\frac{\pi}{2\ (S\ \epsilon\ (1-\delta) + X\ \pi)} < \frac{\Delta v}{\Delta p}. \tag{3.7}$$

When increased viability accompanies increased allocation to outcrossing pollen ($\Delta v > 0$) selection always favours reallocating unused pollen to outcross pollen, as expected. However, if involving more unused pollen in outcrossing is correlated with a decrease in viability ($\Delta v < 0$), this reallocation can only occur when the sensitivity of viability (relative to the sensitivity of pollen allocation) is sufficiently weak. In addition, when $\Delta v < 0$ selection can initially favour an increase in pollen allocated to outcrossing but may not cause all unused pollen to be re-allocated. To see this, note that high allocation to outcrossing (X) makes the left-hand-side less negative, and the conditions for re-allocation become more strict. Therefore, when $\Delta v < 0$ selection can initially favour reductions in unused pollen but

may not cause re-allocation of all unused pollen to export. The fact that N_{ij} evolves towards a non-zero level reflects a diminishing returns benefit from increasing pollen export when exported pollen must compete more severely with other exported pollen.

3.4.2 Shifting Pollen from being Unused to Self-Pollination

In the second case, I consider the evolution of increased self-pollination by shifting pollen that remains in anthers (N_{ij}) to its use for selfing (S_{ij}); here, mating of a focal individual does not depend on population composition because genotypes export and receive similar quantities of outcross pollen (Harder et al. 2008). This case describes mutations that affect viability and increase selfing without causing pollen discounting.

Now, the *a* allele can invade when $\beta_{NtoS} = \lambda - 1$ is positive, where:

$$\begin{aligned}\beta_{NtoS} = & \frac{(S\,\epsilon\,(1-\delta) + 2\,h\,\pi\,X)}{2\,(S\,\epsilon\,(1-\delta) + 2\,\pi\,X)\,(S\,\epsilon\,(1-\delta) + \pi\,X)\,(S\,\epsilon + \pi\,X)} \\ & \cdot [\phi\,(\Delta p\,\epsilon\,\pi\,X\,(1-2\,\delta) + \\ & 2\,\Delta v\,(S^2\,\epsilon^2\,(1-\delta) + S\,X\,\epsilon\,\pi\,(2-\delta) + \pi^2\,X^2))].\end{aligned} \tag{3.8}$$

The fraction is always positive. Therefore, increased selfing ($\phi > 0$) can invade when the sign of the term in square brackets is positive.

When a shift in pollen allocation does not affect viability ($\Delta v = 0$), increased selfing evolves when $\Delta p\,\epsilon\,\pi\,X\,(1-2\,\delta) > 0$, so that reallocating pollen from the unused pollen pool to selfing only occurs when inbreeding depression is low ($\delta < \frac{1}{2}$). Even though there is not a direct reallocation between selfing and outcrossing, increasing S does reduce the success of outcross pollen in competition (see equation 3.2). Thus we regain one of the most common predictions in models of mating system evolution (e.g., Lloyd 1979, Lloyd 1992).

With pleiotropic effects on viability, shifting unused pollen to its use in self-pollination evolves when

$$-\frac{\epsilon\,\pi\,(1-2\,\delta)\,X}{2\,(S^2\,\epsilon^2\,(1-\delta) + S\,X\,\epsilon\,\pi\,(2-\delta) + \pi^2\,X^2)} < \frac{\Delta v}{\Delta p}. \tag{3.9}$$

The denominator of the left-hand-side is always positive and is an upward-facing parabola whose minimum occurs at a negative value of S; therefore, as allocation to self-pollen increases from $S = 0$, the denominator always becomes larger.

When inbreeding depression is low ($\delta < \frac{1}{2}$), the left-hand-side of (3.9) is negative, so viability benefits of functional pleiotropy ($\Delta v > 0$) always favour increased selfing. In addition, increased selfing can evolve despite decreased viability ($\Delta v < 0$) when inbreeding depression is low, so long as viability costs are not too great (i.e., $\frac{\Delta v}{\Delta p}$ is sufficiently close to zero). However, for reasons similar to the previous case, selection may not favour re-allocating all unused pollen to self pollen.

Sufficiently high viability benefits ($\Delta v > 0$) allows increased selfing to invade even when inbreeding depression is high ($\delta > \frac{1}{2}$). Now, the conditions for the evolution of increased selfing relax as selfing rates increase (with $\delta > \frac{1}{2}$ the left-hand side of (3.9) is positive, but it becomes smaller as allocation to S increases). Therefore, with high $\Delta v > 0$ selection favours the re-allocation of all unused pollen to self-pollen. Given that (X) is assumed to be unaffected by the trait, the system thus evolves to mixed mating despite high inbreeding depression when (3.9) is satisfied and $\frac{\Delta v}{\Delta p} > 0$, with selfing evolving to as high a level as possible given the amount of unused pollen. In contrast, with viability costs ($\Delta v < 0$) and high inbreeding depression ($\delta > \frac{1}{2}$), increased selfing never evolves (the left-hand side of (3.9) is positive, but $\frac{\Delta v}{\Delta p} < 0$).

3.4.3 Re-Allocation of Exportable Pollen to Self-Pollen

In this third case, I consider mating system evolution when each pollen grain allocated to selfing comes at the expense of an exported pollen grain; in this case complete pollen discounting occurs. Like the first case, mating of a focal individual depends on the population composition because pollen export differs among genotypes. As an example, viability selection due to a short growing season could favour shortened floral longevity and reduced dichogamy (temporal separation of sex function within flowers;

Mazer et al. 2004), which could increase the incidence of geitonogamous selfing (see Harder et al. 2000).

Invasion of selfing can occur when $\beta_{XtoS} = \lambda - 1$ is positive, where,

$$\begin{aligned}\beta_{XtoS} = & \frac{(S\,\epsilon\,(1-\delta) + 2\,h\,\pi\,X)}{2\,(S\,\epsilon\,(1-\delta) + 2\,\pi\,X)\,(S\,\epsilon\,(1-\delta) + \pi\,X)\,(S\,\epsilon + \pi\,X)} \\ & \cdot [\phi\,(-\Delta p\,\pi\,(S\,\epsilon - X\,(\epsilon\,(1-2\,\delta) - \pi)) + \\ & 2\,\Delta v\,(S^2\,\epsilon^2\,(1-\delta) + S\,X\,\epsilon\,\pi(2-\delta) + X^2\,\pi^2))].\end{aligned} \tag{3.10}$$

Again, the fraction is always positive and increased allocation to selfing at the expense of exportable pollen ($\phi > 0$) can invade when the term in square brackets is positive.

It is instructive to first consider mating system evolution when pleiotropy does not affect viability ($\Delta v = 0$) because this collapses to cases considered previously (Holsinger 1991, Harder et al. 2008).

Setting $\Delta v = 0$ and substituting $X = 1 - S - N$ into the term in square brackets in (3.10) shows that increased selfing can invade when:

$$\Delta p\,\pi\,((1-N)\,(\epsilon\,(1-2\,\delta) - \pi) - S\,(2\,\epsilon\,(1-\delta) - \pi)) > 0. \tag{3.11}$$

Note that this expression has either zero or one root for S between $0 < S < 1$. Setting $S = 0$ and solving for when (3.11) is true yields the conditions that allow a mutant with a small amount of selfing to invade a purely outcrossing population:

$$\delta < \frac{1 - \frac{\pi}{\epsilon}}{2}. \tag{3.12}$$

Furthermore, it can be shown that mutants increasing outcrossing ($\phi < 0$) can always invade completely selfing populations, so mixed mating evolves whenever selfing can invade outcrossing populations (this re-derives results from Holsinger 1991, Harder et al. 2008). Selfing can invade whenever reallocating pollen to selfing from outcrossing increases the likelihood that a plant's pollen will reach ovules (i.e., $\epsilon > \pi$), so long as high inbreeding depression does not reduce the fitness contribution of a selfed seed below that of an outcrossed seed (the source of the "$\frac{1}{2}$" in relation (3.12)).

Mixed mating evolves whenever (3.12) is satisfied because increased allocation to selfing enhances competition among a plant's own pollen grains for its own ovules (Harder et al. 2008). This local mate competition ensures that complete selfing does not evolve, even if inbreeding depression is absent. Therefore, only complete outcrossing or mixed mating are possible evolutionary outcomes (see also Holsinger 1991, Harder et al. 2008).

Now consider mating system evolution with functional pleiotropy between pollen use and viability ($\Delta v \neq 0$). Inspecting the term in square brackets in equation (3.10) immediately reveals two insights. First, the Δv term is multiplied by a quadratic equation whose value is always positive, which implies that pleiotropy that increases viability with higher allocation to selfing will always increase selection for selfing. Likewise, pleiotropy that decreases viability always favours outcrossing.

Second, because the term in square brackets in (3.10) is a quadratic with respect to S, pleiotropic effects on viability create evolutionary scenarios that otherwise are not possible. Now there may be zero, one or two roots for S between $0 < S < 1$, whereas we find at most one root when $\Delta v = 0$; I denote the two roots as S^- and S^+ (the superscript refers to the radical's sign for S as calculated by the quadratic formula; see Appendix B.2). In addition, the shape of the term in square brackets in (3.10) can take two general forms: either a parabola with upwards curvature (e.g., Figure 3.1 A-F) or downwards curvature (e.g., Figure 3.1 G-L). The combination of having either zero, one or two roots in the range $0 < S < 1$ and two forms of curvature introduces a rich diversity of evolutionary scenarios for mating system evolution that are not possible with $\Delta v = 0$, as illustrated in Figure 3.1.

To interpret this figure, recall that increased selfing ($\phi > 0$) can invade when the term in square brackets is positive but cannot when it is negative. Therefore, if the function is positive for all $0 < S < 1$ then complete selfing evolves (e.g., Figure 3.1A), whereas complete outcrossing evolves if it is always negative (e.g., Figure 3.1C). In addition, mixed mating can evolve if increased selfing is favoured for low allocation to selfing rate but not at a higher one (e.g., Figure 3.1D, E). Finally, "bistability" is possible

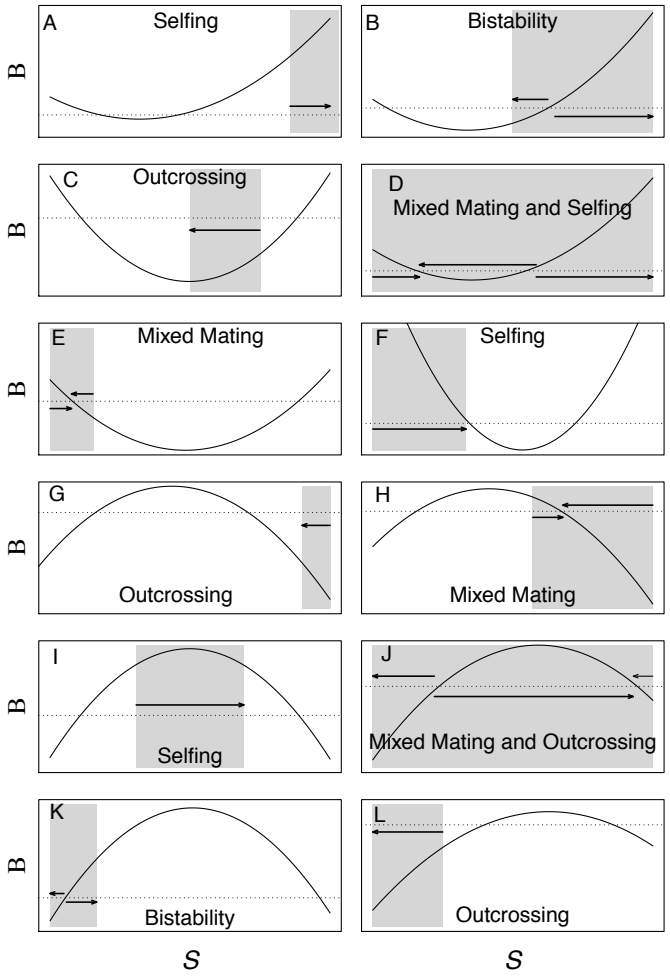

Figure 3.1: Evolutionary scenarios for mating system evolution under complete pollen discounting. Shaded regions indicate biologically relevant allocations to self-pollen (i.e., $0 \leq S \leq 1$). Arrows show the direction of evolution. Curve indicates the value of the portion of (3.10) in square brackets, making β_{XtoS} positive or negative. The dotted line indicates neutrality (i.e., the portion of (3.10) in square brackets equals zero); selection favours increased allocation to selfing for a given S when the curve lies above the dotted line (as indicated by arrows pointing towards higher allocation to selfing); decreased allocation to selfing evolves when the curve lies beneath the dotted line.

where more outcrossing is always favoured below some critical allocation to self-pollen and selfing is always favoured above it (e.g., Figure 3.1B); in this case, either complete selfing or complete outcrossing will evolve, depending on the population's initial allocation to self-pollen.

To determine which outcome illustrated in Figure 3.1 would occur for different parameter values, I set $X = 1 - S - N$ in the square bracket term of (3.10). As the analysis considers shifts only between X and S, I re-scaled S and Δp as S' and $\Delta p'$, respectively, to obtain results independent of N, where $S' = S/(1-N)$ and $\Delta p' = \Delta p/(1-N)$. (Note that I drop the $'$ notation beyond this paragraph.) By re-scaling S and Δp the analysis can focus on pollen used for either selfing or outcrossing (i.e., $X' + S' = 1$, where $X' = X/(1-N)$). Therefore, complete outcrossing or selfing occur when $S' = 0$ or $S' = 1$, respectively. Re-scaling Δp ensures that a given magnitude of $\Delta p'$ affects mating system evolution similarly for any N. Note that this approach provides the same result as assuming that $N = 0$.

Upon rescaling, increased allocation to selfing can invade when

$$\begin{aligned}\Delta p\ \pi\ (\epsilon\ (1 - 2\ S\ (1-\delta) - 2\ \delta) - \pi\ (1-S)) \\ + 2\ \Delta v\ ((\epsilon - \pi)\ (\epsilon\ (1-\delta) - \pi)\ S^2 + \pi\ (\epsilon\ (2-\delta) - 2\ \pi)\ S + \pi^2) > 0.\end{aligned} \tag{3.13}$$

Next, I determined the conditions that yield all combinations of $S^{-,+} < 0$, $0 < S^{-,+} < 1$, or $S^{-,+} > 1$ for S^- and S^+, and upwards or downwards curvature (*Mathematica* file available on request). Tables 3.2 and 3.3 summarize the conditions.

Given that $S^{-,+}$ are real, we can define four compound-parameters, ζ, κ, τ and ω that describe two aspects of the fitness landscapes depicted in Figure 3.1 and determine the results in Tables 3.2 and 3.3. First, ζ and τ describe the slope of the curve described by (3.13) at points of complete outcrossing ($S = 0$) and complete selfing ($S = 1$), such that the slopes at these points are positive when $\zeta > 0$ and $\tau > 0$, respectively (see Figure 3.1). Second, κ and ω are the intercepts of (3.13) at $S = 0$ and $S = 1$, respectively, which indicate when selfing or outcrossing can invade completely

outcrossing or selfing populations, respectively. Selfing can invade a purely outcrossing population when $\kappa > 0$ (as written in Tables 3.2 and 3.3), or equivalently:

$$\frac{-\epsilon\,(1-2\,\delta)+\pi}{2\,\pi} < \frac{\Delta v}{\Delta p}. \tag{3.14}$$

Similarly outcrossing can invade a population with $S = 1$ when $\omega < 0$, or

$$\frac{\Delta v}{\Delta p} < \frac{\pi}{2\,\epsilon\,(1-\delta)}. \tag{3.15}$$

Tables 3.2 and 3.3 reveals three primary results. First, complete outcrossing is the most common mating system to evolve, likely because local mate competition promotes the invasion of outcrossing. Second, functional pleiotropy that enhances viability with allocation to selfing ($\Delta v > 0$) can overcome local-mate competition and cause the evolution of complete selfing; indeed, complete selfing can evolve even with high inbreeding depression ($\delta > \frac{1}{2}$). Third, mixed mating can arise in three different scenarios.

In the first, simplest scenario, mixed mating evolves when selfing can invade a purely outcrossing population (3.14) and vice-versa (3.15), as seen in Figures 3.1E, 1H. Mixed mating can evolve in ths scenario despite $\Delta v < 0$, however $\Delta v < 0$ limits the invasion of selfing into outcrossing populations and therefore reduces opportunties for mixed mating to evolve, relative to a model with $\Delta v = 0$. Importantly, mixed mating can evolve despite high inbreeding depression when $\Delta v > 0$. However, viability benefits that accompany selfing cannot be too strong, or else complete selfing evolves instead (when (3.15) is not satisfied).

In the other two scenarios, mixed mating arises when both S^- and S^+ lie between 0 and 1. Mixed mating occurs with either complete selfing (upward curvature; e.g., Figure 3.1D) or complete outcrossing (downward curvature; e.g., Figure 3.1 J) as alternate stable states; in each case, the selfing rate that evolves depends on the population's initial allocation to selfing, S. Such mixed mating only arises when viability increases with the allocation to self-pollen ($\Delta v > 0$) and when pollen allocated to selfing has a greater probability of fertilizing ovules than pollen allocated to outcrossing ($\epsilon > \pi$;

Table 3.2: Conditions for evolutionary scenarios predicted by pleiotropy between viability and allocation to self-pollination, with complete pollen discounting (Figure 3.1), for cases with upward curvature (downward curvature results in Table 3.3). S^- and S^+ refer to the two roots for the initial allocation to self-pollen in equation (3.13). S_{inv} and O_{inv} indicate whether selfing or outcrossing can invade completely outcrossing or selfing populations, respectively. "Result" includes Bistability, in which selection favours complete outcrossing or complete selfing below and above a threshold value of $0 < S < 1$, respectively (B), or selection for either: Complete Selfing (S), Complete Outcrossing (O), either Mixed Mating or Complete Selfing (M, S), either Mixed Mating or Complete Outcrossing (M, O), or only Mixed Mating (M). "Fig." refers to the panel in Figure 3.1 with an exemplary illustration of the given scenario. The final column indicates the percent of parameter space with the given outcome for a numerical search over parameters with the following ranges: $0.05 \leq \Delta p, \epsilon, \pi, \delta \leq 0.95$, and $-0.95 \leq \Delta v \leq 0.95$ in increments of 0.1, assuming $N_{ij} = 0$. Δv, ϵ and δ were offset by 0.00001 (e.g., $\Delta v = -0.94999$) to avoid numerical errors.

