

EVOLUTIONARY CONSEQUENCES OF DIOECY IN ANGIOSPERMS: THE EFFECTS OF
BREEDING SYSTEM ON SPECIATION AND EXTINCTION RATES

by

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ABSTRACT

Dioecy, the breeding system with male and female function on separate individuals, may affect the ability of a lineage to avoid extinction or speciate. Dioecy is a rare breeding system among the angiosperms (approximately 6% of all flowering plants) while hermaphroditism (having male and female function present within each flower) is predominant. Dioecious angiosperms may be rare because the transitions to dioecy have been recent or because dioecious angiosperms experience decreased diversification rates (speciation minus extinction) compared to plants with other breeding systems. Many traits that might affect diversification rates are different between dioecious and hermaphroditic plants, namely seed dispersal, pollination, geographical distribution, and growth habit (woody versus herbaceous). This thesis is composed of four separate projects that attempt to describe and explain the patchy distribution of dioecy among the angiosperms. In all the chapters, dioecy is examined from a fresh perspective that considers the evolutionary consequences of dioecy rather than from the traditional angle that focuses on the forces involved in the evolution towards dioecy. The first project compares the species richness of dioecious groups and their most closely related sister-groups. Dioecious groups are, on average, smaller than their sister-groups, indicating that dioecious angiosperms experience higher extinction rates or lower speciation rates. Following from this observation, the next two projects use computer simulations and mathematical theory to explore how (a) the limited seed dispersal of dioecious angiosperms (in which only females can disperse seeds) and (b) the differing pollination dynamics of dioecious angiosperms (where males may become more attractive to pollinators than females) may affect extinction rates, finding that both processes are potential causes of the low representation of dioecy among the angiosperms. Finally, using a comparative phylogenetic framework, I explore the reported ecological correlations of dioecy with small, white flowers, fleshy fruits, tropical distribution, and woody growth form. I confirm the presence of these correlations but, contrary to previous theories, find evidence that the cause of the correlations is due to the ability of these traits to alleviate some of the aforementioned disadvantages (in terms of dispersal and pollination) of dioecy.

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PREFACE

Three of the chapters of this thesis have been either published or submitted for publication with much help from an abundance of sources (see Acknowledgments). The full details of the publications of these chapters, including details of the contributions of co-authors Katriina L. Ilves and Sarah P. Otto to the publication (submission) of Chapters II and III are as follows:

1) Chapter I was published as a single-author paper.

Heilbuth, J. C. (2000) Lower species richness in dioecious clades, *American Naturalist* 156:221-241

Except for a vast quantity of discussion and editing by many, especially my supervisor, the paper consisted of ideas, analysis, and writing that were largely my own.

2) Chapter II was published as:

Heilbuth, J. C., K. L. Ilves, and S.P. Otto. (2001) The consequences of dioecy for seed dispersal: modeling the seed-shadow handicap. *Evolution* 55:880-888.

For this chapter, I contributed the original idea for the paper, wrote the computer program, ran a number of simulations, analyzed the data, and wrote the majority of the paper. Katriina Ilves helped with the writing of the program, ran the majority of simulations and helped with graphical display of the data. Sarah Otto contributed by applying a model of spread along a continuous axis. For that section of the paper, indicated in the chapter, Dr. Otto was entirely responsible for the idea, the analysis and writing.

I acknowledge the above statement to be true:

3) Chapter III was submitted for publication as:

Heilbuth, J. C. and S. P. Otto. When looks can kill: the evolution of sexually-dimorphic floral display and the extinction of dioecious plants. *Science* (submitted)

For this chapter, I contributed the original idea for the paper, came up with the preliminary model used in the paper, and co-authored the paper. Sarah Otto helped develop the models further and co-authored the paper.

I acknowledge the above statement to be true:

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CHAPTER I: INTRODUCTION AND OVERVIEW

The reproductive systems of angiosperms can be divided into three classes (Barrett 1989): (i) habitual outbreeders, (ii) habitual inbreeders, (iii) mixed outbreeders and inbreeders. Angiosperms can also practice asexual reproduction (apomixis) and do so to varying extents via (i) vegetative reproduction or (ii) agamospermy (producing seeds from unfertilized ovules). These breeding systems can vary from population to population, from plant to plant or even within one individual plant at different times of its life. Breeding systems inevitably influence population substructure, fitness, and spatial dynamics, and thus are likely to influence the diversification of lineages. Flowering plants provide scientists with a very powerful system to study the interactions among these factors because they display such a wide diversity of reproductive systems (Darwin 1876, 1877 as cited by Barrett 1989), allowing for many hypotheses to be tested in a comparative fashion.

Dioecy constitutes the breeding system whereby male and female reproduction is allocated onto separate individuals. Dioecy is therefore referred to as a dimorphic breeding system (having two types, male and female) while other plants have a monomorphic (one type) breeding system. Although dioecy is the main breeding system in animals, it makes up only a small percentage (6%; Renner and Ricklefs 1995) of flowering plants, where it is taxonomically distributed among most of the major orders of angiosperms (Thomson and Barrett 1981). The low representation of dioecy could potentially be due to high reversion rates, low origination rates, higher extinction rates or lower speciation rates. Despite extensive research on the potential benefits of dioecy to individuals, little is known about the long-term success of dioecious lineages in relation to their hermaphroditic or monoecious relatives. The first project (Chapter Two) examines the distribution of dioecy amongst the angiosperms in a phylogenetic framework. By performing a sister-group comparison of families in angiosperms, I revealed a pattern of lower species richness for most dioecious groups when compared to their non-dioecious sister-groups, indicating that dioecy is associated with higher extinction rates or lower speciation rates.

Dioecious plants, by definition, are obligate outbreeders and this outbreeding may be what is causing the low species richness observed. The evolutionary effect of a switch to an inbreeding mating system was summarized by Baker (1959): "A different set of genes, adapted to a very restricted number of particular backgrounds, may be superior in the inbreeder. In an inbreeding population, these genes will quickly become homozygous and rise to fixation or become eliminated according to the selective advantage or disadvantage they produce. Secondly, the population, in its early stages, will be small, and there may be chance loss of genes. In total, the sum of adaptation

or drift may produce rather suddenly the morphological and genetical diversity which we associate with speciation.” In effect, the switch to inbreeding is thought to lead to rapid reproductive isolation and speciation. Contrary to the case of inbreeders, a dioecious individual has to mate with another individual resulting in a high level of outbreeding maintained in dioecious populations. Dioecy is thought by many (e.g., Uyenoyama and Waller 1991, Charlesworth and Charlesworth 1978) to evolve for the genetic benefits that this increased outbreeding and gene flow offers either because: (i) deleterious mutations are prevented from becoming homozygous, the *partial dominance hypothesis*, or (ii) outbreeding results in an increase in heterozygotes, which may be more fit than either homozygote, the *overdominance hypothesis* (Charlesworth *et al.* 1990). A consequence of this increased outbreeding may be to slow the fixation within different populations of genes for pre- or post-zygotic isolation, resulting in lowered speciation for obligate outcrossers, like dioecious taxa.

Whether inbreeding and the ability to create isolated colonies is the cause of low representation of dioecious taxa was tested in Chapter Two. If the pattern of low species richness is caused by higher outbreeding, then groups of angiosperms with other methods of outbreeding should show the same pattern. Accordingly, another set of sister-group comparisons was performed, this time with clades of angiosperms that were outbreeding via a mechanism other than dioecy, such as gametophytic or sporophytic self-incompatibility or heterostyly. When such sister-group comparisons were performed, no such trend for lower species richness was observed for these monomorphic outbreeders. The fact that only dimorphic outbreeders show this pattern of lower species richness indicates that there is something very specific about having the sexes spatially separated that results in lower diversification rates.

Dioecious individuals have divided the costs of reproduction onto separate male and female individuals resulting in only half the population setting seed, as observed by Darwin (1877). Division of labor theory constitutes another explanation for the evolution of dioecy, which argues that separate males and females may perform their specific functions more efficiently than a hermaphrodite, especially if maternal and paternal costs are completely separate (i.e., no overlap between male and female function) (Charnov 1982) or if there is conflict between parental functions (Lloyd 1982). Division of labor could lead to less speciation or more extinction if having only half the number of seed dispersers (the females) results in lowered dispersal. This may lead to lowered speciation rates as populations that are not widely distributed in space may have less potential to evolve pre- or post- mating isolation. Conversely, the limited distribution may lead to a higher extinction risk because dioecious populations may be outcompeted by hermaphroditic populations that can disperse better and establish in space-limited systems. The strength of this

“seed-shadow handicap”, as it is referred to here, was tested in Chapter Three using spatially-explicit computer simulations of an island where both dimorphic (dioecious) or monomorphic (hermaphroditic or monoecious) plants were in direct competition for space. The results indicate that the seed-shadow handicap could be a very significant force in the increased extinction or decreased speciation rate of dioecious angiosperms. Having fleshy fruits, as many dioecious angiosperms do (Bawa 1980), may be a mechanism that can compensate for the seed-shadow handicap as birds, a common fruit-eater, can disperse the seeds much farther. As well, multi-seeded fruits can also allow for the co-dispersal of male and female seeds so that they arrive together at a new locale (Baker 1958).

In addition to fleshy fruits, dioecious angiosperms often have small, white flowers (Bawa and Opler 1975; Charlesworth 1993). Proposed theories of why this correlation exists include that (i) dioecy evolves more often in lineages with a low allocation to attraction of pollinators (i.e., small, white flowers), and (ii) that white flowers evolve more often in dioecious lineages. Chapter Four introduces and explores the possibilities of yet another cause for the correlation - that dioecious plants that evolve showiness may suffer increased extinction risk. The results of analytical and computer simulation models suggest that unisexual lineages can evolve attractive structures. However, in a dioecious plant, the possibility exists for males to become showier than females by a large margin, especially if pollinators are very abundant. This sexually-dimorphic floral display can be favored because male fitness is limited by the number of mates obtained while female fitness is more often limited by the resources she can devote to offspring. This sexually-dimorphic floral display can lead to pollinators visiting only males, however, especially if pollinators subsequently become scarce, and this can result in a high extinction risk of showy dioecious plants.

The final project, Chapter Five, examines several of the ecological correlates of dioecy and places them in a phylogenetic framework to see if previously reported correlates are maintained. Using maximum-likelihood analysis, I also tried to discern the order in which the inter-correlated traits evolved. Ecological factors are known to be correlated with certain mating systems more than others (Bawa 1980) and should be characterized further to determine what effects these correlations are having on diversification rates. Dioecy, for example, has correlations with fleshy fruits and small, inconspicuous flowers (Bawa 1980), woody growth form (Fox 1985), and generalist or wind pollination (Fox 1985, Thomson *et al.* 1989; but see Renner and Feil 1993, Bawa 1994 regarding this contentious issue). Each of these factors could in turn affect speciation or extinction rates. For example, woody species characteristically develop few interspecific sterility barriers (Baker 1959). Wind pollination could ensure more long-distance gene flow which

might limit genetic differentiation and speciation among sub-populations. Similarly, fleshy fruits are thought to be dispersed longer distances, which may have an effect on population substructure (Carlquist 1974). Shifts in generalist pollinators are less likely to be pronounced enough to cause speciation than shifts in specialized pollinators. Furthermore, Chapter Three and Chapter Four deal with reasons why many dioecious species may go extinct and suggest that certain dioecious species, those that have fleshy fruits, and small, white flowers, may remain extant, a pattern which could both explain the correlations observed and the lower species richness of dioecious angiosperms. Indeed, Chapter Five indicates that the ecological correlates of dioecy are caused by an increased evolutionary success of dioecious clades with these correlated traits rather than by an increased rate of transition to dioecy in clades with these traits.

In short, this thesis examines the evolutionary consequences of having a dioecious breeding system. The projects I have completed use comparative methods, computer simulations, and mathematical analyses to characterize the dioecious condition in angiosperms by asking the following questions:

(i) Is the pattern of lower species richness caused by the increased outbreeding that occurs in dioecious angiosperms?

(ii) Is the spatial component of seed distribution from the mother plant a factor in competition between dioecious and non-dioecious plants?

(iii) Can the floral display of males and females become so sexually-dimorphic as to substantially raise the extinction risk of dioecious plants?

(iv) What are the causes of the observed ecological correlates of dioecy, and how might they be associated with reduced speciation or increased extinction?

Investigation of these questions has indicated that, although the conditions under which dioecy can evolve may be relatively unconstrained (Charlesworth and Charlesworth 1978), the conditions under which dioecy can persist and proliferate are extremely strict. This investigation into dioecy reveals several interesting interactions between breeding system, dispersal, pollination and diversification rates amongst the angiosperms. Further investigation into the abundant and diverse angiosperms will surely reveal even more insight into what determines species diversity.

CHAPTER II: LOWER SPECIES RICHNESS IN DIOECIOUS CLADES¹

INTRODUCTION

Dioecy, the separation of male and female function into separate individuals, is taxonomically distributed among most of the major orders of angiosperms, both primitive and advanced (Thomson and Barrett 1981). Although there has been considerable discussion regarding the evolutionary forces leading to dioecy in plants (e.g., Carlquist 1966, 1974; Bawa 1980; Thomson and Brunet 1990), little work has been done to determine whether dioecy is a successful evolutionary strategy. Dioecy is hypothesized to have evolved as a mechanism to reduce inbreeding (Baker 1959; Carlquist 1966, 1974; Charlesworth and Charlesworth 1978) or to improve resource allocation (Bawa 1980; Givnish 1980). Various authors (Baker 1959; Williams 1975) have argued that dioecy is an easier route to outbreeding, both genetically and physiologically, and empirical evidence suggests that it is a more efficient means of avoiding inbreeding than gametophytic self-incompatibility (Anderson and Stebbins 1984). As well, theoretical studies show that plants carrying a mutation conferring male or female sterility can be favored in a hermaphroditic population (Charnov 1982) when a division of labor into males and females allows each individual to fulfill its roles more efficiently.

Despite the many possible advantages of dioecy, dioecious flora comprise only ~6% of the world's angiosperms (Renner and Ricklefs 1995). The low representation of dioecy is consequently a puzzle and might be caused by dioecious species experiencing a higher extinction rate, lower speciation rate, high reversion rates back to the monomorphic state (Richards 1997), or low origination rates. A possible explanation for the low representation of dioecy might be that a recent environmental shift has favored the evolution of dioecy, so that dioecious groups are fairly young but not necessarily speciating or going extinct at different rates. This explanation would agree with the commonly-held belief that dioecious species occur mostly on the tips of phylogenetic trees as they "are usually more closely related to hermaphrodite species within their own genus or family than they are to each other" (Lebel-Hardenack and Grant 1997). This study is the first to examine whether the infrequent occurrence of dioecy among angiosperms is due to a series of recent adaptations that arise on the tips of trees. Using phylogenetic evidence, I performed sister-group comparisons of species richness using clades that are entirely or mostly dimorphic (those with dioecious, polygamodioecious, gynodioecious or subdioecious breeding systems; see Table 2.1 for terms) as the focal group and monomorphic lineages (those with

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bisexual, monoecious, andromonoecious, gynomonoecious or polygamomonoecious breeding systems) as the sister-group to identify whether dioecious clades are more or less speciose than their hermaphroditic sister taxa. A sister-group comparison (Fig. 2.1) uses a phylogeny to determine whether the difference in number of species in a focal group (in this case, a dimorphic clade) and its sister-group (a monomorphic clade) are significantly different. If dioecy is a recent adaptation, then both the dimorphic and the monomorphic clades in the sister-group comparison should be small but equally speciose. These sister-group comparisons were performed at the family and generic levels. At both levels of analysis, dioecious clades were found to be far less speciose than their non-dioecious sister-groups, indicating that dioecious lineages have lower speciation rates or higher extinction rates.

The possibility that dioecious lineages experience lowered speciation rates is consistent with Baker's Law (Baker 1953), which states that a self-compatible hermaphrodite will be a better colonizer than a dioecious plant due to the fact that it does not need a mate. Long-range dispersers can found new populations that may diverge and form new species allopatrically. If dioecious populations accomplish this long-range dispersal less often than hermaphroditic populations, it could potentially lead to a lower speciation rate among dioecious clades. To examine this possibility, I have again used the sister-group comparison approach; this time using self-incompatible taxa as the focal group against self-compatible sister taxa. Self-incompatibility is achieved in approximately half of the non-dioecious angiosperms through a variety of morphological (e.g., heterostyly) and molecular (e.g., gametophytic and sporophytic self-incompatibility) means. Self-incompatible species are subject to the same difficulties as dioecious ones when dispersing to a new area where mates may be either non-existent or in short supply. If Baker's Law plays a large role in determining diversification rates, these incompatible groups should also show lowered species richness than dioecious clades when examined phylogenetically using the same methods. However, this study found no evidence for this as the self-incompatible lineages were as speciose as their sister-groups implying that Baker's Law is not a large factor in determining divergence rates.

