

POLLINATOR-MEDIATED SELECTION AND DIVERSITY IN FLOWERING
PLANTS

by

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Abstract

This thesis examines the role pollinators have played in the diversification of flowering plants. The extent to which animal pollinators drive the formation of new angiosperm species remains unresolved. Animal pollinators may drive higher rates of diversification because they promote reproductive isolation via specialization on certain floral forms. In Chapter II, using sister group comparisons, I demonstrate that flowering plant lineages possessing monosymmetric (=bilaterally symmetrical) flowers, tend to be more species rich than their radially symmetrical sister lineages. This result supports an important role for pollinator-mediated speciation and indicates that floral morphology plays a key role in angiosperm speciation.

The degree to which flowers should evolve to attract one type of pollinator or a suite of pollinators is unclear. In Chapter III, I develop a population genetics model that examines the effects of local species richness on the evolution of pollinator specialization. The model predicts that local species richness plays a role in determining whether or not plants evolve to specialize on one type of pollinator. This model connects the number of species competing for pollinator attention and the probability of a plant receiving conspecific pollen to show that generalist flowers are more likely to evolve when a species is numerically dominant.

In addition to morphological diversity, angiosperm species also exhibit a wide diversity of mating strategies. In Chapter IV, I develop a population genetic model to explore the evolutionary forces that contribute to the evolution of dichogamy, a mating strategy whereby pollen dispersal and stigma receptivity are separated in time. The model suggests that factors such as anther-stigma interference and inbreeding depression

tend to select for dichogamy, while factors such as the fitness advantage of self-fertilization and selection to match the timing of ovule and pollen production tend to select against dichogamy.

Lastly, In Chapter V, I test the hypothesis that pollination mode (i.e., wind or animal) is evolutionarily correlated with the form of dichogamy using a maximum likelihood program designed to detect correlated trait evolution on phylogenetic trees. The results suggest that protandry and protogyny have evolved in response to different modes of pollination; specifically, in animal-pollinated species flowers evolve protandry, while in wind-pollinated species flowers evolve protogyny.

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Co-authorship Statement

My senior supervisor, Dr. Sarah P. Otto, is the co-author of chapters III, IV and V. For all three chapters, I had the original idea, did the majority of the analysis, wrote the first draft of the manuscript, and am responsible for its final form. Dr. Otto provided guidance with the analyses and revisions of the manuscripts.

Chapter I – Introduction & Overview

1.1 Introduction

The angiosperms have experienced an astonishing radiation since their first appearance in the fossil record approximately 135 to 140 million years ago (Sanderson and Donoghue 1994). This group appears to be a classic example of an adaptive radiation, with an estimated 300 to 3600-fold difference in species number between the angiosperm clade and its most likely sister group, the Gnetales, ginkgos and cycads (Coyne and Orr 2004). Much of this diversification is embodied in floral morphology. A survey by Grant (1949) demonstrated that most traits used for species-level taxonomic distinction in the angiosperms were floral traits, rather than vegetative traits, which display considerably less lability. This finding suggests that speciation in angiosperms has been largely driven by selection on floral traits. Indeed, flowering plant species display an extraordinary degree of variation in floral architecture, mating strategy, and mode of pollination. The role that pollinators have played in generating this variation is the central theme of this thesis.

It has often been assumed that pollinators play an integral role in the evolution of floral form. However, the nature of this role, and its importance in driving floral diversification, is still debated (e.g., Waser 2001). Early floral biologists focused on morphological and ecological descriptions of flower-insect interactions (Faegri and van der Pijl 1978). It was from these observations that the concept of the “Pollinator Syndrome” was born (Stebbins 1970). A pollinator syndrome is a suite of floral traits that tend to be shared by plants that are serviced by the same type of pollinator (e.g., hummingbird pollinated flowers tend to be red, have copious nectar and a long corolla

tube). The existence of syndromes seems to imply that pollinator-mediated selection is so important to floral evolution that the same type of pollinator is able to select for recognizably similar “types” of flowers, even among distantly related species. The concept of the pollinator syndrome has recently become controversial, with researchers disagreeing over its importance, and even its existence (Waser 1996, Kay and Schemske 2004). Certain pieces of evidence, such as the now famous example of differential pollination of F1 hybrids of *Mimulus guttatus* and *M. cardinalis* by bumblebees and hummingbirds (Schemske and Bradshaw 1999), seem to lay to rest any doubts about the importance of pollinators in the evolution of floral morphology. However, because both species of pollinators are known to visit both species of *Mimulus* occasionally (i.e., pollinator isolation is not complete), Waser (2001) argues that there must have been additional factors that contributed to the divergence (see also Ramsey *et al.* 2003).

The first two chapters of this thesis are explicitly concerned with pollinator-mediated selection on flowering plants. An easily recognizable evolutionary trend in floral architecture has been the fusion of petals into a coherent corolla, also known as sympetaly (Stebbins 1974). The evolution of sympetaly appears to enable subtle differences in meristem growth to result in changes in floral architecture and pollination mode (Endress 1997). Sympetaly also allows the possibility of bilaterally symmetrical (or monosymmetric) flowers. Bilaterally symmetrical flowers restrict the direction in which pollinators can enter the flower, and monosymmetry is therefore associated with precision in pollination. Because of this association, monosymmetry in corolla shape has been suggested to be responsible for increasing speciation rates in the lineages where it evolves (Cubas 2004). In Chapter II, I test the hypothesis that monophyletic clades

exhibiting bilaterally symmetrical corollas are more species rich than their radially symmetrical sister clades. I found that of 19 sister groups, 15 support the hypothesis of higher species richness (Figure 2.1). The possible explanations for this include: increased likelihood of precise pollen transfer to monosymmetric flowers, increased visitation by specialist pollinators to monosymmetric flowers and/or higher extinction rates in radially symmetrical lineages. I explore these explanations further in Chapter II.

Floral specialization to attract specific pollinators has been demonstrated to be an important factor in reproductive isolation in angiosperms (Schemske & Bradshaw 1999, Ramsey *et al.* 2003) and is therefore a candidate trait for speciation. However, little is known about the evolutionary and ecological processes that drive flowering plant species to specialize on one or a few pollinators. Indeed, the relative frequency of specialization over generalization has become the focus of debate in the literature (Waser 1996, Johnson & Steiner 2000). In Chapter III, I present a population genetic model that explores the role of a focal species' density relative to other animal-pollinated flowering plants in its vicinity in affecting the propensity for the species to evolve towards specialization or generalization. The results suggest an important, yet currently under-explored role for local species composition in the evolution of floral specialization.

In addition to their morphological diversity, angiosperms exhibit remarkable across-species diversity in mating strategies. Most flowering plants have perfect (hermaphroditic) flowers (Proctor *et al.* 1996), and much of the diversity in mating strategies appears to have arisen in order to offset the special costs associated with pollen dispersal and receipt in a hermaphroditic flower (reviewed by Barrett 2002). The two remaining chapters of this thesis are concerned with the evolution of dichogamy, a

flowering plant mating strategy, and the way in which pollination mode may affect which form of dichogamy evolves.

Dichogamy is the temporal separation of pollen dispersal and stigma receptivity within a perfect (hermaphroditic) flower or between male and female flowers on a monoecious plant (Barrett 2002). This mating strategy has previously been described as a mechanism to improve outcrossing success in hermaphroditic flowers. Although there is much experimental and survey data to support the claim that dichogamy is a mechanism to improve outcrossing success, there has been very little explicit theoretical exploration of the forces influencing the evolution of dichogamy. In Chapter IV, I use a population genetic model to explore the interplay between four factors thought to play integral roles in the evolution of dichogamy: the avoidance of anther-stigma interference, the intrinsic advantage of self-fertilization, the cost of inbreeding depression, and advantages of having overlapping ovule availability and pollen dispersal at the population level. I found that all of these factors may play a role in the evolution of dichogamy, although some are more important in the evolution of further temporal separation than they are in the initial evolution of dichogamy from adichogamy. Several testable predictions arise from the model, and I believe it is unique in elucidating the relative importance of the four factors in the evolution of this intriguing floral mating strategy.

Dichogamy itself is found in a diversity of forms (Lloyd and Webb 1986). Two main forms have been identified: protandry, where pollen is dispersed prior to stigma receptivity, and protogyny, where stigma receptivity precedes pollen dispersal (Figure 4.1). Several studies have indicated evidence for a correlation between the form of dichogamy exhibited by a species and its mode of pollination. However, it was

previously unknown whether the pattern was caused by phylogenetic relatedness between the species or whether it was evidence for correlated evolution between the two traits. In Chapter V, I present the results of a phylogenetically corrected test of the hypothesis that the form of dichogamy and the mode of pollination exhibited by a species are evolutionarily correlated. I found evidence to suggest that there is correlated evolution, with protogynous species more likely to be wind or water-pollinated, and protandrous species more likely to be animal-pollinated. However, a closer examination of the pattern revealed interesting complexities. For example, previous studies had assumed that mode of pollination would drive changes in the form of dichogamy that evolves, but I found evidence for the converse, that the form of dichogamy affects the mode of pollination exhibited by a species. I also found stronger evidence for a role of pollination in the evolution of protogyny from other mating strategies than for the evolution of protandry. The evidence presented in this chapter identifies a need for a reexamination of some of the underlying assumptions about the role of pollination mode in the evolution of dichogamy.

Finally, in *Conclusions and Future Directions*, I summarize the findings of my thesis, how it fits into current ideas about flowering plant diversity, and some implications for future researchers in this field.

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Chapter II – Floral Symmetry Affects Speciation Rates in Angiosperms¹

2.1 Introduction

One of the fundamental objectives of evolutionary biology is to understand why there are such vast differences in speciation rates across taxonomic lineages (Futuyma 1998). The biological species concept emphasizes reproductive isolation as the key factor in speciation. Consequently, traits that promote reproductive isolation among adjacent populations are considered key to the origin of new species (Grant & Grant 1965; Schluter 2001).

One prominent evolutionary trend in flowering plants is the fusion of petals and overall reduction in the number of stamens and carpels (Endress 1997a). The adaptive explanation for these changes is that they have allowed more precise pollination by specialist insect pollinators and, consequently, less expense of pollen and nectar (Regal 1977; Takhtajan 1991). From the plant's perspective, the selective advantage of specialist pollination is clear; plants are less likely to receive incompatible pollen or to have their pollen transferred to an incompatible stigma. Indeed, selection for pollinator specialization has been invoked to explain divergence in several floral traits including: animal pollination, nectar guides, nectar spurs, bilateral symmetry and secondary pollen presentation (Bawa 1995; Waser 2001). Grant (1949) suggested that in the angiosperms, floral morphology has diverged more rapidly than vegetative characteristics, explaining its widespread preference as a basis for taxonomic classification. Many authors hypothesize that this divergence has been driven largely by selection via pollinators (Faegri & van der Pijl 1979; Grant 1949; 1994; Stebbins 1970; however see Waser 1998;

¹ A version of this chapter has been published as "Sargent, R. D. 2004. Floral symmetry affects speciation rates in angiosperms. *Proc. Roy. Soc. Lond. B.* **271**, 603-608.

2001). Accordingly, the occurrence of animal pollination has been invoked to explain differences in diversification rates across angiosperm lineages (Eriksson & Bremer 1992; Dodd *et al.* 1999).

The importance of pollinator-mediated selection in angiosperms is well supported by theory (Kiestler *et al.* 1984) and experimental data (Galen 1996). In the genus *Mimulus*, evidence suggests that discrimination by specialist pollinators (bees and hummingbirds) is responsible for reproductive isolation between two sympatric species (Schemske & Bradshaw 1999). In the genus *Aquilegia*, differences in the form of nectar spurs are correlated with differences in pollinators that visit a flower; the size and placement of the spurs affect reproductive isolation by reducing visitation by some pollinators and increasing visitation by others (Hodges & Arnold 1994). The presence of spurs has also been shown to correlate with the degree of diversification in other clades, supporting the hypothesis that they play a general role in reproductive isolation (Hodges & Arnold 1995).

Floral symmetry was one of the earliest traits used to relate morphology to function in the pollination of angiosperms (Neal *et al.* 1998). There are two main forms of symmetry described in the angiosperms: bilateral symmetry (zygomorphy) and radial symmetry (actinomorphy). Actinomorphy is considered to be the ancestral form (Takhtajan 1969) with zygomorphy having originated several times independently (Takhajan 1991; Donoghue *et al.* 1998). Several theories have been put forth for the adaptive significance of zygomorphy (reviewed by Neal *et al.* 1998). The pollen position hypothesis posits that in zygomorphic flowers, pollinators are restricted in the directionality of approach and movement within and between flowers (Leppik 1972;

Ostler & Harper 1978; Cronk & Moller 1997). In contrast, actinomorphic flowers can be approached from any direction and are not able to restrict pollinator movement within the flower. Hence, in zygomorphic flowers the specificity of pollen placement is improved greatly. Once precise placement of pollen on the pollinator is achieved, reproductive isolation is possible.

Wherever a trait change has occurred convergently in several lineages there is opportunity to compare the resulting differences in diversity between the lineage and its sister lineage (Futuyma 1998). Given sufficient comparisons one can test the hypothesis that the evolution of the trait has had a consistent, replicable effect on diversification. Several studies have tested hypotheses about which traits may be responsible for the differences in diversity among angiosperm lineages (e.g. Farrell *et al.* 1991; Hodges & Arnold 1995; Dodd *et al.* 1999; Heilbuth 2000; Verdu 2002). However, the relationship between floral symmetry and speciation remains untested (Waser 1998). Here I examine whether zygomorphy has the effect of increasing species richness in the angiosperm lineages where it occurs.

2.2 Methods

2.2.1 Data Collection

I tested the null hypothesis that species numbers in zygomorphic clades were lower than or equal to the numbers in their actinomorphic sister clades. I considered symmetry only at the level of the corolla, ignoring the symmetry of the pistil and stamens. Although it is possible to have an actinomorphic corolla and zygomorphic gynoecium or androecium (e.g. *Hibiscus*), or vice-versa (Neal *et al.* 1998), I limited the study to corolla morphology because it is the level of symmetry most likely to affect the

pollination process (Stebbins 1974). Families in which corolla morphology was defined as zygomorphic were identified using Judd *et al.* (2002). If the information in that source was inadequate, I referred to Watson & Dallwitz (1992) or Mabberley (1997). Families described as having radially symmetrical, polysymmetric or regular corolla morphology were considered actinomorphic; those described as having bilaterally symmetrical, monosymmetric or bilabiate corolla morphology were considered zygomorphic. Only animal-pollinated families were considered.

2.2.2 Sister-group comparison

Once I had exhausted the listed family descriptions I identified the phylogenetic relationships between these families using the angiosperm phylogeny created by Soltis *et al.* (2000). All the families I had identified as having primarily zygomorphic flowers were found on this tree. Upon identifying a zygomorphic clade I used the Soltis *et al.* (2000) tree to identify the actinomorphic sister clade. This process revealed that several of the zygomorphic families were in fact part of the same lineage. In the end, 40 zygomorphic families yielded 19 sister group comparisons (Figure 2.1).

Once the appropriate sister groups had been identified I used Mabberley (1997) to determine the number of species in each family. In cases where Mabberley (1997) disagreed with the taxonomic divisions in the Soltis *et al.* (2000) phylogeny, I used other sources (Watson and Dallwitz 1992 or Judd *et al.* 2002) to determine the number of species in the lineage. Occasionally, the zygomorphic families (e.g. Fabaceae) contained some actinomorphic members. Using methodology described in Farrel *et al.* (1991) and Heilbuth (2000), I reported the number of species for the sister group as the total minus the number of actinomorphic species (Figure 2.1). Similarly, in one case (Zingiberales) a

group of taxa having wind-pollinated flowers (Poales) was removed from the zygomorphic sister group total for the comparison. This procedure was conservative and could only bias the results against rejecting the null hypothesis. The reciprocal procedure (subtracting zygomorphic species from actinomorphic clades) was not performed; this also ensured that the test was conservative. While most sister groups represented independent comparisons, I included one sister pair (Polygalaceae – Surianaceae) that fell within the zygomorphic sister lineage of another pair (Fabaceae and its sister group). I controlled for any possible bias that this approach could have caused by subtracting the species from the Polygalaceae-Surianaceae comparison from the more inclusive sister group (leaving only the Fabaceae), thus assuring that one large group was not providing the basis for more than one positive comparison. Removing this additional pair does not, however, change the significance of the results reported below.

2.2.3 Statistical Tests

To determine whether there was a significant effect of the evolution of zygomorphy on the diversification rate of a lineage, I subtracted the number of species from the zygomorphic lineage from the number of species in the actinomorphic sister lineage. I tested whether there was a detectable trend in the direction of the differences using a one-tailed sign test and by testing whether the mean difference in species number between sister groups differed from zero using the non-parametric Wilcoxon signed rank test. Means are reported as +/- one standard error.

2.3 Results

In 15 of 19 sister-group comparisons the lineage with zygomorphic flowers was more diverse than its sister group (Table 2.1; Figure 2.1: $P = 0.0096$, one-tailed sign test).

Furthermore, the mean difference in species number between the sister groups was significantly greater than zero (Table 2.1: $N=19$, $P = 0.003$, Wilcoxon signed rank test). The mean negative difference (actinomorphic clade contains more species) was 847.75 ± 758.17 and the mean positive difference (zygomorphic clade contains more species) was 3318.53 ± 1688.07 .

2.4 Discussion

The sister group analysis leads to the rejection of the null hypothesis in favour of the alternative hypothesis that bilaterally symmetric (zygomorphic) clades are more species rich than their radially symmetric (actinomorphic) sister clades.

This conclusion is consistent with field studies reporting an association between zygomorphy and species richness. In their study of 25 flowering plant communities, Ostler & Harper (1978) found that zygomorphy was correlated with increased plant diversity. Their explanation for this result is that in species-rich communities, zygomorphy should be favoured because it promotes increased fidelity between flowers of a given species and their pollinators.

It has been hypothesized that the evolution of zygomorphy will lead to increased speciation rates because it affects the precision of pollen transfer and hence the probability of reproductive isolation arising among slight variants (Neal *et al.* 1998). If this were true, we would expect zygomorphy to be correlated with either specialist pollinators or the placement of pollen on specific parts of a pollinator's body. Additionally, I predict that other traits that require precise pollen transfer in order to have a selective advantage, such as lower pollen-ovule ratios, will be correlated with zygomorphy.

Indeed, an association between zygomorphy pollination by specialist bees has been reported in several angiosperm taxa (Donoghue *et al.* 1998; Goldblatt *et al.* 2000). Specialist pollinators clearly have the potential to increase diversification rates. Bumblebee pollinators may prefer zygomorphic to actinomorphic forms (Neal *et al.* 1998). In addition, bees may be inefficient pollinators of actinomorphic flowers (Cronk & Moller 1997). Moreover, reversals to actinomorphy may accompany a switch from specialist to generalist pollinators (Cronk & Moller 1997; Donoghue *et al.* 1998). There is also evidence suggesting that in some species with zygomorphic flowers, pollen placement is so precise that the same pollinator can visit multiple species and preserve reproductive isolation because the pollen is placed on different parts of the pollinator (Brantjes 1982; 1985). While further exploration is required to confirm the trend, a correlation between zygomorphy and specialist pollinators further supports the hypothesis that higher species richness in zygomorphic lineages is a result of pollinator-mediated speciation.

If zygomorphy promotes efficient pollination we would predict that zygomorphic species would have lower pollen-ovule ratios. It has been demonstrated that the amount of pollen produced by a species (measured as the pollen-ovule ratio) is negatively correlated with the likelihood that the plant's pollen grains will reach a compatible stigma. For example, animal-pollinated plants have lower pollen-ovule ratios than wind-pollinated plants (Sharma *et al.* 1992), and plants that are obligately selfing (autogamous) have lower pollen-ovule ratios than those that obligately outcross (Cruden 1977). If zygomorphy promotes reproductive isolation via improved placement of pollen we would expect that the pollen-ovule ratio in species with zygomorphic flowers would evolve to

be lower than in species with actinomorphic flowers. There is indeed some evidence that species with zygomorphic flowers have lower pollen-ovule ratios. For example, in the Orchidaceae, pollen is packaged into units known as pollinaria, which results in a pollen-ovule ratio that is several orders of magnitude smaller than plants that lack these structures. The evolution of pollinaria has been directly attributed to the improved specificity accompanying the evolution of zygomorphy (Johnson and Edwards 2000). The pollinaria have been touted as a key innovation that allowed the rapid diversification of the orchid clade (Johnson & Edwards 2000). However, without a preceding adaptation to ensure highly specific pollination, pollinaria would be disadvantageous. In the Asterales, lineages that develop zygomorphy have often undergone a subsequent decrease in anther number (Endress 1997b). While there are other possible explanations for this trend, it is an intriguing observation that deserves further exploration.

A potential problem with any sister group analysis is that the examined trait (in this case zygomorphy) could be correlated with a different trait that drives diversification rather than be the actual cause of the diversification. This is an intrinsic problem with all correlative studies. The presence of secondary pollen presentation, i.e. the presentation of pollen on floral structures other than the anther sacs (Yeo 1993), is also correlated with low pollen-ovule ratios (Cruden 2000), reportedly due to its ability to facilitate highly specific placement of pollen grains (Howell *et al.* 1993). Because of its purported role in improving pollination efficiency, secondary pollen presentation is another candidate trait that may play a role in angiosperm speciation. In addition, many families that display secondary pollen presentation also have zygomorphic flowers. Therefore I repeated the sister group comparison, excluding species or families that displayed secondary pollen

presentation (Table 2.1) in order to test whether secondary pollen presentation could have driven the association between zygomorphy and species richness. When these species are removed, only one comparison (Fabaceae and its sister lineage) is reversed, and the sign-test remains significant ($P = 0.0155$). Because secondary pollen presentation is not strongly correlated with zygomorphy (Table 2.1), it is unlikely to be driving the observed patterns of diversification. Secondary pollen presentation may also work in conjunction with zygomorphy in some families to ensure precise pollen placement (Yeo 1993).

A major weakness of a sister group analysis is that it cannot distinguish whether differences between sister lineages in species richness are caused by more speciation events in one lineage or by more extinction events in the other. In the present case, however, there is no reason to expect that actinomorphy would increase extinction rates. Rather, actinomorphy may lead to lower extinction rates because of its association with generalist pollinators (Bond 1994; Johnson & Steiner 2000).

In conclusion, I have argued that the correlation between zygomorphy and increased species richness in angiosperms is caused by the ability of this trait to promote reproductive isolation through improved precision of pollen placement and tendency for specialist pollinators to be attracted to zygomorphic flowers. This study is distinctive in that it investigates a trait long suspected to be important in reproductive isolation and confirms a hypothesis central to evolutionary biology: traits that promote reproductive isolation are correlated with increased diversification rates.

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2.6 Figure Legend

FIGURE 2.1: Phylogeny of zygomorphic angiosperm families and their sister taxa adapted from Soltis et al. (2000). Brackets indicate the 19 sister group comparisons. The number opposite each bracket indicates the difference in species number between the two sister groups (zygomorphic species – actinomorphic species); † indicates zygomorphic families, * indicates actinomorphic families.