S^-	S^+	Result	S_{inv}	O_{inv}	Fig.					
Upward Curvature: $4\,\Delta v\,(\epsilon-\pi)\,(\epsilon\,(1-\delta)-\pi)>0$										
$S^-<0$	$S^+<0$	S	Yes	No	A	$\zeta>0$	$\kappa>0$			1.69%
$S^-<0$	$0<S^+<1$	B	No	No	B		$\kappa<0$	$\tau>0$	$\omega>0$	1.46%
$S^-<0$	$1<S^+$	O	No	Yes	C		$\kappa<0$		$\omega<0$	21.58%
$0<S^-<1$	$0<S^+<1$	M, S	Yes	No	D	$\zeta<0$	$\kappa>0$	$\tau>0$	$\omega>0$	0.04%
$0<S^-<1$	$1<S^+$	M	Yes	Yes	E	$\zeta<0$	$\kappa>0$		$\omega<0$	12.79%
$1<S^-$	$1<S^+$	S	Yes	No	F			$\tau<0$	$\omega>0$	5.27%
Imaginary		S								7.18%

$\zeta = \tau - 4\,\Delta v\,(\epsilon-\pi)\,(\epsilon\,(1-\delta)-\pi)$; slope of (3.13) at $S=0$
$\kappa = \Delta p\,\pi\,(\epsilon\,(1-2\,\delta)-\pi)+2\,\Delta v\,\pi^2$; intercept of (3.13) at $S=0$
$\tau = -\Delta p\,\pi\,(2\,\epsilon\,(1-\delta)-\pi)+2\,\Delta v\,\epsilon\,(2\,\epsilon\,(1-\delta)-\pi\,(2-\delta))$; slope of (3.13) at $S=1$
$\omega = -\Delta p\,\epsilon\,\pi+2\,\Delta v\,\epsilon^2\,(1-\delta)$; intercept of (3.13) at $S=1$

Table 3.3: Conditions for evolutionary scenarios predicted by pleiotropy between viability and allocation to self-pollination, with complete pollen discounting (Figure 3.1) for cases with downward curvature. See caption of Table 3.2 for additional details. The percentage of parameter space that yields a given outcome pools results from this Table and Table 3.2

S^-	S^+	Result	S_{inv}	O_{inv}	Fig.					
Downward Curvature: $4\,\Delta v\,(\epsilon-\pi)\,(\epsilon\,(1-\delta)-\pi)<0$										
$S^-<0$	$S^+<0$	O	No	Yes	G	$\zeta<0$	$\kappa<0$			10.56%
$0<S^-<1$	$S^+<0$	M	Yes	Yes	H		$\kappa>0$	$\tau<0$	$\omega<0$	5.16%
$1<S^-$	$S^+<0$	S	Yes	No	I		$\kappa>0$		$\omega>0$	6.47%
$0<S^-<1$	$0<S^+<1$	M, O	No	Yes	J	$\zeta>0$	$\kappa<0$	$\tau<0$	$\omega<0$	0.10%
$1<S^-$	$0<S^+<1$	B	No	No	K	$\zeta>0$	$\kappa<0$		$\omega>0$	0.58%
$1<S^-$	$1<S^+$	O	No	Yes	L			$\tau>0$	$\omega<0$	16.15%
Imaginary		O								10.98%

$\zeta=\tau-4\,\Delta v\,(\epsilon-\pi)\,(\epsilon\,(1-\delta)-\pi)$; slope of (3.13) at $S=0$
$\kappa=\Delta p\,\pi\,(\epsilon\,(1-2\,\delta)-\pi)+2\,\Delta v\,\pi^2$; intercept of (3.13) at $S=0$
$\tau=-\Delta p\,\pi\,(2\,\epsilon\,(1-\delta)-\pi)+2\,\Delta v\,\epsilon\,(2\,\epsilon\,(1-\delta)-\pi\,(2-\delta))$; slope of (3.13) at $S=1$
$\omega=-\Delta p\,\epsilon\,\pi+2\,\Delta v\,\epsilon^2\,(1-\delta)$; intercept of (3.13) at $S=1$

results not shown; *Mathematica* file available on request).

With upward curvature ($\delta < 1 - \frac{\pi}{\epsilon}$), mixed mating evolves in populations with relatively low inbreeding depression ($\delta < \frac{1}{2}$; results not shown, *Mathematica* file available on request) and low or intermediate initial allocation to selfing (Figure 3.1D). Selfing can invade outcrossing populations (in part due to low δ), but local mate competition and pollen discounting arrest selection for further allocation to selfing at some intermediate selfing rate, despite a viability advantage of increased selfing (i.e., $\Delta v > 0$). In populations with a high initial allocation to selfing (S), the benefits of increased viability ($\Delta v > 0$) can outweigh these costs of selfing and favour the evolution of complete selfing.

The third scenario for the evolution of mixed mating, involving downward curvature ($\delta > 1 - \frac{\pi}{\epsilon}$; Figure 3.1J), only arises in populations with high inbreeding depression ($\delta > \frac{1}{2}$; results not shown, *Mathematica* file available on request), and sometimes occurs in populations with very high inbreeding depression (e.g., $\delta = 0.95$). High δ prevents selfing from invading purely outcrossing populations, so populations with low initial S evolve complete outcrossing. However, mixed mating evolves in populations with higher initial allocation to selfing because at intermediate S selection favours increased selfing due to pleiotropic increases in viability ($\Delta v > 0$), but at high S inbreeding depression costs imposed on a large fraction of offspring negate these benefits so selection favours increased outcrossing.

3.5 Discussion

Functional pleiotropy between viability selection and allocation of pollen to selfing or export offers novel insights into the evolution of plant mating systems and can potentially explain empirical findings that are not predicted by models that lack pleiotropic effects on viability. I considered three different scenarios that represent different forms of pleiotropy: re-allocation of unused pollen to either selfing or export, and re-allocation of pollen for selfing that removes it from the pool for export. Differences in the conditions for mating system evolution among these scenarios highlight the

importance of the form of pleiotropy for the evolution of selfing rates.

The model revealed three main results. First, as expected, pleiotropy that decreased viability with increased allocation to selfing ($\Delta v < 0$) favours the evolution of complete outcrossing. Second, viability benefits that accompany increased allocation to selfing ($\Delta v > 0$) allows the evolution of complete selfing with complete pollen discounting, which was not possible when pleiotropy did not affect viability ($\Delta v = 0$; e.g., see Holsinger 1991). Therefore, viability benefits can overcome costs of local-mate competition and pollen discounting to cause the evolution of complete selfing, even in the face of high inbreeding depression. This result supports verbal arguments for the role of abiotic selection for the evolution of selfing (e.g., Runions and Geber 2000). Third, increased viability with allocation to self-pollen expands the opportunities for mixed mating to evolve; in particular, $\Delta v > 0$ allowed mixed mating to evolve despite high inbreeding depression, which is rarely predicted by theory (and not by previous models of competing selfing, e.g., Harder et al. 2008). These results are similar to those reported in Chapter 2, but one difference deserves mention. With prior-selfing, mixed mating could not evolve when pleiotropy decreased viability with selfing unless the rate of pollen discounting also decreased (Chapter 2); but with mass-action mixed mating can evolve with $\Delta v < 0$, even though the analysis did not consider an evolving discounting rate. Overall, these studies suggest that pleiotropy between viability selection and factors that alter selfing rate may affect mating system evolution in a similar manner, regardless of whether mating is frequency-dependent (prior-selfing and frequency-independent mating: Chapter 2; facilitated- and competing-selfing, geitonogamy, and frequency-dependent mating: Chapter 3).

As expected, whether or not allocation of pollen to selfing involves pollen discounting greatly affects mating system evolution (i.e., whether self pollen comes from the unused pool or the export pollen pool). Consider the evolution of mixed mating in populations with high inbreeding depression. Without pollen discounting (i.e., shifting pollen between non-use and selfing), mixed mating evolves whenever selfing can invade an outcrossing

population, without major constraints on the range of parameters (in particular, ϵ and π). This case might help explain the maintenance of mixed mating in species like *Aquilegia canadensis*, which gains little reproductive assurance from selfing, has a high selfing rate (76%), very high inbreeding depression, but little geitonogamy (which involves complete pollen discounting) (reviewed by Eckert and Herlihy 2004).

In contrast, when mating system evolution involves complete pollen discounting with high inbreeding depression, mixed mating can only evolve for a restrictive range of ϵ and π. Numerous studies have measured the fraction of pollen removed from flowers that reaches conspecific stigmas, which typically equals less than 1% for species with granular pollen (reviewed by Harder and Johnson 2008). These data suggest that π is small, and inform a general estimate of π (say, within an order of magnitude), particularly if the post-pollination component of π is large. For illustration, if $\pi = 0.01$ mixed mating only evolves in the scenario with one root for S between 0 and 1 (e.g., Figure 3.1E) either when self-pollen has a low probability of reaching ovules (say, $\epsilon < 0.05$ for $\delta = 0.95$) or inbreeding depression approaches unity (say, $\delta > 0.999$ to allow mixed mating with $\epsilon \approx 0.5$). Similarly, in the scenario with two roots for S between 0 and 1 and downward curvature (Figure 3.1J), mixed mating can only evolve for relatively low ϵ, but the conditions are less restrictive than in the single-root case (Table 3.4). Mixed mating evolves more easily under both scenarios when outcross pollen reaches stigmas more successfully (e.g., $\pi = 0.1$, which may be common for species with aggregated pollen, Harder and Johnson 2008; Table 3.4). Therefore, with high inbreeding depression and complete pollen discounting, mixed mating is most likely to evolve in species with residual self-incompatibility and/or traits associated with high π (e.g., aggregated pollen). In addition, mixed mating can only evolve in the case with downward curvature (Figure 3.1J) when a sufficient fraction of pollen is initially used for selfing (S sufficiently high; e.g., *Leptosiphon jepsonii*, Goodwillie and Ness 2005).

Although I have treated mating system evolution with and without pollen discounting separately, both scenarios can operate within a given

Table 3.4: Example parameter ranges in which mixed mating arises, exemplified by Figures 3.1D and 3.1J. Parameter ranges are derived from a numerical search for combinations that satisfy the conditions for mixed mating given $\pi = 0.01$; the search considered Δp, Δv, ϵ, δ that ranged from 0.05 to 0.95 in increments of 0.05 (δ (and ϵ for $\pi = 0.1$) was offset by 0.00001 to avoid rounding errors when subtracting values that should equal zero). The ranges of Δp and Δv explored were arbitrary, and need not be restricted to values less than 1. Note that these values present parameter ranges over all combinations, so that the specific combinations that yield mixed mating will be more restrictive than implied by the ranges, themselves (e.g., for $\pi = 0.01$, 122 and 84 combinations yielded mixed mating out of 130321 combinations tested for upward and downward curvature, respectively).

Case	Δp	Δv	ϵ	δ
$\pi = 0.01$				
$\Delta v\,(\epsilon - \pi)\,(\epsilon\,(1-\delta) - \pi) > 0$	0.25 - 0.95	0.05 - 0.25	0.05 - 0.45	0.25 - 0.45
$\Delta v\,(\epsilon - \pi)\,(\epsilon\,(1-\delta) - \pi) < 0$	0.05 - 0.95	0.05 - 0.95	0.05 - 0.1	0.9 - 0.95
$\pi = 0.1$				
$\Delta v\,(\epsilon - \pi)\,(\epsilon\,(1-\delta) - \pi) > 0$	0.15 - 0.95	0.05 - 0.35	0.25 - 0.95	0.15 - 0.45
$\Delta v\,(\epsilon - \pi)\,(\epsilon\,(1-\delta) - \pi) < 0$	0.05 - 0.95	0.05 - 0.95	0.15 - 0.9	0.65 - 0.95

population. For instance, imagine that when selfing initially invades an outcrossing population, evolution occurs through changes in an underlying trait that does not cause pollen discounting. If selection favours it, allocation to increased selfing can continue along this trait axis until no unused pollen remains in anthers ($N_{ij} = 0$), at which point further increases in allocation to selfing must involve pollen discounting. However, the conditions that favour increased allocation to selfing are more stringent under pollen discounting than without (the term in square brackets in (3.10) is smaller than that in (3.8) by the amount $\Delta p\ \pi\ (\epsilon\ S + \pi\ X)$), so selection that favoured a complete re-allocation of unused pollen to selfing may not be sufficient to cause further increases in selfing. In general, equation (B.1; see Appendix B.1) can be used to determine evolutionary trajectories when pollen is reallocated among the three pools (N, X, S).

Several parameters in these models are likely to evolve with the selfing rate and affect the outcome of mating system evolution. Principally, an increase in selfing is expected to purge deleterious alleles and reduce inbreeding depression (Byers and Waller 1999, Crnokrak and Barrett 2002). Purging can facilitate the evolution of complete selfing and reduce opportunities for the evolution of mixed mating. However, theory suggests that purging may not always occur appreciably (Charlesworth et al. 1990, Lande et al. 1994, Morgan 2001), and some species maintain high inbreeding depression despite mixed mating (e.g., *Aquilegia coerulea*: selfing rate $= 0.59 \pm 0.06$ and $\delta = 1.03 \pm 0.07$, Brunet and Sweet 2006). Characters that determine ϵ and π could also evolve as allocation to self-pollen increases. For instance, an increase in the probability that a self-pollen grain reaches the ovules (ϵ) and / or a decrease in the success of exported pollen (π) may accompany the evolution of selfing, which could facilitate the evolution of complete selfing; however, recall that complete selfing cannot evolve unless $\Delta v > 0$. Additionally, the sensitivity of viability to changes in the underlying trait will also likely evolve. For example, consider the evolution of smaller flowers, which may both increase viability and opportunities for selfing ($\Delta v > 0$) but also reduce ovule number (Delph et al. 2004), so viability benefits of producing smaller flowers may decrease with flower size. This reduction in

viability benefits can weaken selection for increased selfing at intermediate selfing rates and contribute to the evolution of mixed mating.

Attractiveness to pollinators will likely change with the evolution of many underlying traits, which will also affect mating system evolution. Again, consider a reduction in flower size, which causes $\Delta v > 0$, but also reduces attractiveness. Reduced attractiveness will likely limit outcross-pollen receipt and thereby increase the effective selfing rate, which may oppose selection for selfing in populations with high inbreeding depression. On the other hand, reduced attraction may exacerbate pollen limitation, which occurs frequently in plant populations (Knight et al. 2005, but see Aizen and Harder 2007). Models that consider how interactions between either pollen limitation or attraction and viability selection affect mating system evolution remain to be explored.

Although not explicitly examined here, this model enables predictions for mating system evolution when deposition of self-pollen and pollen export are positively correlated. For example, consider a species where the resident genotype does not self-pollinate but also does not export all of its pollen: if a mutant that both increases pollen export and selfing simultaneously can invade, mixed mating necessarily evolves. This positive correlation may exist for some species, including *Eichhornia paniculata* (in the absence of the S-morph; Kohn and Barrett 1994; see Harder 2000 for discussion) and *Erythronium grandiflorum* (Harder and Thomson 1989). Results here (and expression (B.1); see Appendix B.1) suggest that, because increased viability ($\Delta v > 0$) can facilitate the allocation of unused pollen to both exportable pollen (when S is constant) and self-pollen (when X is constant), viability benefits should also aid the invasion of mutants that simultaneously increase pollen export and self pollination (see also Harder and Wilson (1998) for additional results with $\Delta v = 0$).

Collectively, this model and that in Chapter 2 suggest that functional pleiotropy between viability and pollen allocation can greatly affect mating system evolution for most modes of selfing described by Lloyd and Schoen (1992). However, it is unlikely that viability selection will promote the evolution of the remaining mode, delayed selfing (selfing occurs

at the end of a flower's life). The reason is simple: some models (e.g., Schoen and Brown 1991, Lloyd 1992) predict that delayed selfing should be favoured universally because it can provide reproductive assurance without costs of either reduced pollen export or ovule discounting (when self-pollen usurps ovules available for outcrossing; Lloyd 1992). Therefore, functional pleiotropy is unlikely to expand opportunities for the evolution of delayed selfing, but it could reduce them (e.g., if $\Delta v < 0$).

To understand mating system evolution, one must determine the sources of selection that favour allocation to selfing versus outcrossing. Traditionally, efforts towards this end have classified sources of selection as either "ecological factors", which typically refer to reproductive assurance, details of pollination, and pollen discounting, or "genetic factors", that focus on inbreeding depression and a transmission bias for alleles that increase selfing (Johnston 1998, Barrett and Harder 1996, Kalisz et al. 2004). However, the current results and previous analyses (e.g., Lloyd 1979, Johnston 1998) suggest that functional pleiotropy can blur the line between "ecological" (viability selection, in this study) and "genetic" factors, because functional pleiotropy can render multiple selection pressures non-independent. Indeed, an allele that increases selfing can invade a population due to transmission bias, even if functional pleiotropy decreases viability ($\Delta v < 0$), suggesting that increased selfing can evolve even when fitness decreases by some "ecological" metric. Studies that consider how functional pleiotropy can integrate multiple sources of selection on traits that affect selfing rate will greatly improve our understanding of mating system evolution.

Chapter 4

The Potential for Sexually Antagonistic Polymorphism in Different Genome Regions

4.1 Summary

Sex differences in the fitness effects of alleles at a single locus (intra-locus sexual antagonism, or SA) have several evolutionary consequences, including a potentially important role in the evolution of suppressed recombination between the sex chromosomes through SA polymorphism at genes partially linked to the sex-determining region of the sex chromosome pair. The conditions under which polymorphism can exist at such partially sex-linked genes (pseudo-autosomal, or PAR loci) can help predict when we may expect potentially empirically detectable allele frequency differences between the sexes, and should increase understanding of the evolution of recombination between sex chromosome pairs. Models so far published have concluded that PAR genes can maintain SA polymorphisms over a wider range of selection coefficients than autosomal ones, but have used restrictive assumptions. We expand the modelling of SA alleles at a single locus with the full range of degrees of linkage to the male-specific region, to

include strong or weak selection and the possibility of different dominance coefficients in the two sexes. We confirm the previous major conclusion that SA polymorphisms are generally maintained in a larger region of parameter space if the locus is in the PAR than if it is autosomal.

4.2 Introduction

Mutations may be deleterious, advantageous or neutral, but may also combine these properties through trade-offs, such that fitness advantages via one function are accompanied by pleiotropic disadvantages in another organism function or process. Costs of mutations conferring disease resistance are an example of such trade-offs, and it is well known that this can lead to balancing selection (e.g. Bonsall and Raymond 2008; Kwiatkowski 2005). An example is the human sickle-cell mutation, whose heterozygous carriers are protected against malaria, relative to wild-type (HbA) homozygotes, but HbS homozygotes suffer a strong disadvantage due to sickling of the red blood cells (Allison 1955; Jones 1997). Another situation in which trade-offs are likely is the allocation of resources to male and female functions in simultaneously hermaphroditic or cosexual species: when resources are used for one sex function, the other sex function is expected to suffer a loss of resources. This concept is central to understanding why outcrossing organisms have higher allocation to male functions than inbreeders; if there were no cost of male functions, inbreeders' low pollen output requirement would not lead to their evolving lower levels of such traits (Charlesworth and Charlesworth 1981; Charlesworth and Morgan 1991; Charnov 1987; Lloyd 1984).