The evidence gained in this study agrees with studies done on local floras, such as that on the Hawaiian flora (Sakai *et al.* 1995), which suggest that Baker's Law does not reduce the amount of dioecious colonizers. In fact, Sakai *et al.* (1995) found that the high proportion of dioecy in the Hawaiian flora is due in part to a disproportionately high (~10%) percentage of dioecious colonizers. However, these results do differ from Sakai *et al.* (1995) in that their comparisons of lineage size in the Hawaiian Islands (Sakai *et al.* 1995) show no trend for higher extinction rates or lower speciation rates: dimorphic genera are as speciose as monomorphic genera. Several factors

may contribute to dioecy being relatively more successful on the Hawaiian islands. The prevalence of dioecy has been shown to be correlated with tropical environments (Renner and Ricklefs 1995); a correlation that may result from dioecious species having higher speciation rates or lower extinction rates in tropical environments. Furthermore, the numbers of dimorphic and monomorphic species on Hawaii may not reflect long term expectations given the relatively recent (maximum 5.7 MYA; MacDonald *et al.* 1983) nature of the present-day islands. For example, dioecious plants may be more successful in species-poor habitats (see Chapter Three). As well, Sakai *et al.* (1995) did not employ the sister-group comparison approach and therefore did not control for the ages of the lineages compared.

The distribution of the dioecious families included in this study spanned all 15 of the major sub-classes of angiosperms (Takhtajan 1997) with the exception of Triurididae, which has no dimorphic family representatives. The analysis at the generic level spans 10 of the 15 sub-classes. The subclasses Triurididae, Alismatidae, Lamiidae, Ranunculidae, Magnoliidae either have no dioecious genera or have dioecious representatives that could not be found on any published phylogeny. This survey of worldwide lineages gives us a better idea of when and where dioecy is a successful breeding system. Using the sister-group comparison approach controls for the age of the lineages since sister-groups are, by definition, the same age, and the trait in question (i.e. dioecy) is assumed to have evolved only once. As a final control for the possibility that the lower species richness found in dioecious clades may be an artifact of the methods used in this study or other taxonomic biases, sister-group comparisons were performed using clades of angiosperms with monoecious breeding systems as the focal group as well. Sister-group comparisons at the genus level with only monoecious focal groups revealed no trend for lowered species richness. Monoecy, where male and female organs are separated on different flowers on the same plant, is represented in ~5% of angiosperms and is an adaptation thought to reduce inbreeding (Richards 1997). As monoecy is as unique and morphologically distinct as dioecy, these sister-group comparisons can be used to assess the bias that may occur in systematic studies, which may bestow unique family or genus status to a group that shares an unusual breeding system. The results of the monoecious sister-group comparisons also clarify the causes of the low representation of dioecy, as monoecy and dioecy share some of the same ecological correlates as well as other features involved in having unisexual flowers. I conclude that the low speciosity observed in dioecious clades is likely caused by factors that are unique to dioecy alone and not factors shared by both monoecious and dioecious species.

METHODS

Family level data collection

Families in which the breeding system was defined as “mostly” or “completely” monoecious or dioecious were found by referring to Takhtajan (1997). When this source was ambiguous, other sources were found, usually those given on the DELTA website (Watson and Dallwitz 1992) or in Judd *et al.* (1999). In the dioecious sister-group comparison, bisexual, monoecious, polygamomonoecious and gynomonoecious were considered equivalent monomorphic species while dioecious, polygamodioecious, gynodioecious, and subdioecious species were considered dimorphic species following the system developed by Lloyd (1980). Species described as polygamous were omitted. For the self-incompatible (SI) comparisons, information regarding the outbreeding status of a plant species is not as readily available as its sexual status (i.e. dioecious, monoecious or hermaphrodite), because outbreeding status can depend on chemical as well as morphological attributes. Finding families in which all or most of the members have some form of self-incompatibility (be it from gametophytic or sporophytic self-incompatibility, dichogamy, or heterostyly) was accomplished by referring to Judd *et al.* (1999). Monoecious families that were reported to be self-incompatible due to dichogamy were also included in this analysis as not enough of them were found to warrant a separate sister-group comparison at the family level. Because Judd *et al.* (1999) covers only the major families of angiosperms, other reviews on self-incompatibility (Fryxell 1957; Charlesworth 1985) and heterostyly (Ganders 1979) were checked so as to not bias the comparisons in favor of large, major angiosperm families. These extra sources of information revealed Eupomatiaceae, Plumbaginaceae, Hypericaceae, Resedaceae, and Valerianaceae as additional SI focal groups.

The majority of families used in the analysis were found on the recently published multi-gene phylogeny created by Soltis *et al.* (1999). The dioecious families Phytaceae (Morton *et al.* 1997), Achatocarpaceae (Brown and Varadarjan 1985), Gyrostemonaceae (Rodman *et al.* 1996), Nolinaceae, and Didieraceae (Rice *et al.* 1997), which were not included in the phylogeny of Soltis *et al.* (1999), were found on other existing phylogenies to guard against any biases that might arise from their exclusion. Sister-group comparisons entailed comparing the number of species in the focal clade (e.g. dimorphic groups) to the number of species in the sister-group (Fig. 2.1). When the number of species in a family was given as a range, the average between the lowest possible number of species and highest number of species was used.

Commonly, the sister groups of the dimorphic families were families that included some dimorphic members. When this problem was encountered, I used a protocol similar to that of Farrell *et al.* (1991); the number of species reported for the sister group was the total number of

species minus the number of dimorphic species within that family. Because the sister-groups to dimorphic clades tended to be more speciose, this procedure led to a conservative estimate of the number of species in the sister group. For example, the family Ranunculaceae is in the sister-group comparison for the dioecious family Menispermaceae. Ranunculaceae has 2500 species, all of which are hermaphroditic with the exception of those in the genus *Thalictrum* (which has 330 species). Therefore, the number 2170 [$2500 - 330 = 2170$] was the number of species reported for the family Ranunculaceae. The reciprocal procedure (subtracting monomorphic species from dimorphic clades) was not performed on the focal group since this would have increased the significance of the results. In the SI sister-group comparison, however, it became clear that the self-incompatible groups were more often larger than their sister-groups, and hence the conservative approach was the opposite, with any known self-compatible genera being subtracted from the largely self-incompatible focal groups.

Dimorphic and self-incompatible groups were mapped onto the consensus tree of Soltis *et al.* (1999) in order to maximize confidence in reported relationships among families. Further complications arose due to unresolved relationships and paraphyletic groupings on the consensus tree of Soltis *et al.* (1999). The protocol for unresolved relationships was to only include the family if any sister-group comparison made between the focal group and any other member of the polytomy resulted in the same sign (e.g., Phellinaceae, Table 2.2). In the few cases where paraphyly was indicated for the sister group, the group reported as sister to the focal group consisted only of those genera listed in the phylogeny (e.g., Salicaceae, Table 2.2).

Genus level data collection

In most respects, the genus level analysis was identical to the family level analysis with the addition of monoecious genera (including genera that are andromonoecious, gynomonoeious and polygamomonoeious) as the focal groups in a new set of sister-group comparisons (see Appendix I). Phylogenies that used molecular, morphological, or a mixture of both types of data were collected from the literature. If the literature article contained a number of different types of phylogenies, all showing slight differences in the relationships of its members, the strict consensus cladogram was preferred. Phylogenies for all three sets of sister-group comparisons (i.e., dioecious, SI, and monoecious) were found through independent literature searches, but some phylogenies are used in more than one set of sister-group comparisons.

The breeding system status of genera was found by referring to Yampolsky and Yampolsky (1922), Hutchinson (1964), and Heywood (1981). Care was taken to confirm the breeding system status of genera by referring to more recent sources, such as Uhl and Dransfield

(1987; for Arecaceae), a database used by Weller *et al.* (1995), Jarvie and Ermayanti (1996), and Mabberley (1997). These efforts resulted in >90% of the genera (and 100% of the dioecious focal genera) used in this study being confirmed in regards to their breeding system by very recent sources. Self-incompatible genera were identified from the compilations in Fryxell (1957), Ganders (1979), Charlesworth (1985), Watson and Dallwitz (for Poaceae only; 1992), and Weller's database (Weller *et al.* 1995). The number of species reported in each genus was taken from Mabberley (1997) unless more up-to-date information could be found in Judd *et al.* (1999).

Phylogenies were accepted or rejected for sister-group analysis based on their completeness for the genus in question and its relatives. Phylogenies that are absolutely complete for all the genera in a family are rare. Therefore all the sister-groups reported in this study may not reflect the true relationships among the genera. Phylogenetic inaccuracies should tend to randomize the size of the sister group in comparison to the focal group. Nevertheless, there might be biases such that focal groups tend to be smaller in size than their sister groups due to the methods employed. The analyses with self-incompatible and monoecious plants as focal groups suggest that such biases are not strong. To minimize such inaccuracies, phylogenies were used only if they reported sister-groups that were in the same taxonomic order. Exceptions were made to this rule, however, when the sister-group comparison involved an order that consisted of only one genus. In this case, the sister-group to this genus is inevitably a member of another order. Similarly, there are many families of angiosperms that contain only one genus. If this was the case, then the sister-group comparison was made with the nearest clade, which would consist of another family. As the family level analysis would also include these families (or orders) with only one genus, the family and genus level analyses should not be considered as completely independent. The family and genus level analyses were kept separate, instead of combined, to show that the pattern of lowered species richness can be seen at more than one taxonomic level.

Occasionally a genus could be found on more than one phylogeny. If this was the case, the phylogeny that was most complete for the family to which that genus belonged was used. If both phylogenies were equally complete and gave opposite results as to whether the sister-group was larger or smaller, then both sister-group comparisons were listed. If the two phylogenies gave the same result, then only one was listed. In a few rare cases, the genus was listed in more than two phylogenies with different sister groups in each, giving a mix of positive and negative results. When this was the case, the relationships of the genus were deemed too ambiguous for analysis, and the group was omitted from the data set.

As in the family-level sister-group comparison, careful attention was paid to ensure that the number of species reported for the sister group was not artificially inflated due to the inclusion of dimorphic members. If the sister group contained dimorphic members, the number of dimorphic species was determined and subtracted from the total number of species reported for the sister-group. To be conservative, monomorphic species were not excluded from the focal group. Occasionally, a sister-group engulfed a smaller sister-group comparison within it. In these cases, both the species contained within the focal-group and the sister-group of the smaller sister-group comparison were removed from the analysis of the larger sister-group comparison.

Statistical analysis

All species richness values were tabulated for the focal dimorphic groups and their corresponding sister-groups (see Tables 2.2 and 2.3; Appendix I). Two-tailed sign tests were performed to see whether the proportion of dimorphic groups that have higher species richness values were significantly different from one half as one would expect if equivalent processes were governing species richness values in the focal and sister groups. The sign test was chosen over other statistical tests available for analyzing key innovations [i.e. signed-ranks test (Wiegmann *et al.* 1993), randomization test for matched pairs (Barracough *et al.* 1995) and the Slowinski-Guyer test (Slowinski and Guyer, 1993)] both because its lack of power makes it the most conservative test available (see De Queiroz, 1998 for discussion) and because the other tests require more exact information on the numbers of species in each group. As well, Fisher's exact tests were performed to determine whether the proportion of self-incompatible or monoecious clades having higher species diversity values was significantly different from that obtained in the sister-group comparisons using dimorphic clades.

RESULTS

Family level analysis

The results of all the sister-group comparisons and statistical analyses are presented in Table 2.4. Dimorphic clades were far less speciose than their sister taxa. Of the 28 families included in molecular phylogenies (Table 2.2), only six were more speciose than their sister taxa ($p = 0.0066$; two-tailed sign test), far fewer than the half expected if there was no difference in speciation or extinction rates between dimorphic and monomorphic lineages. If the shortest tree of Soltis *et al.* (1999) is used instead of the consensus tree, several more relationships are resolved and the results become even more convincing, with only seven of 36 sister-group comparisons showing larger lineage size in the dimorphic clade.

By contrast, obligate outbreeders did not have significantly different species richness values than their selfing sister-groups overall. Of the 22 sister-group comparisons performed, the self-incompatible group was more speciose than its self-compatible sister group in 13 cases ($p=0.524$; two-tailed sign test), which is not significantly different from the expectation of no difference in speciation or extinction rates between self-incompatible and self-compatible groups. The number of self-incompatible families with higher species diversity values was, however, significantly greater than the proportion of dioecious families with higher species richness ($p = 0.009$; two-tailed Fisher's exact test). If the shortest tree of Soltis *et al.* (1999) is used instead of the consensus tree, the results are much the same, with 17 out of 32 comparisons showing the self-incompatible group to be more speciose, which is again not significantly different from one-half ($p = 0.860$; two-tailed sign test) but is significantly different from the number of dioecious families that have higher speciosity ($p = 0.005$; two-tailed Fisher's exact test). The shortest tree also resolved a few additional monoecious families, which enabled a separate sister-group comparison to be performed. Nine out of 16 comparisons showed the monoecious focal group to be more speciose than its sister-group, which is not significantly different from one-half ($p = 0.804$; two-tailed sign test) and is significantly different from what found in the dioecious sister-group comparison ($p = 0.020$; two-tailed Fisher's exact test).

Genus level analysis

The lowered species richness of dioecious clades is also reflected at the genus level. Out of 66 sister-group comparisons (Appendix I), only 22 showed the dimorphic clade to be more speciose than its sister-group, which is significantly less than expected ($p = 0.009$; two-tailed sign test). As can be seen in Tables 2.2 and Appendix I, the conditions of gynodioecy, androdioecy, polygamodioecy and subdioecy are relatively rare so this result mainly reflects attributes of dioecious clades. In contrast to the results of the sister-group comparison with dimorphic genera, the self-incompatible genera seem to be as speciose as their self-compatible sister-groups on average. In 56 sister-group comparisons, 29 showed the self-incompatible group to have greater species richness ($p = 0.894$; two-tailed sign test). As in the family level analysis, this is significantly different from the proportion of dimorphic genera found to have more species than their sister groups ($p = 0.045$; Fisher's two-tailed exact test).

The low species richness associated with dioecy is further demonstrated by a sister-group comparison done using monoecious genera. The non-monoecious genera used as the sister-groups were predominantly hermaphroditic although some comparisons were between monoecious and dioecious genera. Out of the 52 sister-group comparisons between monoecious and non-monoecious genera, 28 showed the monoecious sister-group to be more speciose ($p = 0.678$; two-

tailed sign test), which is not different from what is expected. This was again significantly different from what was found in the sister-group comparison of dimorphic genera ($p = 0.0385$; Fisher's two-tailed exact test). Even if only the comparisons with hermaphroditic sister-groups are used in the analysis, the results are much the same with 21 out of 41 comparisons showing the monoecious clade to be larger than its sister group ($p = 0.875$; two-tailed sign test), which results in a nearly significant difference from the dioecious sister-group comparison ($p = 0.067$; Fisher's two-tailed exact test).

DISCUSSION

Key innovations are expected to correlate with a radiation in the abundance and variation among species (Farrell *et al.* 1991; Hodges and Arnold 1994). Although there is some evidence that the combination of dioecy and animal dispersed seeds leads to increased species diversity in non-angiosperms (Donoghue 1989), this does not seem to be the case with dioecy in angiosperms. These results demonstrate that dioecious groups are less speciose than their sister-groups and therefore must have higher rates of extinction or lower rates of speciation due to particular constraints involved with the dioecious condition. Among the 28 family sister-group comparisons, 22 showed the dimorphic lineage to have a lower species richness. This was further exemplified at the genus level, where 44 of the 66 sister-group comparisons showed the dimorphic lineage to have a lower species richness.

One potential cause of the difference in lineage size between dimorphic and monomorphic clades could be different colonization abilities of dimorphic and monomorphic plants. According to Baker's Law (Baker 1953), self-compatible hermaphroditic plants are better colonizers due to the fact that a single propagule can found a population. If this population receives little to no gene flow from the ancestral population, a new species may form allopatrically. It is not known how often new allopatric populations are founded by single individuals in nature or to what extent dioecious populations have evolved compensatory mechanisms in order to accomplish an equivalent amount of long-range dispersal (e.g. animal-dispersed fruit containing seeds of both sexes or "leaky" dioecious systems that allow for selfing under certain environmental conditions (Baker and Cox 1984)). Furthermore, approximately half (Thomson and Barrett 1981) of the non-dioecious angiosperms are unable to self due to a number of self-incompatibility mechanisms (gametophytic self-incompatibility in Solanaceae, Legumaceae, Liliaceae and Poaceae; sporophytic self-incompatibility in Brassicaceae and Asteraceae; protandry or protogyny in Juglandaceae and Araceae; and heterostyly in Rubiaceae). Self-incompatible species ought to be at a similar disadvantage as dioecious species when colonizing as they too need an individual of the opposite type to mate.

The existence of self-incompatible taxa provides a way to test the extent to which Baker's Law explains the disparity in lineage size between dimorphic and monomorphic lineages. Sister-group comparisons using focal groups that are predominantly self-incompatible did not show any tendency to be either larger or smaller than their sister groups and produced results that were significantly different from the results with the dimorphic focal groups at both the family ($p = 0.009$; two-tailed Fisher's exact test) and genus level ($p = 0.045$; two-tailed Fisher's exact test). The fact that the pattern of low species richness is not continued with other groups that experience the same difficulties in regards to colonization (i.e., needing a mate) indicates that the difference seen between dimorphic and monomorphic lineage size cannot simply be explained by the fact that dimorphic lineages are unable to self.