Figure 2.1

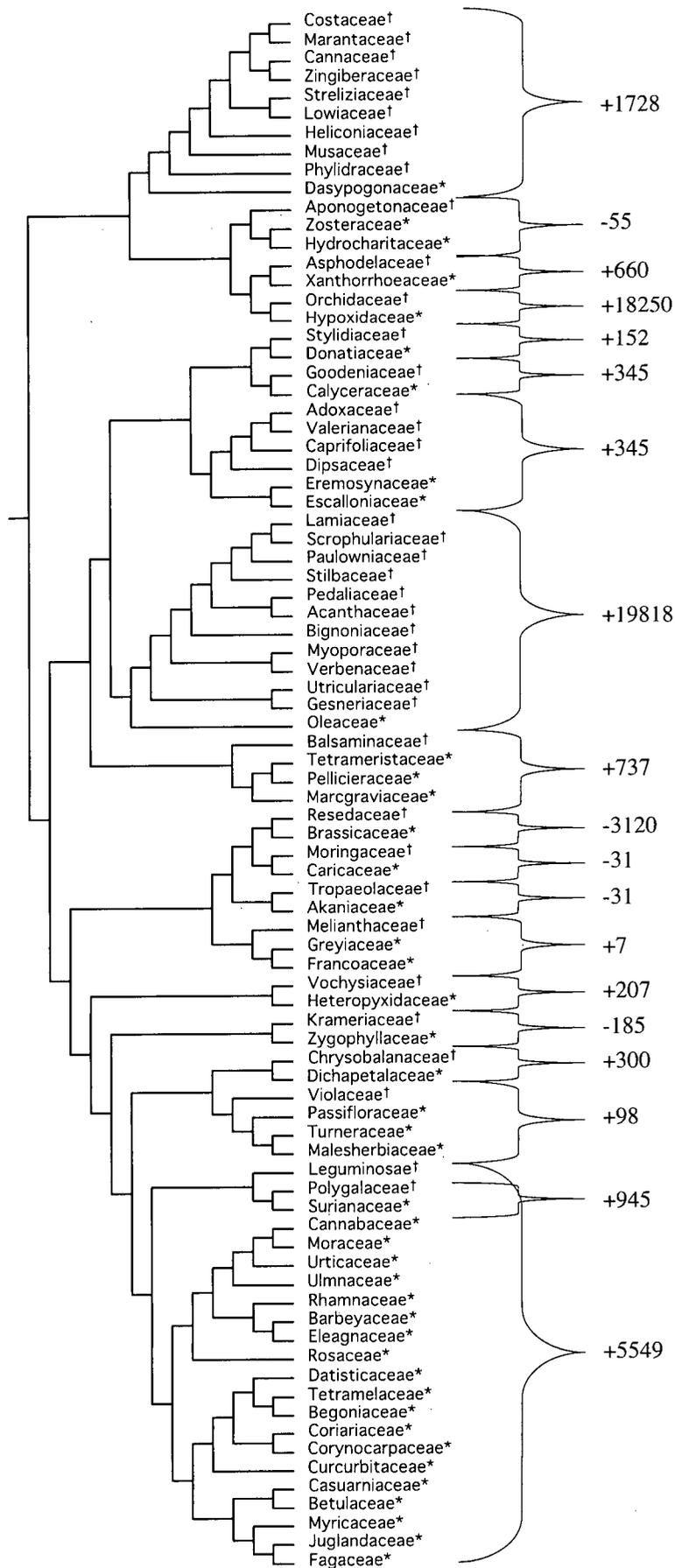


Table 2.1: Sister group comparisons for zygomorphic families

Zygomorphic Family	No.	Sister Group	No.	+/-
Acanthaceae (3450) + Bignoniaceae (750) + Gesneriaceae (2900) + Lamiaceae (6700) + Myoporaceae (235) + Paulowniaceae (6) + Pedaliaceae (85) + Scrophulariaceae (5100) + Stilbaceae (12) + Verbenaceae (950) + Utriculariaceae (245)	20433	Oleaceae	615	+
Adoxaceae (5) + Caprifoliaceae (420) + Dipsacaceae (290) + Valerianaceae (300)	1015	Eremosynaceae (150) + Escalloniaceae (1)	151	+
Aponogetonaceae	43	Hydrocharitaceae ² (80) + Zosteraceae (18)	98	-
Asphodelaceae	750	Xanthorrhoeaceae	90	+
Balsaminaceae	850	Marcgraviaceae (108) + Pellicieraceae (1) + Tetrameristaceae (4)	113	+
Cannaceae ¹ (8) + Costaceae (1100) + Heliconiaceae (80) + Lowiaceae (7) + Marantaceae ¹ (535) + Musaceae (200) + Phylidraceae (6) + Streliziaceae (7) + Xyridaceae ² (300) + Zingiberaceae (1100) – Bromeliaceae (1520)	1823	Dasypogonaceae	95	+
Chrysobalanaceae	460	Dichapetalaceae	160	+
Goodeniaceae ¹	400	Calyceraceae ¹	55	+
Krameriaceae	15	Zygophyllaceae	200	-
Fabaceae ² (15315) – Polygalaceae ² (950) – Surianaceae (5)	14360	Barbeyaceae (1) + Begoniaceae (900) + Betulaceae (110) + Cannabaceae (4) + Casuarنيaceae (95) + Coriariaceae (5)	8811	+

¹ Entire family displays secondary pollen presentation.

² Some members display secondary pollen presentation.

Note: Only animal-pollinated families were used. The numbers given have been corrected against bias by removing the actinomorphic members of the zygomorphic clade from the total (see text for details). The final column indicates the outcome of the sister-group comparison; + indicates the zygomorphic clade had more species; - indicates the actinomorphic clade had more species.

		+ Corynocarpaceae (4) + Cucurbitaceae (775) + Datisticaceae (4) + Eleagnaceae (45) + Fagaceae (700) + Juglandaceae (59) + Moraceae (1100) + Myricaceae (55) + Rhamnaceae (900) + Rosaceae (2825) + Tetramelaceae (4) + Ulmnaceae (175) + Urticaceae (1050)		
Melanthaceae	12	Francoaceae (2) + Greyiaceae (3)	5	+
Moringaceae	12	Caricaceae	43	-
Orchidaceae	18500	Hypoxidaceae	220	+
Polygalaceae ²	950	Surianaceae	5	+
Resedaceae	80	Brassicaceae	3200	-
Stylidiaceae	154	Donatiaceae	2	+
Tropaeolaceae	89	Akaniaceae	1	+
Violaceae	800	Malesherbiaceae (27) + Passifloraceae (575) + Turneraceae (100)	702	+
Vochysiaceae ²	210	Heteropyxidaceae	3	+
Total			15+ / 4-	

Chapter III – The role of local species abundance in the evolution of pollinator attraction in flowering plants.

3.1 Introduction

That floral traits evolve for specialized pollination by certain types of animals is a central tenet in explanations for the astonishing diversity of angiosperms (Grant 1949, Grant 1994, Hodges and Arnold 1995, Dodd *et al.* 1999, Sargent 2004). Specialized pollinators are thought to drive the evolution of phenotypic divergence between insipient plant species, which leads to reproductive isolation and speciation. This concept is supported by evidence for “pollinator syndromes” where suites of floral traits in species with similar pollinators exhibit convergent evolution (Faegri and van der Pijl 1978). Indeed, plant-pollinator specialization has been identified as a key factor in studies of reproductive isolation in flowering plants (Hodges and Arnold 1994, Schemske & Bradshaw 1999, Ramsey *et al.* 2003).

In this chapter we develop a population genetic model to examine the forces affecting the tendency of flowers to evolve traits in order to attract a single pollinator (i.e., specialize) or a suite of several different pollinator species (i.e., generalize).

For the purposes of this chapter, we define “specialization” as a floral strategy to invest in particular traits that increase the relative preference of certain pollinators for the flower. In contrast, a generalist plant invests in a combination of traits so that a broader variety of pollinator species are attracted, but not as keenly. In our model, any pollinators whose preferences can be manipulated by a flower in a manner indistinguishable to floral evolution are grouped together. Hence, specialization can evolve to a pollinator species, or a ‘type’ of pollinators. For example, a flower can evolve specialization to two

phylogenetically distinct bee species if their preferences for certain floral traits are identical. The model addresses the evolution of plant specialization to fixed pollinator preferences, not the evolution of pollinator preferences.

While plants with specialized pollination systems have traditionally been considered the rule in plant-pollinator interactions (reviewed by Johnson and Steiner 2000), others have argued that, rather than being specialized on one or a few pollinators, the majority of plant species are in fact pollinated by several pollinator species and should therefore be considered generalists (Ollerton 1996, Waser *et al.* 1996, Olesen and Jordano 2002). Whether specialist or generalist plant species prevail is currently under debate. To further complicate matters, a flower that receives visits by many pollinator species may be “effectively pollinated” by only a few of the visiting species. Thus, in spite of a high diversity of pollinator visitors, the plant species may in fact be a specialist. This insight makes it difficult to determine whether a plant species is indeed a generalist or a specialist in the absence of very specific data (e.g., Schemske and Horvitz 1984).

While there are reliable examples of both extremes of the generalist and specialist spectrum, the relative frequency of such interactions is poorly understood (Kay and Schemske 2004). In addition, it is unclear which ecological circumstances lead to the evolution of specialization or generalization in floral traits. The lack of theory regarding the factors affecting the evolution of specialization and generalization is surprising, particularly considering that plant pollinator interactions have profound implications for our understanding of floral adaptation and ultimately plant speciation (Johnson and Steiner 2000, Kay and Schemske 2004).

A recent study found a positive correlation between local plant species richness and the extent of specialized plant-pollinator interactions in a community (Olesen and Jordano 2002, although see also Kay and Schemske 2004). In the same study, plants, but not their animal pollinators, were found to be specialists more often in the tropics than at higher latitudes. This supports previous evidence that the frequency of generalized interactions tends to increase latitudinally, with tropical plant species being specialized and generalization increasing towards the arctic (Johnson and Steiner 2000, Olesen and Jordano 2002, Kay and Schemske 2003, but see also Ollerton and Cranmer 2002).

The motivation for predicting a relationship between a species' relative abundance and the evolution of specialization was communicated by Feinsinger (1983):

“If a plant population is quite densely distributed, nearest neighbours are likely to be conspecific. Nearly any visitor, no matter how uncommitted, is likely to bring useful pollen to a plant and to disperse the plant's own pollen to conspecific stigmas. Selection on plants to specialize is relaxed. Consider a population of widely dispersed plants with few flowers each, however. If these flowers invite all comers, then the pollinators may not distinguish the rare species from the more common ones.”

The hypothesis that the frequency at which a plant species occurs plays an important role in the evolution of plant specialization has never been examined theoretically; it has largely been overlooked in favour of the “Most Effective Pollinator Principle” (MEPP, Stebbin's 1970). MEPP predicts that a plant will tend to evolve floral traits that promote specialization on those pollinators that “visit it most frequently and effectively in the region where it is evolving” (Stebbins 1974). There is overlap between MEPP and Feinsinger's model of plant specialization, for example, they make similar

predictions when a plant community consists of two or more species at low frequency. However, we predict that the predictions of the two models should diverge in cases where a plant species of interest exists at intermediate or high frequency. Here, we introduce a population genetic model that incorporates aspects of MEPP and Feinsinger's frequency model in order to explore the influence of local plant species' abundances on the evolution of specialization and generalization in animal-pollinated plant species.

3.2 The Model

The model describes the conditions under which a rare allele k spreads in a focal population of self-incompatible diploid floral morphs where K is the resident allele. Within the focal plant species, KK , Kk and kk are three floral morphs that differ in the degree to which they attract pollinators in the community (Table 3.1). The frequencies of these three diploid genotypes are D (KK), H (Kk) and R (kk). In the plant community at large, the frequency of the focal species is f , and the frequency of all other species of flowering plant (O) is $1-f$. For clarity, we assume that there are two pollinator species, A and B , pollinating the community of flowering plants. It is straightforward, however, to extend the model to incorporate more pollinator types. In accordance with the MEPP (Stebbins 1970), the two pollinators are allowed to differ in abundance and in their pollinating efficiency. Accounting for differences in abundance, we describe the probability of visitation by pollinator A as g and the probability of visitation by pollinator B as $(1-g)$. In our model the efficiency of pollen transport and deposition is described by γ_A for pollinator A and γ_B for pollinator B .

Visitation by a pollinator can only contribute to the male fitness of a self-incompatible genotype when subsequent visits by that pollinator are to a plant of the

same species. This consideration distinguishes our model from previous models of plant specialization (e.g., Waser *et al.* 1996), which implicitly assume self-fertilization. We simplify our model by assuming that the majority of pollen transfer occurs between one plant visit and the next. However, assuming pollen deposition isn't affected by the type of plant species visited in interim steps, the results are the same regardless of whether the focal species is the next plant visited or the n^{th} plant visited (see Appendix 3.1). This assumption allows us to focus on pollinator visits as a sequence of two stop trips, with the first stop representing pollen accumulation and the second stop representing pollen deposition. Each sequence of pollinator visits has a different probability, depending on the frequency of the morphs and the pollinators. Only a sequence where a pollinator visits a flower of the focal species (i.e., morphs KK , Kk or kk) followed by a flower of the same species contributes to the fitness of the focal species.

The two pollinator species have different visitation preferences for the flowering plant forms in the community (Table 3.1), where these preferences depend on the investment, α_i and β_i , by individual plants in the community in attracting pollinators A and B , respectively. We use a relative preference scheme, as used by Kirkpatrick (1982) in models of sexual selection. Specifically, the degree to which an individual of genotype i attracts a pollinator of type j , χ_{ij} , is measured relative to the pollinator's overall attraction to other flowers in the local area, T_j . Therefore, we define the probability that pollinator A visits genotype i (where i refers to D , H , or R) as, $\chi_{iA} = \frac{\alpha_i f_i}{T_A}$, and the probability that pollinator B visits genotype i as, $\chi_{iB} = \frac{\beta_i f_i}{T_B}$. T_A and T_B represent the average strength of attraction of pollinators A and B to the plants in the community:

$$T_A = f(\alpha_D D + \alpha_H H + \alpha_R R) + \alpha_O(1-f), \quad (3.1)$$

$$T_B = f(\beta_D D + \beta_H H + \beta_R R) + \beta_O(1-f). \quad (3.2)$$

One major limiting assumption of our model is that we treat the relative frequency of pollinators *A* and *B* as constants. Clearly, these frequencies could respond to the local plant community through migration of pollinators as well as fitness differences among pollinators. Although further work allowing plant-pollinator coevolution is warranted, it seems reasonable to assume that factors other than local plant abundance, such as density regulation at the larval stage, may be more important predictors of pollinator density. Furthermore, our model provides important insight into the evolutionary forces in the presence of a fixed pollinator pool.

The number of *KK* individuals in the next generation, D' , is determined by summing over the probabilities that pollinators *A* and *B* gather and deposit pollen on flowers of the focal species, times the probability of the visit sequence between a specific maternal genotype and a specific paternal genotype, times the Mendelian probability of those parents producing *KK* offspring. From Table 3.2, a set of recursions can be derived that describe the change in frequency of the three genotypes over a single generation:

$$D' = \frac{1}{\bar{w}} \left(\begin{array}{l} g\gamma_A \chi_{DA}^2 + (1-g)\gamma_B \chi_{DB}^2 + g\gamma_A \chi_{DA}\chi_{HA} + (1-g)\gamma_B \chi_{DB}\chi_{HB} + g\frac{\gamma_A}{4}\chi_{HA}^2 \\ + (1-g)\frac{\gamma_B}{4}\chi_{HB}^2 \end{array} \right), \quad (3.3)$$

$$H' = \frac{1}{\bar{w}} \left(\begin{array}{l} g\gamma_A \chi_{DA}\chi_{HA} + (1-g)\gamma_B \chi_{DB}\chi_{HB} + g\frac{\gamma_A}{2}\chi_{HA}^2 + (1-g)\frac{\gamma_B}{2}\chi_{HB}^2 + 2g\gamma_A \chi_{DA}\chi_{RA} \\ + 2(1-g)\gamma_B \chi_{DB}\chi_{RB} + g\gamma_A \chi_{HA}\chi_{RA} + (1-g)\gamma_B \chi_{HB}\chi_{RB} \end{array} \right), \quad (3.4)$$

and

$$R' = \frac{1}{\bar{w}} \left(g\gamma_A \chi_{RA}^2 + (1-g)\gamma_B \chi_{RB}^2 + g\frac{\gamma_A}{4} \chi_{HA}^2 + (1-g)\frac{\gamma_B}{4} \chi_{HB}^2 + g\gamma_A \chi_{HA}\chi_{RA} \right. \\ \left. + (1-g)\gamma_B \chi_{HB}\chi_{RB} \right), \quad (3.5)$$

where \bar{w} represents the mean pollen fitness, or “average degree to which pollen is successfully gathered and deposited on a conspecific flower”:

$$\bar{w} = g\gamma_A (\chi_{DA} + \chi_{HA} + \chi_{RA})^2 + (1-g)\gamma_B (\chi_{DB} + \chi_{HB} + \chi_{RB})^2. \quad (3.6)$$

We used equations (3.1) – (3.6) to investigate the spread of a new allele k that alters the allocation of floral resources invested in the attraction of one, or both, pollinator species.

3.3 Invasion Criteria

To assess the evolutionary forces acting on the allocation of floral resources to attracting different pollinators, we examined when a resident genotype (KK , $\hat{D} = 1$) could be invaded by a rare mutant allele (k) that differs from the resident in its attractiveness to the two pollinators (Table 3.1). To do so, we performed a local stability analysis of the equilibrium, $\hat{D} = 1$, assuming that Kk and kk were rare. Because of the assumption that k is rare and that selfing does not occur, the frequency of kk individuals does not influence the invasion criteria. Therefore, the population at the time of invasion effectively contains only Kk individuals (invading morph) and KK individuals (resident morph).

If there were no constraints on floral attractiveness, flowers would evolve to be infinitely attractive to all pollinators. In consideration of this we have included a trade-off between investment in attracting one pollinator versus the other, such that $\beta + \alpha = C$, where C is the maximum amount of energy available for attracting pollinators in the focal species. Other species may invest more or less in floral structures, and we take C_o to be the average level of investment over all other species in attraction to pollinators A and B (α_o and β_o , respectively).

Assuming that the frequency of the k allele is rare (on the order of ε , a small term), we determined the leading eigenvalue, λ , where $H' = \lambda H + 0(\varepsilon)$, and

$$\lambda = \frac{\frac{g_e \alpha_D \alpha_H}{(f \alpha_D + (1-f) \alpha_O)^2} + \frac{(1-g_e) \beta_D \beta_H}{(f \beta_D + (1-f) \beta_O)^2}}{\frac{g_e \alpha_D^2}{(f \alpha_D + (1-f) \alpha_O)^2} + \frac{(1-g_e) \beta_D^2}{(f \beta_D + (1-f) \beta_O)^2}} + 0(\varepsilon^2). \quad (3.7)$$

We define $\lambda = 1 + s$, where s can be thought of as the selection coefficient acting on the new floral morph while it is rare. Similarly, we can define a selection gradient as

$$b = \frac{s}{(\alpha_H - \alpha_D)},$$

which describes how strong selection would be as a function of the

effect of the allele on the floral trait. After a bit of algebra, it can be shown that the

selection gradient, b , depends only on α_D . In equation (3.7), $g_e = \frac{g \gamma_A}{g \gamma_A + (1-g) \gamma_B}$ is the

“effective abundance” of pollinator A , a term that combines the relative abundance of each pollinator and its pollination efficiency.

3.4 Results

We varied the relative frequency of the focal species and examined the ability of the rare floral morph allele to invade a population of resident alleles. The intuition behind the results becomes most clear at the extremes, when the focal species is common relative to other species in the community and when the focal species is rare relative to other species. We therefore commence our discussion of the results at these extremes.

When plants are surrounded primarily by conspecifics, (e.g., when a species occurs in dense patches), there is an increased probability that pollen received will be genetically compatible, and we expect relaxed selection to specialize on a single

pollinator (Feinsinger 1983). Substituting $f = 1 - \xi$ where ξ is a small quantity, into (3.7), we find that the selection coefficient equals:

$$s \approx \frac{(\alpha_H - \alpha_D)(Cg_e - \alpha_D)}{\beta_D \alpha_D} + O(\xi), \quad (3.8)$$

assuming that neither α_D nor β_D are zero. Selection on the floral traits is zero when

$\frac{\alpha_D^*}{C} = g_e$, where α_D^* is the amount invested in attracting pollinator A for which $s = 0$. In

other words, when proportion of available resources invested in attracting the A pollinator

equals the effective abundance of the A pollinator. $\alpha_D^* = Cg_e$ represents an evolutionarily

stable strategy (ESS; denoted by a *)), which by definition cannot be invaded by any

other strategy. From (3.8), when $\alpha_D < \alpha_D^*$, a rare allele can invade only if it increases α ,

with the converse holding when $\alpha_D > \alpha_D^*$. These inferences are illustrated in Figure 3.1.

Thus, if floral mutations are assumed to have small effects (so that overshooting α_D^* can

be ignored), the system converges to the ESS at $\alpha_D^* = Cg_e$ through the successive fixation

of mutations. At this ESS, plants invest in attracting all available pollinators in

proportion to each pollinator's effective abundance, rather than specializing on the most

effective pollinator. Thus, we expect specialist plants to evolve to be more generalist in

their attraction of pollinators when a focal species is numerically dominant. The ESS

investment in attracting pollinator A will be higher whenever g_e is higher, either because

pollinator A is more abundant or more efficient.

At the other extreme (i.e., when the focal species is rare relative to other species in the community), incoming pollen is less likely to be genetically compatible, and we expect strong selection on plants to specialize on a pollinator (Feinsinger 1983).

Substituting $f = \xi$ into (3.7), we obtain an equation that describes selection on the rare Kk morph,

$$s = (\alpha_H - \alpha_D) \frac{g_e \frac{\alpha_D}{\alpha_O^2} + (1 - g_e) \frac{\beta_D}{\beta_O^2}}{g_e \frac{\alpha_D^2}{\alpha_O^2} + (1 - g_e) \frac{\beta_D^2}{\beta_O^2}} + 0(\xi) \quad (3.9).$$

An ESS occurs when $s = 0$ at $\frac{\alpha_D^*}{C} = \frac{(1 - g_e)\alpha_O^2}{(1 - g_e)\alpha_O^2 + g_e\beta_O^2}$. In this case, however, invasion occurs when $\alpha_D < \alpha_D^*$ if the invading allele invests less in attracting pollinator A, while invasion occurs when $\alpha_D > \alpha_D^*$ if the allele causes the flower to be more attractive to pollinator A. Thus, populations not initially at the generalist ESS evolve away from it (we call this a *repelling* ESS), and the system evolves towards a specialist on pollinator A (if $\alpha_D > \alpha_D^*$) or B (if $\alpha_D < \alpha_D^*$) through a series of small mutational steps (Figure 3.2). Importantly, plants do not always specialize on the most effective pollinator. Instead, they can specialize on the least effective pollinator if the plant is initially more attractive to that pollinator. Nevertheless, specialists on pollinator A are able to invade a broader range of generalists (i.e., species with a broader range of α_D) when pollinator A has a high effective abundance (Figure 3.2, dashed curve), while specialists on pollinator B are able to invade under a broader range of conditions when pollinator B has a high effective abundance (Figure 3.2, thick solid curve). When the two pollinators are equally abundant (i.e. $g_e = 0.5$), and the other plant species are equally attractive to the two pollinators (i.e., $\alpha_O = \beta_O$), plants tend to evolve towards specialization on whichever pollinator was initially more attracted (i.e., pollinator A if $\alpha_D > \frac{1}{2}$ and pollinator B otherwise). In

contrast, when the other species in the community are specialized on pollinator A (i.e., $\alpha_o = C$, $\beta_o = 0$), the focal species is more likely to specialize on pollinator B.