Trade-offs between male and female functions are also plausible in organisms with separate sexes (dioecious species) and during the evolution of dioecy from hermaphroditism. Sexual antagonism (abbreviated to SA) and sex differences in the fitness effects of alleles at a single locus in a dioecious species, commonly called intra-locus SA, have several evolutionary consequences (Rice 1984; Rice 1987), including affecting the evolution of sexual dimorphism (Mank 2009; Rice 1984), and for sexual selection (Al-

bert and Otto 2005).

Polymorphism at sexually antagonistic loci is estimated to account for a large proportion of fitness variation within populations (Gibson et al. (2002)). An interesting property of intra-locus SA polymorphisms is their potential to cause selection favoring loss of recombination between the sex chromosome pair, which are thought to have evolved from initially normally recombining genome regions (Bengtsson and Goodfellow 1987; Bull 1983; Charlesworth and Charlesworth (1978); Clark 1988; Nei 1969; Rice 1987). Recombining regions of sex chromosomes are termed pseudo-autosomal regions (PAR). When dioecy has recently evolved, the young sex chromosome pair (often called proto-sex chromosomes) may often first evolve suppressed recombination in a region around the sex-determining loci. A large PAR then remains, in which recombination with the sex-determining region can occur in the heterozygous sex. This situation is known in some plants and animals (Liu et al. 2004; Matsubara et al. 2006; McDaniel et al. 2007).

In the sex chromosome pairs of some animal and plant taxa, the PARs have subsequently evolved to become smaller, i.e. further formerly recombining regions have also lost recombination (Bergero et al. 2007; Lahn and Page 1999; Handley et al. 2004; Matsubara et al. 2006; Tsuda et al. 2007). This evolution of suppressed recombination is hypothesized to be a consequence of SA. For the example of an XY chromosome pair (Figure 4.1), an allele that benefits males but is detrimental in females can invade a population more easily if it is linked to the sex-determining region of the Y (Bull 1983; Charlesworth and Charlesworth 1978; Rice 1984). Although the benefit in one sex may sometimes be large enough to outweigh the reduction in fitness in the other sex, allowing fixation (Charlesworth and Charlesworth 1978), fixation is not inevitable. Stable polymorphism may sometimes be established, with linkage disequilibrium (LD) between the sex-determining locus and alleles at the locus with the SA polymorphism (i.e., in the case of an XY system, male-benefit alleles will show LD with the male-specific region of the Y chromosome, the MSY). This LD favours reduced recombination between these loci (Bengtsson and Goodfellow 1987; Bull 1983;

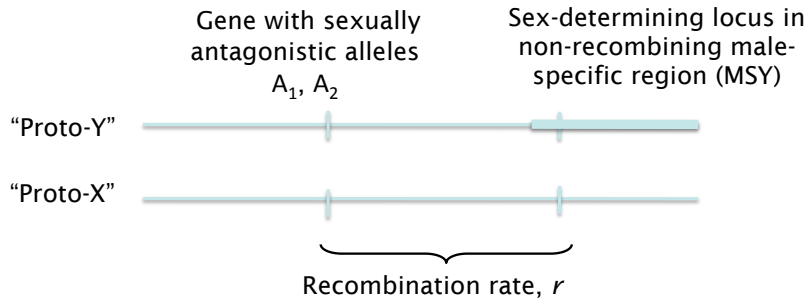

Figure 4.1: The two different regions of a sex chromosome pair. The figure shows the non-recombining male-specific region containing the sex-determining locus (MSY) and PAR regions, with recombination frequency r between the MSY and a locus with SA alleles.

Charlesworth and Charlesworth 1980). If recombination is lost, this leads to a reduced effective population size of the Y-linked region, a major factor leading to Y chromosome genetic degeneration and to sex chromosome sequence divergence and heteromorphism (reviewed in Bachtrog (2008); Bergero and Charlesworth 2008).

It is therefore important to understand the potential for intra-locus SA polymorphism, and how it varies among regions of the genome. In addition to the ideas just outlined for the evolution of reduced recombination of sex chromosomes, the presence of autosomal loci with polymorphism for SA alleles is central to one model for the turnover of sex chromosomes (van Doorn and Kirkpatrick 2007). Rice (1984) provided some influential results for such polymorphisms. However, subsequent analyses (reviewed below) suggest that it is important to examine broader ranges of the strength of selection and the form of dominance of alleles than he considered. Although some of the studies outlined in the next section have made such comparisons, the potential for SA polymorphism in the PAR has been little explored, and comparisons among all genomic regions (autosomes, non-recombining regions of sex chromosomes, and the PAR) have not included

the full range of possibilities with respect to dominance of alleles, an important parameter (see below). Here, we synthesize the conditions for SA polymorphism over all three genomic regions, and specifically we expand the treatment of loci in the PAR.

4.2.1 Models and Previous Results

To model SA, we assume, following most previous authors, that the alleles have opposite fitness effects in the two sexes. Table 4.1 summarizes the notation used to study the potential for maintenance of a polymorphism for SA alleles of loci in recombining genomic regions. The cost in homozygous females of having the male-benefit A_2 allele is t, and s is the selection coefficient against homozygous males with the female-benefit allele. Two types of comparison have previously been made of the potential of different genome regions to support SA polymorphism. One type compares autosomal genes with genes located in the non-recombining part of an XY chromosome pair; such comparisons assume male hemizygosity for X-linked alleles (thus, if there are two alleles, there are only two male genotypes, unlike the case in Table 4.1). The second type of comparison, which is the main focus of our new analyses, is between different recombining regions of genomes, including the PAR of the sex chromosome pairs. Denoting the recombination frequency in males between the male-specific Y chromosome region containing the sex-determining locus and the fitness-determining locus by r, (Figure 4.1), we have $r < 0.5$ for some PAR genes, and $r = 0.5$ for autosomal genes and PAR genes distant from the sex-determining locus (or, equivalently, the PAR boundary).

(i) Comparisons Between Autosomal Genes and Genes in the Non-Recombining Part of an XY Chromosome Pair

Although we are not primarily concerned here with comparisons between fully sex-linked loci and autosomal genes, it is helpful to briefly outline some assumptions and results of such models. An important assumption is male hemizygosity for X-linked alleles (see Table 4.1).

Table 4.1: Genotypes and fitnesses for the case of partially sex-linked or autosomal genes ($r \leq 0.5$). We follow Patten and Haig (2009) and Fry (2010) and assign a fitness of 1 to the best genotype in each sex, rather than to allele A_1, regardless of sex (as in Rice 1984). In both sexes, the fitness reducing allele is assumed to affect heterozygotes fitness, but the dominance coefficients may differ, as explained in the text. Our notation ensures that the selection coefficients affect fitnesses similarly, i.e. $s = 0.1$ has the same fitness effect as $t = 0.1$. This is not true for the notation used by Rice (1984), where the A_1 allele has unit fitness in both sexes, and allele A_2 increases fitness in males by s but reduces it in females by t. For example, with $s = t = 0.5$ in the notation of Rice (1984), the worst male genotype (whose fitness is $w = 1$) has $\frac{2}{3}$ the fitness of the best male genotype ($w = 1 + 0.5 = 1.5$), whereas the worst female genotype has half the fitness ($w = 1 - 0.5 = 0.5$) of the best female genotype. Fry (2010) further compares the approaches used in the different published papers. Our notation for dominance follows Prout (2000). In this notation, for example, $h_m = h_f = 0.05$ means that the A_2 allele is largely recessive in both males and females, whereas $h_m = 1$ and $h_f = 0$ means that A_2 is dominant in males and recessive in females.

	Genotype		
Sex	A_1A_1	A_1A_2	A_2A_2
Female	1	$1 - h_f\, t$	$1 - t$
Male	$1 - s$	$1 - (1 - h_m)\, s$	1

Rice's (1984) comparisons of these two types of genes largely dealt with invasion conditions for SA alleles, rather than maintenance of polymorphism. He found that male-benefit SA mutations in X-linked genes invade more readily than mutations at autosomal loci, if they are sufficiently recessive in females (h in Table 4.1 less than a threshold value), and sufficiently dominant female-benefit SA mutations also invade more readily when X-linked. The X chromosome should thus evolve an excess of genes whose alleles have different fitness effects in the two sexes, which are likely to include genes controlling sexually dimorphic traits. Although this study did not explicitly analyze maintenance of polymorphism for SA alleles,

Rice (1984) notes that "These genes do not typically increase to fixation but have intermediate gene frequencies", and SA allele frequencies in his figures never exceed 0.5. However, subsequent studies have revealed that some of these predictions depend on Rices (1984) specific assumptions.

Patten and Haig (2009) focused on the potential for polymorphism for SA alleles (not just invasion conditions). They relaxed Rice's (1984) favorable assumptions about the dominance of male- vs. female-benefit alleles, and modelled uniformly distributed dominance coefficients (h, where $0 < h < 1$), with alleles' dominance the same in both sexes (as will be seen below, the dominance in the two sexes plays an important role in the conditions for polymorphism, see Kidwell et al. 1977). Averaged over all possible values of the selection coefficient, including very strong selection (which is not generally biologically plausible), they found a higher probability of maintaining SA polymorphism for autosomal loci than for sex-linked ones (see also Curtsinger 1980), the reverse of Rice's (1984) conclusion (which does, however, hold in the more plausible case of selection coefficients < 0.1).

Fry (2010) and Prout (2000) pointed out that there is no biological basis for assuming that male- or female-benefit SA alleles will have the same dominance relationships in both sexes. Following Gillespie (1978), Fry noted that dominance with respect to fitness may often differ from dominance with respect to a phenotypic trait determining fitness, and it is therefore quite possible for each of two SA alleles at a locus to be partially dominant in the sex in which it is beneficial (Fry 2010). This may be particularly likely for alleles with different expression in the two sexes (Connallon and Clark 2010); two such alleles may often show intermediate dominance for expression levels, but in each sex the higher expression alleles may be most dominant in terms of phenotypic and fitness effects. Polymorphism for such sexually antagonistic alleles can occur in a wider region of parameter space for autosomal than for fully X-linked genes (Fry 2010).

For fully sex-linked genes (with males hemizygous), the only stable equilibria are fixation of either of the alleles at the SA locus, or stable polymorphism (see, for example, Rice 1984, Patten and Haig 2009). In contrast,

when males are not hemizygous polymorphism can simultaneously be stable with fixation of one allele (see below).

Also, polymorphism among Y haplotypes is not possible under the fitness model studied (just as, in a haploid population, a temporally variable environment does not yield polymorphism, Nagylaki 1975), though certain special selection models, such as frequency-dependent selection acting on Y haplotypes, could allow this (Clark 1987). It follows that a male-benefit allele that arises as a mutation in a gene in the non-recombining MSY region either does not spread in the Y chromosome population, or else becomes fixed, leaving the X chromosome with the initial allele (since the Y allele cannot cross over onto the X).

(ii) Models of Autosomal versus PAR Genes

Several previous analyses dealing with the second type of comparison have concluded that PAR genes can maintain SA polymorphism over a wider range of conditions than autosomal ones. However, these analyses used restrictive assumptions, such as free recombination between the SA locus and a male-determining locus (Clark 1988), or a completely dominant male-beneficial allele that is lethal in females (Rice 1987). Although both these authors extended their assumptions by computer calculations, no analysis has yet compared autosomal with PAR loci (with a range of degrees of linkage to the male-specific region) allowing different strengths of selection (as in Patten and Haig 2009) and different dominance of the alleles in the two sexes (as in Fry 2010). Our results below expand the modelling of SA alleles to include the needed wider coverage of the parameter space.

4.2.2 Model Description

For simplicity, we concentrate on XY sex chromosome systems (ZW systems should behave similarly, interchanging the sexes, see the Discussion section). To model recombination, we assume that the sex chromosomes carry a non-recombining male-specific region (or MSY), and that alleles of genes in the PAR can be found in haplotypes carrying the MSY, as well as

in X haplotypes. Because the MSY behaves genetically as a single fully sex-linked locus, the situation is equivalent to a two-locus model. We assume that mating between the gamete types is random, except that all matings are between a male and a female.

The fitness expressions allow for all three genotypes in both sexes (Table 4.1). We also assume the same fitness for the two male genotypes A_1Y/A_2X and A_2Y/A_1X, i.e., that there is no difference in the effect of A_2 versus A_1 between genotypes when the alleles are in coupling with the MSY or with the X (no cis-trans effect). We follow the notation of Prout (2000) for the dominance coefficients (see Table 4.1). We assume directional selection in both sexes (i.e. we do not include the possibility of overdominance at the SA locus, so that any polymorphism that arises is not due to such effects; note, however, with some parameter sets with different dominance in the two sexes, the sex-averaged fitness is highest for heterozygotes, see Fry 2010). Like most previous work, our models assume selection acting at the sexually antagonistic locus wholly through viability effects, i.e. not involving any sexual selection on the alleles (we discuss this assumption below). The deterministic recurrence equations are given in the Appendix C (see also Clark 1988).

To find the parameter values allowing protected polymorphism, invasion of an A_2 allele into a population initially fixed for the A_1 allele was tested, and the reverse. These endpoint analyses used the eigenvalues of the Jacobian matrix of the recursion equations for the model just outlined; when the absolute value of the largest eigenvalue exceeds 1, invasion of the new allele is possible. Because restrictive assumptions are necessary to obtain tractable expressions (Rice 1987), we performed numerical analyses in *Mathematica* (file available on request).

We also did deterministic calculations of the dynamics of our recursion equations. These were used to investigate regions of parameter space yielding polymorphic equilibria that co-exist with one stable endpoint, as explained below (i.e. at least one unstable internal equilibrium exists), and to study equilibrium allele frequencies. Kidwell et al.'s (1977) analysis of an autosomal locus with sexually antagonistic alleles found such unstable

equilibria under certain conditions with respect to the dominance coefficients. Thus analyzing invasion conditions from the two end-points underestimates the parameter space in which polymorphism can occur. Kidwell et al. (1977) inferred numerically that the relationship $(1-h_m)+h_f=1$ (using our notation for dominance, see Table 4.1) defines a boundary between situations with different possible outcomes: only when $(1-h_m)+h_f>1$, can unstable internal equilibrium occur (so that a locally stable equilibrium exists that is not identified from endpoint analysis). This was not found with $(1-h_m)+h_f<1$, or perfectly complementary dominance, i.e. $(1-h_m)+h_f=1$. The case of a PAR gene has not previously been investigated.

To more comprehensively study the existence of stable equilibria for the full range of recombination rates with respect to the sex-determining locus, the initial frequency of the A_1A_1 or A_2A_2 genotype was 0.0001 in both sexes, and heterozygotes were introduced at a frequency of 0.00005. The population was run to equilibrium (all haplotype frequencies in both sexes changing by $< 10^{-13}$ between consecutive generations) for the full range of h_m and h_f values and of selection coefficients s and t. We determined the equilibrium frequencies of the male-beneficial A_2 allele starting with this allele rare and also starting near fixation. When the equilibria reached from these two starting states differ (e.g. one endpoint resists invasion by the other allele, or multiple internal equilibria are found), this indicates the existence of at least one stable equilibrium that is not identified by the endpoint analysis for those parameters.

For the stable equilibria, we calculated the frequencies of the male-benefit allele (A_2) among the Y- and X-chromosomes of parents after selection and before reproduction (denoted, respectively, by p_Y and p_X). Using the notation in Appendix Table C.1 with primes indicating genotype frequencies at the relevant stage in the life cycle, we have:

$$p_Y = \frac{m'_{23} + m'_{24}}{m'_{13} + m'_{14} + m'_{23} + m'_{24}} \tag{4.1}$$

$$p_X = \frac{\frac{1}{2}\left(m'_{14} + m'_{24} + f'_{34}\right) + f'_{44}}{\frac{1}{2}\left(m'_{13} + m'_{14} + m'_{23} + m'_{24}\right) + f'_{33} + f'_{34} + f'_{44}} \tag{4.2}$$

4.3 Results

4.3.1 Invasion Analysis for Autosomal Genes, or Sex Chromosomal Genes Loosely Linked to the PAR Boundary

Figure 4.2 shows the regions of parameter space in which the simple invasion analysis suggests that stable polymorphism can exist when dominance is the same in both sexes (i.e. $h_m = h_f$; this assumption is relaxed below; see also Kidwell et al. 1977, Prout 2000, Patten and Haig 2009, Fry 2010). The figure shows a range of selection values, including very strong selection. Although male- or female-benefit alleles often go to fixation (when the cost to the other sex is below a threshold value), protected polymorphism is indicated in some of the parameter space. For autosomal genes (or, more generally, $r = 0.5$), the dominance coefficient of the male-benefit allele, A_2, does not affect the outcomes when its dominance is similar in males and females (Kidwell et al. 1977; Prout 2000).

Consistent with previous studies (e.g. Patten and Haig 2009), and with expectations from models of opposing selection (Prout 2000), weaker selection reduces the region of polymorphism for autosomal loci, and also for genes in the PAR (s and t in Figure 4.2 both restricted to < 0.1, as indicated by the box close to the origins of the plot, see also Figures 1 and 2 of Rice 1987).