If Baker's Law is not the reason why dioecy is correlated with small lineage size, the question of why dioecious lineages are so poorly represented remains a mystery. The possibility that these results could be because of separate genus or family status is more readily bestowed on plants with different breeding system has been ruled out by the sister-group comparisons performed using monoecious focal groups (which were more speciose than their sister-groups in 28 out of the 52 comparisons). This observation is useful as it forces us to look for factors that are unique to dioecy for clues as to what may cause the decreased species richness of dioecious clades. Renner and Ricklefs (1995) examined the worldwide distribution of dioecy and found correlations between dioecy and climbing growth form, biotic dispersal, abiotic pollination, and tropical distribution, which, with the exception of tropical distribution, were also ecological correlates of monoecious species. Interestingly, tropical climate has putatively been named as the cause of high diversity values of many angiosperm taxa (e.g., Qian and Ricklefs 1999), so one might think that the correlation of dioecy and tropical climate would result in higher species richness values for dioecious clades. It may be that dioecious angiosperms go extinct at a higher rate in both temperate and tropical environments but that this process occurs less rapidly in tropical environments, which would explain both the low overall representation of dioecy and its higher prevalence in the tropics. Understanding the relative speciation and extinction rates of dioecious groups in and out of the tropics requires further investigation but may illuminate what environmental factors are necessary for a dioecious clade to persist or speciate.

Several other factors are unique to dioecy and provide avenues for future investigations into why the pattern of decreased speciation/increased extinction exists in dioecious clades. Extinction may be more common in dioecious clades since only half the population (the females) sets seed, and females may not be able to compensate for the loss. The amount by which a dioecious species

suffers from this “cost of males” is only beginning to be understood (Lloyd and Webb 1977; Richards 1997 and references therein; Pannell 1997). Furthermore, demes of dioecious plants may go extinct more often if they are segregated into small populations in which there are no members of the opposite sex (Pannell and Barrett 1998). The likelihood of this problem may increase when differences exist between the sexes in ecological tolerances (Meagher 1984, references therein). Investigations into resource allocation and demographic stochasticity questions like these have always been framed with regards to how they might contribute to the evolution of a dioecious breeding system. A change in the focus of studies to how they might affect a dioecious system that has already evolved may reveal how these factors could influence speciation and extinction rates. Recent work by Weller and Sakai (1999) and Weiblen *et al.* (2000) elucidates the strengths of phylogenetic approaches in providing new insights into both the evolution of, and causes of ecological correlations with different breeding systems in angiosperms. It seems clear that phylogenetic approaches will be of use in studying how speciation and extinction rates are correlated with changes in breeding system as well.

This study gives evidence that dimorphic clades have fewer species on average than their monomorphic relatives even when origination and reversion rates are controlled for. This analysis was performed on a wide range of angiosperm taxa and seems to be robust at both the family and genus levels. However, I am unable to determine whether dimorphic lineages go extinct at a more rapid pace or speciate at a slower one than their monomorphic relatives. There are many consequences to having a dioecious breeding system that may contribute to its lack of success, such as a possible decrease in seed set or different ecological tolerances between the sexes (see Chapters Three and Four). Although the ability to self and the possibility of taxonomic bias have been shown to be less likely explanations for the pattern of lowered species richness in dioecious clades, further study using more complete phylogenies as they arise, along with more detailed studies within specific families, may help illuminate where and when dioecy is a successful breeding system and, in doing so, clarify why dioecy is commonly an evolutionary dead-end.

TABLE 2.1. Quick reference for breeding systems in angiosperms

| BREEDING SYSTEM | DEFINITION |
|-------------------------------|--|
| Dimorphism: Dioecy | male and female plants |
| Gynodioecy | hermaphroditic and female plants |
| Androdioecy | hermaphroditic and male plants |
| Subdioecy | male and female plants, and rare hermaphrodites |
| Polygamodioecy | plants with male and hermaphroditic flowers, plants with female and hermaphroditic flowers |
| Monomorphism: Hermaphroditism | bisexual flowers |
| Monoecy | male and female flowers on each plant |
| Andromonoecy | male and bisexual flowers on each plant |
| Gynomonoecy | female and bisexual flowers on each plant |
| Polygamomonoecy | male or female and bisexual flowers on each plant |
| Polygamous* | polygamomonoecious or polygamodioecious |

*see Judd *et al.* (1999)

TABLE 2.2: Sister-group comparisons for dimorphic families. The number given for the non-dioecious families in the sister-clade have been corrected against bias by subtracting the number of dioecious members in the family from the total. All the focal groups here are entirely or mostly dioecious with the exception of Nyssaceae, which has some polygamodioecious members. The final column states the outcome of the sister-group comparison; + indicates the dimorphic clade had more species, - indicates the monomorphic clade had more species.

| <u>Dioecious Clade</u> | # | <u>Non-Dioecious Clade</u> | # | +/- |
|---|------|---|-------|-----|
| Aextoxicaceae | 1 | Berberidopsidaceae | 7000 | - |
| Amborellaceae | 1 | eumagnoliids (>5000) + Nymphaeaceae (70) + Austrobaileyaceae(1) + Schisandraceae (50) | >5000 | - |
| Anacardiaceae (600) + Burseraceae (550) | 1150 | Sapindaceae (2215 - <i>Xerospermum</i> (2) - <i>Distichostemon</i> (6) - <i>Dodonaea</i> (68)) + Simaroubaceae (100 - <i>Simarouba</i> (6) - <i>Alvaradoa</i> (5) - <i>Castela</i> (15) - <i>Picramnia</i> (45) - <i>Picrolemma</i> (3)) + Meliaceae (950 - <i>Guarea</i> (40) - <i>Cedrelopsis</i> (8) - <i>Aphanamixis</i> (3) - <i>Lansium</i> (3)) + Rutaceae(1650 - <i>Amyris</i> (40) - <i>Araliopsis</i> (3) - <i>Empleuridium</i> (1) - <i>Lunasia</i> (10) - <i>Orixa</i> (1) - <i>Phellodendron</i> (10) - <i>Teclea</i> (30)) | 4616 | - |
| Balanopaceae | 9 | Chrysobalanaceae (495) + Dichapetalaceae (180)+ Trigoniaceae(26) | 701 | - |
| Barbeyaceae | 1 | Elaeagnaceae (50 - <i>Hippophae</i> (3) - <i>Shepherdia</i> (3) | 44 | - |
| Cannabaceae | 4 | <i>Trema</i> (12) OR <i>Celtis</i> (100) of Celtidaceae | ≥12 | - |
| Didiereaceae | 11 | Portulacaceae | 450 | - |
| Didymelaceae | 2 | Buxaceae | 70 | - |
| Dioscoreaceae | 650 | Taccaceae | 13 | + |
| Garryaceae (13) + Aucubaceae (1) + Eucommiaceae (1) | 15 | <i>Icacina</i> of Icacinaceae | 6 | + |
| Griselinaceae | 6 | Melanophyllaceae (<i>Melanophylla</i> alone (8)) OR Apiaceae (3150 - <i>Acronema</i> (25) - <i>Arcuatopteris</i> (3) - <i>Trinia</i> (10) - <i>Xanthosia</i> | ≥8 | - |

| | | | | |
|--|-----|---|------|---|
| | | (25) - <i>Aciphylla</i> (39)) + Araliaceae(1200 - <i>Aralidium</i> (1) - <i>Meryta</i> (30) - <i>Oreopanax</i> (80))+ Pittosporaceae (246) | | |
| Gyrostemonaceae | 17 | Resedaceae | 80 | - |
| Helwingiaceae (5) + Aquifoliaceae (400) | 405 | Phyllonomaceae | 4 | + |
| Heteropyxidaceae | 3 | Vochysiaceae | 200 | - |
| Menispermaceae | 450 | Berberidaceae (600) + Ranunculaceae (2500- <i>Thalictrum</i> (330)) | 2770 | - |
| Montiniaceae | 4 | Hydroleaceae (<i>Hydrolea</i> alone) | 11 | - |
| Myricaceae | 50 | Juglandaceae (60 - <i>Alfaroa</i> (7)) | 53 | - |
| Myristicaceae | 370 | Annonaceae (2400 - <i>Ephedranthus</i> (5)) + Eupomatiaceae (2) + Magnoliaceae (240 - <i>Kmeria</i> (2)) + Himantandraceae (2) + Degeneriaceae(2) | 2639 | - |
| Myrothamnaceae | 2 | Gunneraceae | 40 | - |
| Nolinaceae | 50 | Ruscaceae (13 - <i>Ruscus</i> (6)) | 7 | + |
| Nyssaceae | 7 | Cornaceae (55 - <i>Torricellia</i> (3) - <i>Aucuba</i> (4) - <i>Afrocrania</i> (1)) OR Loasaceae (300) | ≥47 | - |
| Pandanaceae | 700 | Cyclanthaceae | 235 | + |
| Phellinaceae | 10 | Alseuosmiaceae (12) OR Argophyllaceae (11) OR Menyanthaceae (60) OR Calyceraceae (50) + Goodeniaceae (420) OR Asteraceae (23000) | ≥11 | - |
| Physenaceae | 2 | Asteropeiaceae | 5 | - |
| Restionaceae | 400 | Poaceae (10500 - Panicoideae (3319) - Chloridoideae (1409)) | 5772 | - |
| Salicaceae | 400 | <i>Flacourtia</i> (60) + <i>Abatia</i> (12) of Flacourtiaceae | 72 | + |
| Simmondsiaceae | 1 | Asteropeiaceae (5) + Caryophyllaceae (2200 - <i>Silene</i> (700) - <i>Schiedea</i> (22))+ Amaranthaceae (1000 - <i>Amaranthus</i> (60) - <i>Iresine</i> (80) - <i>Celosia</i> (45) - <i>Chamissoa</i> (2))+ Molluginaceae (120) + Portulacaceae (450 - <i>Ceraria</i> (4) - <i>Tiliacora</i> (22)) + Cactaceae (500) + Nyctaginaceae (400 - | 6143 | - |

| | | | | |
|---------------|---|--|-----|---|
| | | <i>Pisonia</i> (40) - <i>Grajelesia</i> (1)) + | | |
| | | Phytolaccaceae (30) + Aizoaceae (2500 - | | |
| | | <i>Tetragonia</i> (85) - <i>Glischrothamnus</i> (1)) | | |
| Tetramelaceae | 2 | Cucurbitaceae (391 monecious spp.) OR | ≥31 | - |
| | | Begoniaceae (950) OR Coriariaceae (18) + | | |
| | | Corynocarpaceae (13) | | |

TABLE 2.3: Obligate outbreeders and their sister taxa. The number of species given for the self-incompatible (SI) families in the focal group have been corrected against bias by subtracting the number of self-compatible (SC) members in the family from the total. The final column states the outcome of the sister-group comparison; + indicates the SI clade had more species, - indicates the SC clade had more species.

| SI CLADE | # | SC CLADE | # | +/- |
|---|------|---|------|-----|
| Betulaceae | 85 | Casuarinaceae | 96 | - |
| Cactaceae (- <i>Opuntia</i> (200)) | 1300 | Portulacaceae | 950 | + |
| Caryophyllaceae (- <i>Cerastium</i> (100) - <i>Corrigiola</i> (11) - <i>Dianthus</i> (300) - <i>Honckenya</i> (2) - <i>Moenchia</i> (3) - <i>Polycarpon</i> (16) - <i>Sagina</i> (20) - <i>Scleranthus</i> (15) - <i>Spergula</i> (6) - <i>Spergularia</i> (25) - <i>Stellaria</i> (175) | 1527 | Amaranthaceae | 1000 | + |
| Cyperaceae (- <i>Elocharis</i> (120) - <i>Eriophorum</i> (12)) | 5168 | Juncaceae | 355 | + |
| Eupomatiaceae | 2 | Annonaceae | 2400 | - |
| Gentianaceae (- <i>Centaurium</i> (20) - <i>Cotylanthera</i> (4)) | 1176 | Loganiaceae | 15 | + |
| Geraniaceae (- <i>Geranium</i> (300)) | 450 | Vivianiaceae (6) OR Greyiaceae (3) + Francoaceae (2) + Melianthaceae (14) | ≤19 | + |
| Hypericaceae | 550 | Podostemaceae | 275 | + |
| Illiciaceae | 42 | Schisandraceae | 50 | - |
| Juglandaceae | 60 | Myricaceae | 50 | + |
| Lythraceae | 600 | Onagraceae | 680 | - |
| Malvaceae (- <i>Gossypium</i> (39) - <i>Hibiscus</i> (300) - <i>Urena</i> (6)) | 1205 | Sarcolaenaceae (62) + Dipterocarpaceae (700) + Cistaceae (200) + Tiliaceae (450) OR Thymelaeaceae (700) OR Bixaceae (4) OR Neuradaceae (10) | ≤962 | + |
| Myrsinaceae | 1000 | Primulaceae (1000) + | 1110 | - |

| | | | | |
|---|-------|------------------------|------|---|
| | | Theophrastaceae (110) | | |
| Orchidaceae (- <i>Bletilla</i> (9) - | 18457 | Blandfordiaceae (4) OR | ≤521 | + |
| <i>Cephalanthera</i> (14) - <i>Coeloglossum</i> | | Anthericaceae (575) OR | 6 | |
| (1) - <i>Epipactis</i> (22) - <i>Epipogium</i> (3) - | | Asteliaceae (35) OR | | |
| <i>Goodyera</i> (55) - <i>Habenaria</i> (600) - | | Hypoxidaceae (220) OR | | |
| <i>Herminium</i> (30) - <i>Nigritella</i> (2) - | | Asparagales (families | | |
| <i>Ophrys</i> (25) - <i>Orchis</i> (33) - <i>Phaius</i> | | totaling ~5216 spp) | | |
| (45) - <i>Pseudorchis</i> (3) - <i>Spathoglottis</i> | | | | |
| (30) - <i>Sprianthes</i> (30) - <i>Vanilla</i> (100) | | | | |
| - <i>Zeuxine</i> (26) - <i>Zygopetalum</i> (15)) | | | | |
| Oxalidaceae | 700 | Cunoniaceae (250) + | 618 | + |
| | | Elaeocarpaceae (325) + | | |
| | | Tremandraceae (43) | | |
| Passifloraceae | 600 | Malesherbiaceae(35) + | 185 | + |
| | | Turneraceae (150) | | |
| Plumbaginaceae | 650 | Polygonaceae | 1100 | - |
| Platanaceae | 10 | Proteaceae | 1050 | - |
| Resedaceae | 80 | Brassicaceae | 3200 | - |
| Rutaceae (- <i>Citrus</i> (20) - <i>Ptelea</i> (11) - | 1366 | Meliaceae (1300) OR | ≤130 | + |
| <i>Triphasia</i> (3) - <i>Zanthoxylum</i> (250)) | | Simaroubaceae (100) | 0 | |
| Valerianaceae | 400 | Linnaeaceae(250) + | 810 | - |
| | | Caprifoliaceae(260) + | | |
| | | Dipsacaceae(300) | | |
| Winteraceae | 80 | Canellaceae | 16 | + |

TABLE 2.4: Summary of the different sister-group comparisons performed and their results. The results show the proportion of times that the focal group in question was more speciose than its sister group. The result is analyzed with respect to (1) how different it is from the one-half expected due to random processes using a binomial test (see methods) and (2) how different the result is from the dioecious sister-group comparison (DSGC) using the Fisher-exact test (where applicable).

| Sister-group comparison | Family level | | | Genus level | | |
|-------------------------|--------------|-------------------------------|-------------------------------|-------------|-------------------------------|-------------------------------|
| | Result | Different from half (p-value) | Different from DSGC (p-value) | Result | Different from half (p-value) | Different from DSGC (p-value) |
| <u>Consensus Tree</u> | | | | | | |
| Dioecious (DSGC) | 6/28 | 0.007 | - | 22/66 | 0.009 | - |
| Monoecious | - | - | - | 28/52 | 0.678 | 0.038 |
| Self-Incompatible | 13/22 | 0.524 | 0.009 | 29/56 | 0.894 | 0.045 |
| <u>Shortest Tree</u> | | | | | | |
| Dioecious | 7/36 | 0.0003 | - | - | - | - |
| Monoecious | 9/16 | 0.804 | 0.020 | - | - | - |
| Self-Incompatible | 17/32 | 0.860 | 0.005 | - | - | - |

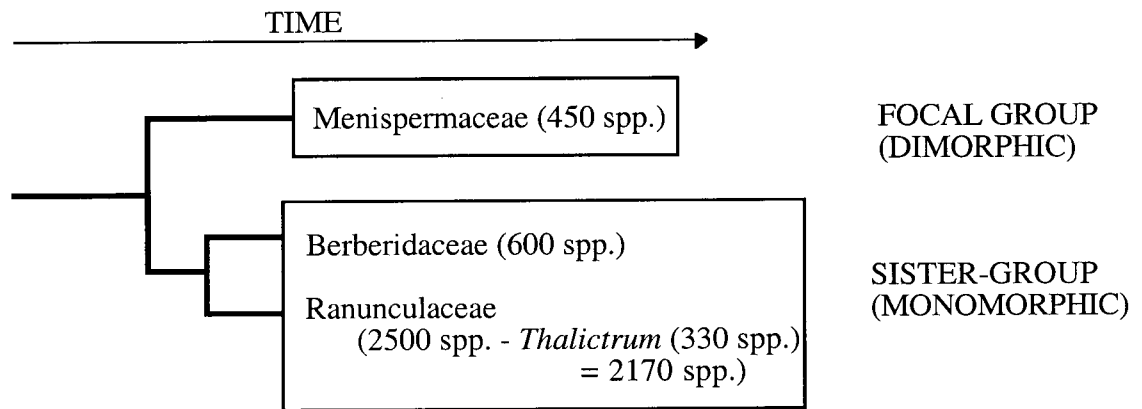


FIGURE 2.1: A sister-group comparison. The focal group and sister-group have existed for the same amount of time (i.e., since their divergence from a common ancestor) and hence should have equal numbers of species on average unless the trait in question (here, dimorphic breeding system) has a direct or indirect effect on speciation or extinction rates.