The above cases represent the two extremes of local flowering plant diversity (i.e., $f \approx 0$ and $f \approx 1$). It is of particular interest to examine the evolutionary forces acting in the more biologically realistic case of communities with intermediate species richness (i.e., $0 < f < 1$). Unfortunately, it is difficult to interpret the general equation describing the ESS's of (3.7) as it is a cubic polynomial. From Figures 3.1 and 3.2 we inferred that as f varies from 0 to 1, there comes a point, f_{crit} , at which the selection gradient crosses zero with a slope that crosses from being positive to negative

($\left. \frac{db}{d\alpha_D} \right|_{b=0} = 0$) at the generalist ESS. Below this point, evolution of floral investment

leads to extreme specialization (either $\alpha_D = 0$ or $\alpha_D = C$, which we say are *attracting ESS*), while the generalist ESS is repelling. For f above f_{crit} , however, there is an additional attracting ESS with a generalist strategy (Figure 3.3). Next we focus on the question: what is the value f_{crit} where the slope of b at the generalist ESS changes from positive to negative allowing the generalist ESS to be attracting?

We answer this question by focusing on two floral attraction strategies of the other plants in the community. In the first case, the attractiveness of the flowers in the community is well matched to the pollinators' effective abundance; in the second, the plants in the community are highly specialized to attract only one of the pollinators. In both cases, the analysis simplifies and sheds light on the conditions favouring the evolution of specialization.

No under-utilized pollinators – In order to find f_{crit} we first made the simplifying assumption that pollinator investment among the non-focal species (α_o, β_o) is proportional to the effective abundance of the pollinators, i.e. $\frac{\alpha_o}{g_e} = \frac{\beta_o}{(1-g_e)}$. In other words, the rest of the plant community is well matched to the pollinator community and there is no under-utilized pollinator. In this case, there is again a generalist ESS at $\alpha_D^* = Cg_e$. Recalling that $\alpha_o + \beta_o = C_o$ we determined the critical value of f at which the slope of the selection gradient at the generalist ESS equals zero, $\left. \frac{db}{d\alpha_D} \right|_{\alpha_D = Cg_e} = 0$. This has one solution between 0 and 1: $f_{crit} = \frac{C_o}{C_o + C}$. Thus we find that when other species invest less in floral attraction ($C_o < C$), $0 < f_{crit} < \frac{1}{2}$, and there is a larger range of communities that allow for a generalist ESS. Conversely, when other species invest more heavily in floral attraction ($C_o > C$), $\frac{1}{2} < f_{crit} < 1$, and there is a smaller range of communities allowing for a generalist ESS (Figure 3.4).

An under-utilized pollinator – In this second case, we explored a scenario where other species in the community are specialists on only one pollinator (pollinator B , for the purposes of this description), such that there is a very under-utilized pollinator (pollinator A). Substituting $\alpha_o = 0$ and $\beta_o = C_o$ into the selection gradient, we determined that specialization on pollinator A was always attracting but that specialization on pollinator B never was. Furthermore, a generalist ESS exists and is attracting if the frequency of the

focal species was greater than $f > f_{crit} = \frac{2g_e C_o}{2g_e C_o + C(1 - g_e)}$, but only when the effective abundance of pollinator *A* is sufficiently low,

$$g < g_{crit} = \frac{C^2 f^2}{C^2 f^2 + 4C C_o(1 - f)f - 4C_o^2(1 - f)^2}. \text{ If pollinator } A \text{ was so abundant or so}$$

efficient that this second criterion was not met, then the focal species always evolved to specialize on the under-utilized pollinator *A*. Hence, if all other plants in the focal species' community are specialists on pollinator *B*, the focal species either evolves specialization on pollinator *A*, evolves towards generalization, depending, as in previous cases, on the initial floral investment of the population.

Evolution of specialization vs generalization – We should note that specialists on *A* and specialists on *B* are attracting ESS under all conditions except when the focal species is numerically dominant ($f \approx 1$). Therefore, it is critical to ask whether plants evolve towards a generalist ESS, assuming that it exists, starting from a broad range of initial levels of investment in attracting pollinator *A*. In Figure 3.5, we show that the generalist ESS is often attracting over a broad range of initial investment strategies and that the plant must be nearly invisible to the other pollinator for it to evolve greater specialization rather than toward generalization.

3.5 General Predictions

The main prediction stemming from our model that a species that is numerically rare relative to other animal-pollinated plant species in its vicinity is more likely to exhibit specialist floral traits that are attractive to only one or a few species of pollinators. In

contrast, a species that is relatively common will be more likely to exhibit generalist pollinator traits that are attractive to many species of pollinators.

When a species exists at an intermediate frequency, we predict that the ESS reached (i.e. generalist or specialist) depends on the initial state of the population (Figures 3.5A and B). Thus, a plant that finds itself at an intermediate frequency in a new environment will be more likely to become a specialist if it already invests heavily in attracting a pollinator that is locally abundant, but a generalist if it tends to attract several local pollinators or attracts a locally rare pollinator.

To date, most studies of pollinator specificity in a species of interest have not measured or otherwise accounted for the abundances of the other flowering plant species in the community. Our results suggest that the predictive power of future studies could be improved by accounting for the composition of plant species in the community.

3.6 Discussion

Our model of the evolution of floral morphology makes a clear prediction linking local species abundance and the evolution of floral traits that influence pollinator specificity. Our results indicate that plants evolve to be pollinator specialists in communities where the focal species is relatively rare, because in such communities there exists an increased probability that random pollinator visits will result in the deposition of genetically incompatible pollen. In communities where the focal species occurs at a high density, we found that plants evolve to be pollinator generalists because most pollinators carry compatible pollen. In this case, there is an advantage to mutations that attract under-utilized pollinators because they preferentially visit the mutant plant but are still likely to carry compatible pollen. We found that in communities with an intermediate density of

the focal species, multiple evolutionarily stable strategies (ESS) are possible. According to our model, in such communities specialization evolves over a broader set of conditions in a focal species that invests less in pollinator attraction than surrounding species, while generalization is more often favoured in a focal species that invests more in pollinator attraction than its neighbours (Figure 3.4). Interestingly, not all populations can reach any particular ESS, because the direction of evolution often depends on the initial level of floral investment in attracting different pollinators, which indicates that the history of floral evolution affects the evolution of plant specialization to pollinators.

Most studies of plant-pollinator interactions focus on the relationship between a single plant species and its pollinator(s) (Vazquez and Aizen 2003). Consequently, our current understanding of how the plant and/or pollinator community affects the evolution of generalization or specialization is underdeveloped (Olesen and Jordano 2002). Stebbins' (1970) "most effective pollinator principle" (MEPP) places an emphasis on the efficiency with which a pollinator removes and deposits pollen but does not consider local plant species abundance. The MEPP states that floral traits evolve towards specialization on the pollinator that transports pollen most effectively (Mayfield *et al.* 2001), either because the pollinator transports pollen frequently (i.e., a pollinator at high density), or is a particularly high quality pollinator (i.e., each visit has a high likelihood of transferring pollen to another plant), or both. For example, a plant that is visited by two pollinator species, one more effective than the other, should evolve specialized floral traits corresponding to the preferences of the most effective pollinator. Conversely, if two equally effective pollinators visit a plant, floral traits evolve such that the plant is attractive to both, and the plant would therefore be considered a generalist (Wilson and

Thomson 1996). Our results are distinct from the predictions of MEPP in several important ways. In keeping with the MEPP hypothesis, we predict that when specialization is favoured, floral traits should be selected to increase the plant's attractiveness to the most effective and/or abundant pollinator, but only if the species already tends to be more attractive to that pollinator. In stark contrast to MEPP, our model predicts that selection can drive a plant towards specialization on the *least* effective and/or abundant pollinator, if the species already possesses traits that are attractive to that pollinator (Figure 3.2). Furthermore, we expect specialization to evolve only when the species is rare relative to other species in the community.

The predictions of MEPP do not account for the attributes of the plant's community, and the model has found mixed support (e.g. Aigner, 2001, Aigner 2004, Mayfield *et al.* 2001, Wilson 1995). Interestingly, our results suggest that a species' relative abundance should be a better predictor of its pollinator specificity than the effective abundances of pollinators. In contrast to what MEPP predicts, we found that plants should only evolve to specialize on the most effective pollinator when the focal species is rare, and even then only when the current allocation to attracting that pollinator is already reasonably high. If a species is numerically dominant, our model predicts the evolution of floral traits that are of intermediate attractiveness to all available pollinators, with the most effective/abundant pollinators being attracted more often (in proportion to their effective abundance). For example, when a plant exists in a low diversity community that is visited by both bees that prefer pink corollas and hummingbirds that prefer red corollas, we predict corolla colour will evolve to some intermediate level determined by the effectiveness and abundance of the two pollinators. Conversely, if this

same plant exists in a high diversity community we predict that the ESS that it evolves towards will depend on the initial corolla length of residents in the population. Thus our model should have improved predictive power over MEPP.

Olesen and Jordano (2002) recently reported finding that the level of generalization declined with increasing species richness in a study of several pollinator networks (however, see also Kay and Schemske 2004), which is consistent with our key prediction. Our results also predict that specialist plant-pollinator interactions should evolve under a broad array of conditions. This prediction contradicts a previous review suggesting that specialization is rare (Waser *et al.* 1996) but is consistent with a recent study by Vazquez and Aizen (2003). The plant communities examined by Vazquez and Aizen varied considerably in the number of extreme specialists and generalists, with both extreme specialists and extreme generalists more prevalent than expectations generated using a null model.

Our model also has implications for predicting the establishment of introduced plant species. A positive correlation between the existence of a generalist pollination system and a plant's propensity to invade a community has been observed previously (Pheloung *et al.* 1999, Olesen *et al.* 2002). The "natural enemy escape hypothesis" purports that invasive plants should be able to invest more resources into traits such as pollinator attraction because in its new habitat it has escaped from the requirement of investing in anti-herbivory defences (Myers and Bazely 2003). Our findings suggest that it would be worth exploring empirically whether plant species with increased investment in pollinator attraction have a greater tendency to evolve to be generalist (Figure 3.4).

Recently, the predicted association between specialization and increased extinction risk has been contradicted by data showing that generalist and specialist plants are equally affected by habitat fragmentation (Ashworth *et al.* 2004). Our model results suggest that rare plants that have evolved specialization should have a lower risk of extinction than rare generalist species. In a natural system, a rare plant attracting a broad variety of pollinator species risks a higher probability of extinction if it receives little or no compatible pollen prior to evolving to be a specialist. However, because of our assumption that all ovules receive sufficient pollen for fertilization (i.e., pollen is not limiting), rare generalists evolve to be specialists rather than facing extinction. It would be worth relaxing this assumption in future explorations. On the other hand, it is precisely the strong selection on rare plants to specialize that drives some of our most interesting results.

Based on the results of our model, we contend that local plant species richness may play an important, yet largely overlooked, role in the evolution of floral traits that influence pollinator specificity. Although species richness was historically considered to be an important variable, it has received little theoretical or empirical attention. We hope that our findings will inspire those preparing future studies in this flourishing field to consider the frequency of the focal species when constructing their hypotheses and interpreting their data.

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Table 3.1 Preferences of two pollinator species for the different forms of flowering plants in the field.

Plant Morph	Description	Pollinator A Preference	Pollinator B Preference
KK	Resident	α_D	β_D
Kk	Invading Heterozygote	α_H	β_H
kk	Invading Homozygote	α_R	β_R
O	Other Species	α_O	β_O

Table 3.2 Probability of and genetic contributions of four possible visit sequences by pollinators *A* and *B*.

Visit Sequence	Probability of Sequence for <i>A</i>	Probability of Sequence for <i>B</i>	Proportion of Offspring <i>KK</i>	Proportion of Offspring <i>Kk</i>	Proportion of Offspring <i>kk</i>
<i>KK-KK</i>	$g\gamma_A \left(\frac{\alpha_D f D}{T_A} \right)^2$	$(1-g)\gamma_B \left(\frac{\beta_D f D}{T_B} \right)^2$	1	0	0
<i>KK-Kk</i>	$2g\gamma_A \frac{\alpha_D \alpha_H f^2 D H}{T_A^2}$	$2(1-g)\gamma_B \frac{\beta_D \beta_H f^2 D H}{T_B^2}$	$\frac{1}{2}$	$\frac{1}{2}$	0
<i>Kk-KK</i>					
<i>KK-kk</i>	$2g\gamma_A \frac{\alpha_D \alpha_R f^2 D R}{T_A^2}$	$2(1-g)\gamma_B \frac{\beta_D \beta_R f^2 D R}{T_B^2}$	0	1	0
<i>Kk-Kk</i>	$2g\gamma_A \frac{\alpha_H \alpha_H f^2 H^2}{T_A^2}$	$2(1-g)\gamma_B \frac{\beta_H \beta_H f^2 H^2}{T_B^2}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$
<i>kk-Kk</i>	$2g\gamma_A \frac{\alpha_H \alpha_R f^2 H R}{T_A^2}$	$2(1-g)\gamma_B \frac{\beta_H \beta_R f^2 H R}{T_B^2}$	0	$\frac{1}{2}$	$\frac{1}{2}$
<i>kk-kk</i>	$g\gamma_A \left(\frac{\alpha_R f p}{T_A} \right)^2$	$(1-g)\gamma_B \left(\frac{\beta_R f p}{T_B} \right)^2$	0	0	1

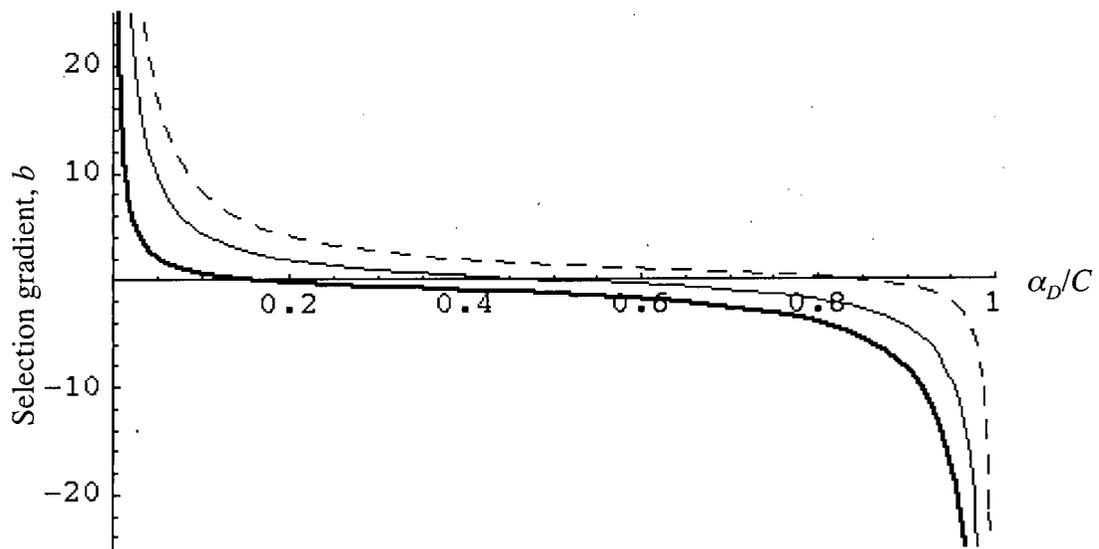


Figure 3.1. Selection gradient (b) on the k allele in a resident population that invests a proportion $\frac{\alpha_D}{C}$ of its resources in attracting Pollinator A when the frequency of the focal species is high ($f \approx 1$). Each curve represents a different effective abundance of pollinator A : $g_e = 0.85$ (dashed), $g_e = 0.5$ (thin) and $g_e = 0.15$ (thick).

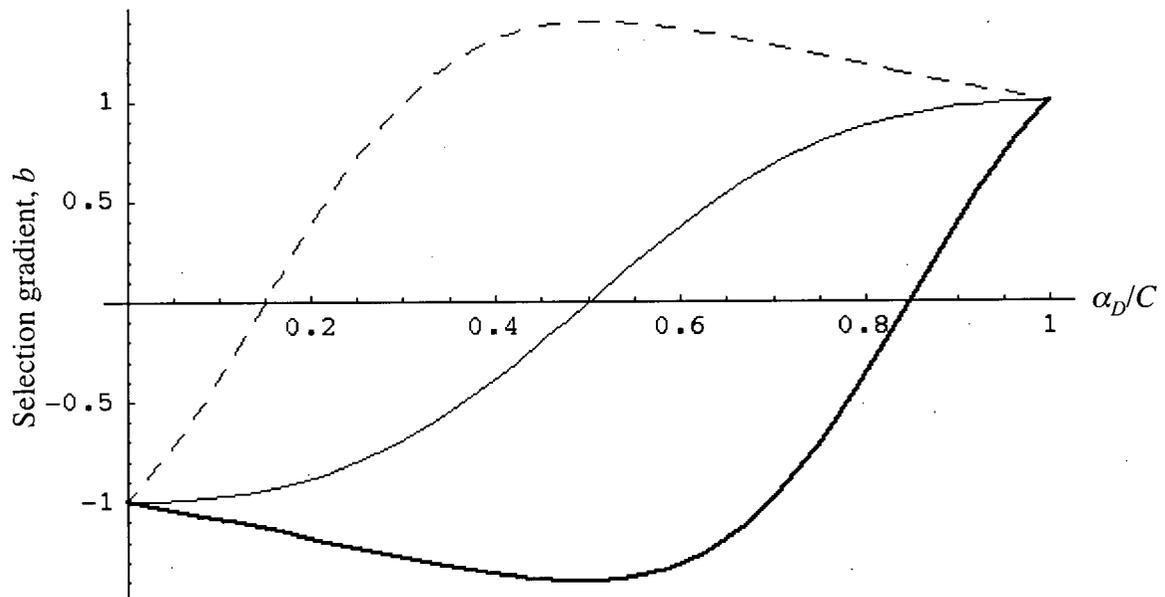


Figure 3.2. Selection gradient (b) on the k allele in a resident population that invests a proportion $\frac{\alpha_D}{C}$ of its resources in attracting Pollinator A when the frequency of the focal species is low ($f \approx 0$). Each curve represents a different effective abundance of pollinator A: $g_e = 0.85$ (dashed), $g_e = 0.5$ (thin) and $g_e = 0.15$ (thick).

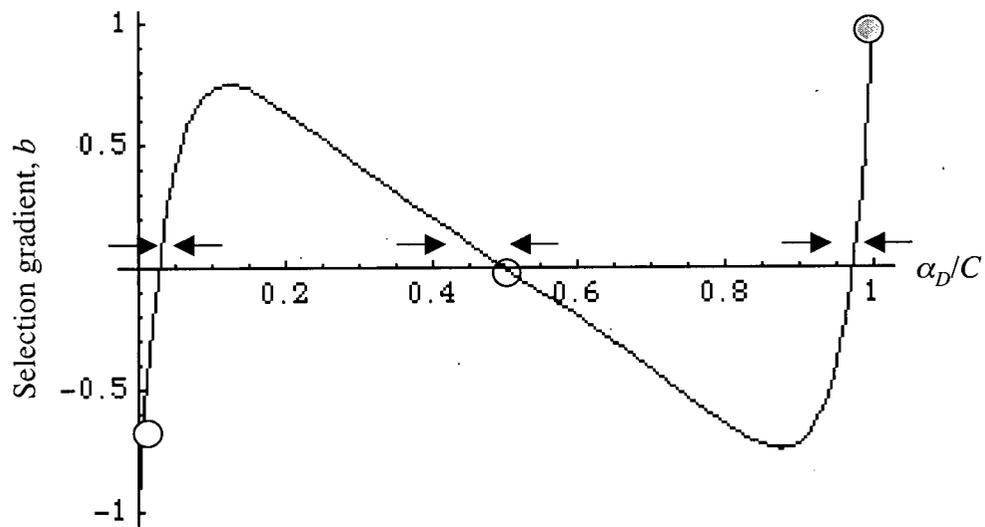


Figure 3.3. Selection gradient (b) on the k allele in a resident population that invests a proportion $\frac{\alpha_D}{C}$ of its resources in attracting Pollinator A when $f = \frac{3}{4}$. An open circle indicates the generalist ESS at $\alpha_D^* = Cg_e$. The shaded circles indicate the two specialist ESS's ($\alpha_D = 0$ and $\alpha_D = C$). Arrows indicate the direction selection is expected to drive a population for which the initial allocation to investment in pollinator A is α_D (x -axis).

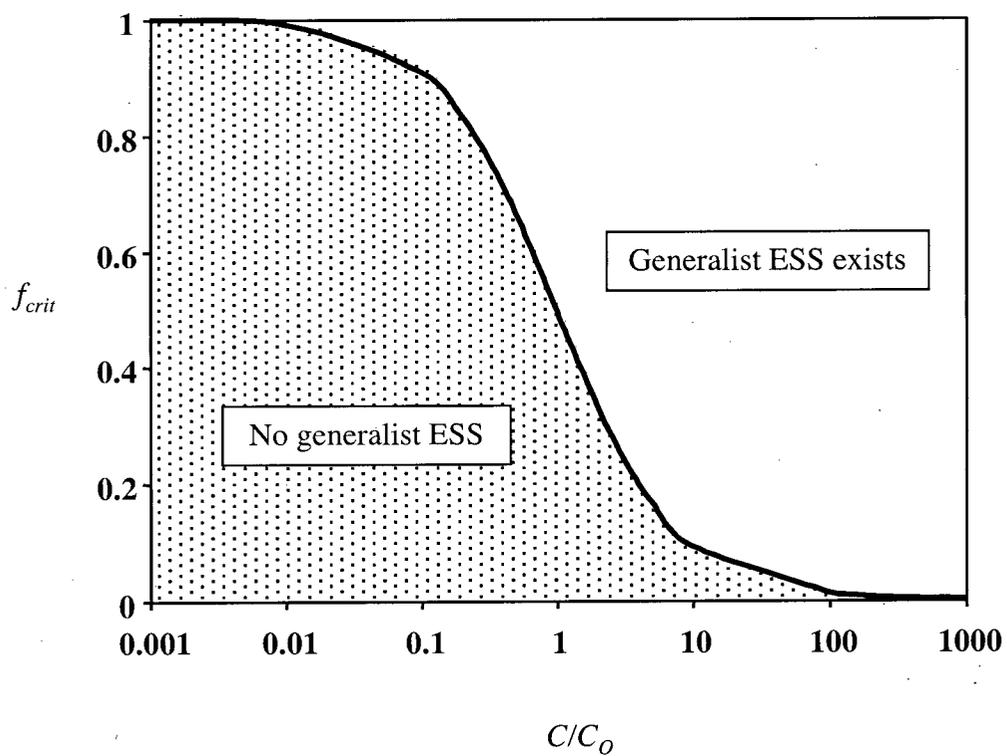


Figure 3.4. The relative values of C_0 and C affect the evolution of a generalist ESS. f_{crit} (y-axis) represents the frequency of the focal species above which a generalist ESS exists at $\alpha_D = Cg_e$ for a given set of values of C_0 and C (x-axis, log scale).