4.3.2 PAR With the Same Dominance of the Male-Benefit Allele in Both Sexes

Figure 4.3 shows the effects of departures from this simplest model. For a PAR gene with restricted recombination with the MSY ($r < 0.5$), the symmetry of invasion conditions between male- and female-benefit alleles is lost; there is only a small reduction of the parameter region where the male-benefit allele fixes, but the area of parameter space in which female-benefit

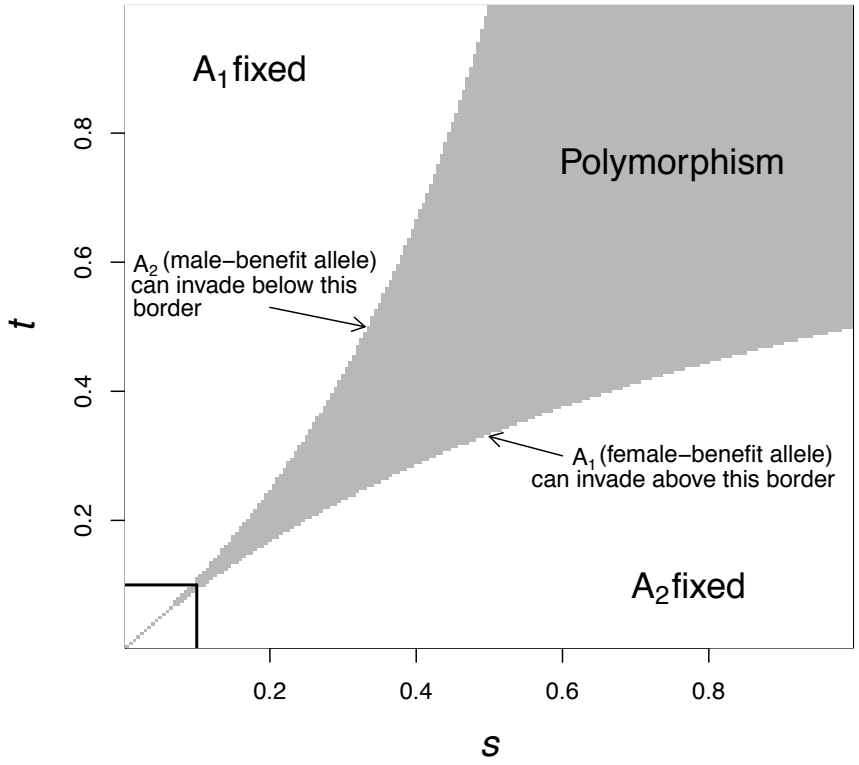

Figure 4.2: The regions of parameter space in which endpoint invasion analysis suggests that protected polymorphism occurs (i.e. A_2 alleles can invade a population fixed for A_1, and vice versa), when dominance is the same in both sexes. The figure shows results for autosomal alleles (or genes with $r = 0.5$, where r is the recombination frequency with the sex-determining locus).

alleles become fixed is greatly reduced. Thus, overall, compared with alleles at an autosomal locus (Figure 4.2), polymorphism becomes possible in a larger region of parameter space, mostly because male-benefit (A_2) alleles can invade despite a higher cost to females, especially when the male-benefit allele is dominant (Figure 4.3); tight linkage ($r < 0.001$) largely eliminates the region in which the female-benefit allele fixes, unless this allele is dominant (i.e. $h_m < 0.5$), and further slightly shrinks the region where the male-benefit allele fixes, allowing polymorphism in a very large region

when the male-benefit allele is dominant, even when selection is not very strong. For PAR genes loosely linked to the MSY ($0 << r < 0.5$), strong selection and increased dominance of the A_2 allele both increase the opportunity for polymorphism (primarily by decreasing the region in which the female-benefit allele fixes), and tighter linkage favours polymorphism (Figure 4.3).

4.3.3 Different Dominance in the Two Sexes

When the dominance of the two alleles at the fitness locus differs between the sexes, the existence of polymorphism when $r = 0.5$ depends greatly on the pattern of dominance, as shown in Figure 4.4A. In the left- hand plots (Figure 4.4Ai, iii and v), the male-benefit allele is more recessive in males than in females (h_m always $< h_f$) and the region of parameter space that allows polymorphism is decreased, whereas the opposite is the case in the right-hand plots, Figure 4.4Aii, iv and vi (with the same dominance coefficients, but $h_m > h_f$, and again with $r = 0.5$). As noted by Fry (2010), polymorphism is particularly likely if the advantageous allele in each sex is dominant in that sex (Figure 4.4A ii). However, Figure 4.4B illustrates for $r = 0.1$ that, other things being equal, PAR genes have larger polymorphic regions of parameter space than autosomal ones, i.e. close linkage to the MSY ($r << 0.5$) helps maintain such polymorphisms. With favorable dominance, polymorphism occurs in a very large proportion of the parameter space with s and $t < 0.1$, even with r as high as 10% (compare Figure 4.4Bii with the top row of Figure 4.3). However, at the limit where each allele is fully dominant in the sex it benefits ($h_m = 1$ and $h_f = 0$), polymorphism occurs for all levels of selection, even with free recombination (Kidwell et al. 1977).

Tables 4.2 ($h_f = h_m$) and 4.3 ($h_f \neq h_m$) summarize the overall results in terms of the percentages of parameter space that the endpoint invasion analysis suggests can support polymorphism, under different recombination rates and dominance coefficients. With the same dominance of the male-benefit allele in both sexes ($h_m = h_f$), although the ranking of the

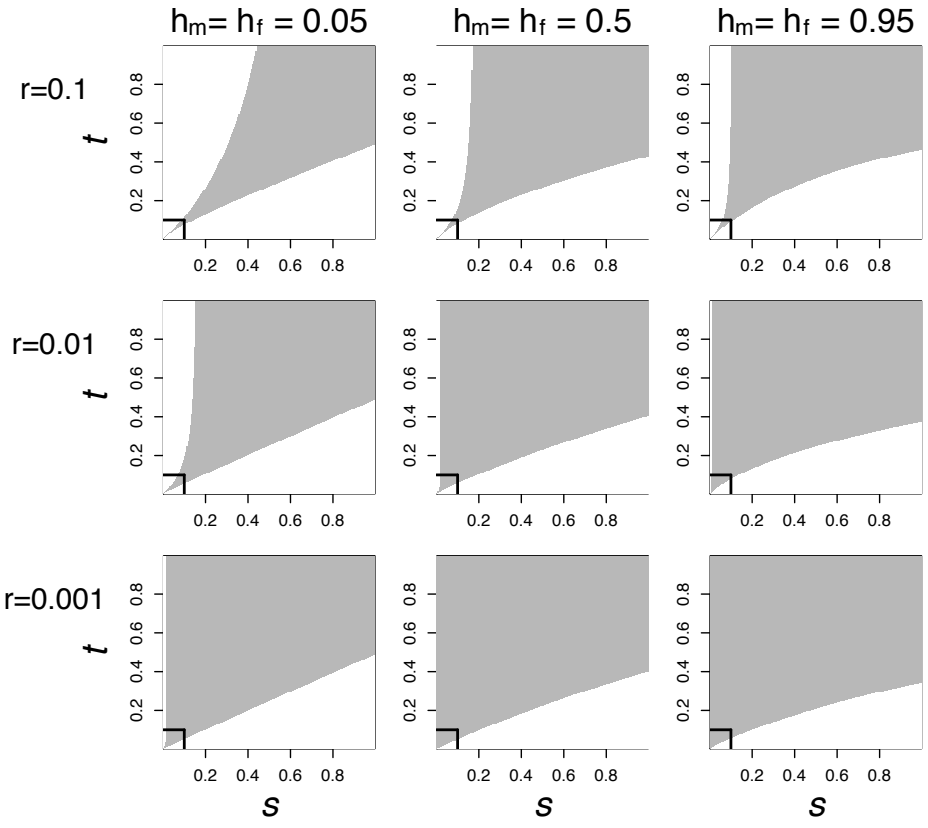

Figure 4.3: The regions of parameter space in which endpoint invasion analysis suggests that protected polymorphism occurs for a gene with restricted recombination with the MSY, showing the loss of the symmetry between male- and female-benefit alleles. Note that, for $r = 0.001$, there is a very narrow region close to $s = 0$ where the A_1 allele is fixed which is not visible in the plots, due to the scale used (steps of 0.005 for s and t values).

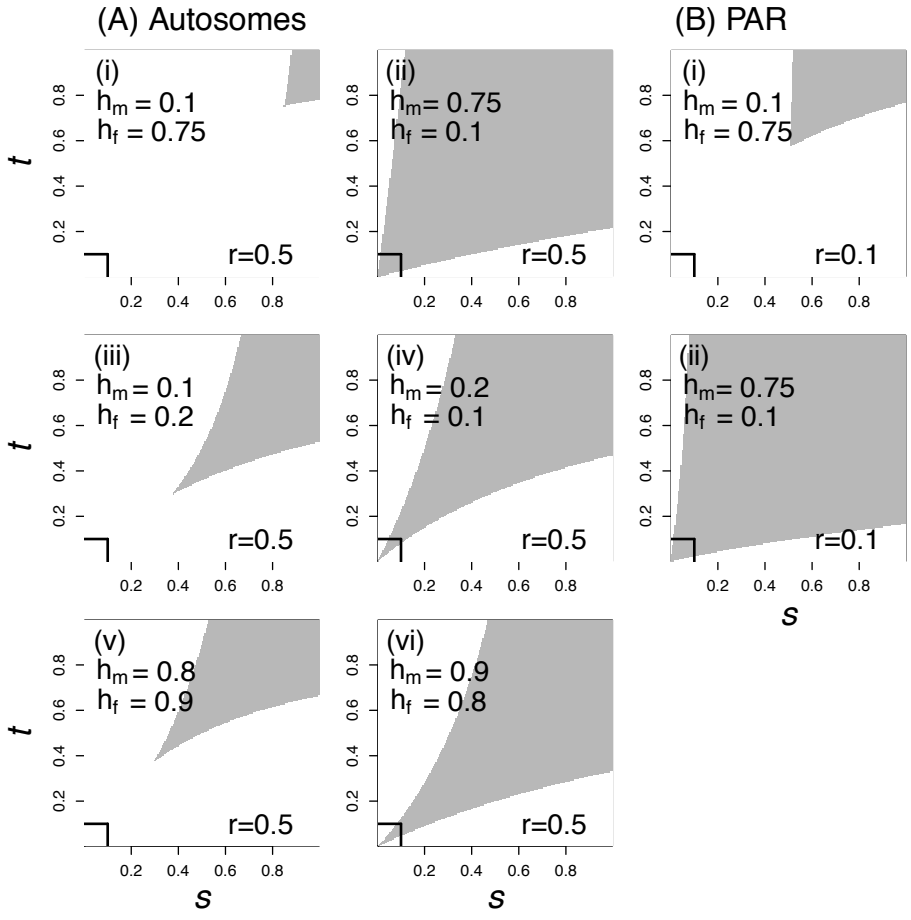

Figure 4.4: The effect of different dominance coefficients in the two sexes on the regions of parameter space in which endpoint invasion analysis suggests protected polymorphism.

genome regions depends on the parameters, genes with low r (i.e., PAR genes closely linked to the MSY) always yield the largest region of parameter space allowing polymorphism. When h_m and h_f vary independently between 0 and 1, closely linked PAR genes still have the greatest potential for SA polymorphism, with autosomal and X-linked loci intermediate and least likely to exhibit polymorphism, respectively, regardless of the selection strength. When alleles are partly dominant in the sex they benefit, both autosomal and PAR loci readily yield polymorphism, even for weak selection, but again $r < 0.5$ facilitates this (e.g. Figure 4.4).

4.3.4 Additional Equilibria

To more completely analyze the parameter space supporting stable equilibrium of SA alleles, including cases when only local equilibria exist (see Methods and Kidwell et al. 1977), we calculated the full allele frequency dynamics, starting from low frequencies of the A_1 and A_2 alleles. We found such equilibria for both free and restricted recombination. For autosomal loci ($r = 0.5$), our results agree with those of Kidwell et al. (1977) in suggesting that, when $(1 - h_m) + h_f > 1$, at least one internal stable local equilibrium can exist that cannot be reached by invasion from one end-point. For PAR loci, the conditions for additional equilibria appear to be slightly broader. We examined the case of $r = 0.1$ between the selected locus and the sex-determining gene, and found that the boundary of $(1 - h_m) + h_f = 1$ no longer applies, and that locally stable internal equilibria can exist for $(1 - h_m) + h_f < 1$, as well as greater than 1. Overall, therefore, when $r = 0.5$, end-point invasion analysis alone, without exploring the dynamics, correctly identifies the biologically relevant set of selection coefficients that allow the possibility of stable polymorphism because an expanded polymorphic region of parameter space due to the existence of unstable equilibria requires the rather unlikely situation of deleterious alleles tending to be dominant; either both alleles must be at least mildly dominant in the sex where they are deleterious, or one allele must be highly dominant in the sex where it is deleterious. Furthermore, our numerical results for $r = 0.1$ sug-

Table 4.2: Percentage of the parameter space where endpoint invasion analysis indicates stable polymorphism at a sexually antagonistic locus when $h_f = h_m$. The parameter values were sampled evenly across the ranges of values indicated. Different ranges of dominance coefficients are shown separately, as are situations with strong and weak selection, as defined in the text. For comparison with cases of $h_f \neq h_m$ and for an X-linked gene in a system with hemizygous males, see Table 4.3.

			Dominance Coefficients				
Dominance pattern	Selection strength	Recombination rate, r	Partially recessive $0 - 0.5$	Near additive $0.25 - 0.75$	Partially dominant $0.5 - 1$	$h_f \leq 0.5$, $0.5 \leq h_m$	Full range of dominance, $0 - 1$
$h_f = h_m$[1]	Strong	0.5	0.38	0.39	0.38	-	0.38
		0.1	0.54	0.61	0.63	-	0.57
		0.01	0.71	0.77	0.77	-	0.73
	Weak	0.5	0.07	0.07	0.07	-	0.06
		0.1	0.15	0.17	0.17	-	0.16
		0.01	0.46	0.55	0.54	-	0.49

[1] All combinations of 49 values of each of s and t, and 51 values of $h_f = h_m$.

Table 4.3: Percentage of the parameter space where endpoint invasion analysis indicates stable polymorphism at a sexually antagonistic locus when $h_f \neq h_m$ and at an X-linked gene in a system with hemizygous males (from Patten and Haig 2009); for this latter case h refers to the dominance of the male-benefit alleles in female genotypes. See Table 4.2 for additional details.

			Dominance Coefficients				
Dominance pattern	Selection strength	Recombination rate, r	Partially recessive $0-0.5$	Near additive $0.25-0.75$	Partially dominant $0.5-1$	$h_f \leq 0.5$, $0.5 \leq h_m$	Full range of dominance, $0-1$
$h_f \neq h_m$[1]	Strong	0.5	0.38	0.39	0.38	0.73	0.40
		0.1	0.51	0.60	0.59	0.82	0.54
		0.01	0.68	0.75	0.73	0.91	0.67
	Weak	0.5	0.20	0.16	0.20	0.66	0.27
		0.1	0.23	0.21	0.25	0.69	0.30
		0.01	0.43	0.52	0.49	0.81	0.46
X-linked	Strong	-	0.49	0.25	0.08	-	0.28
	Weak	-	0.42	0.12	0	-	0.21

[1] 25 values of each of s and t, and 26 values of each of h_f and h_m.

gest an expanded range of dominance coefficients allowing local equilibria, including the biologically relevant case when $(1 - h_m) + h_f < 1$. Therefore our conclusions from the results in Tables 4.2 and 4.3 (noting also that, for fully sex-linked genes, no local equilibria are possible, see Methods section) are likely to be conservative: the PAR supports stable polymorphism for a larger range of selection coefficients than other genome regions.

4.3.5 Allele Frequencies at Equilibrium

The frequencies of SA alleles in PAR regions of sex chromosome are also of great interest because empirical data are potentially obtainable, and might allow inferences about the strength of selection involved, given information about the recombination rate between a locus and the non-recombining region of the sex chromosome. It is thus important to investigate whether the model studied here leads to large sex differences in allele frequencies that could be detected in feasible data sets. As explained above, at a PAR locus, allele frequency differences between the chromosomes with and without the MSY region reflect LD between the SA locus and the non-recombining region (Charlesworth and Charlesworth 1978), which produces selection for reduced recombination (Bengtsson and Goodfellow 1987; Bull 1983), and this selection will be stronger, the larger the allele frequency difference between the chromosomes. We therefore calculated the frequencies of both alleles at the SA gene (see Methods) for illustrative parameter values.

When a polymorphism is maintained, the SA alleles are often at intermediate frequencies, often > 0.2 (Figure 4.5). For r = 0.5, in agreement with Kidwell et al. (1977), we found that, where equilbria with stable polymorphism occurred, allele frequencies were usually intermediate (minor allele frequency > 0.2). This remains true for $r < 0.5$; because close linkage to a Y-linked male sex-determining region increases the parameter space with polymorphism, intermediate allele frequencies are commonly observed for loci with $r < 0.1$. For such PAR loci, we also calculated the difference in frequency of the male-benefit allele on the Y chromosomes of male individuals vs. on X chromosomes (of either sex, see equations 4.1 and 4.2

above). Even with the weaker selection studied ($s, t < 0.1$), very close linkage (when $r = 0.01$) allows the frequency of the male-benefit allele on the Y chromosomes of male individuals to differ greatly from that on X chromosomes, as intuitively expected (Figure 4.6), whereas if the SA locus is unlinked ($r = 0.5$) or loosely linked ($r = 0.1$) the male-benefit allele tends to form strong associations with the MSY only for very strong selection. Such differences in SA allele frequencies between the sex chromosomes could allow detection of SA loci in natural populations.

4.4 Discussion

4.4.1 Theoretical Conclusions

The results of our analyses show that, other things being equal, SA polymorphisms are stably maintained in a larger region of parameter space if the locus is in the PAR and $r << 0.5$ than if it is autosomal (and also compared with the fully sex-linked case in which males are hemizygous, see Tables 4.2 and 4.3). This is consistent with the previous major conclusions about the capacity of genome regions to harbour polymorphism for sexually antagonistic alleles (Bengtsson and Goodfellow 1987; Bull 1983; Clark 1988; Rice 1987), but we include a more detailed analysis of the PAR, which is of most interest with respect to sex chromosome evolution, and of the dominance coefficients, and we examined the possibility of unstable equilibria (local internal equilibria, not reached by alleles invading from both end-points); this analysis shows that the existence of such equilibria is unlikely to affect this conclusion. Clark (1988) also included PAR genes in his study, and found that polymorphism was more likely with closer linkage to the MSY. However, he studied randomly chosen fitness models, and did not examine what characteristics of the fitness models were associated with this effect, so his study did not define which regions of biologically relevant parameter space produce this effect, and which do not.