CHAPTER III: THE CONSEQUENCES OF DIOECY FOR SEED DISPERSAL: MODELING THE "SEED-SHADOW HANDICAP"²

INTRODUCTION

Dioecy constitutes the breeding system where sexual function is partitioned into male and female individuals. Dioecious angiosperms make up only ~6% (Renner and Ricklefs 1995) of flowering plants but are taxonomically distributed among both basal and derived families (Bawa 1980). By definition, only females produce seeds in dioecious species. The consequences of this fact are that the evolution of dioecy requires that reduced inbreeding depression (Charlesworth and Charlesworth 1978) or increased division of labor be beneficial, (Charnov 1979, Bawa 1980, Lloyd 1982) to outweigh the reduction in number of individuals producing seeds (Bawa 1980, Lloyd 1982) and the decrease in probability of ovule fertilization (Lloyd 1982, Pannell 1997, Pannell and Barrett 1998). A recent phylogenetic analysis of dioecy found that dioecious angiosperms are less speciose than sister clades of equal age, suggesting that they experience higher extinction rates or lower speciation rates than cosexual (i.e., hermaphroditic or monoecious) species (Chapter Two). Why dioecious lineages are less speciose remains a mystery. In this paper, we explore the influence that the reduction in seed bearers has on the spread and extinction risk of dioecious lineages.

To offset the fact that the number of seed bearers in a dioecious species is half that of a cosexual species, dioecious females must produce more or better seeds than their cosexual counterparts (Maynard Smith 1978) or disperse these seeds to an equivalent number of suitable sites as cosexuals to ensure the same number of progeny (Charnov 1976; Bawa 1980; Lloyd 1982; Lloyd and Bawa 1984). If seeds of dioecious females were twice as numerous (to account for the lack of investment in male structures), then dioecious "seed shadows", the areas surrounding the seed bearers to which seeds are dispersed (Janzen 1971), would be half as numerous yet twice as dense compared to those of hermaphrodites with equal dispersal characteristics (Fig. 3.1). This results in greater local resource competition (Clark 1978) among the dioecious offspring and diminishing gains for investment in female function, all else being equal (Charnov 1976; Lloyd and Bawa 1984). Both local resource competition and diminishing female gain curves have been shown to hinder the evolutionary transition between cosexuality and dioecy (Charnov 1976; Lloyd and Bawa 1984). However, the fact that dioecious populations can sometimes overcome these barriers is evidenced by the existence of dioecious species. Nevertheless, the increase in local

² Published as: Heilbut, J. C., K. L. Ilves, and S. P. Otto (2001) The consequences of dioecy on seed dispersal: modelling the seed shadow handicap. *Evolution* 55: 880-888. [In order to give credit to the other authors, I have chosen to keep the original text used in the publication for this chapter, using the tense "we" instead of "I", etc.].

resource competition experienced by dioecious populations could contribute to an increased extinction risk in dioecious populations (see Chapter Two).

Insight into the impact of a “seed-shadow handicap” can be gained by considering results from spatially-explicit models that have examined the conditions necessary for species coexistence (Tilman 1994; Bolker and Pacala 1999). These models have identified a competition-colonization trade-off; if an organism has a lower ability to disperse and colonize, it must have a higher competitive ability to persist. The seed-shadow handicap can be seen as a factor reducing the dispersal ability of dioecious species because seeds are clumped around females. Consequently, on the basis of these spatial models, we would expect that dioecy should only persist when there are compensating advantages in terms of competitive ability or dispersal ability. Furthermore, we can also predict how the dispersal distribution of seeds around parental plants might affect the magnitude of the seed-shadow handicap. Recent studies have shown that most plant dispersal systems follow a long-tailed, or leptokurtic, distribution (e.g. LeCorre *et al.* 1996; Higgins and Richardson 1999; Donoghue 1998), with most seeds landing extremely near the parental plant and a few going very far away. We predict that a more leptokurtic distribution of seeds will result in yet more sib-competition, thereby placing dioecious populations at an even greater disadvantage.

Further examination of the traits among surviving dioecious species may provide clues as to how dioecious lineages can offset the disadvantages created by the seed-shadow handicap. Correlations between dioecy and ecological factors may arise because (1) dioecy evolves more often in lineages that have these features or (2) when dioecy does evolve in these lineages, it is more likely to persist. Dioecy is an evolutionarily successful strategy for some very ancient families of angiosperms (e.g., Sargentodoxaceae and Menispermaceae) as well as a large number of ancient non-angiosperm divisions (e.g., Cycadophyta, Gnetophyta, and Ginkgophyta). Furthermore, dioecy is not always associated with increased extinction risk (e.g., in the Hawaiian islands [Sakai *et al.* 1995a]) and is relatively prevalent in many other tropical islands (Costa Rica 23%; New Zealand 14.5% [Bawa 1981]) despite Baker's Law (1955, 1959), which states that dioecy should be rare on islands due to the fact that dioecious individuals cannot successfully colonize an island without a mate. In addition to being correlated with oceanic islands, dioecy has been found to be correlated with perennial life history, tropical environment, biotic dispersal (fleshy fruits), and abiotic pollination (reviewed by Renner and Ricklefs, 1995). Each of these correlations, or combinations thereof, may be critical for offsetting the disadvantages incumbent on dioecious lineages.

This study aims to investigate (1) how much of a disadvantage is imposed on dioecious

populations because seed dispersal is limited to the seed-bearing females, (2) how the seed-shadow handicap changes depending on the mode of seed dispersal, and, finally, (3) how other factors, such as the number of seeds dispersed, life history, seed bank, and per-seed fitness and dispersal, may offset these disadvantages. In an approach very similar to that of Hamilton and May (1977), who investigate the evolution of dispersal when offspring compete for space, we first examine the effects of having a lower number of seed bearers on a dioecious population by designing a computer simulation of a two-dimensional "island" where females and cosexuals are identical in every way except that dioecious females produce twice the number of seeds as cosexuals but distribute them to equal-sized seed shadows (Fig. 3.1). Simulations estimate the competitive ability of the dioecious populations when the two types (i.e., dioecious and cosexual) colonize the island simultaneously and when one type establishes and the other is introduced. We then approach the problem analytically using a one-dimensional model of population spread (Lewis 2000) and compare the spread rates of dioecious versus cosexual populations. Results from both methods suggest that when all other factors are equal, cosexuality is far more successful due to the greater efficiency of seed dispersal. Our models do not incorporate gene flow between dioecious and cosexual individuals and are, instead, a measure of competition between dioecious populations and similar cosexual populations. Despite the widely recognized importance of the competitive ability of species in structuring communities (Goldberg 1996) and the large amount of theory developed to explore the relative effects of colonization ability versus competitive ability (Tilman 1994, Bolker and Pacala 1999), simulation of interspecific competition between plants with differing breeding systems is a neglected area of study (but see Durrett and Levin 1998). Investigating the effects of dioecy on competitive ability can expand our knowledge of macroscale phenomena such as the prevalence and distribution of breeding systems.

METHODS

The Basic Model

The goal of this study was to apply a simplified version of classical competition-colonization trade-off theory directly to competition between dioecious and cosexual plants. The program (available from the authors upon request) was written in Pascal and was run using CodeWarrior IDE Version 3.2 on a PowerMac computer. To begin, we explored a basic set of simulations. Later, we describe further simulations that explored altering various attributes of the basic set. In the basic model, we assumed equal viability (i.e., equal germination rates) and equal seed dispersal from the parental plant for dioecious and cosexual plants alike. Mate assurance is guaranteed for cosexuals at all times and is guaranteed for dioecious females as long as at least one male exists on the island. The model is started with one female, one male, and two cosexuals placed in random cells of a 50 x 50 lattice array (as in Pannell 1997). The seed set of each plant (n)

and the direction (seeds were only allowed to move in the directions: North, South, East, West, North-East, North-West, South-East, South-West) and distance that each seed travels were chosen at random for each seed using the random number generator "marsini" (Marsaglia *et al.* 1990). To isolate the effects of the seed-shadow handicap from the "cost-of-males" in dioecious species, the average number of seeds produced by dioecious females ($2n$) was twice that of cosexuals (n). The value of n varied as a random variable with mean n for each parental plant according to either a Poisson (if $n < 30$) or normal (if $n \geq 30$) distribution with variance equal to the mean. The fecundity (n) was set to 3, 4, 5, 6, 7, 8, 9, 10, 15, or 25. The low mean fecundities give an indication of how the stochastic deviations from equal sex ratio affects the success of dioecious populations because these low values of n give a higher chance of all male or all female progeny at the beginning of the simulation. At these low fecundities, both the cosexual and dioecious populations went extinct occasionally, in which case, the simulation was not counted.

The distance traveled by each seed and its parental plant followed a Poisson or a leptokurtic distribution. Given the same mean dispersal distance, a leptokurtic distribution generates more sib-competition because most seeds are clumped together near the parent. In the Poisson-dispersed simulations, the average distance that a seed moved was one cell away from the mother plant. For a seed traveling in the diagonal direction, this corresponds to a Euclidean distance of $\sqrt{1 + 1} = 1.4$ units (where one unit is the average distance between adjacent cells in a row or in a column). The average Euclidean distance traveled by seeds in the Poisson simulations is thus 1.2 units away from the parental plant. Effort was taken to make the average dispersal distance in the leptokurtic dispersal distribution roughly equivalent to that in the Poisson dispersal distribution (i. e., the average Euclidean dispersal distance was also 1.2 units when we let 97.8% of seeds fall randomly within the nine cells around the parental plant (including the parental site) and 2.2% disperse to random cells on the island, including the sites around the parent).

It was assumed that the boundaries of the island represent transitions to inhospitable environments, i.e., a seed does not germinate if the direction and distance traveled place it outside the island's dimensions. When a seed lands on the island, it is tabulated as "waiting" for that particular site. After seed dispersal, the adults died. For every site where multiple seeds have landed, one seed was chosen at random to germinate and occupy that site in the next generation, and the other waiting seeds died. The sex of dioecious individuals was chosen randomly with a 50% chance of being female. For more biological reality, the program also included environmental stochasticity by randomly making 10% of the sites on the island uninhabitable each generation. (10% environmental stochasticity was chosen arbitrarily but further simulations indicate the level has little effect on the qualitative results .) The simulations were run until either the cosexuals or

dioecious individuals went extinct.

Extensions. To explore the characteristics that reduce the seed-shadow handicap, we made several extensions to our simulations (see Table 3.1 for descriptions). In particular, we examined the influence of perenniality, a seed bank, and high average dispersal distance on the survival and spread of dioecy. We also measured the seed-shadow handicap in terms of the susceptibility of dioecious populations to invasion by cosexuals and their ability to invade cosexual populations. Finally, we tested how much of an advantage dioecious individuals would need to have, in terms of either per-seed competitive ability (W) or average dispersal distance (Δ), in order to constitute a majority ($\geq 50\%$) of the population after 100 generations. Note that competitive ability of dioecious seeds (W) could be greater than that of cosexual seeds if the per-seed investment of females is greater or if they suffer from less inbreeding depression. Regardless of the mechanism, if there were x dioecious seeds and y cosexual seeds on a site, we modeled competition by altering the probability of successful germination for a dioecious seed to $Wx / (Wx + y)$.

RESULTS

The Basic Model

Examples of the population growth curves of the simulated dioecious population with and without cosexual competitors are shown in Figure 3.2. Results for the basic model with seed sets of $n = 5, 10$, and 25 (10 simulations run for each) are presented in Table 3.2 (data for $n = 4, 6, 7, 8, 9, 15$ are similar and are not shown). Dioecious populations were extremely poor competitors. On average, it took dioecious individuals fewer than 100 generations to go extinct in the Poisson simulations and fewer than 50 generations to go extinct in the leptokurtic simulations. In contrast, cosexuals never went extinct before dioecious individuals. Indeed, dioecy was at a disadvantage for every fecundity tested for both seed dispersal distributions.

Perenniality, seed bank, and increased dispersal

Adding perenniality, a seed bank, or a longer average dispersal distance to the model extended the persistence time of dioecy on the island (Table 3.2), but these factors did not prevent the eventual extinction of dioecy. Although the dioecious population always went extinct even at the highest dispersal distances tested, they do as well as cosexuals (i.e., comprise $\sim 50\%$ of the population after 10000 generations) if dispersal is entirely random over the island, as expected (data not shown).

Invasibility Differences

Results of the invasibility simulations indicate that dioecious individuals are unable to

invade cosexual populations and are susceptible to invasion by cosexuals. Dioecious invaders never drove the cosexual residents to extinction, regardless of the average fecundity or the distribution of seed dispersal (Table 3.3). Cosexual invaders, on the other hand, were often able to drive dioecious residents to extinction. With leptokurtic seed dispersal, dioecious populations were more often invaded by cosexual colonizers, which is consistent with the expectation that more seeds fall near the parent than with a Poisson dispersal of seeds with the same average dispersal distance.

Fitness Advantages and Dispersal Advantages of Dioecious Plants

The percentage of dioecious plants after 100 generations (averaged over 10 simulations) increased dramatically when dioecious per-seed competitive advantage was increased in both the Poisson (Fig. 3.3a) and leptokurtic (Fig. 3.3b) seed dispersal models. When simulations were run with low fecundity values ($n < 5$), the stochastic loss of one sex early on caused the dioecious population to go extinct often, resulting in a low average proportion of dioecious individuals after 100 generations even when dioecious individuals were twice as fit as cosexuals. At higher fecundities ($25 \geq n \geq 5$), the competitive advantage of dioecious seeds had to be about 1.2 - 1.3 times that of cosexuals in the Poisson simulations and 1.4 - 2.0 times that of cosexuals in the leptokurtic simulations in order for dioecy to comprise the majority of individuals on the island after 100 generations (Figs. 3.3 and 3.4). When dioecious females were not given a two-fold fecundity advantage (i.e., when both dioecious and hermaphroditic plants produced n seeds), the competitive advantage of dioecious seeds over cosexual seeds necessary for dioecy to persist increased dramatically, to at least three-fold and at least four-fold, for Poisson and leptokurtic simulations respectively (results not shown). This confirms that the seed-shadow handicap is substantial and is, in terms of competitive ability, equivalent to a major reduction in fitness.

When dioecious females were able to disperse their offspring farther on average than cosexual plants, dioecy became more successful. In contrast to when dioecy had a higher relative fitness, the percent of dioecious individuals after 100 years was relatively insensitive to fecundity (n ; $n \leq 5$ excluded). With a Poisson dispersal distribution (Fig. 3.4a), the percentage of dioecious plants in the population after 100 generations increased to 50% when dioecious plants dispersed between 1.6 - 1.8 times farther, on average, than cosexual plants. When the average dispersal distance was too high (> 5 units away), decreases in the proportion of dioecious occupants were observed as more seeds disperse off the island. In contrast, the leptokurtic simulations resulted in very little increase of dioecious plants after 100 generations until dioecious seeds dispersed approximately five times further than cosexuals (Fig. 3.4b). Note that, in the leptokurtic simulations, sites were chosen randomly on the island so that increasing dispersal distance did not

lead to increased seed loss to an inhospitable environment. In both the Poisson and leptokurtic simulations, no amount of dispersal advantage resulted in dioecious populations constituting a majority on the island when dioecious females and hermaphrodites had equal fecundity (n).

ALTERNATIVE ANALYSIS³

Although we have focused on the spread of dioecy within a two-dimensional island population of limited size, the seed-shadow handicap will slow the spread of dioecy whenever sibling seeds compete directly with one another. In this section, we briefly explore a different model, using theory developed to estimate the rate at which a new species spreads over continuous space.