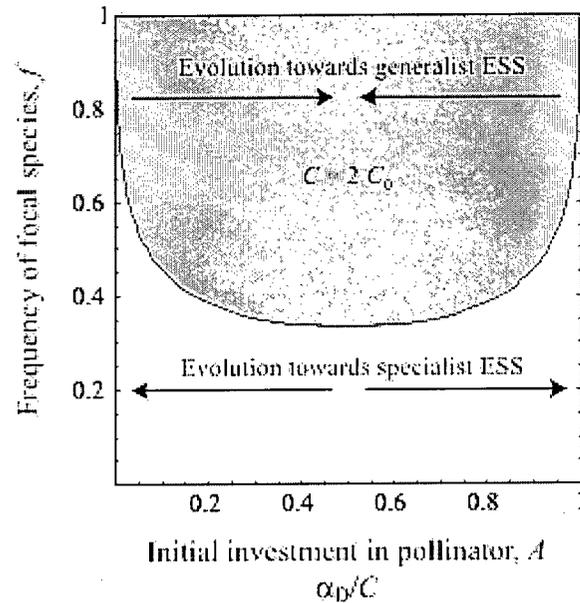


Figure 3.5. The focal species evolves toward a generalist or a specialist ESS depending on its frequency in the community, f (y -axis), and the initial investment by the focal species in attracting the A pollinator, (x -axis). The area below each curve indicates the parameter space where the focal species is predicted to evolve towards specialization on one or the other pollinator; the area above each curve indicates the parameter space where the focal species is predicted to evolve towards generalization. We assumed that

$$\frac{\alpha_o}{g_e} = \frac{\beta_o}{(1-g_e)}, \text{ in which case the generalist ESS is } \alpha_D^* = Cg_e \cdot A)$$

When $C = 2C_0$, (i.e., investment in pollinator attraction by a focal species' is two times greater than that of the other species in the community), the focal species is predicted to evolve towards the generalist ESS (shaded area), except where the focal species is rare or has a high initial investment in one pollinator over the other (unshaded area).

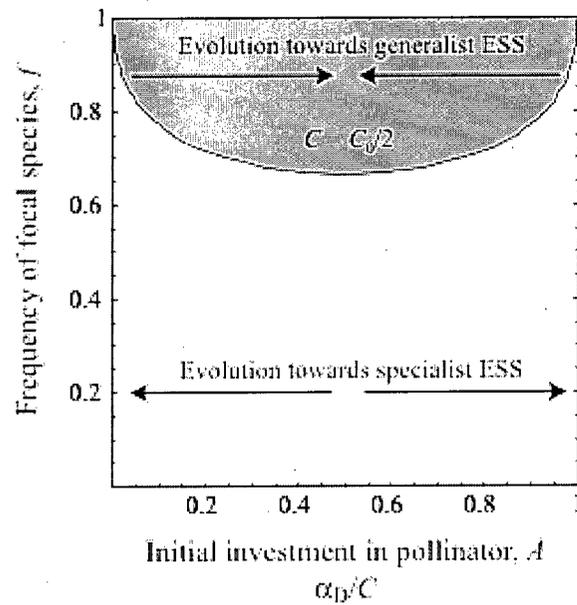


Figure 3.5. B) When $C = \frac{C_0}{2}$, (i.e., investment in pollinator attraction by a focal species' is half that of the other species in the community), the focal species is predicted to evolve towards the specialist *ESS* (unshaded area), except where the focal species is very common and its initial investment does not favour one pollinator over the other (shaded area).

Appendix 1

To prove that the model applies regardless of the number of visits we consider a pollinator that makes n visits after picking up pollen from a focal plant and deposits a proportion, $f[X]$ at the X^{th} flower stop after the pollen grains are picked up. Using this logic the overall probability of pollen transfer from a KK to another KK individual (Table 3.2) is:

$$P[KK \rightarrow KK] = g\gamma_A \frac{\alpha_D fD}{T_A} \left(f[1] \frac{\alpha_D fD}{T_A} + f[2] \frac{\alpha_D fD}{T_A} + \dots f[n] \frac{\alpha_D fD}{T_A} \right). \quad (1.1)$$

Since $f[X]$ is a probability distribution, by definition $\sum_{X=1}^n f[X] = 1$. Thus, assuming that the proportion of pollen deposited, $f[X]$, is not dependent on the sequence of flowers visited, this indicates that the results are the same regardless of whether the focal species is the next plant visited or the X^{th} plant visited.

Chapter IV – Modeling the evolution of dichogamy.

4.1 Introduction

Flowering plants exhibit a remarkable diversity of mating strategies. Explanations for this diversity have focused largely on two main processes: selection for efficient pollination and selection to avoid inbreeding depression associated with self-fertilization (Barrett 2003). In flowering plants, mating strategies often involve the separation of male and female function. This separation is often spatial, such as the placement of anthers and pistils in separate positions in the same flower (herkogamy) or in separate flowers on the same plant (monoecy), or indeed, on different plants (dioecy). Another category of mating strategies, generally known as dichogamy, involves the separation of male and female function in time, rather than space. Dichogamy has two main forms: pollen presentation may precede pistil receptivity (protandry) or vice-versa (protogyny) (Lloyd and Webb 1986, Figure 4.1). This chapter is an examination of the selective forces that may have contributed to the evolution of dichogamy.

Although an exact estimate of its frequency is unavailable, dichogamy is extremely common (Lloyd and Webb 1986, Barrett 2003). In a literature survey of 4277 species, Bertin and Newman (1993) found that 3716 species (~87%) exhibited some form of dichogamy. Indeed, the timing of pollen presentation and stigma receptivity is rarely simultaneous in hermaphroditic flowers. Despite the widespread occurrence of dichogamy, there have been relatively few empirical and/ or theoretical explorations of its causes and consequences. Across-species comparisons have revealed considerable variability in the degree of separation of the timing of pollen presentation and stigma receptivity (Lloyd and Webb 1986, see also Chapter V).

From an evolutionary perspective, dichogamy is puzzling because frequency-dependent selection causes the rare sex in a population to have a fitness advantage, and thus population sex ratios are predicted to evolve towards equal numbers of males and females (Fisher 1930). Not only does dichogamy reduce the overlap between pollen production and pistil receptivity within a flower, it also causes a mismatch between the timing of the availability of pollen and ovules at the population level (Brunet and Charlesworth 1995, Sargent and Roitberg 2000). Consequently, dichogamy can decrease the likelihood of pollen transfer to early or late-blooming flowers (Brunet 1996, Huang *et al.* 2004).

Historically, dichogamy was described as a mechanism to avoid self-pollination (Darwin 1876). This explanation is complicated by the fact that self-pollination confers a potential fitness benefit, relative to outcrossing. Because a selfing plant can provide both pollen and ovule for its own offspring, as well as pollen to seeds of other plants, it can pass on more copies of its genes to the subsequent generation (Fisher 1941). If, however, inbred seeds have lower fitness than outcrossed seeds, this transmission advantage can be negated. The phenomenon whereby inbred offspring have lower fitness than outbred offspring is known as inbreeding depression. How inbreeding depression operates to reduce fitness is currently under investigation, but one common explanation is that deleterious alleles are predominantly recessive (or partially recessive) and their deleterious effects are thus compounded in homozygous inbred offspring (Charlesworth and Charlesworth 1999).

Inbreeding depression has been invoked to explain the evolution of many aspects of plant reproductive biology, including mating system evolution (Husband and

Schemske 1996). The role of inbreeding depression in the evolution of dichogamy is currently unclear. While some studies have found support for high inbreeding depression and reduced selfing in dichogamous species (e.g. Dudash *et al.* 2001), others have found no evidence for such a relationship (e.g. Hossaert-McKey 2001).

A second factor thought to contribute to the evolution of dichogamy is the avoidance of physical interference between male (anther) and female (stigma) function (Holsinger *et al.* 1984, Lloyd and Webb 1986, Bertin 1993, Routley and Husband 2003). The cost of anther-stigma interference from the male perspective is a reduction in the total number of offspring sired because 1) pollen is deposited on the plant's own stigmas in excess of the amount used for self-fertilization ("pollen discounting") 2) the pollen is more likely to result in offspring that suffer from inbreeding depression, and/or 3) the removal of pollen by a pollinator is physically obstructed by the stigma.

The significance of anther-stigma interference in the evolution of dichogamy gained attention after a survey of angiosperm species revealed an intriguing pattern: dichogamy is equally common in self-compatible and self-incompatible species (Bertin 1993). It was considered puzzling that species possessing one mechanism to prevent self-pollination (self-incompatibility) would exhibit a second (dichogamy). This observation lends support to the alternative explanation that dichogamy may have evolved to reduce sexual interference between female and male function, rather than selfing avoidance (Lloyd & Webb 1986; Bertin 1993).

The extent to which the timing of pollen and ovule availability at the population level, inbreeding depression, and anther-stigma interference influence the evolution of

dichogamy is unknown. In spite of its potentially important consequences, anther-stigma interference has received little attention in theoretical explorations of plant mating strategies. Here we develop a model that allows us to explore the relative importance of each factor in the evolution of dichogamy in a population of flowering plants.

4.2 The Model

Our model examines the conditions under which an allele for dichogamy invades a population of diploid hermaphroditic plants with perfect (bisexual) flowers and annual (discrete, non-overlapping) generations. The proportion of a plant's ovules that are available for fertilization at time t is modeled as a continuous probability distribution, $F[t]$, where $\int F[t] dt = 1$ (see Table 4.1 for a list of all parameters and variables). We assume that the ovule availability schedule is the same for all genotypes in the population, on average although not all plants need be flowering on a given day. For mathematical convenience, we measure time such that $t = 0$ corresponds to the mean date of ovule availability. Because $F[t]$ is not genotype specific, it can be thought of as the average availability of ovules in the population at time t . In contrast, the amount of pollen dispersed by plants at time t is assumed to depend on a plant's genotype, x , and is given by the probability distribution, $V[r_x, t]$. Thus, the mean date of pollen dispersal depends on the plant genotype, x . Because the timing of ovule availability is fixed, the degree of dichogamy for a plant of genotype x is measured by the average difference in timing between when the ovules become available for pollination and when pollen becomes available, r_x . Although this limits our model to genes that affect the timing of pollen function, it has recently been proposed that one of the most likely developmental

pathways affecting the evolution of protandry alters the timing of anther development (S. Kalisz *pers. comm.*). When $r_x = 0$, the mean date of pollen and ovule production is the same (“adichogamy”). When $r_x > 0$, the average date of ovule availability is earlier than the average date of pollen production, and genotype x is protogynous. Conversely, when $r_x < 0$, genotype x is protandrous. Thus we can track the evolution of dichogamy within a population by following the frequency of genotypes with different values of r_x . We assume that the time delay between pollen dispersal and ovule fertilization is negligible.

In many plants, stigma presentation can interfere with the export of pollen, and this interference is worsened when pollen and ovule availability overlap extensively (Lloyd and Webb 1986). We define the interference function, $C[r_x, t]$, as the proportion of pollen lost due to overlapping anther and stigma development, where interference is a function of both a plant’s genotype, x , and time, t . Similarly, $M[r_x, t] = 1 - C[r_x, t]$ indicates the proportion of pollen contributed to the outcrossing pollen pool by genotype x at time t . We assume that the presence of dichogamy reduces anther-stigma interference, and therefore $C[r_x, t]$ is a decreasing function of $|r_x|$ (i.e., $\frac{\partial C[r_x, t]}{\partial |r_x|} < 0$).

Seed production is the result of either self-fertilization or outcrossing. We make the simplifying assumption that pollen is abundant and that its availability does not limit ovule fertilization. The proportion of selfed seeds of genotype x produced at time t , $S[r_x, t]$, is assumed to be a decreasing function of the degree of temporal separation between pollen and ovule production (i.e., $\frac{dS[r_x, t]}{dr_x} < 0$). The number of selfed seeds

integrated over all time is $S[r_x] = \int_{-\infty}^{\infty} S[r_x, t] F[t] dt$. The proportion of outcrossed seeds produced by genotype x at time t , $O[r_x, t]$, is the proportion of seeds that are not selfed (i.e., $O[r_x, t] = 1 - S[r_x, t]$, and $O[r_x] = 1 - S[r_x]$). When inbreeding depression (δ) exists, only a fraction, $(1 - \delta)$, of selfed seed is viable. This assumes that inbreeding depression remains fixed, which need not be true as dichogamy evolves and alters the amount of selfing.

We consider a population comprised of three genotypes: AA (frequency D), Aa (frequency H) and aa (frequency R) where each genotype exhibits a different degree of dichogamy (r_{AA}, r_{Aa}, r_{aa} , respectively). For the purposes of this description, we assume the species in question is protogynous (i.e., ovules are produced before pollen; $r_x > 0$). However, the model is equally applicable to understanding the evolution of protandry (i.e., $r_x < 0$).

After mating and seed production, the total number of seeds with genotype AA is the sum of the number of selfed seeds (discounted by losses incurred due to inbreeding depression) and outcrossed seeds produced by genotypes containing A alleles (i.e., AA , Aa) multiplied by the frequency of those genotypes in a parental population of size N :

$$N'_{AA} = DN \left((1 - \delta) \int_{-\infty}^{\infty} S[r_{AA}, t] F[t] dt + \int_{-\infty}^{\infty} p_{A,t} O[r_{AA}, t] F[t] dt \right) + HN \left(\frac{(1 - \delta)}{4} \int_{-\infty}^{\infty} S[r_{Aa}, t] F[t] dt + \frac{1}{2} \int_{-\infty}^{\infty} p_{A,t} O[r_{Aa}, t] F[t] dt \right), \quad (4.1)$$

where $p_{y,t}$ is the frequency of pollen containing allele y carried by pollinators at time t .

For example, $p_{A,t}$ includes outcrossed pollen from AA individuals at time t ,

$(DV[r_{AA},t]M[r_{AA},t])$, and half the outcrossed pollen from Aa individuals at time t ,

$(\frac{H}{2}V[r_{Aa},t]M[r_{Aa},t])$, divided by the total amount of pollen carried by pollinators at time

t . $p_{a,t}$ is calculated in a similar fashion:

$$p_{A,t} = \frac{DV[r_{AA},t]M[r_{AA},t] + \frac{H}{2}V[r_{Aa},t]M[r_{Aa},t]}{DV[r_{AA},t]M[r_{AA},t] + HV[r_{Aa},t]M[r_{Aa},t] + RV[r_{aa},t]M[r_{aa},t]},$$

$$p_{a,t} = \frac{RV[r_{aa},t]M[r_{aa},t] + \frac{H}{2}V[r_{Aa},t]M[r_{Aa},t]}{DV[r_{AA},t]M[r_{AA},t] + HV[r_{Aa},t]M[r_{Aa},t] + RV[r_{aa},t]M[r_{aa},t]}$$

The numbers of seeds of the remaining genotypes, N'_{AA} and N'_{aa} , are calculated similarly (Table 4.2). The total number of seeds in the next generation, N' , is the sum of the number of seeds from the three genotypes ($N' = N'_{AA} + N'_{Aa} + N'_{aa}$). The frequency of each genotype in the next generation is thus $D' = \frac{N'_{AA}}{N'}$, $H' = \frac{N'_{Aa}}{N'}$, and $R' = \frac{N'_{aa}}{N'}$. In the following sections, we use these recursions to investigate the spread of a newly introduced allele, A , that alters the timing of pollen availability relative to the timing of ovule availability.

4.3 Invasion Analysis

To assess the evolutionary forces acting on dichogamy, we examined when a resident genotype (aa , $\hat{R} = 1$) could be invaded by a newly introduced allele (A) that causes a shift in the pollen production schedule (i.e., $r_{AA}, r_{Aa} > r_{aa}$). To do so, we performed a local stability analysis of the equilibrium, $\hat{R} = 1$. First, we introduce the parameter

$$\phi = (r_{Aa} - r_{aa})(1 - F) + (r_{AA} - r_{aa})F, \text{ which describes the overall effect of the rare modifier}$$

on dichogamy averaged over heterozygous carriers (frequency $1 - F$) and homozygous carriers (frequency F), where F is the equilibrium inbreeding coefficient within a

population in the presence of inbreeding depression $F = \frac{S[r_{aa}](1 - \delta)}{2 - S[r_{aa}] - \delta S[r_{aa}]}$. Thus, ϕ is

positive for a mutant allele that increases the degree of protogyny. To obtain interpretable solutions, we assumed the genotypic differences in dichogamy are small (i.e., $r_{AA} - r_{aa} = O(\varepsilon)$; $r_{Aa} - r_{aa} = O(\varepsilon)$). As the total selfing rate depends on the level of dichogamy, $S[r_{aa}]$, alleles that cause a small change in dichogamy cause a small change

in selfing that is proportional to $\left. \frac{dS[r_x]}{dr_x} \right|_{x=r_{aa}}$, which we write as $S'[r_{aa}]$.

As described in Appendix 4. 1, we found the leading eigenvalue governing the spread of the rare A allele to be:

$$\lambda = 1 + \frac{\phi \left\{ (1 - 2\delta) S'[r_{aa}] + (\omega + \rho) O[r_{aa}] \right\}}{2(1 - \delta S[r_{aa}])} + O(\varepsilon^2). \quad (4.2)$$

In (4.2), ω measures the expected sensitivity of the mismatch in the timing of pollen and ovules caused by a change in the amount of dichogamy (r_{aa}), where

$$\omega = E \left[\frac{\partial V[r_{aa}, t] / \partial r_{aa}}{V[r_{aa}, t]} \right] = \int_{-\infty}^{\infty} \frac{\partial V[r_{aa}, t] / \partial r_{aa}}{V[r_{aa}, t]} \frac{O[r_{aa}, t] F[t]}{O[r_{aa}]} dt.$$

For example, a plant species with a short flowering season (e.g., alpine species) should exhibit greater sensitivity to a given mismatch in pollen and ovule availability (r_{aa}) than a species with a longer flowering season (e.g., tropical species). This is because the same mismatch for the alpine species represents a larger portion of the flowering season than for the tropical species, and hence there exists less opportunity to recuperate the lost mating opportunity.

Similarly, ρ indicates the expected sensitivity of anther-stigma interference (measured as a decline in the proportion of pollen available for export), $M[r_{aa,t}]$, to a

$$\text{change in dichogamy } (r_{aa}), \text{ where } \rho = E \left[\frac{\partial M[r_{aa},t] / \partial r_{aa}}{M[r_{aa},t]} \right] = \int_{-\infty}^{\infty} \frac{\partial M[r_{aa},t] / \partial r_{aa}}{M[r_{aa},t]} \frac{O[r_{aa},t]}{O[r_{aa}]} F[t].$$

For example, species that exhibit herkogamy (spatial separation of anther and stigma within a flower) may be less prone to anther-stigma interference (Fetscher 2001).

Consequently, the amount of anther-stigma interference should be less sensitive to dichogamy in such species (i.e., ρ closer to 0). In a slightly different context, plants with large inflorescences may suffer greater between flower anther-stigma interference (ρ larger in magnitude) than those with small inflorescences, and therefore be under stronger selection to evolve dichogamy (e.g., Harder *et al.* 2000). Both ω and ρ are calculated by integrating over the distribution describing the proportion of ovules available for outcrossing at time t , $\frac{O[r_{aa},t]}{O[r_{aa}]} F[t]$. Thus, these expectations are weighted by the likelihood that a pollen grain will successfully fertilize an ovule.

The difference between the leading eigenvalue and one, $\lambda - 1$, measures the rate of spread of the A allele and can be thought as a measure of the strength of selection acting on the A allele while rare. When $\lambda - 1 > 0$, the A allele increases in the population because of its effects on dichogamy. In the following section we determine what conditions allow the spread of the A allele, i.e., lead to $\lambda - 1 > 0$.

4.3.1 General Conditions for Invasion

Assuming that the A allele increases the degree of protogyny, ($\phi > 0$), it will spread if the term in braces in (4.2) is positive. This term consists of three parts. The

first part, $(1 - 2\delta)S'[r_{aa}]$, describes the effects of the intrinsic advantage of selfing and the fitness cost of inbreeding depression (δ) on the fate of the allele, where $S'[r_{aa}] = \frac{dS[r_{aa}]}{dr_{aa}}$ describes the change in the total number of selfed seeds with increased protogyny, r_x . We assume that $S'[r_{aa}]$ is negative, implying that dichogamy reduces the level of selfing. Thus, $(1 - 2\delta)S'[r_{aa}]$ is positive when $\delta > \frac{1}{2}$ and negative when $\delta < \frac{1}{2}$. Thus, when inbreeding depression is strong $\left(\delta > \frac{1}{2}\right)$, selfing drives selection for an allele that increases dichogamy. This term is equivalent to the classical condition under which selfing is favoured (Fisher 1941).

The second part of the numerator, $\omega O[r_{aa}]$, describes the effects on outcrossing success of a change in the timing of pollen production (ignoring anther-stigma interference). ω describes the degree to which increasing dichogamy alters the match in timing between the production of pollen for outcrossing and the production of ovules. ω is expected to be negative, because larger amounts of dichogamy result in a greater mismatch. The effect of ω on the evolution of dichogamy is proportional to $O[r_{aa}]$, the proportion of outcrossed seeds. Because ω is typically negative, this second part will be negative, indicating that a mismatch between pollen and ovules drives selection for adichogamy, rather than dichogamy. This part of (4.2) measures the evolutionary advantage of producing an equal sex ratio (Fisher 1930) at every point in time, an evolutionary force selecting against dichogamy.

The last part of the numerator, $\rho O[r_{aa}]$, describes the effect of the sensitivity of anther-stigma interference to changes in dichogamy, on outcrossed seed success. We

expect ρ to be positive, reflecting the assumption that dichogamy causes a decline in anther-stigma interference and an increase in pollen available for export. This final term therefore drives selection for dichogamy, indicating that reduced anther-stigma interference is a selective force favouring dichogamy.

Our general analysis thus describes the relative advantages and disadvantages of dichogamy. Specifically, (4.2) describes how different evolutionary forces balance to affect dichogamy. In particular, (4.2) reflects two well known advantages of dichogamy (avoiding inbreeding through $(-2\delta)S'[r_{aa}]$ and reducing anther-stigma interference through $\rho O[r_{aa}]$) as well as two disadvantages of dichogamy (reducing the intrinsic advantage of selfing through $S'[r_{aa}]$ and increasing the disadvantage of shifting pollen production to times where ovule availability is lower). To determine the net effect of these conflicting selective forces requires empirical data on the factors that we have identified (especially, δ , ω , and ρ).