Overall, quite special conditions (strong selection, or similar selection coefficients in males and females when selection is weak, or favorable dom-

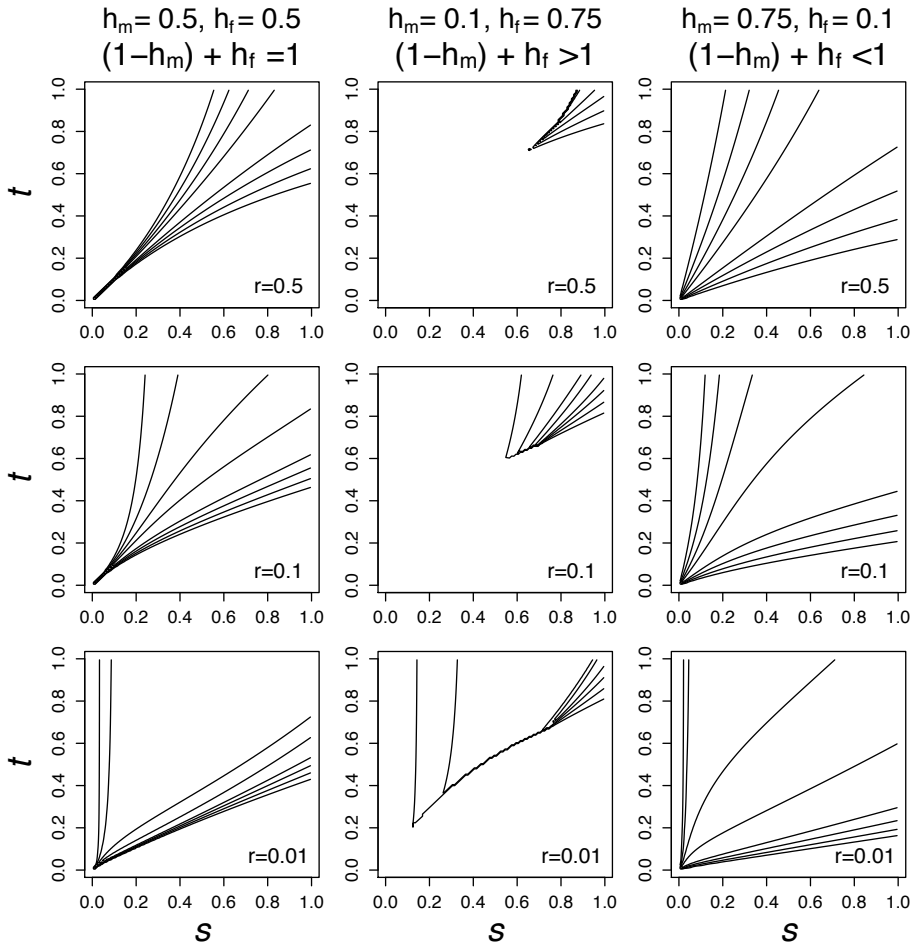

Figure 4.5: Minor allele frequency of the A_1 or A_2 alleles at equilibrium, for various strengths of selection. The eight lines indicate allele frequencies of 0.1, 0.2, 0.3 and 0.4; outermost and innermost pairs of lines correspond to frequencies of 0.1 and 0.4, respectively. Calculations began from both endpoints (i.e. with either A_2 or A_1 initially rare) but the figure presents results with the A_1 allele rare. The existence of locally stable equilibria slightly enlarge polymorphic regions in some of these figures compared with Figure 4.4. When A_2 is initially rare, results differ only in the areas of polymorphic parameter space; the general conclusion, that, when polymorphism occurs, intermediate gene frequencies are often seen, remains true whether A_2 or A_1 is initially rare.

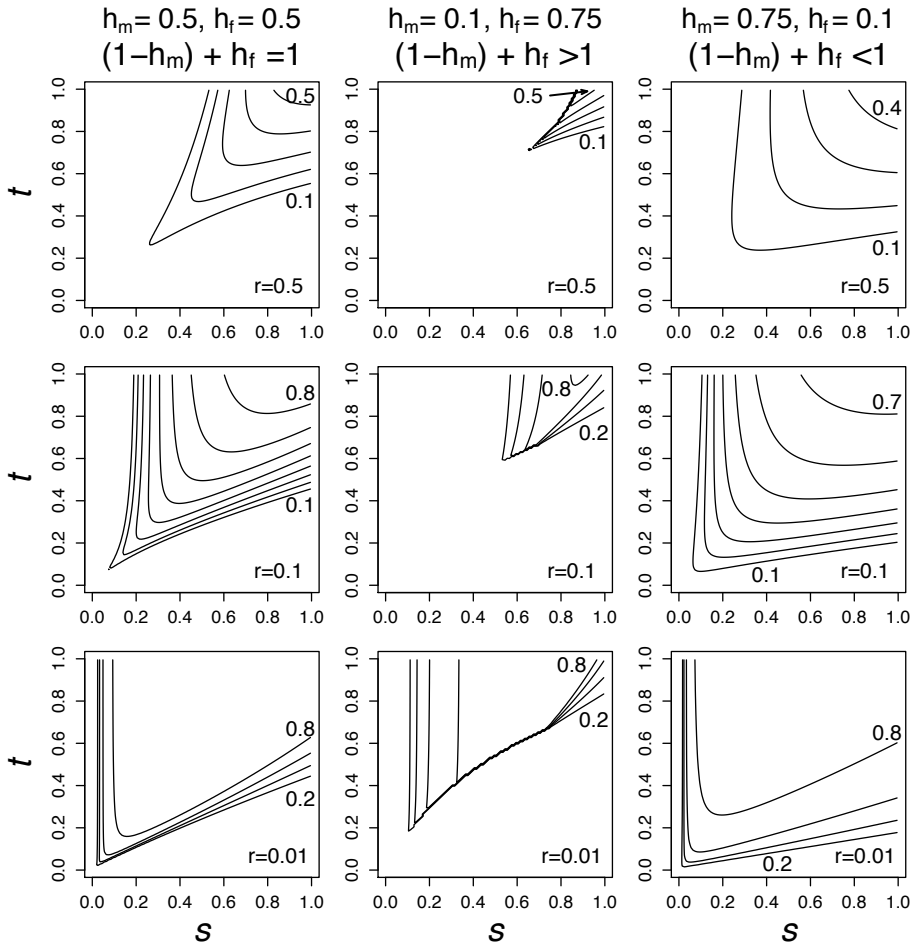

Figure 4.6: The difference in the frequency of the male-benefit allele on the Y-chromosome vs. the X-chromosome following selection and before reproduction (equations 4.1 and 4.2). Lines indicate the frequency difference in steps of 0.1 or 0.2; numbers beside the outermost and innermost lines indicate the smallest and largest frequency difference, respectively. As in Figure 4.5, calculations began with the female-benefit allele rare, but the conclusion remains true that large frequency differences can occur between sex chromosome haplotypes with and without the MSY region, even with weak selection when $r = 0.01$ (for looser linkage, strong selection is required), for either A_2 or A_1 initially rare.

inance) are needed to maintain SA alleles at autosomal loci, so that the conditions for such polymorphisms to trigger changed sex-determining systems (vanDoorn and Kirkpatrick 2007) are likely to be restricted. It has, however, recently been shown that SA polymorphisms can also be maintained under less restrictive conditions when closely linked to another locus with a SA polymorphism, and that this produces LD between the two selected loci (Patten et al. 2010; Úbeda et al. 2011). This suggests that SA effectively strengthens selection at linked SA loci, increasing the opportunity for polymorphism (Patten et al. 2010). This effect will presumably be stronger for a PAR gene than between two SA loci, because one allele at one of the two loci involved (the Y-linked sex-determining locus) is wholly restricted to one sex (and can be assumed to be very detrimental if recombined into in females, e.g. in species where it contains female suppressors; the accumulation of male-function genes on the *Drosophila melanogaster* Y through movement of copies from autosomes (Carvalho 2002), also argues that these genes would have detrimental effects in females). Comparison between our results in Figures 4.3 and 4.4, and those of Patten et al. (2010), does indeed show a stronger effect for the PAR (Patten et al. 2010 assume $h_m = h_f = 0.5$). It remains unclear over what linkage distances variation and LD can be maintained if more than two such loci are segregating in a population, and specifically in the PAR region (which has not yet been modelled in this respect).

Our conclusions are probably not restricted to the situations we have studied here. They should also apply to a ZW system, with the sexes reversed. They are also probably not restricted to cases with fitness differences restricted to viability differences (Charlesworth and Charlesworth 1978), although most work, including ours here, studied models in which selection at the sexually antagonistic locus acts wholly through viability effects, i.e., not involving any sexual selection on the alleles. Sexual selection has not been fully investigated in this regard, but when there are no differences among females in their preferences for male genotypes at the SA locus (or possibly also when male genotypes compete for mates), this can be equated to a form of viability selection on males, simply determining

which males enter the mating pool (S. Otto, personal communication; Albert and Otto 2005). Our overall conclusions for SA loci partially linked to the sex-determining locus may therefore still apply when mating involves sexual selection.

4.4.2 Is SA Polymorphism Responsible for Reduced Recombination Between Sex Chromosomes?

The hypothesis that SA polymorphisms generate the selection that has reduced recombination between sex chromosome pairs is appealing, but as yet not firmly supported. Our results relate to the question of whether SA polymorphisms are likely to be maintained for long enough that selection for reduced recombination can occur, and recombination between the MSY and the gene can become suppressed. We show that intermediate allele frequencies can be maintained, particularly at PAR loci, and it is known that such situations generate selection for reduced recombination (see Introduction 4.2). However, the SA might quickly become resolved by evolution of sex-specific expression of the male- and female-beneficial alleles. Empirical evidence remains lacking to show that SA polymorphisms are actually present in PAR regions. Clearly, such evidence will be difficult to obtain. We discuss some potential sources of evidence for SA polymorphisms below, but first briefly mention some alternative possibilities selecting for reduced recombination that were not discussed in the recent review by Ironside (2010).

Alleles that benefit one sex, but whose effects are not expressed in the other, have also been termed SA (Kirkpatrick et al. 2010), in a broader usage than the one employed here. Such variants can also evolve LD with a sex-determining locus, and this would also be expected to select for tighter linkage between the two loci. However, the selection pressure is, of course, ephemeral, and disappears when the beneficial allele is fixed in the population. Other situations involving different selection in the two sexes can also generate ephemeral selection for tighter linkage (Lenormand 2003).

4.4.3 Testing Empirically for SA

Testing whether SA polymorphism is responsible for reduced recombination between sex chromosomes will require not only models showing that it can produce the selection pressure on recombination modifiers, but also evidence that modifiers reducing recombination exist in natural populations, and could respond to such selection. Evidence for this kind of genetic variation already exists (e.g. Brooks and Marks 1986; Koehler et al. 2002; Korol and Iliadi 1994; Li and W. 2009; Nilsson and Säll 1995; Sanchez-Moran et al. 2002). Evidence that sexually antagonistic effects exist within the *Drosophila melanogaster* genome is also strong (e.g. Pischedda and Chippindale 2006), but it is much less clear that polymorphism for SA alleles exists at individual loci (Bonduriansky and Chenoweth 2009), and it is interesting to consider how such intra-locus differences might be detectable. A recently suggested approach to discovering such loci is to use gene expression differences, and this yielded a first, surprisingly high, estimate that almost 10% of all *D. melanogaster* loci may carry polymorphisms for SA alleles (Innocenti and Morrow 2010).

Another approach is a population genetic one. If selection maintains a polymorphism for SA alleles, allele frequency differences between the sexes are expected at loci partially linked to the Y. For PAR regions closely linked to the sex-determining region, the alleles can be regarded as occupying partially isolated populations, corresponding to the X- and Y-linked alternatives for the sex-determining region, allowing expected allele frequency differences to be studied (Bull 1983; Clark 1988; Rice 1987), including some analytical results for special cases (Bengtsson and Goodfellow 1987; Bull 1983; Clark 1988; Rice 1987). If the selection on a PAR gene's alleles is strong, such frequency differences offer a possible source of empirical information about whether SA effects are important. However, the phase of variants at PAR loci, with respect to the sex-determining region (i.e. whether an allele is carried by a chromosome with the Y region, or on an X chromosome), is generally unknown, and can usually be determined only by genotyping a haploid stage of the life cycle, or sets of parents and

their offspring.

A less laborious alternative is to estimate nucleotide diversity, because different variant frequencies between the sexes at a PAR locus implies LD between the alleles at the locus and the sex-determining region, similarly to the LD expected in a subdivided population (Charlesworth et al. 1997). If X- and Y-associated sequences are pooled, diversity will be increased due to their slightly diverged sequences. This expectation has so far been quantified only for the case of neutral PAR loci linked to a locus at which balancing selection was assumed to occur (Kirkpatrick et al. 2010). Clearly alleles maintained polymorphic at PAR loci by SA should show these effects, as should alleles under the broader definition of SA used by Kirkpatrick et al. (2010). If high diversity is found, further work will be necessary to exclude other possible explanations, such as a polymorphic chromosomal inversion, or some form of balancing selection not involving SA. However, higher diversity at multiple PAR loci than at autosomal loci, would suggest SA selection, because balancing selection is not very commonly found within populations, and is not likely to be concentrated into a single genome region.

4.4.4 What Kinds of Mutations Could Increase Male Viability and Decrease Female Viability, and what Selection and Dominance Coefficients Are Expected?

In studying the conditions for polymorphism at genes with opposing selective effects in different sexes or environments, fitness models are often randomly chosen (Clark 1988; Prout 2000). It would be helpful to understand what kinds of fitness effects are biologically plausible for sexually antagonistic loci. Understanding the kinds of genes and gene action that might lead to intra-locus SA might allow one to infer something about the expected selection and dominance coefficients. To date, very few loci have been identified, and the effects of alleles at specific loci have been estimated only for a few special cases. We next briefly discuss what is currently known. Discussions of male advantage/female disadvantage situations often involve a character that gives a male mating advantage but causes sur-

vival disadvantages (although developmental and physiological trade-offs are also possible). Examples in animals include the male coloration alleles of guppies (Fisher 1930; Lindholm and Breden 2002) or the horn growth in male bighorn sheep cited by Rice (1984). The horned/polled polymorphism in Soay sheep appears to be maintained by a trade-off in males between reproductive success favouring the horned (Ho+) allele, and survival favouring the HoP (polled) allele, together with greater lifetime reproductive success in Ho+/HoP females; dominance also differs between the sexes (Johnston 2010).

In considering SA alleles, one should distinguish between de novo evolution of sex-determining loci, versus the evolution, in a species where different sexes already exist, of a new sex-determining locus (e.g. through a novel sex-determining gene replacing an existing one, resulting in a single sex-determining gene, or by transposition of an ancestral sex-determining locus). Some newly evolving animal sex chromosomes are clearly in the latter category (van Doorn and Kirkpatrick 2007). SA may be involved in both kinds of situations, in the latter through prior establishment of an SA polymorphism that facilitates the change (van Doorn and Kirkpatrick 2007), for example.

The situation in dioecious plants might be different, because de novo evolution of sex chromosomes is probably commoner than changes in established systems (Smith 1969), and, because these are often recent evolutionary changes, development and physiology may differ little between the sexes, making SA effects less likely. However, SA is likely to be involved in the initial evolution of dioecious plants, because the two genders evolved, at least in part, through sterility mutations with advantages in the sex that is not rendered sterile (Westergaard 1958).

Other forms of SA should not, however, be ruled out for dioecious plants until empirical data studies have been done. Competition for pollinators might act somewhat similarly to sexual selection involving male-male competition. Ecological differences between males and females have been documented, some in plants with genetic sex determination (Bierzychudek and Eckhart 1988; Cox 1981), as well as some physiological differ-

ences between the sexes, such as a difference in water use efficiency (Delph et al. 1998), and gender-specific differences in life history are common (Eppley 2001), particularly, but not exclusively, in perennials (e.g., Antos and Allen 1999; EspritoSanto et al. 2003; Sun et al. 2009; Thomas and LaFrankie 1993). Although these are not reproductive characters, and are sometimes expressed during non-reproductive periods of life, such differences may have evolved to benefit reproduction (e.g., to conserve resources that females may need later for support of seeds and fruits, and to deter seed and fruit predators). However, somatic physiological trade-offs may be of less importance than in animals, because genes involved in gender determination could be expressed exclusively in the inflorescences and flowers, at least until dioecy has been established long enough for adaptations to have evolved due to selection differentially affecting males and females.

Very strong selection is generally considered unlikely (Keightley and Eyre-Walker 2010; Wloch et al. 2001), and it is well established that smaller fitness differences between alleles tend to be associated with larger dominance coefficients, whereas strongly deleterious mutant alleles tend to be recessive (Korona 2004; Phadnis and Fry 2005; Simmons and Crow 1977). However, early in de novo sex chromosome evolution, strong selection cannot be ignored, because female suppressors must be necessary as males evolve from hermaphrodites, if constraints due to resource availability prevent enhanced male functions unless female functions are at least partially reduced (Charlesworth and Charlesworth 1978). Such mutations are intrinsically antagonistic and involve strong selection, and the involvement of dominant or largely dominant female suppressors in the evolution of dioecy is supported by genetic results from dioecious plants that recently evolved (Westergaard 1958).

Close enough linkage to the MSY allows invasion of alleles with large selective advantages in one sex (making chance loss while rare unlikely), even if they are strongly deleterious, or even lethal, in the other sex. They can then establish polymorphisms (Charlesworth and Charlesworth 1978). Later modifiers of sex allocation, and modifiers improving one sex function at the expense of the other seem likely to involve weaker selection.

In the context of de novo evolution of sex chromosomes beginning with hermaphroditism, a locus with an allele with effects on male and female functions in a cosexual ancestor (with both sex functions) might be expected to reflect a balance of the advantages through each sex function (Lande 1980). As males evolve (or hermaphrodites evolve greater maleness once females have appeared), new male-benefit alleles at such a locus might arise by mutation, and, if these generally have recessive, minor effects, the results above suggest that polymorphisms may less often be maintained.

Chapter 5

When Can a Stressor Facilitate Divergence by Altering Time to Flowering? A Critical Discussion Using Data From *Mimulus guttatus*

5.1 Summary

Stressors and heterogeneity are ubiquitous features of natural environments, and theory suggests that environmental qualities can alter flowering schedules, thereby causing assortative mating within habitats and promoting divergence. Using a greenhouse experiment that controlled for genetic background and that standardized stress intensity, we tested whether two common stresses (low water and herbivory) influenced time to flowering in *Mimulus guttatus*. Both stresses altered the time to first flower, but in different directions. Furthermore, as expected, additional fitness correlates (plant height, number of flowers produced) differed between stressful and benign treatments. We use these results as a vehicle to discuss how the in-

fluence of stress on these traits may affect the evolution of flowering time, by changing rates and the symmetry of gene flow between habitats, and also by creating additional mechanisms for assortative mating. Therefore, this discussion contributes novel predictions for the evolution of flowering time and conditions for local adaptation.

5.2 Introduction

Gene flow tends to homogenize populations, which reduces the potential for local adaptation (Lenormand 2002), speciation (Coyne and Orr 2004), and range expansion (Kirkpatrick and Barton 1997). In general, high migration relative to the strength of selection prevents population differentiation (reviewed by Lenormand 2002). Migration of an allele from an environment in which it is favoured to one in which it is disfavoured causes migration load in the latter, which can generate selection for traits that reduce gene flow (Lenormand 2002). Similarly, numerous forms of assortative mating can evolve that reduce gene flow and promote diversification (e.g., Doebeli and Dieckmann 2003), including mating within groups due to differential timing (Antonovics 2006) or location of reproduction (Otto et al. 2008), and self-fertilization (e.g., Dickinson and Antonovics 1973, Epinat and Lenormand 2009). In this light, studying mechanisms that reduce gene flow is fundamental to understanding the maintenance and generation of biodiversity.