In a seminal paper, Fisher (1937) showed that the spread through space of a new advantageous allele could be described as a traveling wave radiating out from the source of the new allele. Under certain simplifying assumptions, this traveling wave attains a constant speed (c). Similar results have been obtained for the growth and spread of a population following colonization of an area (e.g. Kot and Schaffer 1986). Here, we use the results of Lewis (2000) to compare the spread rate of a dioecious population to that of a cosexual population. Because Lewis' analysis includes the effects of competition on the spread rate, his results can be applied directly to assess the effects of the seed-shadow handicap on the spread of dioecious populations.

In Lewis' model, there is a continuous, one-dimensional spatial axis (x), over which a population is spreading. In the absence of competition, the population size is assumed to be small relative to the resources available, such that growth at any site is approximately exponential. Individuals produce a number of offspring drawn from a Poisson distribution with mean R_0 . Each offspring migrates away from the parent by a distance drawn at random from a dispersal distribution, $k(\Delta x)$. Starting from an initially clustered population, Lewis (2000) demonstrated that the population would eventually (for certain classes of dispersal distributions) spread at a constant rate, c , to the left and the right. Although an exact solution for c was not found, Lewis derived upper and lower bounds to the spread rate. Lewis also explored the effects of competition, by positing that individuals would be unable to survive and reproduce if other individuals had landed within a small region (ϵ) around them. Competition thus reduced the reproductive output per parent from R_0 to approximately $R_0(1 - \epsilon p)$, where p is the local density of other individuals

³ The "Alternative Analysis" described in this section was provided in its entirety by Sarah P. Otto and no credit for its contents should be given to the author of this thesis. Because it shows further evidence of the seed-shadow disadvantage, this section was not removed from the chapter and remains in the same form as it was presented in the *Evolution* publication.

given the presence of a focal individual. Lewis' analysis showed that populations with higher values of ϵ (stronger local competition) would spread at lower rates.

For our purposes, we set the average reproductive rate of dioecious and cosexual individuals to be equal (R_0). Because only half of the dioecious population produces seeds, this assumes that each female produces twice as much seed as each hermaphrodite. Consequently, near the advancing wave of the population (where density is low), the seeds of a dioecious plant experience approximately twice the local density of competing seeds as the seeds of a hermaphrodite (assuming equal dispersal distributions). The effect of mating system on spread rate can thus be explored by setting ϵp for a dioecious population to twice that of a cosexual population.

For three different seed dispersal distributions (Fig. 3.5), we calculated the rate of spread of a dioecious population relative to that of a cosexual population (Fig. 3.6). These curves are based on Lewis' upper bound for c (eqn 24), which overestimates the spread rate of dioecy relative to cosexuality. Based on simulations presented in Lewis (2000), the curves in Figure 3.6 should actually decline more steeply with ϵ , implying that dioecious populations will spread at an even lower rate relative to cosexual populations.

Several conclusions may be drawn from these results. First, if the average dispersal distance is much larger than the spatial scale over which competition occurs ($\epsilon \ll \kappa$), then the seed-shadow handicap disappears. Second, as found in the island simulations, the seed-shadow handicap is more severe when the dispersal distribution is more peaked (leptokurtic) for a given average dispersal distance, because there is then a higher chance that a seed will fall near the parent and near sibling seeds (compare curves C to A in Fig. 3.5 and 3.6). Finally, it is possible for dioecy to compensate for increased sib-competition if dioecious individuals have a higher fitness, but, as in the island model, the fitness advantage has to be substantial for dioecious and cosexual populations to spread at the same rate (Fig. 3.6).

DISCUSSION

The prevalence of dioecy in animals but not in plants suggests that spatial constraints created by the lack of mobility in plants may be a critical force determining the abundance and distribution of breeding system strategies in extant species. Consequently, it is surprising that stochastic spatial effects have seldom been incorporated into models of the evolution, or subsequent success, of dioecy. Our simulations place dioecious and cosexual plants on a common island where the only limiting factor is space and allow seed dispersal to take place simultaneously

for all plants. Our results indicate that, as Bawa (1980) and Lloyd (1982) suggest and as classical competition-colonization theory predicts (Tilman 1994; Bolker and Pacala 1999), having spatially separated males and females reduces the competitive ability of dioecious populations even if the actual number of seeds produced is the same. Because seeds are more effectively dispersed to different sites by cosexuals, cosexuals spread faster, eventually driving dioecious populations to extinction. The more clumped the distribution of seeds, the more severe local resource competition becomes, as can be seen with both the analytical model and by comparing the leptokurtic (more clumped) and Poisson results. Although, our study only examines seed dispersal, a similar phenomenon might also occur with pollen dispersal, although we would expect the pollen-shadow handicap to be much weaker given that pollen is generally dispersed farther than seeds (Lloyd and Bawa 1984).

The seed-shadow handicap is a disadvantage that is unique to the dioecious condition and may be a major determinant of why dioecious clades seem to experience higher extinction rates or lower speciation rates while other outbreeding mechanisms (such as gametophytic or sporophytic self-incompatibility) do not (see Chapter Two). The difference between the seed-shadow handicap and other disadvantages associated with outbreeding, such as decreased mate assurance, is evident from a comparison of our results to those of Pannell and Barrett (1998). Pannell and Barrett (1998) found that extinction rates and extinction probabilities of self-incompatible outbreeders decreased when perenniality and seed banks were incorporated into an analytical metapopulation model presumably due to the fact that these factors improved the chance that an outbreeder will eventually find a mate. In contrast, our results showed that extinction was slowed but still inevitable for perennial dioecious plants or dioecious plants with seed banks. Decreased mate assurance is a disadvantage experienced by all outbreeders and will occur whether competitors are present or absent (as in Pannell and Barrett 1998). The seed-shadow handicap decreases the spread rate of dioecious populations in the presence or absence of competitors, but, in our model, only results in extinction when the competitor is present (Fig. 3.2). This is not a stochastic process, like the loss of one sex, but rather a deterministic process caused by the increased spread rate of the competitor driving the dioecious species extinct. Hence, the seed-shadow handicap represents a source of group selection that dioecious plants can only overcome by having an advantage over their competitors. Note that if dioecious species had a seed bank or were perennial but the cosexuals were not, then the success of dioecious populations may increase.

Our results indicate that large increases in the relative fitness or dispersal ability of dioecious seeds are required to increase the long-term success of dioecy. These estimates for the minimum fitness and dispersal advantage required by dioecious individuals may be conservative as

dioecious females often do not have a two-fold advantage over cosexuals in producing seeds, as we assumed in the majority of our simulations. For instance, in the gynodioecious species (containing females and cosexuals) of *Plantago lanceolata*, females set 69% more seeds than hermaphrodites, while in the gynodioecious *Stellaria longipes*, females set 60% more seeds than hermaphrodites (based on Richards 1997). If dioecious species do not double the number of seeds they produce relative to their cosexual neighbors, then dioecy must have an even larger increase in per-seed fitness or dispersal than estimated in Figures 3.3 and 3.4 to overcome the disadvantages caused by both the cost-of-males and the seed-shadow handicap. This is further indicated by the simulations in which females did not have a two-fold advantage; when both females and cosexuals produced n seeds, much larger fitness advantages were needed to enable dioecy to persist, and no amount of dispersal advantage could compensate (as, once dispersal is randomized across the entire island, the dioecious populations are left with only half the total number of seeds).

It is, of course, possible that dioecious seeds experience both fitness and dispersal effects together and therefore need less of an advantage in each. Interspecific competition experiments would be extremely useful for estimating the true advantages and disadvantages of dioecy in terms of seed set, dispersal, and competition. Although there have been many interspecific competition experiments (see Goldberg and Barton 1992, for review), few have examined the relative fitness or dispersal abilities of dioecious plants compared to cosexual neighbors in natural communities. Competition studies that have examined the effects of dioecy on seed dispersal have investigated competition between dioecious species (Nanami *et al.* 1999) and have not explored the influence of differing breeding systems. The paucity of competition studies between dioecious plants and their cosexual neighbors may be due, in part, to the presence of so many confounding ecological correlates with dioecy.

Ecological correlates of dioecy are often viewed in terms of how they might influence the initial evolution of dioecy, but they can also be viewed in terms of how they influence extinction rates of existing dioecious species. The ecological correlates of dioecy include perennial life history, large seeds, biotic dispersal (via fleshy fruits), abiotic pollen dispersal, and tropical oceanic islands (Renner and Ricklefs, 1995; references therein). Our results indicate that these correlates may each reduce the risk of extinction caused by the seed-shadow handicap. A perennial life history would improve the chances that some dioecious individuals successfully compete for sites (as mature plants) (Table 3.2). An increase in per-seed fitness can be thought of as an increase in vigor of progeny, which might result from a decrease in inbreeding depression (Charlesworth and Charlesworth 1978) or an increase in maternal investment to the quality/size of seeds (Bawa 1980). Larger seeds tend to have a relatively high fitness but are produced in smaller

numbers. When sib-competition is more severe, dioecious species that produce few seeds of high fitness are less likely to waste seeds because multiple seeds will less often land on the same site. Furthermore, large seeds are more likely to out-compete small seeds for a site (Bawa 1980). In addition, because the large fruit displays on dioecious females may attract far-dispersing birds (Bawa 1980), dispersal may be more efficient for some dioecious plants, thus decreasing competition between sibs. Bird dispersal has been hypothesized to contribute to the disproportionately high number of dioecious immigrants to oceanic islands, such as Hawaii and New Zealand (Carlquist 1974, Godley 1979, Bawa 1980, Sakai *et al.* 1995a, Sakai *et al.* 1995b). Without birds, the correlation between oceanic islands and dioecy is paradoxical as oceanic islands should be poorly colonized by species that require that two sexes make the same long-distance dispersal event (Baker's Law; Baker 1955, 1959). What may further contribute to the correlation between oceanic islands and dioecy is that, once transported there by birds, dioecy may not go extinct as often as it does elsewhere because cosexual competitors are rare or absent. Oceanic islands are thought to have an initially low biological diversity and incomplete vegetation cover (Carlquist 1974), which may give the dioecious individuals opportunity to invade and adapt to local conditions.

In summary, correlations currently observed between dioecy and ecological traits may result from a process whereby dioecious species with these traits are more likely to persist and survive competition than dioecious species without these traits. Phylogenetic analysis of dioecy and its correlates may be able to decipher whether these correlations are observed because of differences between lineages in the transition rates to dioecy or in the success of dioecy. That dioecy would experience low success rates in some lineages seems unavoidable as our model suggests that the conditions for dioecy to persist are extremely stringent, requiring dioecious individuals to have a substantial fitness or dispersal advantage over cosexuals even when the number of seeds per female is twice that of cosexuals. As empirical evidence accumulates about the seed-shadow handicap, it will become clearer how much of a fitness or dispersal advantage is required by a plant that switches to dioecy. Our results indicate that the seed-shadow handicap may impede the evolutionary success of dioecy and could help explain why dioecious lineages, when they do arise, experience higher extinction rates (Chapter Two).

TABLE 3.1: Extensions to the basic model. The basic model simulates one male, one female, and two cosexuals placed on the island and allows them to multiply until one type (cosexual or dioecious) goes extinct. 100 simulations were run at each fecundity (n) with no perennality ($P = 0$), no seed bank ($S = 0$), and an average dispersal distance of one cell ($D = 1$; Euclidean distance = 1.2). Each of these parameters was then altered in turn, as described.

| Extension | Program modification | Simulations |
|---|--|--|
| Perennality | Allowing all plants (dioecious and cosexual alike) to have a certain probability of surviving to reproduce until the following year (P). | As in the basic model, with $P = 0.5$ or $P = 0.99$ ($n = 10$) |
| Seed bank | Allowing each remaining seed on a site to survive until the next year with probability S . | As in the basic model with $S = 0.2$ or $S = 0.8$ ($n = 10$) |
| Increased dispersal of dioecious and cosexual seeds | Increasing the average number of sites traveled away from the parental plant (D). | As in the basic model, with $D = 5$ or $D = 10^*$ ($n = 10$) |
| Invasibility | Placing a male and female on random spots of the island after a cosexual population has had 200 generations to establish (and vice versa with two cosexuals invading an established dioecious population). | As in the basic model with $n = 10$ and $n = 100$ |
| Increasing dioecious relative fitness | Increasing the average fitness of dioecious seeds to W . | 10 simulations run for 100 generations with W ranging from 1.0 to 2.0 in increments of 0.1 with $n = 3, 4, 5, 6, 7, 8, 9, 10, 15, 25^{**}$ |
| Increasing dispersal of dioecious seeds only | Increasing the average dispersal distance of dioecious seeds to Δ . | As in the basic model, with Δ ranging from 1.0 to 5.0 (Poisson) and 1.0 to 8.0 (leptokurtic)*** |

*For leptokurtic simulations, $D = 5$ and $D = 10$ correspond to 82.8% and 62.8% of seeds traveling one cell away from the parent and the remaining seeds traveling to random cells, respectively.

Average Euclidean distances for both cosexual and dioecious seeds were 5.05 ($D = 5$) and 10.2 (D

= 10), whereas for Poisson simulations, $D = 5$ and $D = 10$ corresponded to average Euclidean distances of 5.72 and 11.3, respectively.

**Leptokurtic simulations were performed at the additional fecundity values of $n = 500$, 1000, and 1500. Poisson simulations were not performed at these high fecundities as all simulations with $n > 9$ had similar relationships between fitness and dioecious success.

***In the Poisson simulations, the average dispersal advantage of dioecy was incremented in units of 0.1 cells; in the leptokurtic simulations, the dispersal advantage was increased by allowing extra dispersal to random cells in 5% increments. The effect of increasing the percentage of seeds to random cells on the island by 5% was, on average, equivalent to increasing the Euclidean distance that one seed travels from its parental plant by approximately 1.2 units.

TABLE 3.2: Dioecious extinction. The average number of years \pm SD (over all 100 simulations) until the dioecious population went extinct under both Poisson and leptokurtic models of seed distribution. Dioecious and cosexual populations colonized the area simultaneously in all simulations. Due to the seed-shadow handicap, dioecy always went extinct first.

| | Average # years to extinction | |
|----------------------------------|-------------------------------|--------------------|
| | Leptokurtic | Poisson |
| Basic model (n = 5) | 31.6 \pm 16.0 | 69.3 \pm 33.4 |
| (n = 10) | 34.1 \pm 16.8 | 73.6 \pm 24.9 |
| (n = 25) | 35.2 \pm 6.8 | 70.2 \pm 19.4 |
| With extensions (n = 10): | | |
| perenniality (P = 0.5) | 57.8 \pm 16.9 | 124.8 \pm 39.6 |
| (P = 0.99) | 343.0 \pm 111.0 | 812.1 \pm 68.9 |
| seed bank (S = 0.2) | 56.5 \pm 16.1 | 114.5 \pm 39.8 |
| (S = 0.8) | 642.3 \pm 205 | 1446.7 \pm 476.9 |
| dispersal distance (D = 5) | 40.23 \pm 8.7 | 440.5 \pm 23.1 |
| (D = 10) | 59.9 \pm 12.8 | 120.6 \pm 52.9* |

* increasing the average dispersal distance (D) to 10 resulted in an increase in dioecious extinction rate in Poisson simulations because more seeds disperse off the island.

TABLE 3.3: Comparing dioecious and cosexual invasion success. The table shows the average fraction of 100 simulations that resulted in the extinction of established populations when invaded by the opposite type, and the average length of time \pm SD (in generations) for extinction to occur.

| | | Leptokurtic dispersal | | Poisson dispersal | |
|------------------------------|-----------|-----------------------|------------------|---------------------|------------------|
| | Fecundity | Resident Extinction | # of generations | Resident Extinction | # of generations |
| Dioecious invaders | n = 10 | 0/100 | - | 0/100 | - |
| (Cosexual residents) | n = 100 | 0/100 | - | 0/100 | - |
| Cosexual invaders | n = 10 | 64/100 | 79.5 \pm 12.5 | 47/100 | 149.4 \pm 20.4 |
| (Dioecious residents) | n = 100 | 70/100 | 82.3 \pm 9.0 | 48/100 | 156.4 \pm 21.5 |

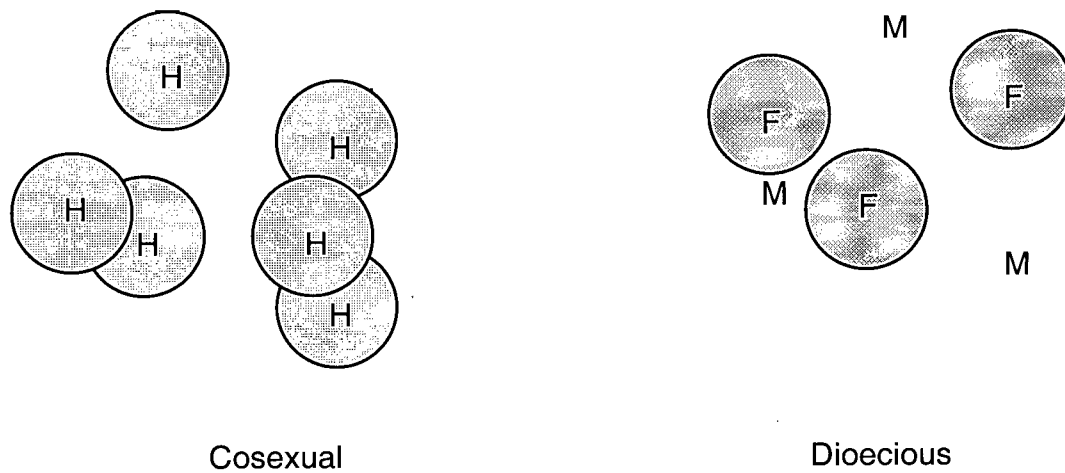


FIGURE 3.1: Representation of the "seed-shadow handicap". Dioecious populations have only half the number of seed dispersers and therefore will have less seed dispersal than an equivalent cosexual population.