4.3.2 Critical Inbreeding Depression

From (4.2) we calculated the critical amount of inbreeding depression, δ^* , above which dichogamy evolves from the current degree of separation (r_{aa}) to a greater degree of separation,

$$\delta^* > \frac{1}{2} + \frac{\rho O[r_{aa}]}{2S'[r_{aa}]} + \frac{\omega O[r_{aa}]}{2S'[r_{aa}]} \quad (4.3).$$

As discussed earlier, $S'[r_{aa}]$ is typically negative and therefore $\frac{\rho O[r_{aa}]}{2S'[r_{aa}]}$ will be negative, indicating that anther-stigma interference decreases the level of inbreeding depression necessary to drive the evolution of dichogamy. Conversely, the mismatch in the timing

of pollen production to the availability of ovules, $\frac{\omega O[r_{aa}]}{2S[r_{aa}]}$, is expected to be positive, indicating that when the mismatch is large, the critical amount of inbreeding depression is higher, making it more difficult to evolve greater dichogamy. It is the interaction between these processes that determines whether a greater degree of dichogamy can evolve. Furthermore, if anther-stigma interference is strong enough, $\frac{\rho O[r_{aa}]}{2S[r_{aa}]} \ll 0$, the right hand side of (4.3) becomes negative, indicating that dichogamy is favoured regardless of the level of inbreeding depression.

4.3.3 Evolution of dichogamy assuming functions are Gaussian

We turn next to an analysis where we specify the forms of the ovule and pollen distributions. Using (4.2), we can then obtain clearer insight as to when dichogamy is expected to evolve. We now assume that pollen production $V[r_x, t]$ (Figure 4.2), ovule availability $F[t]$, anther-stigma interference $C[r_x, t]$, and the selfing rate $S[r_x, t]$ (Figures 4.3A and 4.3B), are Gaussian functions (Table 4.1, Appendix 4.2). The terms needed for (4.2) were then derived using these functions (Table 4.1).

In the previous section we postulated the sign of the two sensitivity functions, ω and ρ , in order to draw general conclusions about their effect on the evolution of dichogamy. Using the Gaussian functions, we confirmed that $\omega < 0$ and $\rho > 0$, corroborating our assumptions about their behaviour (Table 4.1).

Dichogamy is expected to evolve when (4.2) is greater than zero. Evaluating this condition with the Gaussian functions, we expect dichogamy to invade an adichogamous population (i.e., where $r_{aa} = 0$) when the following condition is met:

$$C[0] - S[0](1 - 2\delta(1 - C[0])) > 0. \quad (4.4)$$

It is apparent from (4.4) that if $S[0] = 0$, at least some dichogamy is favoured provided that there is anther-stigma interference (i.e., $C[0] > 0$). Thus, dichogamy can evolve even in the absence of selfing, such as in a species with genetic self-incompatibility.

Interestingly, when deriving (4.4) for pollen and ovule availability functions that are Gaussian (see Table 4.1), the effect of mismatched timing between pollen production and ovule availability, ω , equals zero at $r_{aa} = 0$. This result reflects the fact that, for the Gaussian functions, and allowing only small changes in r_x near $r_{aa} = 0$, the loss in ovules available per pollen grain for pollen produced after the mean date of ovule production ($t = 0$) is very nearly the gain in ovules available per pollen grain for pollen produced before $t = 0$. Nevertheless, ω becomes more important as the species becomes more dichogamous.

According to (4.4), the critical inbreeding depression necessary to drive selection for dichogamy from adichogamy is

$$\delta_{r_{aa}=0}^* = \frac{S[0] - C[0]}{2 S[0](1 - C[0])} \quad (4.5).$$

The denominator of (4.5) is always positive, and thus the critical inbreeding depression, $\delta_{r_{aa}=0}^*$, is greater than zero when $S[0] > C[0]$. Conversely, if anther-stigma interference is greater than the selfing rate for an adichogamous species, $C[0] > S[0]$, dichogamy is always favoured.

4.3.4 Evolutionarily Stable Strategy

An evolutionarily stable strategy (*ESS*) is one that, once adopted by all members of the resident population, cannot be invaded by another mutant strategy (Maynard Smith 1982). When $\lambda - 1 = 0$, the frequency of *A* does not increase or decrease and the resident allele is considered the *ESS*. This *ESS*, by definition, cannot be invaded by any genotype with a slightly larger (or slightly smaller) degree of dichogamy.

In this section we use the Gaussian functions to graphically examine the *ESS* degree of anther-stigma separation (r_{aa}). Equations (4.2) and (4.4) indicate that selfing can either promote selection for adichogamy (because of its associated fitness benefits) or selection for dichogamy (due to inbreeding depression). In order to determine the ultimate level of dichogamy expected, we plotted the *ESS* for combinations of the adichogamy selfing rate ($S[0]$) and inbreeding depression (δ) (Figures 4.4 - 4.5). Figure 4.4 examines the *ESS* when anther-stigma interference is absent ($C[0] = 0$). From Figure 4.4 it is clear that adichogamy, $r_{aa} = 0$, is the *ESS* only when inbreeding depression is less than the critical value, $\delta = \frac{1}{2}$, although little dichogamy is expected at the *ESS* unless the selfing rate of adichogamous species is high (i.e., $S[0] \gg 0$). A different picture emerges in Figure 4.5, where 50% of pollen is lost due to anther-stigma interference in adichogamous species (i.e., $C[0] = 0.5$). Here adichogamy is the *ESS* only when inbreeding depression is low enough and selfing common enough to impart a strong intrinsic advantage. However, when selfing is low these benefits are not enough to offset the cost of anther-stigma interference. Notice also that the *ESS* level of dichogamy is much higher with anther-stigma interference.

To illustrate the combined effects of anther-stigma interference and selfing, Figure 4.6 plots the *ESS* when inbreeding depression is 0.75. This figure shows that some dichogamy is favoured when inbreeding depression is substantial, except where there is no selfing and no anther-stigma interference (i.e., $C[0] = 0, S[0] = 0$). The highest degree of separation is predicted to evolve when $C[0] = 1$, regardless of the amount of selfing, illustrating our finding that anther-stigma interference is a sufficient force to drive the evolution of dichogamy.

4.4 Conclusions

Our model explores several aspects of the reproductive biology of hermaphroditic flowering plants and their role in the evolution of dichogamy. The results stemming from our model confirm that inbreeding depression plays an important role in the evolution of dichogamy. This finding is supported by a large body of theory that implicates inbreeding depression as a major player in several aspects of plant mating systems (Barrett 2002). Furthermore, Husband and Schemske (1996), in a survey of 62 natural plant populations, estimated inbreeding depression and found a range of -0.15 to 0.92 . This indicates it is reasonable to assume that in natural systems inbreeding depression occurs at levels as high as those discussed in our results section.

We also found that anther-stigma interference plays an important role in the evolution of dichogamy. Indeed, in the absence of self-fertilization, anther-stigma interference alone can drive the evolution of dichogamy. Although the importance of anther-stigma interference had been invoked to explain dichogamy in self-incompatible species (Bertin 1993, Routley and Husband 2003), its role in the evolution of dichogamy in self-compatible species has received less attention than inbreeding depression. Here

we have demonstrated that both anther-stigma interference and inbreeding depression act simultaneously to drive selection for dichogamy. Which force is most responsible for the evolution of dichogamy in any particular instance will depend on the specifics of the species. Furthermore, our model highlights a rarely discussed disadvantage of dichogamy: the fitness cost of producing pollen and ovules at a different time than other plants in the population. Overall, we determined that the evolution of dichogamy depends on the balance of its advantages (i.e., reduced production of inbred seeds and decreased anther-stigma interference) and disadvantages (i.e., loss of fitness via selfed seeds and production of pollen on a different schedule than would be optimal given the schedule of ovule availability).

4.4.1 *Predictions*

Our model predicts that both anther-stigma interference and selfing avoidance may drive the evolution of dichogamy within a single species. In a broad survey of 588 angiosperm species, Bertin (1993) found that dichogamy was equally common among self-compatible and incompatible species. This relationship has recently been confirmed using phylogenetic methods (Routley *et al.* 2004). Our model also predicts that anther-stigma interference may be an important factor in the evolution of dichogamy in species with low inbreeding depression. A specific testable prediction is that self-incompatible and self-compatible species with inbreeding depression less than one half should be more likely to evolve dichogamy when anther-stigma interference is present. Furthermore, our *ESS* analysis (Figures 4.4 – 4.6) shows graphically that more dichogamy is expected in groups with greater potential for anther-stigma interference (i.e., higher $C[0]$).

Our model predicts that the mismatch between pollen and ovule availability should drive the evolution of adichogamy in dichogamous species to equalize the sex ratio at every point in time. Besides adichogamy, plants may mitigate the lost mating opportunity associated with dichogamy by producing multi-flowered inflorescences, where individual flowers (particularly the earliest and latest, which tend to suffer the greatest loss of mating opportunity) only represent a small fraction of the plant's overall investment in reproduction. If this is true, dichogamous species are predicted to evolve larger inflorescences than adichogamous or heterodichogamous species. Indeed, there is some anecdotal evidence to suggest this pattern exists. However, we should note that an alternative explanation for the pattern is that dichogamy is more likely to evolve in species with large inflorescences in order to reduce the transfer of pollen between flowers on the same plant (Routley and Husband 2003, Harder *et al.* 2000). It is likely that both processes play a role in driving the evolution of dichogamy in multi-flowered plants.

4.4.2 *Future Directions*

Although it is an important question, we have not modified the model to make predictions concerning the conditions that may lead to the evolution of the different forms of dichogamy (i.e., protandry and protogyny). Our model makes no direct predictions about which form of dichogamy evolves, rather it predicts conditions under which either evolves. One explanation for the existence of the different forms is that in some species there may be an asymmetry in the distribution of pollen and ovule availability. The specific functions we tested (Gaussian) are unimodal and symmetrical, and therefore did not allow an exploration of this explanation. Future explorations using functions with skew might be useful for elucidating the processes that drive these

different forms. The role of pollination mode in the evolution of protandry and protogyny is explored in Chapter V. It is also possible that the form of dichogamy that evolves is constrained by floral developmental pathways (Chapter VI).

Finally, reproductive assurance may have played a role in the evolution of dichogamy in some species (see Herlihy and Eckert 2002). Reproductive assurance is a hypothesis to explain the evolution of self-fertilization as a means to ensure pollination in the event that insufficient outcrossed pollen is received. Reproductive assurance has been invoked to explain the evolution of protogyny because the presentation of the stigma before anthers enables self-pollination to occur after a period of time where outcrossing is possible. Because we assume all ovules are fertilized we have omitted reproductive assurance as a potential benefit that could drive the evolution of protogyny. Future modifications should relax this assumption in order to test the hypothesis that dichogamy evolves because it offers reproductive assurance.

In conclusion, our model offers insight into the interacting forces that influence the evolution of dichogamy in hermaphroditic plant populations. In particular, the model predicts that dichogamy may arise in populations that are prone to inbreeding depression and/or anther-stigma interference. These traits differ widely between species of plants; consequently, the specific forces driving selection for dichogamy will depend on the ecology and genetic background of the species of interest.

4.5 Literature Cited

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Table 4.1 Variables and parameters appearing in the model.

Parameter	Description	Gaussian Functions
$C[r_x, t]$	Amount of pollen lost to anther-stigma interference for genotype x at time t .	$e^{-\frac{(t-\mu[r_{aa}])^2}{2\sigma[r_{aa}]^2}} C[0]$
$C[r_x]$	Amount of pollen lost to anther-stigma interference for genotype x , integrated over all time t (see Appendix 4.2 for explanation).	$\int_{-\infty}^{\infty} e^{-\frac{(t-\mu[r_{aa}])^2}{2\sigma[r_{aa}]^2}} C[0] dt$
$C[0]$	Amount of pollen lost to anther-stigma interference when $r_x = 0$ (Appendix 4.2).	
D, H, R	Frequency of the genotypes AA, Aa, aa , respectively.	
δ	Fraction of selfed seeds that are inviable due to inbreeding depression.	
$F[t]$	Probability distribution of ovule availability at time t .	$\frac{e^{-\frac{t^2}{2}}}{\sqrt{2\pi}}$
$\mu[r_{aa}]$	Date of maximum selfing and anther-stigma interference (Appendix 4.2, Figure 4.3A)	
$M[r_x, t]$	Amount of pollen available for outcrossing for genotype x at time t , where $M[r_x, t] = 1 - C[r_x, t]$	$1 - e^{-\frac{(t-\mu[r_{aa}])^2}{2\sigma[r_{aa}]^2}} C[0]$
$O[r_x, t]$	Proportion of seeds produced by genotype x at time t that are not selfed.	$1 - e^{-\frac{(t-\mu[r_{aa}])^2}{2\sigma[r_{aa}]^2}} S[0]$
$P_{y,t}$	Frequency of pollen	

	containing allele y carried by pollinators at time t .	
r_x	Average difference in timing of pollen and ovule availability for genotype x (also the degree of dichogamy)	
$\sigma[r_{aa}]^2$	Variance of the selfing and anther-stigma interference (Appendix 4.2, Figure 4.3A)	
$S[r_x, t]$	Number of selfed seeds of genotype x produced at time t ($O[r_x, t] = 1 - S[r_x, t]$).	$e^{\frac{-(t-\mu[r_{aa}])^2}{2\sigma[r_{aa}]^2}} S[0]$
$S[0]$	Proportion of selfed seeds when $r_x = 0$ (Appendix 4.2)	
ρ	Expected sensitivity of a change in pollen available for export, $1 - C[r_{aa}, t]$, to a change in dichogamy, r_{aa} .	$\frac{C[r_x] \frac{1 + 2\mu[r_{aa}]^2 + \sigma[r_{aa}]^2 + 3\mu[r_{aa}]^2 \sigma[r_{aa}]^2}{2r_x(1 + \sigma[r_{aa}]^2)^2}}{1 - C[r_x]}$
ϕ	Effect of the rare modifier on dichogamy averaged over heterozygous and homozygous carriers of the allele.	
$V[r_x, t]$	Probability distribution of pollen availability for genotype x at time t .	$\frac{e^{\frac{-(t-r_x)^2}{2}}}{\sqrt{2\pi}}$
ω	Expected sensitivity of the timing of pollen production to a change in the amount of dichogamy, r_{aa} .	$\frac{-r_{aa} \left(1 - \frac{e^{\frac{-\mu[r_{aa}]^2}{2(1+\sigma[r_{aa}]^2)}} S[0] \left(1 - \frac{\mu[r_{aa}]/r_{aa}}{(1 + \sigma[r_{aa}]^2)} \right)}{\sqrt{1 + \frac{1}{\sigma[r_{aa}]^2}}} \right)}{1 - S[r_{aa}]}$

Table 4.2 Number and frequency of *Aa* and *aa* seeds in generation ($t+1$).

Genotype	Number of seeds in generation ($t+1$)	Frequency in generation ($t+1$)
<i>Aa</i>	$N'_{Aa} = D \left(\int_{-\infty}^{\infty} p_{a,t} O[AA,t] dt \right)$ $+ H \left(\begin{aligned} & \left(\frac{(1-\delta)}{2} \int_{-\infty}^{\infty} S[Aa,t] F[t] dt \right. \right. \\ & \left. \left. + \frac{1}{2} \int_{-\infty}^{\infty} p_{A,t} O[Aa,t] dt + \frac{1}{2} \int_{-\infty}^{\infty} p_{a,t} O[Aa,t] dt \right) \right) \\ & + R \left(\int_{-\infty}^{\infty} p_{A,t} O[aa,t] dt \right) \end{aligned}$	$H' = \frac{N'_{Aa}}{N'}$
<i>aa</i>	$N'_{aa} = H \left(\begin{aligned} & \left(\frac{(1-\delta)}{4} \int_{-\infty}^{\infty} S[Aa,t] F[t] dt \right. \right. \\ & \left. \left. + \frac{1}{2} \int_{-\infty}^{\infty} p_{a,t} O[Aa,t] dt \right) \right) \\ & + R \left((1-\delta) \int_{-\infty}^{\infty} S[aa,t] F[t] dt + \int_{-\infty}^{\infty} p_{a,t} O[aa,t] dt \right) \end{aligned}$	$R' = \frac{N'_{aa}}{N'}$

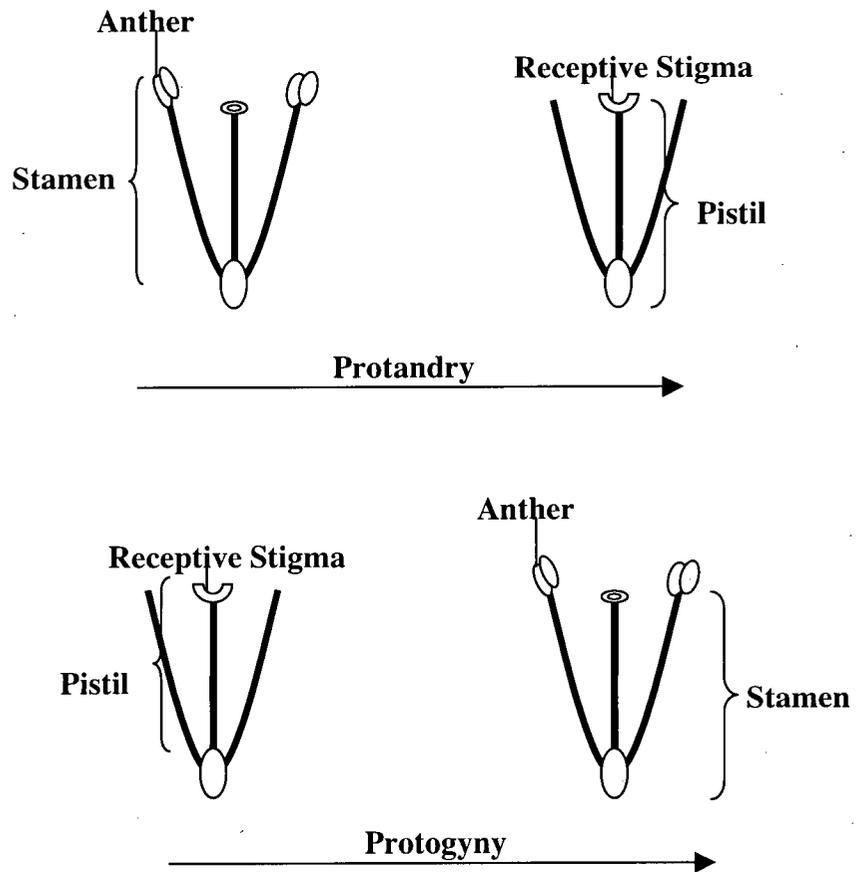


Figure 4.1. The order of anther dehiscence and stigma receptivity for a protandrous (top panel) and protogynous (bottom panel) species.

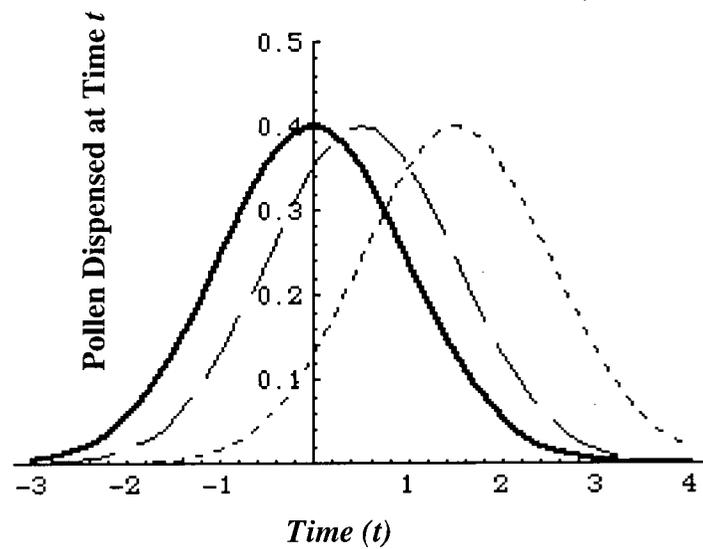


Figure 4.2. Pollen and ovule availability as a function of time according to a Gaussian distribution with a standard deviation of one. Ovule availability is represented by the thick solid line. Long dashed line is pollen availability for the degree of dichogamy $r_x = 0.5$. Short dashed line is pollen availability for the degree of dichogamy $r_x = 1.5$.

For comparison, in Figures 4.4 – 4.6 the *ESS* degree of dichogamy is always less than one standard deviation.

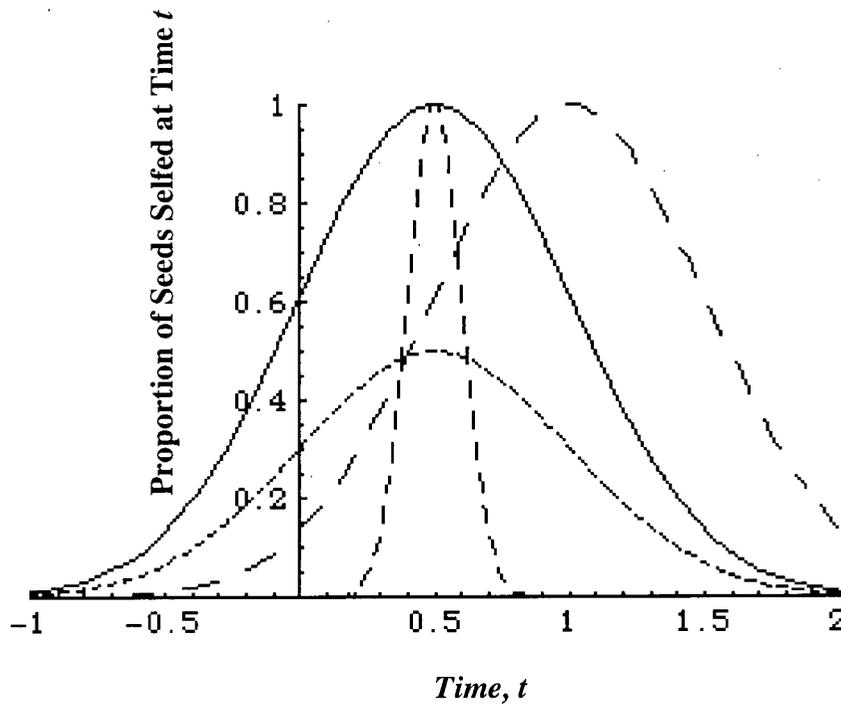


Figure 4.3A. The proportion of selfed seeds at time t ($S[r_{aa}, t]$) for a protogynous species

where $r_{aa} = 1$. Solid curve, $\sigma^2 = \frac{1}{4r_{aa}}$, $\mu = \frac{r_{aa}}{2}$, $S[0] = 1$, short-dashed curve,

$\sigma^2 = \frac{1}{4r_{aa}}$, $\mu = \frac{r_{aa}}{2}$, $S[0] = 0$, medium-dashed curve, $\sigma^2 = \frac{1}{100r_{aa}}$, $\mu = \frac{r_{aa}}{2}$, $S[0] = 1$, long-

dashed curve, $\sigma^2 = \frac{1}{4r_{aa}}$, $\mu = 0.875r_{aa}$, $S[0] = 1$.