The time to first flowering often changes when plants grow in different environments (reviewed by Levin 2009; also see below), and the possibility that an environmentally-mediated phenological shift could facilitate the evolution of assortative mating by habitat type has attracted increasing attention. Stam (1983) modeled this possibility, where a habitat-induced shift in date of first flowering (HISF) causes habitat-specific assortative-mating. Specifically, he considered a population consisting of two patches that were identical, except for an environmental difference that induced an initially small, neutral, non-genetic change in flowering time between patches, for example, causing patch A to flower slightly before patch B, but the envi-

ronmental difference has no effect on the duration of flowering by individuals; as well, no seed dispersal occurred between patches. The patches initially overlapped in flowering time, and genetic variation for flowering time existed in both patches, so that genes for "early" and "late" flowering were initially present in each patch. He showed that HISF caused the early flowering patch (A) to tend to receive pollen with alleles for early flowering from the later-flowering patch (B); likewise, patch B tended to receive pollen with alleles that cause late flowering from A. Thus, HISF caused biased gene flow between patches for flowering time that alone caused genetic divergence for flowering time between the patches and reduced gene flow. Counterintuitively, simulations showed that increasing pollen dispersal between patches aided divergence in flowering time (a form of character displacement), which becomes obvious when one considers that no genetic divergence in flowering time could occur if there were no pollen migration between patches in this model. In contrast, seed dispersal between patches eroded genetic differences in flowering, independently of flowering time.

Recent work has extended Stam's (1983) study. Gavilets and Vose (2007) used spatial simulations of hard selection that differed between two habitats and showed that a small, environmentally-induced phenological shift between habitats (on the order of the effect of a single gene substitution) greatly improved the opportunity for genetic divergence of flowering time between habitats. Finally, Levin (2009) reviewed empirical cases of HISF, and proposed that habitat-specific flowering times could result from plasticity alone, or a combination of plasticity and subsequent genetic differentiation. The theoretical expectation (Fox 2003) and empirical demonstration that within-population variation in flowering time can cause assortative mating (Weis and Kossler 2004) supports the argument that HISF should promote assortative mating by habitat type. Empirical studies have shown that genetic differences in flowering time have evolved among plant habitats, apparently reducing gene flow (e.g., Savolainen et al. 2006, reviewed by Antonovics 2006), and it is possible that HISF could have aided this process.

In order to connect these theoretical predictions to natural populations

it is useful to test how specific environmental factors that reduce fitness (stressors) influence the time to flowering. Many such tests, together, can help answer questions such as, do particular stresses consistently cause earlier (or later) flowering? Are some stresses prone to cause larger changes in flowering time than others? And, how might a stressor's effect on traits other than flowering time affect gene flow and the evolution of flowering time? Many studies demonstrate that the flowering times of different ecotypes differ between environments (reviewed by Levin 2009). Often, however, these studies have not controlled for differences in genetic background, making it difficult to know the extent to which immediate shifts in phenology could contribute to divergence. In Table 5.1 (see Discussion), we summarize results from studies that have controlled for genetic background when examining the effects of stress on flowering time, including the present study.

Using a full-sib design that allowed us to examine similar genotypes in different environments, we tested the effect of two common stresses (low water and herbivory) on time to flowering and other ecologically important fitness correlates (height and number of flowers produced) for *Mimulus guttatus* to address two objectives. First, our data contribute to previous studies to gain a broader sample of effects of stress on time to flowering; we report the interesting result that stress type affects the direction of change in time to flowering and discuss its potential impact for the evolution of flowering time. Second, although our data do not deal directly with gene flow, we use our results as a vehicle to discuss novel hypotheses regarding how a stressor's effect on ecologically important traits (other than flowering time) may affect gene flow, and subsequently affect the evolution of flowering time. Throughout, we follow previous convention that a "stressful" environment is one that decreases fitness (e.g., Fowler and Whitlock 2002, Armbruster and Reed 2005), so that encountering a novel environment may cause stress.

5.3 Methods

5.3.1 Production and Maintenance of Genetic Lines

Our experiment used plants collected from the Wreck Beach population of *Mimulus guttatus*, situated on the edge of the University of British Columbia campus. *Mimulus guttatus* displays showy yellow flowers and occurs as either an herbaceous annual (Hall and Willis 2006) or perennial. Our population has a perennial habit with observable vegetative reproduction through runners, with seed production in this population occuring through a mixture of selfing and outcrossing (selfing rate $\approx$ 59%, Ritland and Ganders 1987). This population lives on a sandy slope, with many plants growing in ground moistened by water fed from above.

In early summer of 2008 we collected a total of 38 plants on two sampling dates (32 and 6 plants), spaced at least 1 meter apart to limit sampling genetically identical individuals produced by vegetative growth. Sampling on the two dates occurred in different areas of the population. We potted these plants in standard potting soil and watered them in the greenhouse as needed. Within each sampling date we randomly assigned individuals to mating pairs, with one member of the pair serving as the sire and the other as the maternal plant to produce 19 full-sib lines.

We began crosses in late May 2008. All maternal flowers were emasculated in the bud phase, and freshly opened flowers were chosen on sire plants whenever possible; flower pedicels were marked with either a tag or liquid paper. We rubbed open anthers onto a stigma using tweezers until the stigma closed (Ritland and Ritland 1989), and wiped the tweezers between pollinations. We monitored each pollination and re-applied pollen from the same donor on later dates if fruiting had not initiated. As our population readily sets fruit by autonomous selfing (personal observation), we occasionally removed excess fruits to aid maturation of our pollinated flowers; we collected fruits when they began to dehisce. All flowers remained uncovered throughout the experiment; however, unwanted pollination by pollinators was unlikely because we only noted three pollinators

in the greenhouse over the course of a year.

The experiment began in mid-October, 2008. We chose approximately 40 filled seeds randomly from each maternal plant (unfilled seeds are unlikely to germinate; Searcy and Macnair 1990). We sowed full-sibs together in single small pots, using a separate pot for each mother's seeds. The seeds germinated and grew for about one month, with the pots arranged randomly on a mist-bench.

After about 4 weeks (November 12) we randomly assigned 10, 5 and 5 seedlings from each seed family to control (C), water-stress (WS) and and herbivory (H) treatments (described below), respectively; we used fewer individuals when germination rates limited seedling availability. Seedlings were transplanted individually into a 10x10x10 cm pot filled with standard potting mix. We randomly assigned pots to trays ($\leq$ 10 pots per tray) with the restrictions that all plants in a tray belonged to the same treatment and that each tray contained only one member from any seed family. The plants were then allowed to recover from their transplant in the mist-bench for one week; on November 20 all trays were moved to the main greenhouse area, where they received unfertilized water, delivered by hose. After two weeks in the greenhouse (December 5), we watered all plants except those in the WS treatment by flooding the bench for seven minutes with fertilized water; beginning the following week, all plants were automatically watered every morning by this method (except WS; see treatment details below). All plants in this experiment received daylight, supplemented with greenhouse lights set for 16-hour days. We moved tray positions randomly within the greenhouse and plant positions haphazardly within trays approximately every 3 days until the beginning of January, 2009; beyond this time we gradually increased the time between randomization, to a maximum of once per week.

5.3.2 Treatment Descriptions

The WS treatment began two weeks after plants were moved to the main greenhouse area (see above), and the H treatment after three weeks (De-

cember 11). WS plants were raised several inches above the flooding bench so they generally experienced the same greenhouse conditions as the other plants. We lowered the WS plants onto the bench for watering by flooding when approximately 50% of WS plants began to wilt. The frequency of WS watering changed as the plants developed; WS plants received water approximately once every 6 days early in the experiment, and once every 3 days towards the end.

Plants assigned to the H treatment experienced "herbivory" once per week: we cut every new leaf greater than 26mm in diameter in half (perpendicular to the main vein) with a pair of scissors, so every leaf was cut once. We cleaned the scissors with ethanol between cutting each plant. In addition, every week we sprayed the top and bottom of leaves of every H plant with a 1 mM solution of methyl-jasmonate, a ubiquitous plant compound that triggers biosynthetic pathways in response to wounding and herbivory (Doughty et al. 1995).

The levels of stress imposed in each treatment were informed by pilot studies, and chosen to be strong enough to affect fitness, as measured by growth and flower production, but weak enough to minimize mortality. This choice helps standardize the strength of the stresses, so that our experiment does not overly confound stress type with stress intensity.

5.3.3 Data Collection

We checked our plants approximately every six days to determine when each plant initiated flowering. At the end of the experiment (the week of March 7, 2009), we counted the number of fruits and flowers initiated by each plant and measured height (cm) after straightening; at this time, all plants showed signs of senescence and flowering had almost ceased. For analyses, we calculated days to first flowering from the date that all plants were transplanted (November 12).

5.3.4 Data Analysis

We analysed our data with the MCMCglmm package (version 2.10; Hadfield (2010) in R (version 2.12.1)). MCMCglmm uses Markov chain Monte Carlo routines to fit generalized linear mixed models in a Bayesian framework. All analyses used expanding priors, which are typically uninformative and facilitate sampling of parameter space by helping to avoid chains becoming stuck at certain values (J. Hadfield, personal communication). Significance is assessed by the posterior distribution of the model's parameters. Likelihood-based mixed models (e.g., lme package in R) produced similar results to those reported here. We tested whether each stressor affected traits relative to the control treatment, which is of most biological interest.

We fitted mixed effects models, with treatment fitted as a fixed effect and line as a random effect; analyses permitted unequal residual variance among treatments. We modeled Line x Treatment (LxT) interactions with a constant correlation / covariance structure, which considers equal genetic variance among treatments and allows correlation for a genotype's response to all treatments, but assumes this correlation is consistent among treatments. All data were ln-transformed to help meet assumptions of the analyses.

Some plants experienced damage due to handling during the experiment (e.g., when randomizing positions). When damage affected measurements of height or the total number of flowers produced we omitted damaged plants from the analyses. All combinations of line and treatment had at least three individuals even after removing damaged plants from the dataset. For the smallest data set, the mean number of plants per line*treatment combination equalled 7.9, 4.6 and 4.4 for the C, WS and H treatments, respectively.

5.4 Results

Water stress caused flowering to occur 1.5 days earlier compared to the control (Figure 5.1A), which was marginally significant ($p = 0.061$) in a model

that included a LxT term. However, there was no evidence in this model for a Line-by-Treatment interaction, as the 95% highest probability density interval of the posterior distribution included zero. Removing the LxT term from the model, the effect of water stress on flowering time is significant ($p < 0.05$). In contrast, simulated herbivory delayed flowering by approximately 1.5 days relative to the control, and this effect was significant whether or not the LxT term was included ($p < 0.05$ and $p < 0.01$, respectively).

Both stresses reduced flower production. Compared to the control, water stress reduced flower production by approximately 45% (Figure 5.1B), which was significant both in models that included and omitted a LxT term ($p < 0.001$ in both models). As above, there was no evidence of Line-by-Treatment interaction for the number of flowers produced. Likewise, the herbivory treatment reduced flower production by 21% relative to the control, in models that included or excluded the LxT term ($p < 0.05$).

Stress also reduced the height of plants in both stress treatments. Plants that experienced the water-stress and herbivory-treated plants were approximately 27 and 14 cm shorter (40% and 21%) than plants in the control treatment, respectively (both stressors significantly different from the control; $p < 0.001$ for models that include or exclude the LxT term; Figure 5.1C). Again, analyses revealed no indication of Line-by-Treatment interaction for height.

5.5 Discussion

5.5.1 Phenological Shifts and Differentiation: Effect Size

Our results demonstrate that environmental heterogeneity can induce phenological shifts, which facilitate the evolution of flowering time and local adaptation, but not all stressors act in the same direction. In general, plant populations connected by gene flow are more likely to diverge genetically when the environment initially induces large differences in flowering time among habitats, increasing variance in time to flowering in the population,

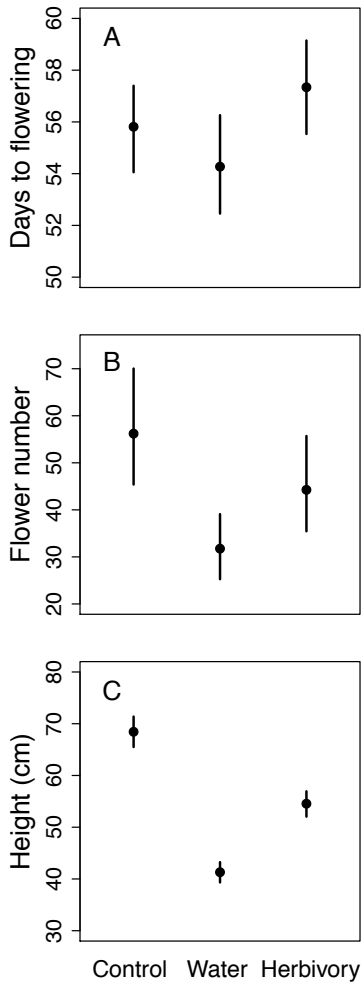

Figure 5.1: Responses of plants from 19 genetic lines of *Mimulus guttatus* to water stress and herbivory, as measured by (A) Number of days to flowering, (B) Total number of flowers produced, and (C) Height. Error bars represent 95% confidence intervals; asymmetric C.I.'s result from back-transformation of the data. Estimates are produced from mixed effects models that include a Line x Treatment interaction. All comparisons between treatment effects and the control are significant, except for the contrast between the water stress treatment and the control for days to flowering, which is marginally significant (see text).

as a whole (Stam 1983; Gavilets and Vose 2007; Levin 2009). Water-stress and herbivory shifted the commencement of flowering in opposite directions in our experiment. Table 5.1 summarizes the direction and magnitude of phenological shifts for studies that, like ours, control for genetic background and contrast ecologically important stresses; it is not intended to be comprehensive because many studies measure the effect of a treatment on flowering time, but serves to illustrate a diversity of results among studies. Table 5.1 reveals that, like ours, multiple studies find contrasting effects of stress type on the direction of a phenological shift, and this result holds important consequences for divergence. When dominant stressors vary spatially among patches within a population, variation in the direction of phenological shifts among stressor-types will enhance initial divergence in flowering time over the whole population. However, species-specific responses to a given stress (Tables 5.1, D.1) suggest that responses to stress may depend on a species' attributes (Stanton et al. 2000) or the intensity of the stress, and complicate predictions for stress on flowering time.

Table 5.1: Effect of stress on time to first flower for various species in laboratory or greenhouse studies that control for genetic background. All shifts in flowering time refer to differences between a control (benign) environment and the given stress; -, +, and ns indicate earlier, later, and non-significant changes in flowering date.

Species	**Stress type**	**Phenology Shift**	**Change (Days)**
Mimulus guttatus[1]	Low water, nutrients	-	1.5
	Artificial herbivory	+	1.5
Mimulus guttatus[2]	Serpentine	-	2
	Low water	-[a]	1.1
Sinapis arvensis[3]	High boron	+[b]	1.5

Continued on next page

Table 5.1 – continued

Species	Stress type	Phenology Shift	Change (Days)
	High salt	ns[b]	.
	Low water	ns[b]	.
	Low light	+[b]	23.2
	Low nutrients	-[b]	0.8
Arabidopsis thaliana[4]	Low nutrients	+	14[c]
	Low water	ns	.
	Low light	+	.
Hordeum spontaneum[5]	Low nutrients	-[d]	2.3[c]
	Low water	+	7.3[c]
Thlaspi caerulescens[6]	Low light	+	10
	High Zn	ns	.
Raphanus raphanistrum[7]	Herbivory	+	2

[1] This study; [2] Murren et al. (2006); [3] Stanton et al. (2000);
[4] Pigliucci et al. (1995); [5] Volis et al. (2002);
[6] Jiménez-Ambriz et al. (2007); [7] Agrawal et al. (1999)
[a] Article contains a typo: words and data (mean ± SE) indicate different directions of effect; I chose the direction suggested by the word "earlier", after receiving no clarification from the article's author
[b] Significance of flowering time inferred from t-tests that used ls-means and SE in Table 4A of Stanton et al. (2000), assuming df=infinity (N=2162 for 6 treatments)
[c] Mean response over 4 populations, estimated from figure.
[d] Data unclear whether low nutrients decrease fitness.

The magnitude of phenology shifts in our experiment were, however,

small. Both stresses shifted flowering time by approximately 1.5 days relative to the control; given that our plants flowered for more than a month, this shift in flowering time represents a small reduction of overlap in flowering between treatments. Effects of this size are not uncommon among similar experiments (Table 5.1). However, our stresses might have induced larger phenology shifts if we had made them stronger (e.g., harsh enough to cause some mortality), and some studies report larger effects of stress on flowering time (Table 5.1), so the small effects seen in this study are not universal (see also Levin 2009). Given that theory suggests that even a small environment-induced phenological change can facilitate differentiation (e.g., of the magnitude of the effect of a single gene-substitution; Gavrilets and Vose 2007), empirical tests are needed to clarify the biological significance of the observed phenological shifts for subsequent divergence in flowering time (see Weis and Kossler (2004) for potential methods).

HISF will likely have a greater impact on species with narrower flowering periods. Interestingly, in an analysis of flowering dates for 49 and 22 animal- and wind-pollinated species of prairie plants, respectively, Rabinowitz et al. (1981) showed that wind-pollinated plants had significantly narrower flowering periods (although their analysis did not consider phylogeny). Therefore, for a given shift in date of first flowering, stress may cause a greater reduction in the overlap of flowering time between two patches for wind-pollinated species, assuming that stress does not alter the duration of flowering.

5.5.2 Hypotheses for the Influence of Flower Number and Height on Flowering Time Evolution

Stressors will often induce changes in other traits (by our definition, will reduce fitness), as we observed with flower number (Figure 5.1B) and plant height (Figure 5.1C). It is important to note that trait changes often accompany changes in phenology (Figure 5.1; see Table D.1 for further examples) which may, themselves, have implications for divergence in flowering time between habitats through a variety of mechanisms.

For instance, a reduction in flower number may affect the nature of di-

vergence in flowering time between environments by changing the relative size of the pollen and seed pools between the environments, thereby affecting the symmetry of responses among habitats. Stam's (1983) model (see Introduction) assumed similar population sizes between patches, which resulted in symmetrical divergence in flowering time: the later-flowering patch received genes for later flowering from the patch that tended to flower earlier to the same extent that the latter patch received alleles for earlier flowering from the former. However, if flower production (or population size) differs between the two habitats, then the magnitude of the pollen and seed-pools will also differ, and gene flow should be biased from the patch with the larger pool to the smaller (see Hendry and Day 2005). Therefore, all else being equal, if HISF causes patch A to commence flowering slightly earlier than patch B and plants in patch A produce fewer flowers, then pollen flow will be biased towards patch A and it will tend to receive alleles for earlier flowering from B to a greater extent than patch B will receive alleles for late flowering times, causing flowering time to evolve more in patch A. The production of fewer flowers may also shorten the overall duration of flowering, and thereby decrease the overlap in phenology between the patches and increase reproductive isolation among them. Asymmetric seed dispersal should have the opposite effect: increased seed dispersal to a patch with a small seed pool will erode the smaller pool's genetic divergence for flowering time, as seed migration is independent of flowering time (following from Stam 1983).