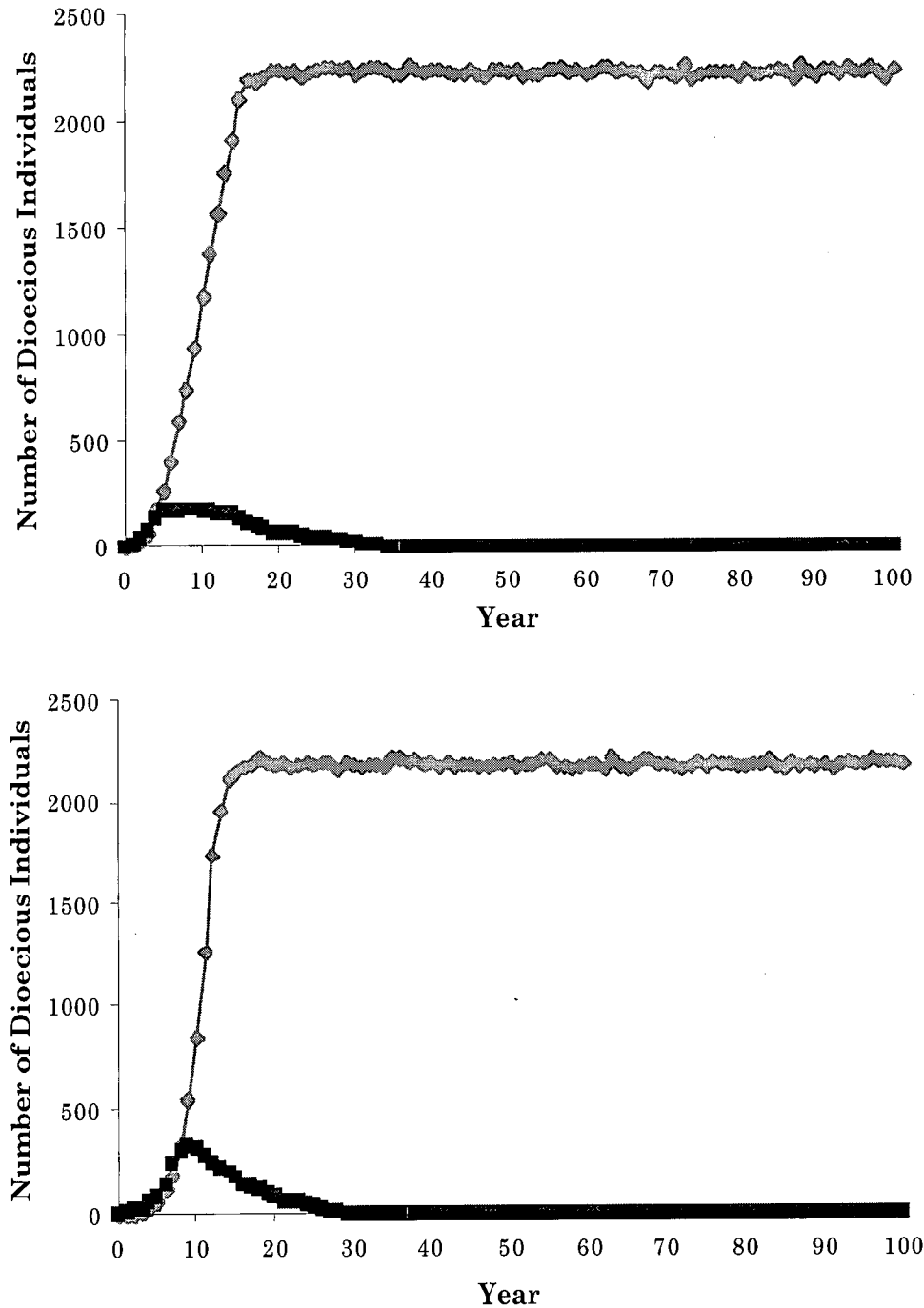


FIGURE 3.2: The dynamics of dioecious population size in two simulations of the basic model with $n = 10$. The upper curves depict growth without competing cosexuals, and the lower curves depict growth with competing cosexuals in (A) Poisson and (B) leptokurtic simulations. Simulations were also performed where mate assurance was guaranteed for both cosexual and dioecious plants alike (i.e., if one sex was missing, it was spontaneously re-created) but the qualitative results were the same.

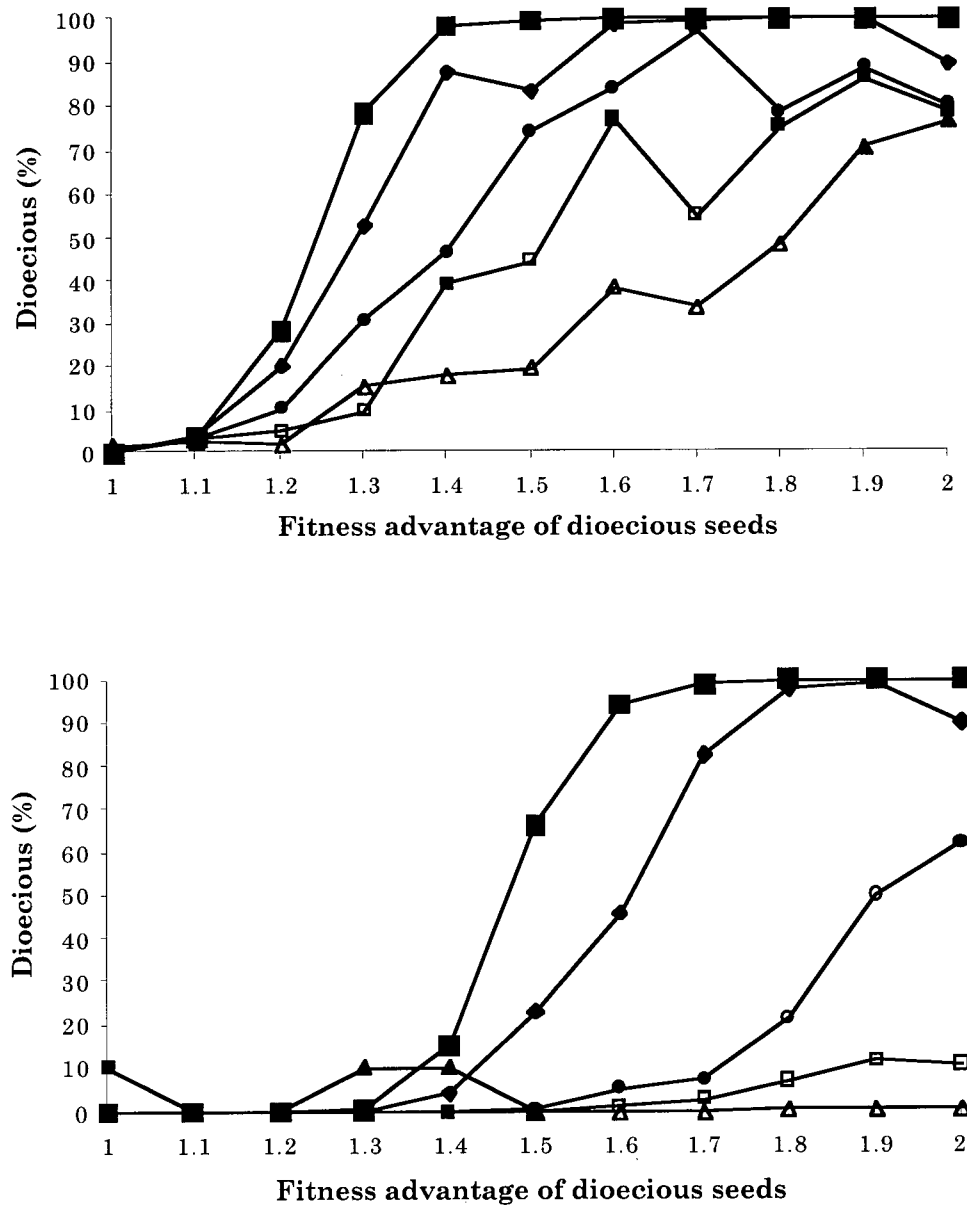


FIGURE 3.3: The success of dioecious individuals with higher fitness. The percentage of dioecious individuals after 100 generations of population growth using different relative fitnesses of the dioecious individuals (higher relative fitness of magnitude W was bestowed upon dioecious individuals). (A) depicts the results of Poisson simulations, (B) depicts the results of leptokurtic simulations. $n = 3$, \blacktriangle ; $n = 4$, \blacksquare ; $n = 5$, \bullet ; $n = 10$, \blacklozenge ; $n = 25$, \blacksquare . Open symbols denote cases where dioecy went extinct in more than 2 of the 10 simulations before 100 generations (these cases were included in the average % dioecy).

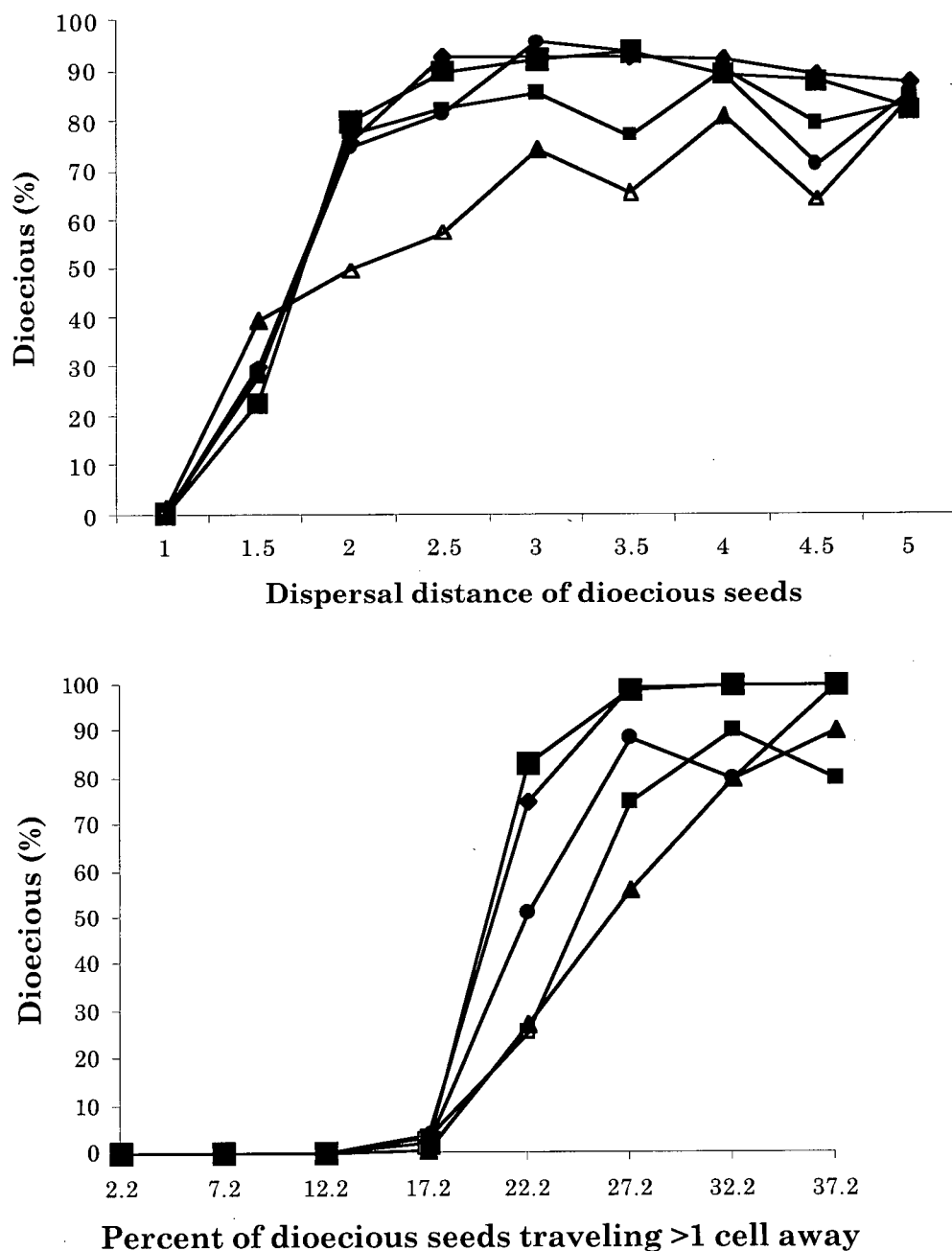


FIGURE 3.4: The success of dioecious individuals with higher dispersal. The percentage of dioecious individuals after 100 generations of population growth with different dispersal advantages of the dioecious individuals. $n = 3$, ▲; $n = 4$, ■; $n = 5$, ●; $n = 10$, ◆; $n = 25$, ■. Open symbols denote cases where dioecy went extinct in more than 2 of the 10 simulations before 100 generations. In the Poisson simulations (A) the x-axis represents the average dispersal distance for dioecious seeds. In the leptokurtic simulations (B), the x-axis represents the percent of seeds traveling > 1 site. For comparison, the cosexuals had 97.8% of seeds traveling to very near sites and 2.2% traveling to random cells. To roughly convert figures A and B, note that 77.8% short-distance travel (22.2% of seeds traveling >1 site away) in the leptokurtic simulations is equivalent to an average dispersal of 5.0 in the Poisson simulations.

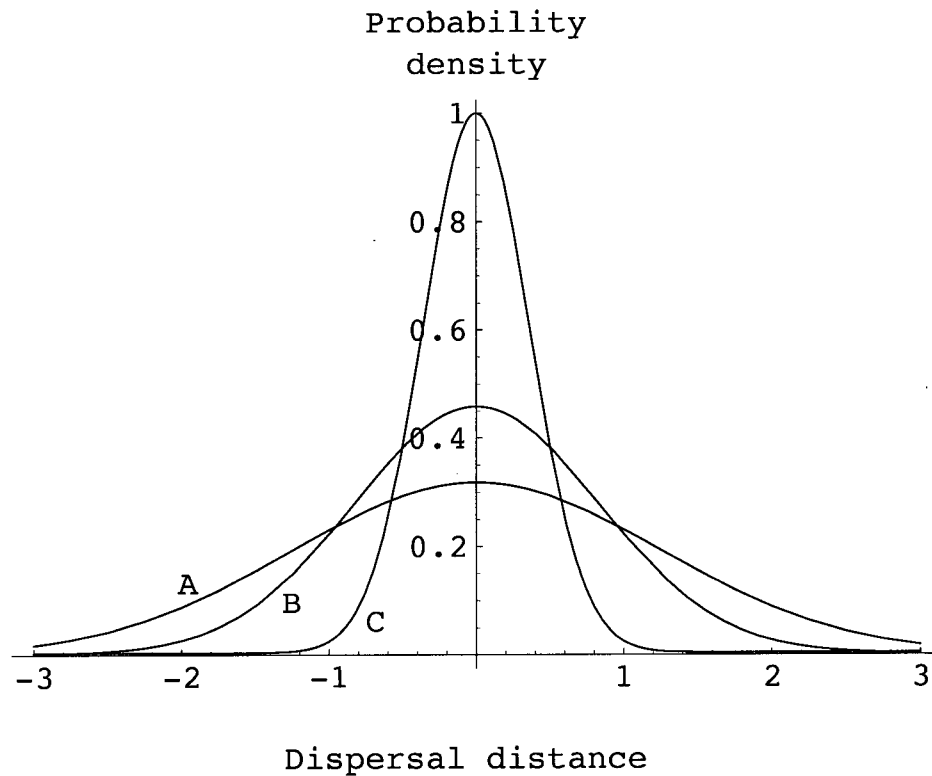


FIGURE 3.5: Dispersal distributions explored in a one-dimensional, continuous model of population expansion. In each case, the seed distribution is centered around the parent, and the average dispersal distance from the parent is one unit (i.e. distance is scaled according to the average dispersal distance of seeds). (A) Normal distribution with $\sigma = 1.2533$ (denoted as $N[0, 1.2533]$). (B) Composite Normal distribution with a 95% chance that distance is drawn from $N[0, 0.8304]$ and a 5% chance that distance is drawn from $N[0, 9.2878]$. (C) Composite Normal distribution with a 90% chance of dispersal drawn from $N[0, 0.3606]$ and a 10% chance that distance is drawn from $N[0, 9.2878]$. Distribution C is more leptokurtic than B, which is more leptokurtic than A.