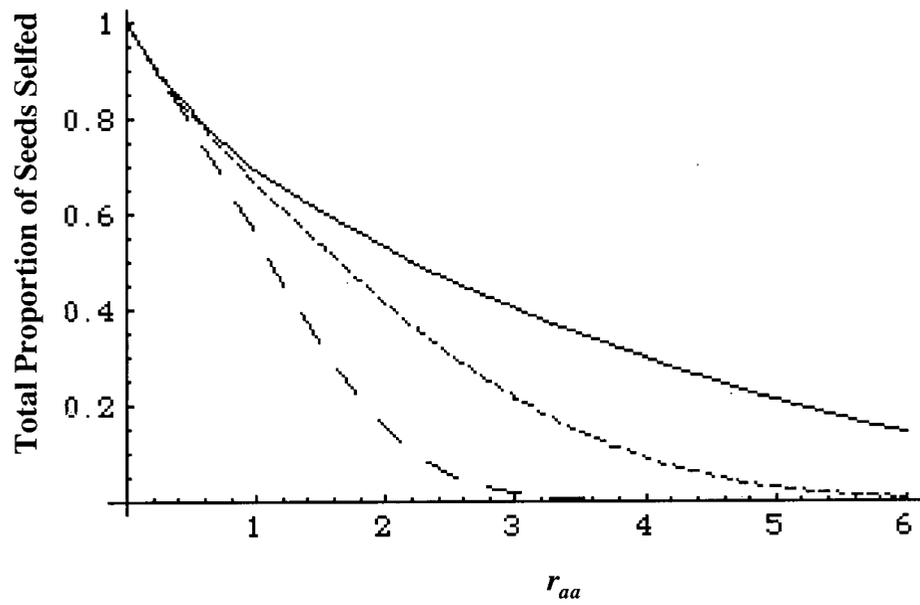


Figure 4.3B. The total proportion of selfed seeds $S[r_{aa}]$ as a function of dichogamy (r_{aa}).

Solid curve $\mu = \frac{r_{aa}}{4}$, short-dashed curve $\mu = \frac{r_{aa}}{2}$, long-dashed curve $\mu = r_{aa}$; $\sigma^2 = \frac{1}{r_{aa}}$.

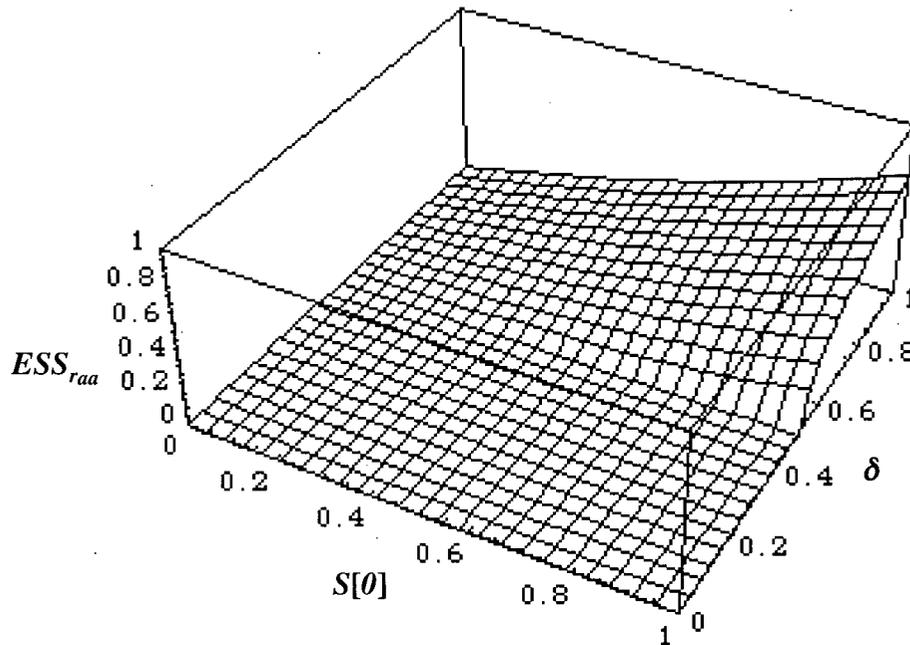


Figure 4.4. The ESS degree of anther-stigma separation as a function of inbreeding depression (δ) and selfing ($S[0]$), for $C[0]=0$, and where $S[0]$ and $C[0]$ are the maximum amount of selfing and anther-stigma interference, respectively, expected when $r_{aa} = 0$ (i.e., adichogamy). Increasing dichogamy causes a reduction the selfing rate and anther-stigma interference according to the functions $S[r_{aa}]$ and $C[r_{aa}]$, respectively (i.e., $S[ESS] \ll S[0]$ and $C[ESS] \ll C[0]$). Height on z-axis indicates the number of standard deviations between pollen production and ovule availability at the ESS (as illustrated in Figure 4.2).

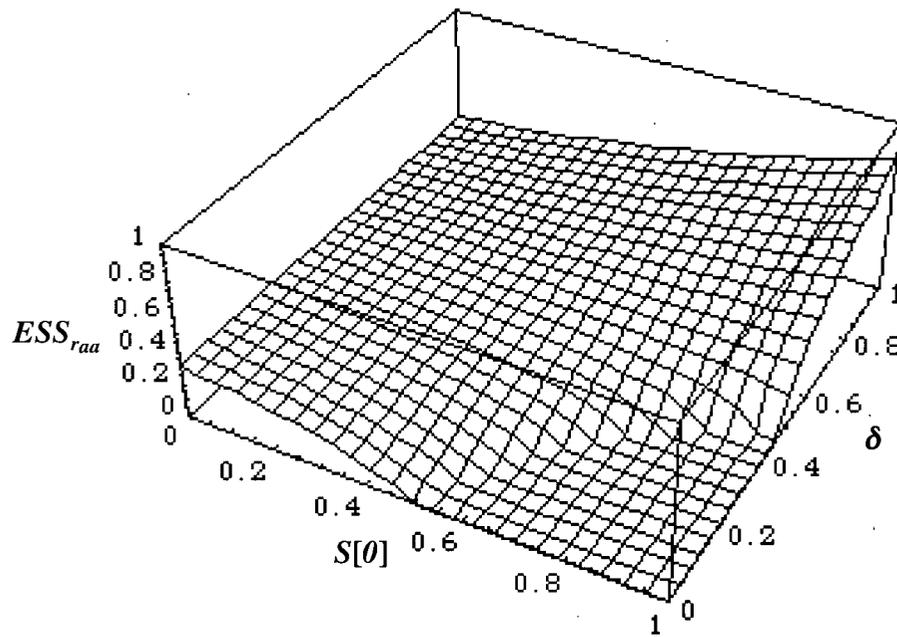


Figure 4.5. The ESS degree of dichogamy as a function of inbreeding depression (δ) and selfing ($S[0]$), where $C[0] = 0.5$.

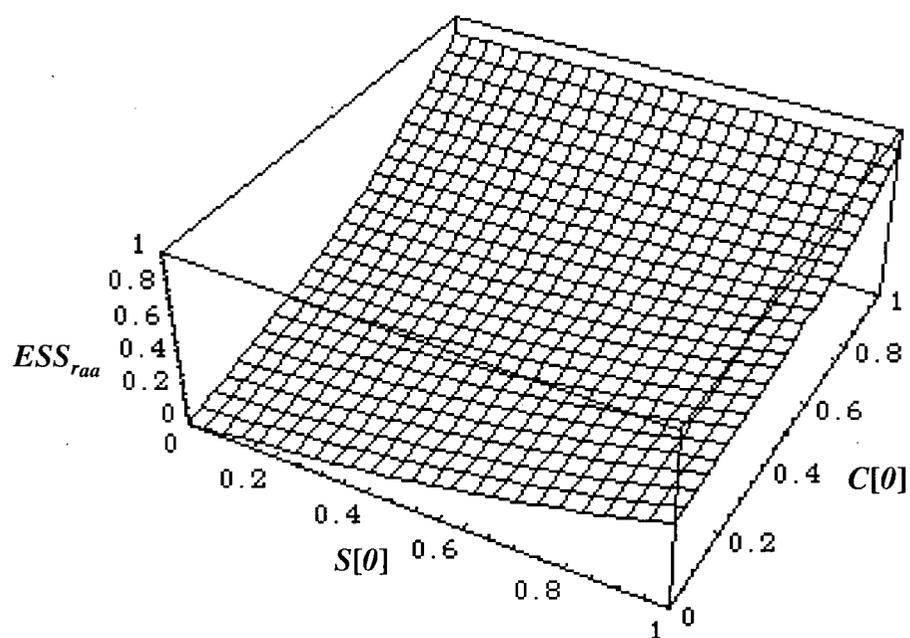


Figure 4.6. The ESS degree of anther-stigma separation as a function of anther-stigma separation ($C[0]$) and selfing ($S[0]$), where $\delta = 0.75$.

Appendix 4.1. Stability Analysis

We performed a local stability analysis of the equilibrium ($\hat{R} = 1$) by analyzing the equations $D' = \frac{N'_{AA}}{N'}$, $H' = \frac{N'_{Aa}}{N'}$, assuming that D and H were close to zero. Replacing R with $1 - D - H$, we performed a Taylor expansion of both $D' = f_1(D, H)$ and $H' = f_2(D, H)$, keeping only linear terms D and H in the expansion.

This procedure generates linear recursions in D and H that are accurate near $\hat{R} = 1$. These recursions can be written in matrix form as $\begin{pmatrix} D' \\ H' \end{pmatrix} = \mathbf{M} \begin{pmatrix} D \\ H \end{pmatrix}$, where

$$\mathbf{M} = \begin{pmatrix} \frac{(1-\delta)S[r_{AA}]}{(1-\delta)S[r_{aa}] + (1-S[r_{aa}])} & \frac{(1-\delta)\frac{S[r_{Aa}]}{4}}{(1-\delta)S[r_{aa}] + (1-S[r_{aa}])} \\ \frac{(1-S_{AA}) + \int_{-\infty}^{\infty} \frac{V_{AA,t}(1-C_{AA})}{V_{aa,t}(1-C_{aa})} O_{aa,t} F_t dt}{(1-\delta)S[r_{aa}] + (1-S[r_{aa}])} & \frac{(1-\delta)\frac{S[r_{Aa}]}{2} + \frac{(1-S[r_{Aa}])}{2} + \int_{-\infty}^{\infty} \frac{V_{Aa,t}M[r_{Aa},t]}{2V_{aa,t}M[r_{aa},t]} O_{aa,t} F_t dt}{(1-\delta)S[r_{aa}] + (1-S[r_{aa}])} \end{pmatrix}$$

Notice that when selfing is absent ($S[0] = 0$), the first row consists of zeros and the AA genotype is not produced at an appreciable frequency.

To evaluate the integrals in \mathbf{M} , we assumed that the A allele caused only a small change in the amount of dichogamy. That is, we assumed that $r_{AA} - r_{aa} = O(\varepsilon)$ and $r_{Aa} - r_{aa} = O(\varepsilon)$, where ε is small. The eigenvalue of \mathbf{M} can then be written in terms of the effect of allele A as $\lambda = 1 + (r_{AA} - r_{aa})X + (r_{Aa} - r_{aa})Y + O(\varepsilon^2)$, where X and Y are terms of order one, that is, they involve only parameters for the resident aa population (e.g., r_{aa}) and do not contain terms involving the AA and Aa genotypes. To find X and Y , we substituted the above equation for λ into the characteristic polynomial and took the

Taylor Series with respect to ε . Because an accurate approximation for λ must cause the characteristic polynomial to be zero (by the definition of an eigenvalue), we set each term in the Taylor Series to zero and solved for X and Y . This procedure is an example of a perturbation analysis, and it resulted in equation (4.2).

Appendix 4.2. Selfing Function and Anther-Stigma Interference (Gaussian)

We chose a selfing function based on the criterion that a greater degree of pollen and ovule overlap would have a higher selfing rate (i.e. selfing is inversely related to the value of r_x). Specifically, we modeled the selfing rate as a Gaussian function, where,

$$S[r_x, t] = e^{-\frac{(t - \mu[r_{aa}])^2}{2\sigma[r_{aa}]^2}} S[0] \quad (4.2.1).$$

$S[r_x, t]$ has the shape of a normal distribution with a maximum selfing rate of $S[0]$, where $S[0]$ is the selfing rate for an adichogamous plant (i.e., $r_x = 0$) (Figure 4.3A). When $S[0] = 0$, no selfed seeds are produced regardless of the degree of overlap of pollen and ovule production (e.g., a self-incompatible species). When $0 < S[0] < 1$, a species is partially self-compatible and when $S[0] = 1$, all seeds are produced by selfing unless dichogamy evolves. The maximum selfing rate occurs at $t = \mu[r_{aa}]$ (4.2.1, Table 4.1, Figure 4.3A) where we assume only that $\mu[r_{aa}]$ and r_{aa} have the same sign. If selfing is at its highest rate when pollen and ovule overlap is greatest, $\mu[r_{aa}] = \frac{r_{aa}}{2}$. Conversely, if selfing is at its highest rate when pollen production is at its maximum, $\mu[r_{aa}] = r_{aa}$. Similarly, the temporal width (variance) of the selfing function is described by $\sigma[r_{aa}]^2$ (Table 4.1, Figure 4.3A). The parameter $\sigma[r_{aa}]$ describes how rapidly selfing declines from its maximum, which we assume to be proportional to the degree of dichogamy. As $\sigma[r_{aa}]$ approaches 0, selfing primarily occurs at points in time near μ , whereas selfing occurs at roughly an equal rate at all points in time as $\sigma[r_{aa}]$ approaches infinity (Figure 4.3A). For genotype x , the total fraction of selfed seeds over all points in time is described by the integral

$S[r_{aa}] = \int_{-\infty}^{\infty} e^{\frac{-(t-\mu[r_{aa}])^2}{2\sigma[r_{aa}]^2}} S[0] F_{aa} dt$ (Figure 4.2A). The derivative

$$\left. \frac{dS[r_x]}{dr_x} \right|_{x=r_{aa}} = \frac{-S[r_{aa}] \left(1 + \sigma[r_{aa}]^2 + \mu[r_{aa}]^2 (2 + 3\sigma[r_{aa}]^2) \right)}{2r_{aa} \left(1 + \sigma[r_{aa}]^2 \right)^2}$$
 is always negative, indicating

that the proportion of selfed seeds declines as a function of dichogamy.

We modeled anther-stigma interference by assuming that overlap in the timing of stigma elongation and anther dehiscence reduces pollen export for genotype aa by a

constant factor, $C[r_{aa}] = \int_{-\infty}^{\infty} e^{\frac{-(t-\mu[r_{aa}])^2}{2\sigma[r_{aa}]^2}} C[0] F_{aa} dt$, at all points in time. Assuming that

anther-stigma interference was constant over time simplified the analysis. We chose this

function for $C[r_x]$ so that the total amount of selfing and anther-stigma interference

would decline in a similar fashion as a function of r_x . In particular, both anther-stigma

interference, $C[r_x]$, and the total selfing rate, $S[r_x]$, are maximal when $r_{aa} = 0$, and their

ratio, $C[r_x] : S[r_x]$, is given by $C[0] : S[0]$. Anther-stigma interference can thus be

included in the model even when selfing is absent by setting $C[0] > 0$ and $S[0] = 0$ (e.g.,

in a self-incompatible species).

Chapter V – A phylogenetic analysis of pollination mode and the evolution of dichogamy in Angiosperms.¹

5.1 Introduction

Dichogamy has long been considered a mechanism that prevents inbreeding in hermaphrodite plants (Darwin 1877, Faegri and van der Pijl 1979). Recent studies suggest that the function of dichogamy may entail more than inbreeding avoidance (Lloyd and Webb 1986, Griffin *et al.* 2000, Harder *et al.* 2000, Fetscher 2001, Mallick 2001, Routley and Husband 2003). Here we examine the evidence for correlated evolution between the form of dichogamy and pollination mode in angiosperms.

Previous studies suggest a role for pollinator-mediated selection in the evolution of dichogamy (Wyatt 1983, Barrett 2003). Indeed, three comparative studies have noted a correlation across species in the mode of pollination and the form of dichogamy. A general survey of angiosperms (Bertin and Newman 1993) and a survey of British flora (Lloyd and Webb 1986) found protandry to be more prevalent than protogyny and observed that protandry was more common in biotically pollinated systems and protogyny more common in wind-pollinated systems. The prevalence of protandry is consistent with the hypothesis that the evolution of protogyny is subject to a developmental constraint, as it requires a reversal in the usual order of the development of floral organs (Waller 1988). However, aspects of reproductive function may also affect the incidence of alternate forms; Wyatt (1983) found protogyny to be twice as common as protandry among species pollinated by wind, beetles, and wasps as opposed to bees and flies.

¹ A version of this chapter has been published as “Sargent, R. D. and S. P. Otto. 2004. A phylogenetic analysis of pollination mode and the evolution of dichogamy in angiosperms. *Evol. Ecol. Res.* 6, 1183-1199.

Protogyny is hypothesized to be an ancestral trait in angiosperms (Endress 1997), as it tends to be clustered in basal angiosperm families (Henslow 1888, Bawa and Beach 1981, Thien et al. 2000). Protogyny is widely thought to have evolved as a means of preventing self-pollination (Lloyd and Webb 1986, Bertin 1993). In a protogynous flower, ovules may be fertilized by pollen from other sources before the flower presents its own pollen (Bawa and Beach 1981, Bertin 1993, Griffin et al. 2000). With protogyny, ovules that remain unfertilized by external sources can be fertilized by self-pollen, assuring some fertilization for self-compatible species (Herlihy and Eckert 2002). Because pollen can remain viable for a period of time in many species, protandry would appear less effective as an adaptation to avoid self-pollination within flowers (Bertin 1993, Barrett 2003). Rather, protandry, when combined with particular inflorescence architectures and stereotypical pollinator behavior, may be an adaptation that reduces self-pollination among flowers (geitonogamy) and the associated reduction in pollen available for export (Jordan 2000, Harder *et al.* 2001, Routley and Husband 2003; for an exception see McKone et al. 1995).

The occurrence of both forms of dichogamy correlates with a variety of ecological conditions (such as latitude and altitude), mating system, pollination mode, and flower size (reviewed by Bertin and Newman 1993). Ecological correlates of protandry and protogyny are difficult to interpret, because both traits also have strong associations with certain plant families, so that many of the patterns described above appear to be at least partly explained by phylogenetic relatedness. In several cases, one form of dichogamy appears to be characteristic of an entire family. For example, protogyny is more common than protandry in monocots (Lloyd and Webb 1986).

Previous studies of the correlates of dichogamy have used species as the unit of comparison, without formal consideration of the evolutionary relationships among these species.

Comparative analyses examining correlated characters across species can pose problems because statistical tests rely on the assumption that observations (data points) are independent. However, data obtained from related species are not independent because the species may have inherited the trait from a common ancestor, rather than having evolved the trait independently, as comparative tests assume (Felsenstein 1985).

Here we test whether transitions between forms of dichogamy evolve in association with specific modes of pollination after accounting for phylogenetic relatedness. We use Pagel's (1994) maximum likelihood program (Discrete) to test the direction of transitions between protandry, protogyny, adichogamy and between biotic and abiotic pollination. In addition, we introduce a new application of Discrete that allowed us to test the hypothesis that pollination mode drives the evolution of dichogamy rather than dichogamy driving the evolution of pollination mode.

5.2 Methods

To test for across-species associations of traits requires a phylogeny, preferably one that is well resolved (Pagel 1994). We used Soltis et al.'s (2000) phylogeny (their figures 1-12B) of relationships among angiosperm taxa. For each hypothesis tested, we pruned the tree according to the species for which we had the necessary data. We then mapped character data for both traits (i.e. pollination mode and dichogamy type) onto the tree. Although this study involves an incomplete sampling of angiosperm taxa, Discrete does not require a complete phylogeny, because it infers the most likely transition rates along those branches that are included in the phylogeny of sampled taxa. As long as taxa are sampled randomly with respect to the traits of interest (as we expect to be true in this study), incompleteness of a phylogeny does not introduce evidence for a correlation if, in fact, the traits have evolved independently. If, on the other hand,

the traits truly evolved in a correlated manner, the power to detect this correlation is reduced by using a less complete phylogeny.

5.2.1 Data Collection

We collected dichogamy and pollinator data for as many species from the Soltis et al. (2000) angiosperm phylogeny as we could find. Much of the data came from a database kindly provided by R. Bertin. The remaining data were found through literature searches. When data for these species were unavailable, we substituted data from closely related species whose phylogenetic position is expected to be similar and for which data were available ($N = 8$). The substituted species were identified using genus and family level phylogenies (see Appendix 5.1). If no family phylogeny or dichogamy/pollination data existed for a species within the same family the species was omitted from the analysis. Depending on the set of characters being tested, different versions of the 'pruned' tree were used to test hypotheses. Of the 560 angiosperm species used to build the Soltis et al. (2000) tree, we obtained sufficient data for 170 species (Appendix 5.1).

5.2.2 Testing for Correlated Evolution

We used Discrete (Pagel 1994) to test for correlated evolution between dichogamy type and pollination mode. Discrete uses maximum likelihood to estimate instantaneous rates of evolution between combinations of states (Figure 5.1). The method estimates transition rates between traits with two discrete states using a continuous-time Markov model. Because the model calculates transition probabilities across all possible character states at each node, hypothesis testing does not require assignment of ancestral states, which are often difficult to infer (Schluter *et al.* 1997).

Discrete calculates the likelihood of two models of evolutionary change for the traits. The four-parameter independent transition model assumes that the two traits (type of dichogamy

and pollination mode) evolve independently. We used a modified independent model that did not allow simultaneous changes in both traits but assumed that the transition rates between states of trait X are independent of the state of trait Y and vice versa (Figure 5.1: $q_{12} = q_{34}$; $q_{21} = q_{43}$; $q_{42} = q_{31}$; $q_{13} = q_{24}$), leaving four parameters. The subscripts for the transition rates indicate the beginning and end states for the X (dichogamy type) and Y (pollination mode) traits, where 1=0,0; 2=0,1; 3=1,0; 4=1,1. Discrete identifies the most likely parameter combination consistent with the observed traits of the species, yielding a log likelihood estimate, $L(I_4)$, for the independent model. The eight-parameter dependent transition model involved no restriction on transition rates, yielding a log likelihood estimate, $L(D_8)$. The likelihood ratio ($LR = 2(L(D_8) - L(I_4))$) measures the extent to which the dependent model fits the data better than the independent model. The significance of the LR can be tested either by comparing it to the χ^2 distribution ($df = 4$) or by Monte Carlo simulation. The search for a maximum likelihood parameter combination was repeated a minimum of 20 times to decrease the chance of accepting a local rather than a global maximum likelihood.