Differences in flower number or height between patches could also affect pollen or seed flow by altering the process of dispersal (as opposed to the amount of material dispersed). Increased plant height tends to cause wind-dispersed pollen (reviewed by Friedman and Barrett 2009) and seeds (Sheldon and Burrows 1973, Soons et al. 2004) to travel farther, so disparity in mean plant height could also result in asymmetric pollen and seed dispersal with consequences for divergence in flowering time similar to those for differences in the size of the pollen and seed pools.

However, such predictions for flowering time evolution are less clear for animal-dispersed pollen. For instance, pollinators are often attracted to

taller plants (e.g., Lortie and Aarssen 1999, Dudash et al. 2011) and floral displays with more flowers (reviewed by Harder et al. 2004) or larger flowers (flower size can also respond to stress; e.g., Murren et al. 2006, Caruso 2006, Strauss 1997). This may not matter if pollinators forage in a manner that is consistent with an Ideal Free Distribution (Fretwell and Lucas 1970), so they equalize the benefits of foraging among patches. Specifically, although visitation frequency to inflorescences by bees increases with display size, the mean number of visits per flower does not vary with display size because bees visit fewer flowers on more attractive displays (reviewed by Ohashi and Yahara 2001, Ishii and Harder 2006), likely because flowers on attractive displays offer less reward due to being visited more frequently. Therefore, even if plants in two patches are not equally attractive, it is not obvious whether asymmetrical pollen flow is likely to occur.

Changes in morphological traits may also cause assortative mating independent of phenology. For instance, when foraging in mixed stands of diploid and tetraploid *Chamerion angustifolium*, pollinators preferentially visited tetraploids, which produced larger displays, and pollinators moved between tetraploids more often than expected by chance (Kennedy et al. 2006); similar effects may arise between stressed and non-stressed plants. Also, if a minimum wind-speed is required to remove pollen from anthers of wind-pollinated plants, then plants of different heights may participate in pollination for different wind conditions because wind speeds tend to be lower near the ground (Friedman and Barrett 2009). Therefore, when plant heights vary greatly among habitats, these effects could cause assortative mating by habitat type via wind-speed. Finally, self-fertilization represents an extreme form of assortative mating, which may be enhanced under some environmental conditions that cause plants to produce smaller flowers or weaken self-incompatibility (Levin 2010).

5.5.3 Costs of Assortative Mating

The models considered by Stam (1983) and Gavrilets and Vose (2007) treated flowering time, per se, as a neutral trait. However, Gavrilets and Vose

(2007) also noted that a change in flowering time can involve fitness costs, such as pollen limitation. In addition, the date of flowering can affect the probability of grazing (Ehrlén and Münzbergová 2009) or reproducing before drought occurs (Hall and Willis 2006), and genes (e.g., *Frigida*) affecting flowering time could have pleiotropic effects on water use efficiency (Mckay et al. 2003, Stinchcombe et al. 2004). The length of the growing season can also constrain the evolution of flowering time (e.g., Olsson and Ågren 2002), and conflicting selection pressures can produce stabilizing selection on flowering time (Colautti and Barrett 2010, Hall and Willis 2006). Such costs should inhibit genetic divergence in flowering time between patches (Gavrilets and Vose 2007), and strong costs could select for convergence in flowering time that negates the HISF. Alternatively, if selection in an environment favours a change in flowering time in the same direction as one caused by HISF, then HISF would facilitate divergence in flowering time.

5.5.4 Consequences for Local Adaptation

As noted in the Introduction, local adaptation can favor the evolution of assortative mating (e.g., Dickinson and Antonovics 1973, Epinat and Lenormand 2009). Conversely, the evolution of assortative mating influences the degree of local adaptation by increasing the frequency of locally adapted alleles in their favoured habitats (e.g., Stam 1983, Gavrilets and Vose 2007). Although a complete review of the interplay between local adaptation and assortative mating is beyond the scope of this paper (see Lenormand 2002), several observations are worth noting in the context of stress' influence on assortative mating.

To illustrate how stress may affect the potential for local adaptation, consider the simple case of an Island-Continent model, where the island may represent a population at the edge of a species' range. In general, local adaptation in the island can occur when the strength of selection for locally beneficial alleles is strong relative to the rate of migration (Lenormand 2002, Yeaman and Otto 2011). If conditions on the island cause stress, then

a decrease in flower production will reduce the island's pool of pollen and seeds, which has the effect of increasing the effective immigration rate of both pollen and seeds from the continent, making local adaptation more difficult (Lenormand 2002). In contrast to these effects that reduce local adaptation, HISF and the evolution of divergence in flowering time will decrease gene flow to the island and facilitate the invasion of locally-adpated alleles. Such differences in flowering time will be particularly important for reducing gene flow when pollen is the major source of immigrant alleles. For example, when strong selection occurs against maladapted alleles from the continent (Levin 2009), most seeds migrating from the continent habitat will have two maladapted alleles and few of these seeds will establish in the new habitat, whereas pollen received from the core will tend to form heterozygous seeds in the novel habitat and experience less selection (Lopez et al. 2008, Levin 2009). In this case, flowering time divergence may effectively shut down gene flow.

The spatial distribution of a stressor will affect both the magnitude of the flowering time difference between potential mates and (consequently) the distribution of alleles that confer local adaptation to the stressor. Following the discussion above, an abrupt shift between stressful and benign conditions will cause the greatest difference in flowering time between two plants that experience different environments. However, spatial heterogeneity will often occur in other forms, such as an environmental gradient (e.g., Stanton et al. 1997). If most pollen dispersal occurs over relatively small distances, then environment-induced changes in phenology between potential mates will be small but will somewhat decrease the distance of gene flow through pollen (J. Sambatti, pers comm.). Consequently, if genetic variation for adaptation to the environmental gradient is segregating along the gradient, an environmentally-induced phenological shift may steepen the cline in these allele frequencies along the environmental gradient (Slatkin 1973).

5.5.5 Conclusions

Studies that consider the role of stress in evolution have traditionally addressed its influence on phenotypic and genotypic variance (e.g., Stanton et al. 2000, Fowler and Whitlock 2002). Stress-induced changes in flowering time present another mechanism for stress to promote evolution in heterogeneous environments, and the current results suggest that future studies need to investigate both the biological significance of small differences in flowering time between stressful and benign environments, and the impact of changes in traits other than flowering time (e.g., height, number of flower, duration of flowering, degree of asexual reproduction) on opportunities for divergence. It is intriguing to note that a number of species best known to have evolved reproductive isolation over short distances and to display different flowering times between habitats (e.g., *Anthoxanthum odoratum* (Antonovics 2006), *Howea* spp. (Savolainen et al. 2006)) are wind-pollinated. Whether characteristics of wind-pollinated species (e.g., extent of pollen dispersal, relatively short duration of flowering) make wind-pollinated species more susceptible to divergent evolution for flowering time between environments would be a fascinating subject for future studies.

Chapter 6

Conclusions

The great diversity of plant reproductive systems has long inspired interest in the forces that generated this diversity. This thesis presents new hypotheses relating to several evolutionary transitions in plant reproduction. Chapters 2 and 3 follow the perspective of several previous models (e.g., Johnston et al. 2009, Johnston 1998, Schoen et al. 1996) that selfing rate (or mode of selfing) can jointly evolve with other fitness traits (e.g., pollen export). The novel contribution of Chapters 2 and 3 is to consider how pleiotropy between selfing rate and viability affects mating system evolution. Notably, these chapters reveal that such pleiotropy allows the evolution of mixed mating in populations with high inbreeding depression and no pollen-limitation, which was previously considered impossible. This novel prediction was shown to be robust across a variety of modes of selfing. These results expand the conditions under which we expect mixed mating to evolve, and suggest explanations for mixed mating in some species that were previously enigmatic.

Chapter 4 revealed that sexually-antagonistic polymorphism can be maintained most easily in a pseudo-autosomal region of sex chromosomes. These results support current hypotheses for the role of sexual antagonism in the evolution of sex chromosomes (Charlesworth et al. 2005), but they are also relevant to the evolution of sexual selection (Albert and Otto 2005, Pischedda and Chippindale 2006) and sexual dimorphism (e.g, Delph et al.

2010). In addition, these predictions for the genomic distribution of sexually-antagonsitic loci can inform empirical work that aims to find and characterize such loci.

Finally, Chapter 5 empirically tested the effect of two stressors on flowering time and ecologically important traits (number of flowers produced and plant height). These data, when combined with previous studies, show that stress types have variable effects on flowering time: a given stress type can cause earlier or later flowering (where the outcome may be species-specific), and the magnitude of a shift in flowering time varies among stressors. Chapter 5 then considered how stress-induced changes in flowering time, flower number and plant height likely affect patterns of gene flow among plant populations, and generated novel, testable predictions for the evolution of flowering time. For instance, the flowering times of neighboring populations may evolve to different degrees due to asymmetrical gene flow between them, caused by differences in flower production.

To close this thesis, I propose directions for future research related to the evolutionary transitions considered here; in particular, I will focus on the areas I feel deserve the most attention. As Chapter 4 already addresses approaches to detect sexually-antagonistic loci (a natural next step for this research), I will not discuss this work further. I will focus on mating system evolution but will touch upon divergence in flowering time, as many of the suggestions directed towards the study mating system evolution apply equally to the study of phenology.

6.1 What Is Needed to Test Theories of Mating System Evolution?

Research on the evolution of selfing rates includes both an abundance of theoretical models (reviewed in Chapters 1, 2, and 3) and empirical study. Table 6.1 summarizes the major foci of empirical research related to mating system evolution; it omits comparative methods (e.g., Goldberg et al. 2010) that describe correlations between a change in mating system and another variable (e.g., diversification rates, or transitions from perennial to annual

life history).

Most empirical studies measure important parameters for mating system models, particularly inbreeding depression and pollen discounting. In addition, some research programs have extensively described ecological and genetic details to provide a robust understanding of the context of mating system evolution within particular species (e.g., *Eichhornia paniculata*, Vallejo-Marín and Barrett 2009; *Aquilegia canadensis*, Eckert and Herlihy 2004; *Collinsia verna*, Kalisz et al. 2004). In some cases these efforts have confirmed (Kalisz et al. 2004) or rejected (Herlihy and Eckert 2002) benefits of selfing through reproductive assurance, which represents a major advance for understanding selection on mating system evolution.

Table 6.1: Major foci of empirical studies of plant mating system evolution; topic headings are not mutually exclusive

Topic	Example Reference
Reproductive Assurance	
Measure pollen vs. resource limitation	Knight et al. (2005)
Test whether selfing provides reproductive assurance	Kalisz et al. (2004)
Measure model parameters	
Inbreeding depression	Husband and Schemske (1996)
Pollen discounting	Chang and Rausher (1998)
Ovule discounting	Herlihy and Eckert (2002)
Probability that pollen removed from anthers reaches conspecific stigmas	Harder and Johnson (2008)
Examine modes of pollination	
Determine contribution of different modes of selfing to selfing rate	Schoen and Lloyd (1992)

Continued on next page

Table 6.1 – continued

Topic	Example Reference
Measure extent of biparental inbreeding	Brunet and Sweet (2006)
Ecological effects	
Local adaptation	Antonovics (1968)
Study of floral development, (e.g., to test correlation between selfing rate and development time)	Elle (2004)
Role of "enemies" (e.g., herbivores)	Steets et al. (2007)
Flower size and pollinator attraction	Abbott and Irwin (1988)
Selfing to avoid heterospecific pollen	Fishman and Wyatt (1999)
Competition between selfed and outcrossed siblings for resources	Schmitt and Ehrhardt (1987)
Genetic factors	
Role for genetic drift in invasion of a selfing variant	Barrett et al. (1989)
Genetic architecture of selfing to determine role of mutations of large effect	Fishman et al. (2002)
Association between selfing trait and inbreeding depression	Takebayashi and Delph (2000)
Genetic basis of inbreeding depression	Charlesworth and Willis (2009)
Environment dependence of inbreeding depression	Armbruster and Reed (2005)
Test whether inbreeding depression can decrease with consecutive generations of selfing	Husband et al. (2008)

Very few studies have directly tested theory, however, rather than deter-

mining whether parameter estimates for a species and its selfing rate match those predicted from a given model. For example, if a species regularly self-pollinates does it also have low inbreeding depression? In some cases, estimates of model parameters can help to reject theoretical explanations for mixed mating. For instance, given extremely high inbreeding depression and strong ovule discounting in *Aquilegia canadensis*, Eckert and Herlihy (2004) concluded that the theory available at that time could not explain mixed mating in this species. The reverse scenario, however, where estimates of parameters for a given species match those predicted by a model, does not provide a rigorous test of a model.

6.1.1 Approaches to Test the Evolutionary Stability of Mixed Mating Systems

Given the current state of knowledge of mating system evolution, I believe that the greatest insights in this field will arise from advances on three fronts. Foremost, we require stronger experimental tests of mating system evolution theory. Such tests may come from experimental evolution approaches, which can be applied to relatively large organisms, like threespine sticklebacks (e.g., Barrett et al. 2008). For example, Morran et al. (2009) used experimental evolution to demonstrate an advantage of outcrossing for *Caenorhabditis elegans* when evolving in a novel environment. Such tests are more difficult in plant systems, but are not impossible: Kohn and Barrett (1994) used experimental arrays of *Eichhornia paniculata* to show that transmission of an allele that increases self-pollination depends on population composition. Similarly, Fishman (2000) and Chang and Rausher (1998) established experimental arrays that varied the frequency of variants with different tendencies to self-pollinate; their approach mimics the mathematical invasion analyses used in this thesis, by testing the fitness of variants when rare vs. common, and therefore can inform whether mixed mating is adaptive. Finally, unlike the preceding examples that considered a single generation, Bodbyl Roels and Kelly (2011) showed that selfing can evolve to reduce pollen limitation within 5 generations in experimental populations of *Mimulus guttatus* that excluded pollinators.

While these approaches offer invaluable tests that complement studies in natural populations, the availability of molecular tools will allow even more rigorous tests of factors that influence the evolution of selfing rate. For instance, Fishman et al. (2002) isolated QTLs associated with selfing rate in *Mimulus* and indicate plans to use these resources to test mating system evolution. Following hypotheses discussed in Chapters 2 and 3, with such tools one could establish experimental arrays and test whether QTL variants that pleiotropically affect viability (say, due to development time) and selfing rate are favoured in arrays that experience drought that shortens the growing season but not in benign arrays. Similarly, experimental populations that manipulate population structure could test the effect of biparental inbreeding on the spread of a selfing variant. Experimental populations also provide opportunities to manipulate environments (e.g., stress type, magnitude, or spatial distribution) and determine its effect on assortative mating and the evolution of flowering time. Such tests, however, rely on standing genetic variation and therefore cannot consider evolution that involves sequential invasion of mutations. Furthermore, they require relatively small population sizes and therefore will only be useful to study variants with large effect. Finally, these tests are limited to the extent that they approximate natural conditions, and studying processes in natural populations will inform and complement these experimental approaches.

6.1.2 Inbreeding Depression in Natural Environments

Due to its central importance in mating system evolution, I believe we require a greater understanding of inbreeding depression, which will also inform the evolution of dispersal, conservation biology and agriculture. In particular, I argue that we require greater understanding of the sources of inbreeding depression under natural conditions.

A large body of experimental work, based largely on laboratory studies or non-natural populations (e.g., agricultural crops), suggests that most inbreeding depression is caused by (partly) recessive, deleterious mutations (reviewed by Charlesworth and Willis 2009). Although such mutations are

undoubtedly important for inbreeding depression in the wild, a variety of processes could contribute to inbreeding depression in nature that may not manifest in laboratory studies (summarized in Table 6.2), so their contribution to inbreeding depression in natural populations is unknown.

An important difference between a number of the factors listed in Table 6.2 and the conventional explanations for inbreeding depression involves the potential for purging of inbreeding depression. For example, inbreeding depression caused by the expression of alleles that are (partly) recessive where they are locally maladapted can be purged to a limited extent because of on-going migration, although barriers to gene flow (e.g., self-fertilization, Dickinson and Antonovics 1973, Epinat and Lenormand 2009, Jordan and Otto unpubl. data; evolution of flowering time, Gavilets and Vose 2007) may evolve to reduce the frequency of such alleles locally.

Table 6.2: Factors that may affect inbreeding depression in the wild but may go undetected in laboratory studies

Source	**Reference**
Alleles involved in local adaptation can cause inbreeding depression when they are recessive where they. are maladaptive.	Epinat and Lenormand (2009)
Stabilizing selection	Lande and Schemske (1985) Ronce et al. (2009)
Population structure; e.g., when different recessive, deleterious alleles drift to high frequency among subpopulations, inbreeding depression for matings within a sub-population can be even more severe relative to matings between sub-populations.	Whitlock et al. (2000)

Continued on next page

Table 6.2 – continued

Source	Reference
Potential overdominance at Resistance genes; e.g., different allelic variants of the *RPP13* gene recognize different allelic forms of pathogen effector molecules, so heterozygous plants can recognize two pathogen types.	Rose et al. (2004) Allen et al. (2004) Hall et al. (2009)

Some factors in Table 6.2, such as stabilizing selection, may have stronger effects on inbreeding depression under natural conditions than under relatively benign experimental conditions. Even when alleles have additive effects on a trait, a non-linear relationship between fitness and phenotype (e.g., stabilizing selection) can cause alleles to exhibit dominance effects for fitness, and inbreeding depression can arise when selfing affects the mean and variance of their progeny's genotypic values (Ronce et al. 2009). Geber and Griffen (2003) surveyed studies of selection on plant traits and found little evidence for stabilizing selection, except for phenology. However, because many studies that measure selection on reproductive traits focus on the reproductive stage, they could miss selection at earlier stages. For example, because fecundity increases with flower size in *M. guttatus*, a study that focused on the reproductive stage would typically conclude selection favours larger flowers (Mojica and Kelly 2010). However, Mojica and Kelly (2010) demonstrated that viability selection disfavoured genotypes that produced large flowers even though selection occurred before flowers were produced (due to slower development). This viability selection was sufficient to cause selection to favour small flowers, even after accounting for fertility differences. They also note, however, that larger flowers may benefit from lower selfing rates and increased male fitness (neither of which they measured), so flower size may experience stabilizing selection. Moreover, a selection experiment on flower size suggests

that balancing selection may help maintain genetic variation in this species (Kelly and Willis 2001). These studies imply that stabilizing selection may be more common than current data suggest; if so, inbreeding depression through stabilizing selection could be strong because inbreeding depression increases with the number of traits under such selection (Ronce et al. 2009).