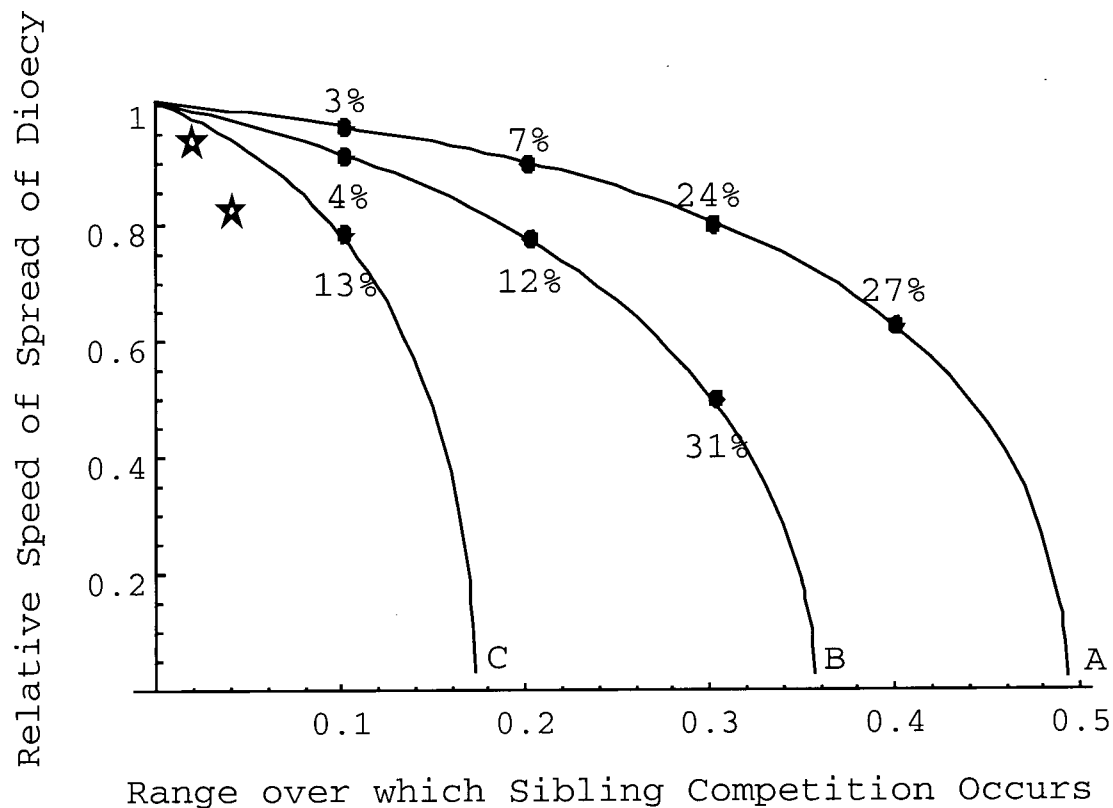


FIGURE 3.6: The relative speed of spread per generation of a dioecious population relative to that of a cosexual population. The x-axis gives the distance (scaled to the average dispersal distance) over which competing sibs will exclude each other (ϵ). The average number of seeds produced per individual (including males) is set to $R_0 = 1.5$, which assumes that each female produces twice as many seeds as each cosexual. Curves A, B, and C refer to the different dispersal distributions described in Figure 3.5. Dioecious populations always spread at a slower rate, especially when dispersal is leptokurtic such that the majority of seeds fall near the parent and experience more severe sib-competition. The numbers indicate the fitness advantage (in terms of R_0) that dioecious individuals would have to have in order to offset the seed-shadow handicap. The curves are based on the upper bound for the wave speed, eqn. (24), from Lewis (2000), which underestimates the seed-shadow handicap. That is, the curves should fall more sharply, as illustrated by the two stars derived from simulation results reported in Lewis (2000) using the dispersal distribution C. Consequently, the true fitness advantage required to balance the seed-shadow handicap is higher than the estimates given.

CHAPTER IV: WHEN LOOKS CAN KILL: THE EVOLUTION OF SEXUALLY-DIMORPHIC FLORAL DISPLAY AND THE EXTINCTION OF DIOECIOUS PLANTS⁴

INTRODUCTION

Sexual selection in angiosperms often involves a third party in the form of a pollinator, which acts as an agent to transport pollen from anther to stigma. Attracting pollinators is thought to be one of the most important functions of flowers (Bell 1985), and flowers have evolved an astounding number of ways to perform this function efficiently. Dioecious plants (having plants with only male or female function) with animal-pollinated flowers, however, typically lag behind their hermaphroditic relatives in the degree of floral display (Bawa 1980; Charlesworth 1993; Richards 1997). Proposed theories of why dioecious plants are correlated with small, white flowers include that (i) dioecy evolves more often in lineages with a low allocation to attraction (i.e., small, white flowers), and (ii) white flowers evolve more often in dioecious lineages (Charlesworth and Charlesworth 1987; Charlesworth 1993). A recent phylogenetic study, however, finds no evidence for either of these two patterns being the cause of the correlation between dioecy and small, white flowers (Chapter Five). Indeed, Sakai (1993) has performed ESS analyses that have shown that the evolution of attractive structures should proceed in much the same way in cosexual and dioecious plants alike. This study puts forth another possibility for the correlation that relies on the fact that showy traits, when they do evolve in a dioecious lineage, may become sexually-dimorphic and express in males more than females. In doing so, these traits may put the resulting showy, sexually dimorphic species at a higher risk of extinction as females may become pollen-limited, especially when pollinator abundance is low. This process would leave more dioecious representatives with small, white flowers extant and, thus, result in an observable correlation between the two traits.

This proposed theory relies on dioecious plants becoming sexually dimorphic for floral display. Sakai (1993) found that sexual dimorphism will not evolve in unisexual flowers unless there was such extreme selection by pollinators for floral display that the plants invested in floral display at the expense of viability. Nevertheless, many unisexual species are known to be sexually dimorphic for floral display (Delph 1996) with males being visited more often than females

⁴ Shortened form of this chapter submitted for publications as: Heilbut, J. C. and S. P. Otto. When looks can kill: the evolution of sexually dimorphic floral display and the extinction of dioecious plants. Submitted to *Science*. This chapter keeps its original tense of "I" instead of "we" as the main contributions of Dr. Otto are not incorporated in this version.

(Bierzychudek 1987; Burd 1994; Schemske and Ågren 1995). This contradiction may be due to the assumptions made by Sakai (1993) that the costs of floral display were the same for males and females and that visitation rates follow a constant logistic function of attraction. The assumption that the cost of increasing floral display is the same for males and females may be erroneous as females are thought to undergo higher costs of reproduction (Webb *et al.* 1999) and showy floral traits may benefit males more than females (Bateman 1948; Bell 1985). According to Bateman's Principle, male flowers should become showier than female flowers as the attraction of pollinators may serve male fitness more than it serves female fitness. Female fitness will be optimized by investing in offspring, while male fitness may be optimized by investing in showiness, resulting in further spread of the pollen (Bell 1985). The assumption in Sakai (1993) that flowers have optimized pollen production may often be violated also as the availability of pollinators usually varies from year to year (Herrera 1989; Eckhart 1992; Utelli and Roy 2000). Using both analytical and computer simulation approaches, this paper investigates how the evolution of dimorphism for floral display changes when differences in male and female fitness costs and pollinator fluctuations are incorporated.

I develop a model that explores the fate of a gene that increases floral display, giving an advantage in terms of pollinator visitation but a disadvantage in terms of the number of ovules and pollen produced in females and males, respectively. For females, however, a decline in ovule production directly affects fitness, whereas, in contrast to the model by Sakai (1993), a decline in pollen production may have little effect on male fitness as long as the male is visited more often by a pollinator. This analytical model was simplified by assuming that reproductive successes is limited by pollinators. However, the number of pollinators per year, as well as how much and how often the abundance varies over time could be important factors to consider for the evolution, and stability, of sexual dimorphism. Should a population be flooded with pollinators, even the less-showy females get visited and even more sexual dimorphism may evolve, assuming that there continues to be selection in favor of increased showiness in males. The greater the extent of sexual dimorphism that evolves, the more the population is left at risk of extinction should pollinators subsequently become scarce during which time the probability of females being stochastically excluded from pollinator visits is high. Therefore, I investigate the consequences of the absolute number of pollinators (pollinator abundance), pollinator movement (the number of visits each pollinator performs before leaving the patch), and pollen carryover on the evolution of showy flowers and sexual dimorphism. With these models of pollination dynamics, I have determined (1) how likely it is for a "showy" gene to evolve within a unisexual populations, (2) what degree of sexual dimorphism is favored in a unisexual population if the costs to each sex differ and how this differs when pollinators are abundant versus scarce, (3) how sexual dimorphism can affect

extinction probability, and (4) how extinction probability depends on the number of pollinators and how many visits each pollinator performs.

MODELING THE EVOLUTION OF A SHOWY TRAIT

Analytical Model

First, to examine the fate of an allele that affects attractiveness of both male and female flowers, I start with a "patch" of flowers that is visited by many pollinators. The model is designed to compare the pollination efficiency of self-incompatible (SI) plants to unisexual plants (UNI) in a very simplified manner as I assume that pollinators only carry pollen from one flower to the next (i.e., female flowers are fertilized by a pollinator only if the pollinator last visited a male). The model assumes that if a female flower is visited after a male flower, then ample pollen is delivered for both SI and UNI flowers regardless of the level of floral display (in this, my model differs from that of Sakai (1993)). The SI population is assumed to have many self-incompatibility alleles and the second flower visited is always compatible with the first. The initial population is fixed for the non-showy allele, P (for plain), which corresponds to white, inconspicuous flowers and a low frequency of visits from pollinators. The model then examines what occurs when an individual with a mutation, S (for showy), appears in the population at frequency q ($q = 1 - p$, where p is the frequency of the P allele). To simplify the analysis, a haploid model is assumed, but an extension to diploids is straightforward. Assuming that the rate of pollinator visitation is not limited by pollinator abundance, flowers expressing the S allele (both male and female flowers) enjoy an increase in pollinator visitation by a fraction V , but, if female or bisexual, suffer in terms of female fitness because of the energetic costs of producing showy flowers. That is, the number of ovules available for fertilization is proportionally reduced by R . The cost of this gene in terms of pollen production is assumed to be negligible as pollen is thought to be comparatively cheap and in large supply (Willson 1979). Viability of the plant is assumed to be unaffected and rewards to pollinators are assumed to be equal. Note that this model assumes that the fertilization rate of females is directly proportional to their attractiveness, which assumes that pollinators are limiting and have not saturated all female flowers with pollen. A showy allele is assumed to have the same relative success in both SI and UNI populations (Table 4.1). Although there is a higher number of visits that do not result in fertilization in the UNI model (caused by male-male, female-female, or female-male sequences of visits), this does not affect the success of the showy allele relative to the plain allele. In this, our model agrees with that of Sakai (1993) in that SI and UNI have the same probability of evolving showy floral traits when mutations are not sex-limited.

When the gene is expressed in both sexes, the change in frequency of the showy allele S in one generation is:

$$\Delta q = \frac{1/2pq(1+V) + 1/2pq(1+V)(1-R) + q^2(1+V)^2(1-R)}{p^2 + pq(1+V) + pq(1+V)(1-R) + q^2(1+V)^2(1-R)} - q. \quad (1)$$

Setting $\Delta q = 0$ and solving for \hat{q} demonstrates that there are three equilibria: $\hat{q} = 0$, $\hat{q} = 1$, and $\hat{q} = (2V-R-VR)/[2V(R-V+VR)]$ for the frequency of the S allele. A stability analysis of the three equilibria revealed that S will only rise in frequency to fixation if R (the cost in ovules) is less than $2V/(1+V)$. If $2V/(1+V) > R > 2V/(1+2V)$, the population reaches a polymorphic equilibrium at $\hat{q} = (2V-R-VR)/[2V(R-V+VR)]$. If $R > 2V/(1+2V)$, then S decreases in frequency to zero (Fig 4.1).

Some mutations affecting floral display could potentially be sex-limited in expression. If there is only expression in females, then the change in frequency becomes:

$$\Delta q = \frac{1/2pq + 1/2pq(1+V)(1-R) + q^2(1+V)(1-R)}{p^2 + pq + pq(1+V)(1-R) + q^2(1+V)(1-R)} - q, \quad (2)$$

as females with the S allele are the only sex to both experience the advantages of increased pollinator visitation and the disadvantages of decreased ovule production. Solving for when $\Delta q = 0$ results in only two equilibria, $\hat{q} = 1$ and $\hat{q} = 0$. Stability analyses reveal that $\hat{q} = 1$ is globally stable when $R < 2V/(1+V)$, and $\hat{q} = 0$ is globally stable when $R > 2V/(1+V)$. Alternatively, if expression of the gene occurs only in males, the recursion equation becomes:

$$\Delta q = \frac{1/2pq(1+V) + 1/2pq + q^2(1+V)}{p^2 + pq(1+V) + pq + q^2(1+V)} - q, \quad (3)$$

as females experience neither the disadvantages in terms of ovule production nor the advantages in terms of pollinator visitation. Note that R is no longer in the equation and that Δq is always positive for $V > 0$. Hence, the equilibrium of $\hat{q} = 1$ is globally stable for all positive values of V . A comparison of equations (1) - (3) indicates that showy genes that are only expressed in males will always be selected for more strongly than those expressed only in females or those expressed equally in both females and males. Nevertheless, while showy males gain an advantage over other males, fixation of such a showy allele will cause a decrease in overall seed set to the extent that males are more often visited by pollinators than females.

To illustrate the disadvantages that male-limited expression of showiness can cause to seed set, consider the following examples. If a population is fixed for the P allele and we assume an equal sex ratio, then 1/4 of pollinator visits involve a male visited first, followed by a female. In

contrast, if a population is fixed for an S allele that has male-limited expression, the fraction of such successful pollinator visits reduces to

$$1/4(1 - \frac{V^2/4}{1+V+V^2/4}). \quad (4)$$

The number of times that a pollinator visits two males in sequence rises with the amount by which males are more attractive than females. If males are twice as attractive as females ($V = 1$), the number of male to female visits reduces to 22.2% of the visits, and, in the extreme, if males are 100 times more attractive to pollinators then only 0.96% of visits involve a male followed by a female. Decreases in male-to-female pollen transfer of these magnitudes may result in not all the females of the population being pollinated, which may contribute substantially to increased extinction risk when sexual dimorphism is extreme. Note that, even though the number of successful pollination events is reduced, there is always selection for males to become showier than other males as long as pollen is produced in excess.

ESS Analysis

In the previous analysis, the original flower is plain, and R and V are held constant. To model the long-term evolution of the system, I allow the resident population to change. In turn, the advantage in terms of pollinator visitation and the disadvantage in terms of ovules should change as a function of the attractiveness of the resident population. This would be because (1) a showy trait will get relatively more visits if the rest of the population is plain than if the resident population is showy and (2) if the resident males are already more showy than females, the cost to females in terms of ovule production would be offset by an increased probability of getting their ovules fertilized. We wish to know whether there is some value of investment in showy flowers (called R^*) that cannot be invaded by any mutation with a different investment into attractive features (R). If there is such an R^* , it represents an evolutionary stable state (ESS).

Of course, if there were no constraints on which mutations are possible (except that $R, V > 0$), then the ESS would involve infinitely attractive flowers ($V = \infty$) that have no cost ($R = 0$). In reality, a trade-off must exist between devoting energy to floral display (V) and to reproduction (R). I assume $(1 - R)$ to be the fraction of the energy that is allocated to offspring/gametes and R to be the fraction devoted to showiness. If $R = 0$, then the flower is plain, and if $R = 1$, it is maximally showy but produces no offspring/gametes. I assume a linear trade-off relationship such that the attractiveness of a flower, $A = 1 + V$, is a linear function of the energy spent on showiness. That is, $A = 1 + cR$, where R is the amount of resources that are not allocated to seeds, and c is a conversion constant -- basically how efficient the plant is at converting energy to floral beauty. If c is large ($\gg 1$), then the plant can easily make much showier flowers (and vice versa).

With this relationship between R and V , we can take our previous recursion equations and put them all in terms of one parameter (R), whose value changes by mutation. If we think of the new R allele as a new showy allele invading an R^* population, the probability of being visited by a pollinator becomes $(1 + cR)/(1 + cR^*)$ as it depends on how attractive the flower is in comparison to the rest of the population, which is in the R^* state. Starting again with the case of showy mutations that are not sex-limited, (eq. 1) becomes:

$$\Delta q = \frac{1/2pq \frac{1+cR}{1+cR^*} (1-R^*) + 1/2pq \frac{1+cR}{1+cR^*} (1-R) + (q \frac{1+cR}{1+cR^*})^2 (1-R)}{p^2 (1-R^*) + pq \frac{1+cR}{1+cR^*} (1-R^*) + pq \frac{1+cR}{1+cR^*} (1-R) + (q \frac{1+cR}{1+cR^*})^2 (1-R)} - q. \quad (5)$$

To determine if this system has an ESS, I define λ as the initial spread of a mutant phenotype R occurring in a resident population R^* , that is

$$\lambda = d\Delta q / dq|_{q=0}$$

An ESS in terms of R^* must then solve

$$d\lambda / dR|_{R=R^*} = 0$$

in terms of R^* . This results in $R^* = 2/3 - 1/(3c)$. As $d^2\lambda / d^2R|_{R=R^*} < 0$, R^* was found to be a maximum and is therefore the value of R^* that cannot be invaded by individuals with either a slightly higher or slightly lower value of R . Assuming that $c = 1$, this gives an ESS R^* value at $1/3$. In the case where mutations affect males and females equally, where there is a linear tradeoff between showiness and seed production, and where increasing attractiveness of a plain flower by 50% reduces seed production by 50% ($c = 1$), the population of flowers should eventually evolve to a point where one-third of the energy available for reproduction is spent on showiness. As c rises above one, and floral display becomes relatively cheaper, more energy is devoted to showiness up to a maximum of $2/3$.