5.2.3 Testing for Directionality of the Correlation

Here we introduce a new method that uses the transition rates estimated by Discrete to detect directionality in the correlated evolution of two traits. In its original version (Pagel 1994), Discrete tested for directionality by fixing two transition rates equal to each other (e.g. $q_{12} = q_{34}$) and determining whether this restriction significantly reduced the log likelihood estimate $L(D_7)$ compared to that of the unrestricted model $L(D_8)$. If the likelihood ratio test finds that the restricted model differs significantly from the full model, the alternative hypothesis is accepted, e.g. $q_{12} \neq q_{34}$, which is interpreted to mean that the state of trait X influences the direction of evolution of trait Y (Pagel 1994). However, it is possible that the rate (the frequency of transitions between the two states), rather than the direction of transitions, is influenced by the

other trait. For example, imagine that transitions from wind pollination to biotic pollination along protandrous branches occur at a much higher rate than along protogynous branches (e.g., $q_{34}=10$ and $q_{12}=1$). One could interpret this to mean that selection for biotic pollination is higher with protandry than with protogyny. However, if the reverse transition rate, biotic pollination to wind pollination, is also higher with protandry than with protogyny (e.g., $q_{43}=10$ and $q_{21}=1$), then the above interpretation would be incorrect. Instead, one should conclude that form of dichogamy does not influence the direction of evolution of pollination mode, but that protandry causes transitions in both directions to occur at a higher rate than protogyny.

To account for the possibility that transition rates are affected by the state of the other trait, M. Pagel modified Discrete for us to test for directionality in evolution using a rate contingency test. Specifically, under the null hypothesis that trait X (e.g., form of dichogamy) does not influence the direction of evolution of trait Y (e.g., pollination mode), we expect $q_{12} q_{43} = q_{21} q_{34}$ even if trait X influences the rate of transitions among states of Y (Figure 5.1). Similarly, one can test whether trait Y influences the direction of evolution of trait X by restricting $q_{13} q_{42} = q_{31} q_{24}$. Note that this reduces the four directionality tests described by Pagel (1994) to two rate contingency tests.

We tested the importance of individual transition rate values (q_{ij}) to the overall likelihood of the dependent model by setting each parameter to zero, rerunning the model, and determining whether this restriction significantly reduced the log likelihood estimate $L(D_7)$ when compared to that of the unrestricted dependent model $L(D_8)$.

5.2.4 Hypothesis Testing

The motivation for this study was to test for a correlation between dichogamy and pollination mode while controlling for phylogenetic relatedness among species. The Discrete program tests for correlated evolution between traits with two discrete categories only.

Unfortunately, the traits of interest, mode of pollination and dichogamy, do not easily fall into dichotomous categories. For example, species may be protandrous, protogynous, heterodichogamous (populations consisting of both protandrous and protogynous individuals) or adichogamous (flowers lacking appreciable temporal separation of anther and stigma presentation). Because of this constraint, we used Discrete to test a series of hypotheses that considered different groupings of species. For each hypothesis, different subsets of the data in Appendix 5.1 were used. Where correlated evolution was detected between two traits, we tested the importance of individual transition rates as described above and in Table 5.4.

1. Protandry vs. protogyny and biotic vs. abiotic pollination

The species for which dichogamy and pollination data were available (Appendix 5.1) were identified as protandrous or protogynous species. The mode of pollination for these species was determined to be primarily biotic (bee, beetle, bird, fly, mammal, moth or wasp) or abiotic (wind or, in one case water). Because some of the species were adichogamous, heterodichogamous or obligate selfers, the sample size for this test dropped to $N = 126$.

2. Protandry vs. non-protandry and biotic vs. abiotic pollination

Species were divided into those that displayed protandry and those that were adichogamous, protogynous, or heterodichogamous. Species were placed into the pollinator categories biotic or abiotic (described previously). The sample size for this test was $N = 167$ (excluding autogamous species).

3. Protogyny vs. non-protogyny and biotic vs. abiotic pollination

Species were divided into those that displayed protogyny and those that were adichogamous, protandrous, or heterodichogamous. Species were placed into the pollinator categories biotic or abiotic (described previously). The sample size for this test was also $N = 167$ (excluding autogamous species).

4. *Dichogamy vs adichogamy and bird/ autogamous vs. other pollination*

Species were divided into those that displayed dichogamy (including heterodichogamy) and those that were adichogamous. Pollination categories were grouped into two categories: species that are bird-pollinated or obligately autogamous and other species (insect, mammal or wind-pollinated). Obligate autogamy has been hypothesized to be associated with adichogamy (Runions and Geber 2000). Similarly, bird pollination is not as effective at reducing geitonogamy in dichogamous species (Harder *et al.* 2001), and thus selection for dichogamy could be reduced if a species experiences a change from insect to bird pollination (see discussion). The sample size for this test was $N = 170$.

5.3 Results

Because all previously reported correlations are based on samples of angiosperm taxa that differ from our own, we first examined whether dichogamy was associated with pollination mode within our dataset regardless of phylogenetic considerations. As in previous studies, protogyny was more common among species exhibiting abiotic (primarily wind) pollination and protandry more common among biotically pollinated species (Table 5.1, $\chi^2 = 15.1$, $df = 1$, $P < 0.001$).

1. *Protandry vs. protogyny and biotic vs. abiotic pollination*

A likelihood ratio test found that dichogamy type (protandry or protogyny) and pollination mode (biotic or abiotic) did not evolve independently (Table 5.2: $P < 0.001$). Monte Carlo simulations (100 replicates) confirmed that a model of correlated evolution fits the data better ($P = 0.01$). The transition rates predict that protogyny and abiotic pollination should become positively associated over time (as they are in the raw data; Table 5.1), as should protandry and biotic pollination. We found no effect of the pollination mode (biotic or abiotic) on the direction of evolution of the type of dichogamy (protandry or protogyny, Table 5.3: $P =$

0.15). In contrast, dichogamy type (protandry or protogyny) affected the direction of evolution of pollination mode ($P = 0.018$). Specifically, the transition rates from abiotic to biotic pollination along protandrous branches (q_{34}) and from biotic to abiotic pollination along protogynous branches (q_{21}) were significantly higher than expected from the opposite transition rates (Table 5.3, Figure 5.1). Table 5.4 shows the likelihood of the eight possible alternative models when compared to the dependent model. The phylogeny examined provides the least evidence for transitions from abiotic to biotic pollination among protogynous species (Figure 5.1, Table 5.4).

2. *Protandry vs. non-protandry and biotic vs. abiotic pollination*

We were unable to reject the hypothesis that protandry versus non-protandry evolved independently of pollination mode (biotic or abiotic; Table 5.2, $P = 0.34$). Monte Carlo simulations (100 replicates) confirmed that a correlated model of evolution fails to fit the data better ($P = 0.56$).

3. *Protogyny vs. non-protogyny and biotic vs. abiotic pollination*

Protogyny versus non-protogyny did not evolve independently from pollination mode (biotic or abiotic; Table 5.2, $P = 0.025$). Monte Carlo simulations (100 replicates) confirm that a model of correlated evolution fits the data better ($P = 0.02$). In this case, the transition rates predict that protogyny and abiotic pollination should become positively associated over time (as they are in the raw data; Table 5.1), as should non-protogyny and biotic pollination. A rate contingency test (Table 5.3) found no evidence that protogyny versus non-protogyny affects the direction of the evolution of pollination mode (biotic or abiotic) or that pollination mode (biotic or abiotic) affects the direction of evolution of dichogamy.

4. *Dichogamy vs. adichogamy and bird/autogamous vs. other pollination*

Adichogamy versus dichogamy did not evolve independently of pollination mode (bird/autogamy vs. other pollination modes; Table 5.2, $P = 0.03$). The rate contingency tests suggested that the type of pollination affected the direction of evolution of dichogamy more than the converse, but neither test was significant (Table 5.3). The transition rates predict that adichogamy and bird pollination should become positively associated over time (as they are in the raw data; Table 5.1), as should dichogamy and other pollination modes. Note that when the correlations are tested separately (i.e. bird vs. other pollination modes or autogamy vs. other pollination modes), neither correlation is significant, reflecting a loss of power.

5.4 Discussion

Associations between dichogamy and pollination appear to be the product of convergent evolution rather than artefacts of phylogenetic relatedness. Tests revealed that the pollination mode (abiotic or biotic) in angiosperms was evolutionarily correlated with the type of dichogamy (protandry or protogyny). Specifically, species that are abiotically pollinated (primarily via wind) are more likely to be protogynous whereas biotically pollinated species are more likely to be protandrous, confirming the results of previous studies (Wyatt 1983, Lloyd and Webb 1986, Bertin and Newman 1993). However, only the evolution of protogyny vs. non-protogyny correlates with pollination mode, with protogyny being strongly correlated with abiotic pollination (Table 5.1, Figure 5.2). We found that the proportions of biotically and abiotically pollinated species are similar among protandrous and adichogamous species (Table 5.1, Figure 5.2). It is therefore not surprising that the association between abiotic pollination and protogyny is strong when contrasted against adichogamous and protandrous species, whereas the association between biotic pollination and protandry disappears when contrasted against adichogamous and protogynous species. Several previous studies compared only protandrous

and protogynous species, and did not compare either to adichogamous species (e.g. Wyatt 1983, Lloyd and Webb 1986). Bertin and Newman (1993) calculated the mean degree of dichogamy (using a continuous scale ranging from protandry through adichogamy to protogyny) for species with different types of pollinators and determined that on average, insect-pollinated species fell between protandry and adichogamy, because insect-pollinated species frequently fall into both categories. This is consistent with our results that transitions from non-protandry to protandry do not depend on pollination mode, in contrast to transitions from non-protogyny to protogyny.

The tests of the causes of the correlations reveal new information about the evolutionary forces acting on dichogamy. We found strong support for correlated evolution between protogyny and abiotic pollination. This correlation is most often explained by strong selection for protogyny in wind-pollinated taxa to prevent self-fertilization (Lloyd and Webb 1986, Barrett 2003). However, we found no evidence that either biotic or abiotic pollination drives increased transitions from protogyny to non-protogyny or the reverse. This could be due to reduced power to detect the cause of correlated evolution because of our use of an incomplete phylogeny. The only significant contingency test found that transitions from biotic to abiotic pollination were more likely among protogynous species than among protandrous species. One explanation for this result is that protogynous species are perhaps less likely than protandrous species to suffer from increased self-fertilization upon a switch to wind pollination.

The results of the rate contingency test are not, however, robust to the inclusion/exclusion of different sub-samples of species. In particular, we tested whether the removal of *Zostera*, the only water-pollinated species in our analysis, altered the result that transitions from biotic to abiotic pollination were more likely among protogynous species than among protandrous species. We found that the result was sensitive to the inclusion of *Zostera* and was no longer significant when *Zostera* was excluded. Rather, in the absence of *Zostera*, there was significant

evidence for the hypothesis that the state of the pollination system affects which type of dichogamy evolves. The reason that the results of the rate contingency test are particularly sensitive to the presence of *Zostera* might be because *Zostera* is an abiotically-pollinated, protogynous species that falls in a large clade of biotically-pollinated, protogynous species. Its phylogenetic position thus shifts the evidence in favor of the hypothesis that the form of dichogamy drives the pollination system, rather than the reverse. In contrast to the results of the rate contingency test, there was significant evidence for correlated evolution regardless of whether or not *Zostera* was included in the analysis.

The association between bird pollination or autogamy and adichogamy provides evidence that the evolutionary interaction between dichogamy and pollination mode is specific to particular types of pollinators and/or floral forms. Movement patterns of bird pollinators on inflorescences differ from those of insects. Insects visiting vertical inflorescences tend to consistently start at either the bottom (in the case of many bees, wasps and moths) or top of the inflorescence (flies) and move upwards or downwards, respectively (Harder *et al.* 2001). In contrast, hummingbirds have been shown to move upwards or downwards on inflorescences with approximately equal frequency (Healy and Hurly 2001). Because movement of bird pollinators among early developing and late developing flowers is less stereotypical, selection on dichogamy to reduce self-pollination among flowers on a plant (geitonogamy) is less effective. This reasoning explains, perhaps, why fewer bird-pollinated species are dichogamous. It is also predicted that obligately autogamous plants should reduce the temporal separation in anther and stigma development to increase the probability of self-fertilization (Runions and Geber 2000). In our study, all three of the obligately autogamous plants were adichogamous. These results should be treated with caution, however, because there were not many bird-pollinated or

obligately autogamous plant species within our study, and their association with adichogamy is not significant when investigated separately.

We were unable to detect any effect of pollination mode (abiotic or biotic) on the transition from non-protandry to protandry (or the reverse), suggesting that this transition may not be as dependent on pollination by animals as has been previously hypothesized (Lloyd and Webb 1986, Wyatt 1983, Bertin and Newman 1993). Indeed, in this and prior studies the association between protandry and biotic pollination is weaker than the association between protogyny and abiotic pollination (Table 5.1; Figure 5.2). A possible explanation for a lack of correlation between protandry and biotic pollination is that forcing the data into the false dichotomy of biotic pollination (which included many types of insects as well as mammals and birds) vs. abiotic pollination may have obscured the true patterns. For example, selection by beetle pollinators may be as different from bee pollinators as they both are from abiotic pollination. Unfortunately, there is no program currently available to detect correlated evolution between characters with more than two states.

One limitation of our methodology is that the tests performed assume that the traits do not affect speciation or extinction rates. However, wind pollination is associated with lower species diversity than biotic pollination (Eriksson and Bremer 1992, Dodd et al. 1999); if this pattern were due to higher extinction of wind-pollinated and non-protogynous species, extinctions rather than transition rate differences could account for the correlations we found. A further caveat of all correlative studies is that the traits in question may in fact be correlated with other traits that are actually responsible for the observed patterns. Other traits that may be correlated with dichogamy include latitude (e.g. protogynous species tend to be found in alpine zones), breeding system (e.g. protogyny is more common among self-compatible species), floral traits (e.g. short-lived flowers are more likely to be protandrous) and many others (Bertin and

Newman 1993). Clearly we have omitted several potential correlates by focusing only on pollination system. However, of the correlates listed in a thorough review by Bertin and Newman (1993), one third are traits related to pollination system. Indeed, the type of pollinator is widely invoked as part of the functional explanation for the existence and form of dichogamy (Wyatt 1983, Lloyd and Webb 1986). Future studies should expand the focus to other candidate traits. A final caveat is that the type of dichogamy has not always held up to experimental validation. Griffin et al. (2000) discovered that *Aquilegia canadensis*, a species considered protogynous in the literature, was functionally adichogamous. If researchers are more likely to label wind-pollinated species as protogynous, such a bias could contribute to the correlations observed in this study.

In conclusion, this study uses phylogenetic evidence to confirm the existence of an association between dichogamy and pollination type. Across the phylogeny of angiosperms, we find little support for the hypothesis that the type of pollinator drives selection for either protandry or protogyny. Rather, our results suggest that the dichogamy may influence the pollination mode that evolves. We also present results that call into question the validity of previous reports of a correlation between biotic pollination and protandry. Protandry does not appear to be significantly more correlated with biotic pollination systems than all other breeding systems considered together. Instead the dichogamy type with an unusual pollination mode is protogyny (and perhaps heterodichogamy), which differs from all other forms in being commonly (but not predominantly) found in wind-pollinated systems.

5.5 Literature Cited

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Table 5.1 Type of pollination and direction of dichogamy for species used in the current study and by Bertin and Newman (1993).

	Protandry ¹	Protogyny ¹	A-dichogamy ¹	Hetero-dichogamy ¹	Protandry ²	Protogyny ²
Abiotic	2	12 ³	1	3	17	133
Insect	62	43	27	3	865	437
Bird	3	1	5	1		
Autogamy	0	0	3	0		
Mammal	1	2	1	0		
Total	68	58	37	7	882	570

¹Indicates species from the current study.

²Indicates species taken from Bertin and Newman 1993.

³All of these species are wind-pollinated except *Zostera marina*, which is water-pollinated.

Table 5.2 Results from tests of independence of two characters using Discrete. *P*-values for likelihood ratio tests (*LR*) are based on a χ^2 distribution with *df* = 4. *P*-values for Monte Carlo simulation are based on *N*=100 replicates.

Test	LI ₄	LD ₈	LR (<i>P</i>)	<i>P</i>
Protogyny/Protandry vs. Abiotic/Biotic	-110.8	-101.4	18.8 (<0.001)	0.01
Protandry/Other vs. Abiotic/Biotic	-151.3	-149.0	4.5 (0.34)	0.56
Protogyny/Other vs. Abiotic/Biotic	-134.5	-128.9	11.2(0.024)	0.02
Dichogamy/Adichogamy vs. Bird/Other	-134.5	-129.1	10.7 (0.03)	N/A

Table 5.3 Comparison of transition rates between type of pollination and form of dichogamy.

The null hypothesis is that the specified transition rates are equal. The test statistic (e.g. $-2(L(D_8) - L(q_{ab}/q_{ba} = q_{cd}/q_{dc}))$) has an approximate chi-square distribution with one degree of freedom.

Test	Hypothesis	LR	P	Description
Protogyny/Protandry vs. Abiotic/Biotic	$\frac{q_{12}}{q_{21}} \neq \frac{q_{34}}{q_{43}}$	5.56	0.018	Dichogamy type (protandry (1) or protogyny (0)) affects the direction of evolution of pollination mode (biotic (1) or abiotic (0)).
			$\frac{q_{12}}{q_{21}} < \frac{q_{34}}{q_{43}}$	
	$\frac{q_{13}}{q_{31}} \neq \frac{q_{24}}{q_{42}}$	2.06	0.15	Pollination mode (biotic or abiotic) has no effect on the direction of evolution of dichogamy type (protandry or protogyny).
Protogyny/ Adichogamy vs. Abiotic/Biotic	$\frac{q_{12}}{q_{21}} \neq \frac{q_{34}}{q_{43}}$	0.38	0.54	Dichogamy type (non-protogyny (0) or protogyny (1)) has no effect on the direction of evolution pollination mode (biotic (0) or abiotic (1)).
	$\frac{q_{13}}{q_{31}} \neq \frac{q_{24}}{q_{42}}$	1.18	0.28	Pollination mode (biotic or abiotic) has no effect on the direction of evolution of dichogamy type (non-protogyny or protogyny).
Dichogamy/ Adichogamy vs. Bird/Other	$\frac{q_{12}}{q_{21}} \neq \frac{q_{34}}{q_{43}}$	2.1	0.15	Presence of dichogamy (adichogamy (1) or dichogamy (0)) has no effect on the direction of evolution of the pollination system (bird/autogamy (0) or other (1)).
	$\frac{q_{13}}{q_{31}} \neq \frac{q_{24}}{q_{42}}$	2.1	0.15	Pollination mode (bird/autogamy or other) has no effect on the direction of evolution of dichogamy (adichogamy or dichogamy).

Table 5.4. Likelihood values for models in which one transition rate is excluded, compared to the likelihood of the full eight-parameter model of dependent evolution between dichogamy (protandry or protogyny) and pollinator type (biotic or abiotic). Stars indicate level of significance (see Figure 5.1).

Model Description	L(D ₇)	Likelihood Ratio	P
$q_{12} = 0$	101.7	0.440	0.5
$q_{13} = 0$	105.0	7.12	0.008**
$q_{21} = 0$	104.8	6.72	0.01**
$q_{24} = 0$	103.1	3.26	0.07*
$q_{31} = 0$	103.3	3.62	0.06*
$q_{34} = 0$	104.0	5.24	0.02*
$q_{42} = 0$	107.2	11.5	<0.001***
$q_{43} = 0$	103.1	3.32	0.07*

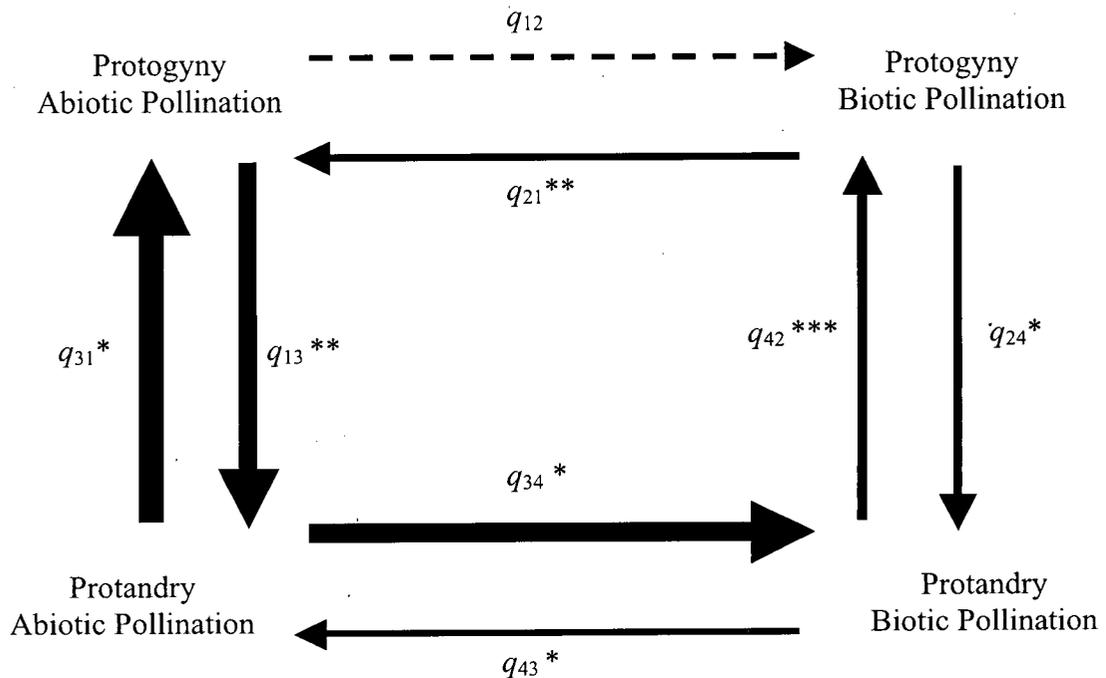


Figure 5.1. Transition rates between two forms of dichogamy and two types of pollinators. The q_{ij} 's indicate transition rate parameters; in this example, 1 = protogyny, abiotic-pollination; 2 = protogyny, biotic-pollination; 3 = protandry, abiotic-pollination; 4 = protandry, biotic-pollination. Dashed lines indicate transition rates that are not significantly different from zero ($P > 0.1$). Line thickness indicates the \log_e of the relative size of the transition rates in the full (LD_8) dependent model with the highest likelihood ratio (LR). Stars indicate that the transition rate differs from zero, where * indicates marginal significance ($P < 0.1$); ** indicates significance at $P < 0.01$; *** indicates significance at $P < 0.001$.