Interestingly, inbreeding depression often varies among environments, and this variation presents an opportunity to understand the causes of inbreeding depression. For instance, the observation that inbreeding depression is often stronger in more stressful environments (Armbruster and Reed 2005) might be explained by an increase in the number of sources of selection in stressful habitats, but it could also be due to changes in dominance (Agrawal and Whitlock 2010, Cheptou and Donohue 2011). Schmitt and Gamble (1990), examined inbreeding depression in natural populations of *Impatiens capensis*, and showed that inbreeding depression increased with distance (up to 12 m, the maximum distance they considered) from a maternal plant; these results suggest an influence of local adaptation on inbreeding depression, but other factors, like maternal effects, could also play a role. Future studies that explain variation in inbreeding depression among environments will contribute greatly to understanding the basis of inbreeding depression, and therefore will inform the potential for purging.

A greater understanding of inbreeding depression will also inform future theoretical work. With a lack of data for the causes of inbreeding depression in natural populations, current theoretical studies of mating system evolution that include purging of genetic load are forced to make assumptions regarding the purging process. Furthermore, it is likely that inbreeding depression differs for male and female function (Chang and Rausher 1999, Ivey and Carr 2005), but most models of mating system evolution assume that inbreeding depression affects only viability differences (or equivalently, reduced fitness through male and female function equally) (but see Rausher and Chang 1999). Few studies examine inbreeding depression through both sex functions under natural conditions; if future studies show that inbreeding depression often differs between the sex

roles (which seems likely), then theoretical studies will likely need to account for this fact (e.g., Rausher and Chang 1999).

6.1.3 Measure Siring Success

Finally, we require a greater understanding of male fitness through siring success. A common thread through much of this thesis concerns the joint evolution of pollen flow or siring success with other plant traits, such as phenology, selfing rate, or viability. Therefore, an understanding of male fitness is critical to this research area.

Most research on mating system evolution focusses on female fitness (e.g., seed production) because of difficulties in measuring siring success. However, recent years have seen advances for measuring siring success, and techniques now permit estimates of selection on plant traits through siring success (e.g., Hodgins and Barrett 2008, van Kleunen and Burczyk 2008). With respect to studies of mating system evolution, a few studies have compared siring success between different treatments or classes of genotypes within experimental arrays (e.g., Kohn and Barrett 1994, Chang and Rausher 1998). However, molecular markers that allow one to measure an individual's siring success can provide greater resolution, for example, by allowing one to determine how siring success varies with an individual's individual selfing rate. These methods can be applied to measure the extent of pleiotropy between male-fitness and other plant traits (Lloyd 1979, Harder and Wilson 1998, Johnston et al. 2009, Chapters 2 and 3), as well as to study the influence of environmental conditions on assortative mating. Furthermore, they may can help address questions of importance in evolutionary biology, such as whether plants mate assortatively by fitness, as has been suggested for *Drosophila* (Sharp and Agrawal 2009).

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Appendix A

Additional Details for Model of Prior Selfing (Chapter 2)

A.1 Calculating f_v and f_d in Empirical Studies

Here we show how to calculate f_v and f_d, even if the trait under selection is unknown.

Suppose the selfing rate and viability are linear functions of the underlying trait, ϕ, so that:

$$\theta = \bar{\theta} + \Delta\theta\ \phi \tag{A.1}$$

$$v = \bar{v} + \Delta v\ \phi. \tag{A.2}$$

It follows that pollen export is proportional to:

$$1 - (\bar{d} + \Delta d\ \phi)\ (\bar{\theta} + \Delta\theta\ \phi). \tag{A.3}$$

Accounting for only small deviations from the means (i.e., ϕ small), then (A.3) suggests that pollen export is approximately proportional to

$$1 - \bar{d}\ \bar{\theta} + (\Delta d\ \bar{\theta} + \Delta\theta\ \bar{d})\ \phi. \tag{A.4}$$

Let $cov_A(x,y)$ equal the additive genetic covariance between traits x

and y, and $var_A(z)$ equal the additive genetic variance of trait z. It follows from (A.2) that, $cov_A(v,\theta) = cov_A(\bar{v} + \Delta v\ \phi, \bar{\theta} + \Delta\theta\ \phi)$, which equals $\Delta v\, \Delta\theta\, var_A(\phi)$. Also, $var_A(\theta) = \Delta\theta^2\, var_A(\phi)$. Therefore, $cov_A(v,\theta)/var_A(\theta) = \Delta v/\Delta\theta$, which is consistent with our interpretation of f_v, the rate of change in viability relative to change in the selfing rate due to selection on the underlying trait.

Similarly $cov_A(pollen\ export,\theta) = cov_A(1 - \bar{d}\ \bar{\theta} + (\Delta d\ \bar{\theta} + \Delta\theta\ \bar{d})\ \phi, \bar{\theta} + \Delta\theta\ \phi)$, so that

$$\frac{cov_A(pollen\ export,\theta)}{var_A(\theta)} = \frac{(\Delta d\ \bar{\theta} + \Delta\theta\ \bar{d})\ \Delta\theta\ var(\phi)}{\Delta\theta^2\ var(\phi)}, \tag{A.5}$$

or, $\frac{cov_A(pollen\ export,\theta)}{var_A(\theta)} = \frac{\Delta d}{\Delta\theta}\ \bar{\theta} + \bar{d}$. Therefore, we can solve for $f_d = \frac{\Delta d}{\Delta\theta}$ if we know the average selfing rate and pollen discounting rate.

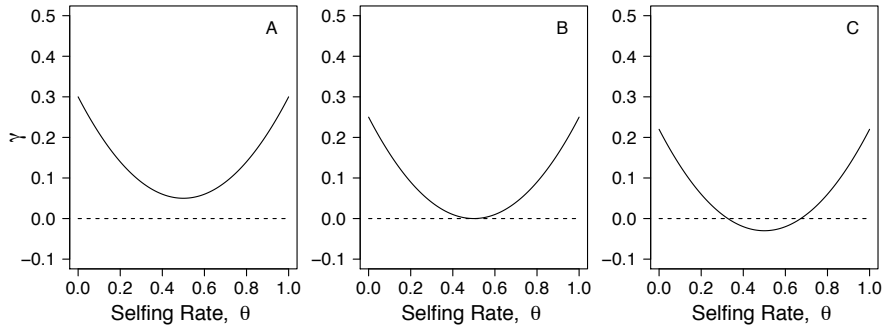

Figure A.1: Illustration of transition from zero to two roots of θ. A) No roots for θ; B) Transition from zero to two roots; our analysis identifies the value of inbreeding depression, δ, as a function of pollen discounting, d, at this transition point, as plotted in Figures 2.3 and 2.4 (dashed curves); C) Two roots for θ. Further explanation: γ (equation 2.8) has two roots with respect to θ, where $\theta = \frac{-b \pm \sqrt{b^2 - 4\ a\ c}}{2\ a}$, and $a = (f_d + 2\ d\ \delta\ f_v)$, $b = (2\ d\ \delta - f_d - 2\ f_v\ (d + \delta))$, and $c = (1 - d - 2\ \delta + 2\ f_v)$. When the value in the radical is negative, the roots for θ are imaginary, as illustrated in A. In contrast, when the value in the radical is positive, the roots are real, as illustrated in C. Therefore, we solved for when the value in the radical equals zero to determine the transition between zero and two roots (see B), and the dashed curves in Figures 2.3 and 2.4 depict these solutions (i.e., the combinations of d and δ for given values of f_d and f_v where the radical equals zero). As a result, scenarios in Figures 2.3 and 2.4 that involve two roots for $0 \leq \theta \leq 1$ (i.e., Figure 2.2D, E) lie on one side of the dashed curve and scenarios with imaginary roots (i.e., Figure 2.2A, B) lie on the other. In cases where the dashed curve touches a straight line, both roots can pass beyond $\theta = 0$ or beyond $\theta = 1$ and become biologically irrelevant.

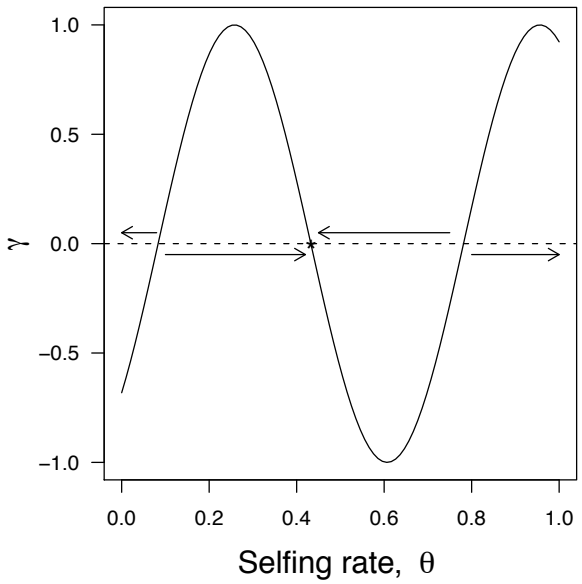

Figure A.2: Hypothetical example of conditions that would allow mixed mating to evolve in the "Bistability" regions of Figures 2.3 and 2.4. Selection favours increased or decreased selfing for $\gamma > 0$ and $\gamma < 0$, respectively; arrows indicate the direction of evolution for ranges of initial selfing rates, θ. Maintaining "Bistability" requires that selection favours increased outcrossing for low selfing rates but increased selfing at high selfing rates, as seen in this example. Three roots for θ exist in this example (i.e., initial selfing rates leading to $\gamma = 0$), and mixed mating evolves for the center root (marked by "*") because selection favours increased selfing below this root and decreased selfing above it. Note that around each root for θ the direction of evolution switches: this fact requires the curve (γ) to have at least 3 roots between 0 and 1 to both maintain Bistability and introduce a root with stable mixed mating (compare this plot to Figures 2.2A, B for examples with no roots, Figures 2.2C, F for examples with 1 root, and Figures 2.2D, E for examples with 2 roots to see this). However, as noted in the text, introducing three roots is impossible because γ is only a quadratic in θ (equation 2.8). Therefore, mixed mating cannot evolve in the parameter space that yields Bistability.

Appendix B

Additional Details for Mass-Action Model of Mating System Evolution (Chapter 3)

B.1 General Conditions for Invasion

Here I provide the general conditions for the invasion of an allele that can shift pollen among any combination of N_{ij}, X_{ij}, and S_{ij}. In general, I assume that the mutant allele shifts pollen from the unused pool to either (or both) the pool for export or selfing, and the sensitivities for these shifts need not be equal. For simplicity I assume the dominance of all effects are equal; more general results will be provided on request. Viability varies as $W_{AA} = 1$, $W_{Aa} = 1 + h\ \Delta v\ \phi$, $W_{aa} = 1 + \Delta v\ \phi$. Genotypes allocate the following fractions to export and selfing: $S_{AA} = S$, $X_{AA} = X$, $S_{Aa} = S + h\ \Delta p_S\ \phi$, $X_{Aa} = X + h\ \Delta p_X\ \phi$, $S_{aa} = S + \Delta p_S\ \phi$, $X_{aa} = X + \Delta p_X\ \phi$. Assuming that the a allele has small effect, it can invade when:

$$\begin{aligned}\beta = & \frac{S\,\epsilon\,(1-\delta)+2\,h\,X\,\pi}{2\,(S\,\epsilon\,(1-\delta)+2\,X\,\pi)\,(S\,\epsilon\,(1-\delta)+X\,\pi)\,(S\,\epsilon+X\,\pi)} \\ & \cdot[\phi\,(\Delta p_X\,\pi\,(S\,\epsilon+X\,\pi)+\Delta p_S\,X\,\epsilon\,\pi\,(1-2\,\delta)+ \\ & 2\,\Delta v\,(S\,\epsilon\,(1-\delta)+X\,\pi)\,(S\,\epsilon+X\,\pi))]>0.\end{aligned} \tag{B.1}$$

The fraction is always positive, so invasion occurs when the term in square brackets is positive. Complete discounting occurs when $\Delta p_S > 0$ and $\Delta p_X = -\Delta p_S$, which retrieves the condition (3.10).

B.2 Values of S when S and X Trade-Off

The quadratic formula yields the roots for S that cause equation (3.10) to equal zero (setting $X = 1 - S - N$):

$$S^{-,+} = \frac{-b \pm \sqrt{b^2 - 4\,a\,c}}{2\,a} \tag{B.2}$$

where,

$$a = 2\,\Delta v\,(\epsilon - \pi)\,(\epsilon\,(1-\delta) - \pi) \tag{B.3}$$

$$b = \pi\,(2\,\epsilon\,(-\Delta p\,(1-\delta) + \Delta v\,(1-N)\,(2-\delta)) + \pi\,(\Delta p - 4\,\Delta v\,(1-N))) \tag{B.4}$$

and,

$$c = -\pi\,(1-N)\,(-\epsilon\,\Delta p\,(1-2\,\delta) + \pi\,(\Delta p - 2\,\Delta v\,(1-N))). \tag{B.5}$$

Appendix C

Recursion Equations That Determine Conditions for Polymorphism at a Sexually Antagonistic Locus (Chapter 4)

Assume a biallelic locus with alleles A_1 and A_2. Each diploid genotype, is denoted by two subscripts, indicating the two gametes involved in their formation (see Table C.1 below). There are 7 distinct genotypes, four in males, and 3 in females (making no distinction between the genotypes formed from uniting a paternal gamete of type 3 (A_1 X) and a maternal gamete of type 4 (A_2 X), and the reciprocal combination). Within each sex, the genotype frequencies sum to 1.

After viability selection, the genotype frequencies are normalised within each sex to obtain the frequencies among surviving mature individuals, which we denote by the same genotype notation as above, with primes. Expressions for the allele frequencies in male and female gametes were then derived, taking into account recombination at rate r between the gene and the MSY, as follows. Male gamete frequencies are denoted by S (for sperm), and female gamete frequencies by E (for eggs), and are given by the follow-

ing expressions, where the subscripts correspond to the haplotype numbers in Table C.1:

Male gametes:

$$\begin{aligned} S_1 &= \frac{1}{2}\, m_{13}^{'} + \frac{1}{2}\, m_{14}^{'}\,(1-r) + \frac{1}{2}\, m_{23}^{'}\, r \\ S_2 &= \frac{1}{2}\, m_{14}^{'}\, r + \frac{1}{2}\, m_{23}^{'}\,(1-r) + \frac{1}{2}\, m_{24}^{'} \\ S_3 &= \frac{1}{2}\, m_{13}^{'} + \frac{1}{2}\, m_{14}^{'}\, r + \frac{1}{2}\, m_{23}^{'}\,(1-r) \\ S_4 &= \frac{1}{2}\, m_{14}^{'}\,(1-r) + \frac{1}{2}\, m_{23}^{'}\, r + \frac{1}{2}\, m_{24}^{'}, \end{aligned} \tag{C.1}$$

Female gametes:

$$\begin{aligned} E_3 &= f_{33}^{'} + \frac{1}{2}\, f_{34}^{'} \\ E_4 &= \frac{1}{2}\, f_{34}^{'} + f_{44}^{'}, \end{aligned} \tag{C.2}$$

Matings were assumed to occur randomly between the genotypes of the two sexes to yield the zygote genotype frequencies in the new generation. Zygote genotypes sum to 1 following random mating; therefore, to restore genotype frequencies that sum to 1 within each sex we multiplied the zygote genotype frequencies by 2. The system was analyzed using *Mathematica* (see text).

Table C.1: Genotypes of parental gametes and progeny genotypes and sexes

Male gamete haplotypes	Frequencies of gametes produces	Progeny sex	Female gamete haplotypes and genotypes in the progeny			
			$A_1\ X$ (Gamete frequency E_3)	Genotype frequency	$A_2\ X$ (Gamete frequency E_3)	Genotype frequency
A_1Y	S_1	Males	A_1Y/$A_1\ X$	m_{13}	A_1Y/$A_2\ X$	m_{14}
A_2Y	S_2		A_2Y/$A_1\ X$	m_{23}	A_2Y/$A_2\ X$	m_{24}
$A_1\ X$	S_3	Females	$A_1\ X/A_1\ X$	f_{33}	$A_1\ X/A_2\ X$	f_{34}
$A_2\ X$	S_4		$A_2\ X/A_1\ X$	-[1]	$A_2\ X/A_2\ X$	f_{44}

[1] Within females, two genotypes are assumed to have identical fitness (see text), and therefore f_{34} is the sum of the the $A_1\ X/A_2\ X$ and $A_2\ X/A_1\ X$ frequencies

Appendix D

Effect of Stressors on Fitness Related Traits in a Variety of Plant Species (Chapter 5)

Table D.1: Effect of various stressors on exemplary traits other than time to first flower for various species in laboratory or greenhouse studies that control for genetic background. See Table 5.1 for effect of stressors on time to flowering.

Species	**Stress type**	**Additional Traits Affected**
Mimulus guttatus[1]	Low water, nutrients	Ht, NoF
	Artificial herbivory	Ht, NoF
Mimulus guttatus[2]	Serpentine	FS, SD, LN, L
	Low water	FS, SD, L
Sinapis arvensis[3,b]	High boron	F, HRGR, LRGR
	High salt	F, Ht, HGR, L, LN

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Table D.1 – continued

Species	Stress type	Additional Traits Affected
	Low water	F, Ht, HGR, L
	Low light	F, Ht, HGR, L, LGR, LN
	Low nutrients	F, Ht, HGR, L, LGR, LN
Arabidopsis thaliana[4]	Low nutrients	F, Ht, NL, LW, NB, LS
	Low water	F, Ht, NB
	Low light	Ht, LS
Hordeum spontaneum[5]	Low nutrients	Ht, L, SW, others
	Low water	Ht, L, SW, others
Thlaspi caerulescens[6]	Low Light	F, Ht, FD
	High Zn	F, FD
Raphanus raphanistrum[7]	Herbivory	F

Ht=height, NoF=number of flowers produced, FS=flower size, SD=stem diameter, F=fecundity, HRG=relative rate of height growth, L=leaf length, LGR=relative rate of increase in leaf length, LN=leaf number, LW=leaf weight, NB=number of branches, LS=life span, SW=spikelet weight, FD=flowering duration

[1] This study; [2] Murren et al. (2006); [3] Stanton et al. (2000);

[4] Pigliucci et al. (1995); [5] Volis et al. (2002);

[6] Jiménez-Ambriz et al. (2007); [7] Agrawal et al. (1999)

[b] Significance of responses (except fecundity) inferred from t-tests that used ls-means and SE in Table 4A of Stanton et al. (2000), assuming df=infinity (N=2162 for 6 treatments)