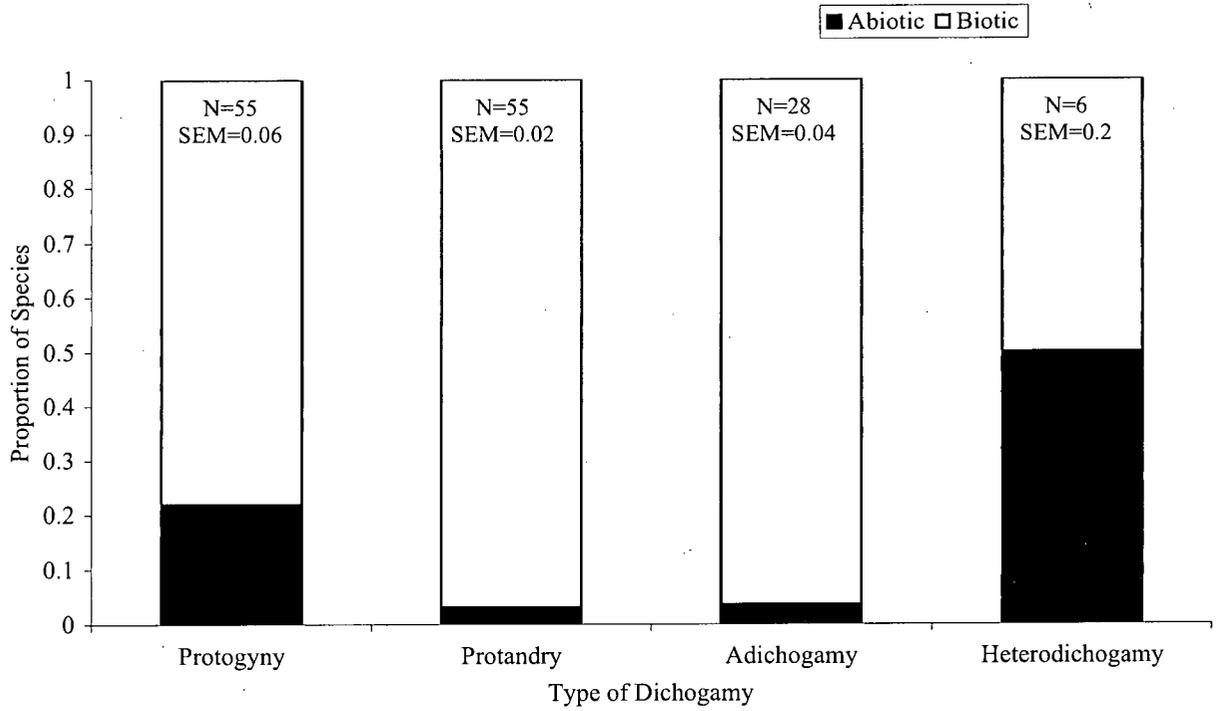


Figure 5.2. Proportion of species from Appendix 5.1 with abiotic (solid portion) and biotic (open portion) pollination as a function of the form of four types of dichogamy.

Appendix 5.1: Species and traits used for analyses. Numbers in parantheses indicate the number of species in the genus that were found to have the dichogamy and pollinator combination listed out of total number of species in the genus (from Mabberley 1997). It is possible that more species in the genus fit the pattern; the dichogamy data (in particular) is difficult to find and thus the values should be interpreted as the minimum for the genus. † Indicates species that were substituted for species on the Soltis et al. (2000) tree; original species is indicated in parantheses. Data is from a data set compiled by R. Bertin unless otherwise indicated.

Family	Species	Type of Dichogamy	Primary Pollinator
Acanthaceae	<i>Justicia squarrosa</i>	Protandry	Bee
	<i>Thunbergia grandiflora</i>	Adichogamy	Bee
Agavaceae	<i>Agave sp.</i>	Protandry	Insect
Aizoaceae	<i>Tetragonia expansa</i> †	Protogyny	Insect
	(<i>Delosperma echinatum</i>)		
Alliaceae	<i>Allium fistulosum</i>	Protandry	Bee
Amaranthaceae	<i>Amaranthus retroflexus</i> †	Protogyny	Wind
	(<i>Celosia argentea</i>)		
Annonaceae	<i>Annona sp.</i> (11/137)	Protogyny	Beetle
	<i>Asimina triloba</i>	Protogyny	Fly
Apiaceae	<i>Apium graveolens</i>	Protandry	Fly
Apocynaceae	<i>Vinca major</i> †	Adichogamy	Fly
	(<i>Apocynum androsaemifolium</i>)		
	<i>Nerium oleander</i>	Adichogamy	Insect
Araceae	<i>Spathiphyllum friedrichsthalli</i>	Protogyny	Bee
Araliaceae	<i>Hedera helix</i>	Protandry	Insect
	<i>Panax quinquefolius</i>	Protandry	Bee/Fly
	<i>Iriarteia sp.</i> (2/2)	Protandry	Bee
Arecaceae	<i>Sabal sp.</i> (3/16)	Protogyny	Fly
	(<i>Phoenix canariensis</i>)		
Aristolochiaceae	<i>Asarum canadense</i>	Protogyny	Fly
	<i>Aristolochia sp.</i>	Protogyny	Fly
Balsaminaceae	<i>Impatiens capensis</i>	Protandry	Bee
Begoniaceae	<i>Begonia sp.</i> (3/900)	Protandry	Insect
Betulaceae	<i>Alnus sp.</i> (2/25)	Protogyny	Wind
Bignoniaceae	<i>Campsis radicans</i>	Protandry	Bird
	<i>Catalpa speciosa</i>	Adichogamy	Insect
Blandfordiaceae	<i>Blandfordia grandiflora</i>	Protandry	Bee

Boraginaceae	<i>Hydrophyllum appendiculatum</i>	Protandry	Bee
	<i>Borago officinalis</i>	Protandry	Bee
Brassicaceae	<i>Brassica sp. (5/35)</i>	Protogyny	Bee/Fly
Bromeliaceae	<i>Aechmea lasserii</i>	Adichogamy	Bird
	<i>Tillandsia ixioides</i> [†] (<i>Glomeropitcairnia penduliflora</i>)	Protandry	Bird
	<i>Pitcairnia altensteinii</i> [†] (<i>Puya raimondii</i>)	Adichogamy	Insect
Buxaceae	<i>Buxus sempervirens</i>	Protogyny	Bee/Fly
Cabombaceae	<i>Brasenia schreberi</i>	Protogyny	Wind
	<i>Cabomba caroliniana</i>	Protogyny	Insect
Calycanthaceae	<i>Calycanthus floridus</i>	Protogyny	Beetle
	<i>Chimonanthus fragrans</i> [†] (<i>Idiospermum australiense</i>)	Protogyny	Beetle
Campanulaceae	<i>Campanula trachelium</i>	Protandry	Insect
	<i>Lobelia angulata</i>	Protandry	Bee
Caprifoliaceae	<i>Sambucus nigra</i>	Adichogamy	Insect
	<i>Symphoricarpos alba</i>	Adichogamy	Insect
	<i>Viburnum opulus</i>	Adichogamy	Insect
Caryophyllaceae	<i>Stellaria media</i>	Protandry	Fly
Celastraceae	<i>Euonymus europaeus</i>	Protandry	Fly
Chloranthaceae	<i>Chloranthus spicatus</i>	Protogyny	Thrips
	<i>Sarcandra sp. (2/2)</i>	Protogyny	Thrips
Chrysobalanaceae	<i>Maranthes polyandra</i> [†] (<i>Licania tomentosa</i>)	Protandry	Insect
Cistaceae	<i>Helianthemum sp.</i> (4/110)	Protogyny	Bee/Fly
Clusiaceae	<i>Hypericum perforatum</i>	Protandry	Insect
Colchicaceae	<i>Colchicum autumnale</i>	Protogyny	Bee/Fly
Combretaceae	<i>Quisqualis indica</i>	Adichogamy	Moth/Bee
Commelinaceae	<i>Tradescantia virginiana</i>	Protandry	Bee
Convallariaceae	<i>Convallaria majalis</i>	Protandry	Bee
Convolvulaceae	<i>Ipomoea purpurea</i>	Protogyny	Bee
	<i>Convolvulus arvensis</i>	Heterodichogamy	Insect
Coriariaceae	<i>Coriaria myrtifolia</i>	Protogyny	Wind
Costaceae	<i>Costus spiralis</i>	Adichogamy	Bird
Cucurbitaceae	<i>Cucumis sativus</i>	Protandry	Insect
	<i>Cucurbita pepo</i>	Protandry	Insect
Cyclanthaceae	<i>Cyclanthus bipartus</i>	Protogyny	Beetle
	<i>Sphaeradenia hamata</i>	Protogyny	Beetle
Cyperaceae	<i>Cyperus sp. (2/300)</i>	Protogyny	Wind
Datisceae	<i>Datisca glomerata</i>	Protogyny	Wind
Degeneriaceae	<i>Degeneria vitiensis</i>	Protogyny	Beetle
Dipsacaceae	<i>Dipsacus silvestris</i>	Protandry	Bee/Fly

Dipsacaceae	<i>Scabiosa sp.</i>	Protandry	Insect
Droseraceae	<i>Drosera sp.</i> (2/110)	Adichogamy	Bee/Fly
Elaeagnaceae	<i>Elaeagnus angustifolia</i>	Protandry	Insect
Elaeocarpaceae	<i>Elaeocarpus hookerianus</i>	Protandry	Bee
Ericaceae	<i>Arbutus unedo</i>	Protogyny	Insect
Euphorbiaceae	<i>Euphorbia sp.</i> (9/2000)	Protandry	Bee/Fly
Fabaceae	<i>Bauhinia unguolata</i>	Protogyny	Bat
Fagaceae	<i>Quercus sp.</i> (5/400)	Protogyny	Wind
Frankeniaceae	<i>Frankenia sp.</i> (2/81)	Protandry	Insect
Geraniaceae	<i>Geranium sanguineum</i>	Protandry	Bee/Fly
Geraniaceae	<i>Pelargonium sp.</i> (14/280)	Protandry	Fly
Graminae	<i>Zea mays</i>	Heterodichogamy	Wind
Gunneraceae	<i>Gunnera sp.</i> (2/40)	Protogyny	Wind
Haloragidaceae	<i>Myriophyllum exalbescens</i>	Protogyny	Wind
Heliconiaceae	<i>Heliconia sp.</i> (10/~150)	Adichogamy	Bird
Hydrangeaceae	<i>Philadelphus coronarius</i>	Protogyny	Insect
Illiciaceae	<i>Illicium parviflorum</i>	Protogyny	Fly
Iridaceae	<i>Gladiolus sp.</i> (2/195)	Protandry	Bee/Fly
Juncaceae	<i>Juncus effuses</i>	Protogyny	Wind
Juglandaceae	<i>Carya sp.</i> (3/14)	Heterodichogamy	Wind
Juglandaceae	<i>Juglans sp.</i> (3/21)	Heterodichogamy	Wind
Lamiaceae	<i>Clerodendrum thomsoniae</i>	Protandry	Bee/Fly
Lamiaceae	<i>Lamium amplexicaule</i>	Adichogamy	Autogamy
Lauraceae	<i>Cinnamomum camphora</i>	Protogyny	Insect
Lentibulariaceae	<i>Pinguicula vulgaris</i>	Adichogamy	Autogamy
Liliaceae	<i>Clintonia borealis</i>	Protogyny	Bee
Liliaceae	<i>Scilla sp.</i> (2/40)	Adichogamy	Bee
Limnanthaceae	<i>Floerkea proserpinicoides</i>	Adichogamy	Autogamy
Limnanthaceae	<i>Limnanthes douglassii</i>	Protandry	Bee
Linaceae	<i>Linum sp.</i> (3/36)	Protandry	Bee/Fly
Loasaceae	<i>Caiphora laterita</i>	Protandry	Bee
Lowiaceae	<i>Orchidantha inouei</i>	Adichogamy	Beetle
Lythraceae	<i>Lythrum salicaria</i>	Adichogamy	Bee
Magnoliaceae	<i>Magnolia tripetala</i>	Protogyny	Beetle
Malvaceae	<i>Theobroma cacao</i>	Adichogamy	Fly
Malvaceae	<i>Tilia americana</i>	Protandry	Fly
Marantaceae	<i>Calathea timothei</i>	Adichogamy	Bird
Meliaceae	<i>Swietenia mahagoni</i>	Protandry	Insect
Moraceae	<i>Ficus sp.</i> (20/750)	Protogyny	Insect
Moraceae	<i>Morus alba</i>	Adichogamy	Wind
Musaceae	<i>Musa acuminata</i>	Protogyny	Mammal
Myrsinaceae	<i>Ardisia escallenooides</i>	Protogyny	Bee
Nelumbonaceae	<i>Nelumbo lutea</i>	Protogyny	Insect

Nyctaginaceae	<i>Mirabilis jalapa</i>	Protogyny	Insect
Nymphaeaceae	<i>Nuphar variegata</i>	Protogyny	Insect
	<i>Nymphaea odorata</i>	Protogyny	Insect
Oleaceae	<i>Jasminum sp.</i> (2/200)	Protandry	Insect
Onagraceae	<i>Clarkia xantiana</i>	Protandry	Bee
	<i>Chamerion angustifolium</i>	Protandry	Bee
Orchidaceae	<i>Cypripedium calceolus</i>	Adichogamy	Bee
Oxalidaceae	<i>Oxalis stricta</i>	Adichogamy	Bee
Paeoniaceae	<i>Paeonia californica</i>	Protogyny	Insect
Papaveraceae	<i>Dicentra spectabilis</i>	Adichogamy	Bee
Parnassiaceae	<i>Parnassia palustris</i>	Protandry	Fly
Passifloraceae	<i>Passiflora quadrangalis</i>	Protandry	Bee
Pedaliaceae	<i>Proboscidea louisianica</i>	Protandry	Bee
	<i>Sesamum indicum</i>	Adichogamy	Insect
Piperaceae	<i>Piper nigrum</i>	Protogyny	Wind
Plantaginaceae	<i>Plantago lanceolata</i>	Protogyny	Wind
Plumbaginaceae	<i>Limonium sp.</i>	Protandry	Bee
Poaceae	<i>Oryza sativa</i>	Protandry	Wind
Polemoniaceae	<i>Cobaea scandens</i>	Protandry	Bat
	<i>Gilia sp.</i> (8/25)	Protandry	Bee/Fly
	<i>Phlox sp.</i> (5/67)	Protandry	Moth
Portulacaceae	<i>Portulaca sp.</i> (2/40)	Adichogamy	Insect
Primulaceae	<i>Anagallis tenella</i>	Adichogamy	Insect
	<i>Androsace sp.</i> (4/100)	Adichogamy	Insect
Ranunculaceae	<i>Coptis trifoliata</i>	Protandry	Insect
	<i>Ranunculus sardous</i>	Protogyny	Bee/Fly
Resedaceae	<i>Reseda sp.</i> (2/60)	Protandry	Bee
Rhamnaceae	<i>Ceanothus thrysofloris</i>	Protandry	Insect
	<i>Rhamnus frangula</i>	Protandry	Insect
	<i>Trevoa quinquenenia</i>	Protandry	Bee
Rhizophoraceae	<i>Brugiera exaristata</i>	Protandry	Bird
Rosaceae	<i>Prunus persica</i>	Adichogamy	Insect
Rubiaceae	<i>Cephalanthus occidentalis</i>	Protandry	Insect
	<i>Cneorum pulverulentum</i>	Adichogamy	Bee/Fly
Rutaceae	<i>Citrus paradisi</i>	Protandry	Insect
	<i>Acer saccharum</i>	Heterodichogamy	Insect
Sapindaceae	<i>Aesculus pavia</i>	Protogyny	Insect
Sapotaceae	<i>Manilkara bahamaens</i>	Protogyny	Bird
Sarraceniaceae	<i>Sarracenia flava</i>	Adichogamy	Bee
Scrophulariaceae	<i>Digitalis sp.</i> (5/19)	Protandry	Bee
	<i>Pedicularis lanceolata</i>	Protandry	Bee
	<i>Scrophularia sp.</i> (25/200)	Protogyny	Wasp
	<i>Verbascum thapsus</i>	Protogyny	Bee
Solanaceae	<i>Veronica beccabunga</i>	Protogyny	Fly
	<i>Nicotiana tabacum</i>	Protogyny	Bee

	<i>Solanum sp.</i> (11/1700)	Protandry	Insect
Sparganiaceae	<i>Sparganium sp.</i> (2/14)	Protandry	Wind
Sterculiaceae	<i>Sterculia chica</i>	Adichogamy	Fly
Staphyleaceae	<i>Staphylea trifoliata</i>	Protogyny	Bee/Fly
Strelitziaceae	<i>Phenakospermum</i> <i>henakospermum</i>	Adichogamy	Bat
	<i>Ravenala</i> <i>madagascariensis</i>	Heterodichogamy	Bird
Stylidiaceae	<i>Stylidium graminifolium</i>	Protandry	Bee
Tecophilaeaceae	<i>Cyanella alba</i>	Protandry	Insect
Thymelaeaceae	<i>Thymelaea hirsuta</i>	Protandry	Insect
Tofieldiaceae	<i>Tofieldia calyculata</i>	Protogyny	Insect
Trilliaceae	<i>Trillium sp.</i> (2/42)	Protandry	Bee
Tropaeolaceae	<i>Tropaeolum sp.</i> (2/87)	Protandry	Bee
Urticaceae	<i>Pilea pumila</i>	Protogyny	Insect
Valerianaceae	<i>Valeriana sp.</i> (2/200)	Protandry	Fly
Velloziaceae	<i>Barbacenia flava</i>	Adichogamy	Bird
Verbenaceae	<i>Phyla incisa</i>	Adichogamy	Insect
Vitaceae	<i>Vitis vinifera</i>	Adichogamy	Insect
Winteraceae	<i>Drimys brassiliensis</i>	Protogyny	Insect
Zingiberaceae	<i>Zingiber</i>	Heterodichogamy	Insect
Zosteraceae	<i>Zostera marina</i>	Protogyny	Water

Chapter VI – Conclusions and Future Directions

6.1 Conclusions

In this thesis I applied an evolutionary framework to the exploration of four aspects of angiosperm evolution: the evolution of bilateral corolla symmetry and its effects on angiosperm diversification, the evolution of floral traits that confer specialization to pollinators, the evolution of dichogamy, and the evidence for correlated evolution between pollination mode and the form of dichogamy. These seemingly distinct topics are connected in that they all address the larger question: why do flowering plants display such incredible diversity in form and mating strategy?

The hypotheses presented in the various chapters of the thesis reveal my bias towards the theory that pollination mode is integral to explaining flowering plant diversity. In all four chapters, pollinators are assumed or hypothesized to play a role in the particular part of floral diversity that I am addressing.

The mechanism by which pollinators may drive plant speciation is currently an area of intense investigation, and direct evidence for pollinator preference driving speciation is rare (Waser 2001). In the now famous example of apparent pollinator-mediated speciation in *Mimulus* (Schemske and Bradshaw 1999), pollinator isolation alone is not sufficient to explain the absence of hybridization between the parental species (Ramsey *et al.* 2003).

While no chapter in this thesis offers direct evidence that plant speciation is driven by pollinator isolation, Chapter II provides convincing evidence that the evolution of a floral trait that improves the specificity of pollen placement on the pollinator (in this case via bilaterally symmetrical corollas) is associated with higher species richness.

Future studies should explore other traits that are thought to induce greater specificity of pollen placement, such as secondary pollen presentation. Another hallmark of a high degree of specificity of pollen placement is a low pollen to ovule ratio. I would expect that if pollen specificity is a key factor in angiosperm speciation, low pollen-ovule ratios, particularly in outcrossing species, should show a similar association with increased speciation rate.

In order for speciation to occur via pollinator isolation, natural selection must favour the evolution of floral traits that attract some pollinators over others. Although early pollination biologists believed that the existence of pollinator syndromes was sufficient evidence for plant-pollinator specialization (Faegri and van der Pijl 1978), it has been argued more recently that the specialization of floral traits to one pollinator is the exception rather than the rule, and that classifying a species as a specialist according to its floral traits, in the absence of data regarding visitation, is potentially misleading (Waser 2001). In Chapter III, I present a model that explores the ecological circumstances under which we should expect plants to evolve floral traits specific to a few, rather than several, types of pollinators. Confirming earlier suggestions (e.g. Feinsinger 1983), the results of the model indicate that the density of the floral species is a key factor in determining whether floral traits should evolve towards a single type of pollinator (i.e., specialize) or several types (i.e., generalize). One important role of theory in biology is to develop hypotheses that can be tested experimentally. The main result presented in Chapter III, that there is stronger selection to specialize when a floral species occurs at low density, could be tested experimentally by directly examining pollinator-

mediated floral trait evolution while manipulating the relative density of the focal plant species.

The final two chapters of my thesis investigate the evolution of dichogamy. In Chapter IV I explore how dichogamy evolves as a function of its advantages (i.e., lower anther-stigma interference, less inbreeding depression) and disadvantages (i.e., loss of fitness through selfed offspring, production of gametes at a different time than other plants in the population). One factor that is an issue in any study of dichogamy is that it is an extremely variable (i.e., non-discrete) trait. Until now, this fact has been ignored or overlooked by most researchers in the field. Indeed, in Chapter V, I force the species into discrete classes in order to test for correlated evolution. The model in Chapter IV is an improvement in that it predicts the level of anther-stigma separation expected to evolve in a species, given information about its level of inbreeding depression and anther-stigma interference. In other words, rather than predicting dichogamy or adichogamy, it predicts a continuous axes of possible levels of dichogamy, depending on the strength of the different selective forces acting. This is a unique feature of the model, and one of the first explorations to treat dichogamy as a continuous trait, and it offers the opportunity to examine not just whether dichogamy should evolve, but to what degree it should evolve.

Lastly, Chapter V uses phylogenetically correct methods to examine the long-standing observation that protandrous plants tend to be animal-pollinated while protogynous plants tend to be wind-pollinated. Prior to this study, it was unknown whether this pattern was caused by selection or phylogenetic constraint. The results show that while the pattern holds generally, there is stronger evidence for a correlation between the evolution of protogyny and wind-pollination than for the evolution of protandry and

animal pollination. This finding was a bit surprising, although upon closer inspection of the raw data it was evident that protogynous animal-pollinated species are much more common than protandrous wind-pollinated species (Figure 5.1). The results indicate that there may be strong selection for wind-pollination to evolve in protogynous species. The reasons for this pattern are unclear. While Chapter V is an important contribution in that it clarifies that it is indeed ecology and not phylogenetic constraint that caused the pattern, it does not address the mechanism that has produced the association between wind-pollination and protogyny. In fact, most studies to date have assumed that wind pollination is the selective pressure that drives the evolution of protogyny. We found the opposite: wind-pollination is more likely to evolve in protogynous lineages than the reverse. This result puts a new perspective on the manner in which this association is viewed, and will hopefully inspire further work.

This thesis explored two seemingly disparate topics, the role of pollinators in flowering plant diversity (in particular floral symmetry, Chapters II and III) and the evolution of dichogamy (Chapters IV and V). In an interesting twist, recent developments in the field of the evolution of floral development have initiated new studies connecting the evolution of dichogamy and floral symmetry. There is now evidence to support a theory that genes such as *CYCLOIDEA* (*CYC*) and *DICHOMATA* (*DICH*) that are known to affect the development of bilaterally symmetrical (zygomorphic) corolla shape (Cubas 2004), are also responsible for the differences in anther development that may contribute to the evolution of dichogamy (S. Kalisz, unpublished data). This finding should inspire new explanations for the evolution of both dichogamy and floral symmetry, and why they occur in certain lineages and not others. I

have included this new advancement here in order to highlight the fact that floral evolution is still a blossoming field, and that in the true spirit of pure research, it is impossible to know how today's findings will be relevant in the future.

6.2 Literature Cited

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