Sex-limited showy alleles can be examined in a similar manner. First consider the long-term evolution of floral display when mutations affect only males. Males will eventually suffer from too little investment in pollen, however, and thus it was assumed that pollen production follows a hyperbolic function, $(1-R)/((1-R)+aR)$, instead of being constant, where a is roughly a pollen grain: ovule cost ratio. If $R = 0$ and the male flower is completely plain, then its fitness through pollen is one. If $R = 1$, however, the male flower is devoting all of its energy to showiness and thus its fitness is zero. When $a \ll 1$, however, a male flower can be quite showy before there is a substantial effect on its fitness through pollen given that pollination occurs (e.g., if $a = 0.01$, then male fitness remains above 95% until more than 84% of the male's resources for

reproduction are devoted to showiness). Now the fitness for a male that invests R in attractive features in a population where males are more attractive than females by a factor of $(1 + cR^*)$ is equal to $(1 + cR^*) / [(1/2)(1 + cR^*) + 1/2] (1-R)/((1-R)+aR)$. Manipulating these equations in the same manner described above gives an ESS of

$$R_m^* = \frac{c - \sqrt{ac - a^2c + ac^2}}{c - ac} \quad (6)$$

for showy alleles that are only expressed in males. If we assume that $c = 1$ and $a = 0.01$, then, if mutations are male-limited in expression, the proportion of resources allotted to attractiveness reaches an ESS when males devote 86.7% of their resources to showiness. Male flowers become even more showy at the ESS as c rises above or a falls below these values (Fig. 4.2). A similar analysis done with female-limited expression reveals the ESS to be $1/2 - 1/(2c)$. As long as $c \leq 1$, then females devote zero percent of their resources to showiness. As the efficiency of turning resources into showiness (c) increases, however, the ESS for R^* in females rises to a maximum of $1/2$. Note that as a approaches one in the male-limited case, (6) approaches $1/2 - 1/(2c)$, which equals the female-limited ESS because the costs of attractiveness are linear functions in both cases as in the model of Sakai (1993).

What is the ESS if mutations produce a mix of alleles that express in both sexes or are male or female-limited? If mutations can result in any of these options, we must estimate the ESS for sexual dimorphism itself. The amount of sexual dimorphism that will arise, however, will depend on whether pollinators are abundant or scarce. To calculate what R_m^* and R_f^* will be under these scenarios requires the same steps as above in addition to finding two derivatives ($\partial\lambda/\partial R_m$ and $\partial\lambda/\partial R_f$). If pollinators are abundant and every flower receives many visits, then every female will be pollinated, and there is no selection to increase floral display in females. On the other hand, selection still acts on male flowers to increase floral display so that they benefit from being visited more often than other males. The ESS for male investment in floral display under this scenario evolves towards eqn (6) while the female investment remains at zero. If pollinators are rare and female reproductive success is linearly related to the number of pollinator visits (which depends on attractiveness), the evolutionarily stable strategy for female investment was found to be equal to the ESS found when mutations were limited to one sex (i.e., where $R_f^* = 1/2 - 1/(2c)$, and R_m^* is equal to eqn(6)). R_f^* is valid as long as $c > 1$ ($R_f^* = 0$ if $c < 1$) and R_m^* is valid as long as $c > 1$ and $0 < a < 1$ ($R_m^* = 0$ if $c < 1$), Figs. 4.2a and 4b). By plotting how much more attractive males can be than females [sexual dimorphism (D) = $(1 + cR_m)/(1 + cR_f)$] versus c (Fig 4.3), it can be seen that males are never more than twice as attractive to pollinators as females at the ESS when pollinators are rare but can be much more attractive when pollinators are abundant. This also assumes that the

efficiency with which the plant converts resources into floral display (c) is equal for both males and females. When c_m is allowed to be larger than c_f , the amount of sexual dimorphism at the ESS can be much larger than what was found when $c_m = c_f$.

Simulation Model - the effect of pollen carryover

The previous ESS analysis assumed that there was no pollen carryover, i.e., that a female could only be fertilized when visited right after a male, and this may have influenced the extent to which sexual dimorphism for floral display could evolve. In this section, I discuss simulation results that explore the importance of pollen carryover to the evolution of sexual dimorphism. The simulation model is identical to the analytical model in having P and S alleles that differ in the level of attractiveness and ovule production, with the alteration that the number of pollinators (B) that entered the patch, the number of flowers that each pollinator visited (N), and the amount of pollen that could remain on the pollinator for use in future visits (pollen carryover; L) could be varied. When $L = 0$, pollen only remains for one flower transfer. In contrast to the analytical model ($L = 0$ or $N = 2$, effectively), when $L = 0.5$ and $N > 2$, the pollen on the pollinator represents all the visits from the males in the following fashion. If two males are visited in sequence, then the second visit replaces half of the first male's pollen with the second male's pollen. If a third male is then visited, half of the pollen again falls off (which is half the pollen from male #1 and half the pollen from male #2) and is replaced by pollen from male #3. Using $L = 0.5$ is somewhat arbitrary but is approximately in the middle of the range (0.37 - 0.65) of estimates found in a number of plants for the average proportion of pollen that adheres to pollinators (de Jong *et al.* 1992 and references therein). Accordingly, a proportionate number of ovules are fertilized by pollen with the S allele when the pollinator visits a female flower. It is assumed that enough pollen is present on the pollinator to fertilize all of a female's ovules as long as at least one male was visited previously. Other assumptions include that all ovules within a flower are fertilized on the first visit and that pollen is unlimited ($a = 0$).

The computer program, written in Pascal, is available from the author. I first simulated pollinators visiting a patch of showy and non-showy flowers within a population of 2500 unisexual flowers to check the results obtained in Fig. 4.1 - 4.3. Simulations were then run that investigate the conditions under which a showy mutation S with increased attractiveness to pollinators, V , and disadvantage in terms of ovules, R , fixed in a population when pollinators only visited two flowers (i.e., $N = 2$) before leaving the patch. The simulation results matched what was found in the analytical model. When N is increased but sexual dimorphism is not allowed, the results are much the same as when $N = 2$ in the analytical model.

Increasing visit number had a major effect on the evolution of sexual dimorphism. This was seen in a simulation program that investigated the fate of a modifier gene that can turn off the showy gene in females when introduced into a population that is fixed for a showy gene that is not sex-limited. The number of times (out of 100 simulations) that a modifier gene causing males to be 10 times as attractive to pollinators than females ($D = 10$) rose from an initial frequency of 0.01 to fixation was recorded with ($L = 0.5$) and without ($L = 0$) pollen carryover (with N ranging from 2 to 10). Having many visits per pollinator with pollen carryover results in a greater probability that this high level of sexual dimorphism will be fixed in the population (Fig 4.4), a pattern similar to increasing the absolute number of pollinators (Fig. 4.3). Simulations were also run that started with a plain population and introduced a modifier gene that made males showier than females by a factor of 10. This gene was able to fix in 100% of the simulations for all values of N and L (results not shown). Starting with either a plain or showy population gives different results because the benefits (in terms of increased ovules) of switching to a low allocation in floral display in females can only materialize if these females are visited (i.e., when N is high and $L \neq 0$) whereas turning showiness on in males results in an immediate benefit of more visits.

Extinction risk analysis

Sexual dimorphism places a dioecious population at a greater risk of extinction if pollinator abundance subsequently declines because pollinators may fail to visit less attractive female flowers. To assess this risk, we consider a patch of flowers fertilized by B pollinators that each visit N flowers. A conservative estimate can be made if we assume that the only risk of extinction arises from pollinators failing to visit females and that the population persists at large size even when only one female is fertilized (more realistic population dynamics would make sexually-dimorphic populations more prone to extinction). The probability of extinction is then f^B , where f is the probability that a pollinator fails to fertilize any females. Pollinator failure will occur whenever a pollinator first visits females and then males, which in a population with an equal sex ratio and a level of sexual dimorphism, D , equals:

$$f = \sum_{i=0}^N \left(\frac{1}{1+D}\right)^i \left(\frac{D}{1+D}\right)^{N-i} = \frac{D^{N+1} - 1}{(1+D)^N (D-1)} \quad (7)^5$$

(For populations with an unequal sex ratio, the risk of extinction can be found by multiplying D in the above by the ratio of males to females.) The extinction risk over 100 generations is dramatically higher with sexual dimorphism than in populations with equally attractive male and female flowers (Figure 4.5). For example, if ten pollinators each visit four flowers, a population with no sexual

⁵ Although the initial idea to model extinction risk due to pollinator limitation was my own, Drs. S. Otto and M. Whitlock should be credited for developing this extinction risk model up until this point.

dimorphism has a very small probability of going extinct within 100 generations (0.0009), while a population with four-fold more attractive males has a 21% risk of extinction and a population with ten-fold more attractive males is almost certain to go extinct (0.999). Notice that the extinction risk does not depend on the number of plants in the population, although the level of sexual dimorphism expected will be higher if, in the past, the number of pollinators exceeded the number of plants. Although these calculations assume that the number of visits per pollinator and the number of pollinators are constant, a stochastic model with B and N drawn from probability distributions generates qualitatively similar results.

This calculation also assumes an annual life history and many dioecious species are perennial (Renner and Ricklefs 1995). The reason for this correlation may lie with the increase in survival probability that perennial sexually-dimorphic dioecious species might experience. Extending this model to a perennial life history is straight-forward. The extinction probability depends on f^B and the life span of the individual (G) and pollinator failure now equals f^{BG} . If a plant species has $D = 20$, $N = 2$, and $B = 150$, an annual plant ($G = 1$) will have a 10% chance of going extinct within 100 years, whereas a plant species that lives for two years ($G = 2$) will have only a 1% chance and a plant species that lives for ten years ($G = 10$) will have only a 1.01×10^{-10} chance of going extinct (Figure 4.5).

DISCUSSION

Showy floral displays are likely to be associated with a cost (Schemske and Ågren 1995; LeCorff *et al.* 1998; Ashman 2000), and there is evidence that female fitness may be compromised more by these costs as the burden of developing fruit is placed on them alone (Lloyd and Webb 1977; Webb *et al.* 1999). The number of pollinators that respond to these showy floral traits has also been shown to vary between years and localities (Herrera 1989; Eckhart 1992; Utelli and Roy 2000). Models of the evolution of sexual dimorphism that assume equal costs of floral traits to males and females and a constant pollinator availability (e.g., Sakai 1993) have found that unisexual lineages are no more likely to evolve showy floral traits than bisexual lineages and are unlikely to evolve sexual dimorphism. I have created a new model, with unequal costs of floral traits to reexamine whether pollinator dynamics affect both (1) the likelihood of unisexual populations evolving showy floral traits and (2) the degree to which sexual dimorphism (differences in attractiveness between male and female flowers) is favored. In an analytical model tracking pollinators that visit two flowers and then leave a patch, I find, in agreement with the results of Sakai (1993), that a unisexual population is not prevented from evolving attractive traits. However, in contrast to the results of Sakai (1993), sexual dimorphism did not require that an upper limit on the size of floral structures be imposed. The evolutionary stable state for this model

with no pollen carryover and with equal efficiency of converting energy into floral display for males and females was for male flowers to be, at most, twice as showy as female flowers when pollinators are rare. If pollinators are abundant, however, a much higher degree of sexual dimorphism can evolve. The results of computer simulations were consistent with those of the analytical models, with the additional finding that the population is more likely to evolve sexual dimorphism if pollinators visit many flowers and pollen carryover is possible, basically because females are more likely to be pollinated even with low investment in floral display. Finally, one further analysis shows that if extreme dimorphism has evolved when pollinators were abundant, and pollinator abundance subsequently is severely decreased, unisexual populations with sexual dimorphism experience a much higher risk of extinction than their counterparts that are not sexually dimorphic.

These results differ from that of Sakai's (1993) because Sakai assumed a linear tradeoff between allocation to floral display and reproductive structures in both males and females and that the fitness of males and females are similarly limited by pollinator abundance. By altering these assumptions, the evolution of sexual dimorphism is much more likely, and this may agree more with some of the patterns seen among extant unisexual organisms. The rarity of dioecious species, attributable to higher extinction rates or lower speciation rates (see Chapter Two), may be related to the exacerbated pollinator limitation that develops when pollinators suddenly become rare. That is, extreme sexual dimorphism can evolve when pollinators are abundant in a dioecious species, but this then places that population at risk of extinction during years of few pollinators because pollinators disproportionately visit male flowers. Many studies have found that sexual dimorphism for flower number and flower size exists among unisexual species (Lloyd and Webb 1977; Delph 1996), but a higher extinction risk of sexually dimorphic species will only occur if this sexual dimorphism is accompanied by differential visitation rates. Although studies of visitation rates in dioecious species are few, these studies reveal that there is much variation in the amount by which males are visited over females. Dioecious males are visited more often than females by a factor of 1.5 in *Wurmbea dioica* (Vaughton and Ramsey 1998), two in *Fragaria virginiana* (Bell 1985), three to four in *Silene dioica* (Carlsson-Granér *et al.* 1998), and four in *Antennaria parviflora*. (Bierzuchudek 1987). According to our study, species such as *S. dioica* and *A. parviflora* are at greater risk of extinction if they experience large swings in pollinator abundance.

Prevalent theories as to why unisexuality and small, inconspicuous flowers are so strongly correlated (Lloyd 1982; Bawa 1980) have involved unisexuality evolving more often in white, inconspicuous lineages (Lloyd 1982; Charlesworth and Charlesworth 1987; Morgan 1992; Charlesworth 1993) or showy flowers evolving less often in unisexual lineages (Charlesworth and

Charlesworth 1987). The fact that showy unisexual species may be more subject to extinction than inconspicuous, unisexual species could also provide a potential explanation for this correlation. Interestingly, dioecious tropical species often have female flowers that are bigger than male flowers, possibly because the female perianth has a protective role in the development of large fleshy fruits (Bawa and Opler 1975; Delph 1996). This trend may be related to why so many extant dioecious species are found in the tropics (Bawa 1980; Renner and Ricklefs 1995) and why tropical dioecious species do not seem to experience higher extinction rates or lower speciation rates than their sister groups (Sakai *et al.* 1995).

The role of pollinator abundance and behavior in the evolution of breeding systems and floral display is starting to receive a lot of attention (Herrera 1989; Eckart 1992; Utelli and Roy 2000), but its role in extinction of plants has rarely been addressed. In one particular study of pollinator movements in *Acontium lycotonum* (Utelli and Roy 2000), high variability was observed in the number of visits (N) from a variety of pollinators (N ranged from 3-18 visits per pollinator with an average of 7.3 flower visits per pollinator). As differences of up to an order of magnitude have been recorded in the abundance of single pollinators between years (Eckart 1992; Utelli and Roy 2000), a sexually dimorphic unisexual species that evolves floral displays to attract one or a few, select pollinators will be more susceptible to extinction than one that attracts many generalist pollinators. This could be a potential reason why surviving unisexual species are polyphilic (pollinated by a wide variety of insects; Bawa 1979, Richards 1997).

The results found in this study may apply to both monoecious and dioecious species. Studies of pollinator behavior (e.g., Richards 1997, references therein; Utelli and Roy 2000) have found that pollinators tend to visit more than one flower on the same plant, a factor which will result in different susceptibility of monoecious and dioecious species to pollinator limitation. Monoecious species are often sexually dimorphic (van der Pijl 1978; Delph 1996), sometimes even to a greater extent than dioecious plants (e.g., in the monoecious *Begonia urophylla* and *Begonia tonduzii* male flowers are visited by a factor of 8.6 and 15.4, respectively (LeCorff *et al.* 1998)). Sexual dimorphism in monoecious species may not have the same consequences in terms of extinction rates perhaps because the less-attractive female flowers are visited more often due to their closeness to attractive male flowers. This may be why monoecious groups: (1) have more showy members than dioecious groups (e.g., members of Musaceae and Begoniaceae), and (2) unlike dioecious plants, do not have higher extinction rates or lower speciation rates (Chapter Two) than their sister-groups.

This is the first theory developed to explore the connection between the observation that dioecious species are so often small and white and, when any degree of showiness is present, females are often less showy than males. Unlike most theories that focus on how the correlation between white flowers and unisexuality arose (reviewed in Charlesworth 1993), I postulate that the correlation results from an increased extinction rate in dioecious species that evolve showiness, as these will also tend to evolve sexual dimorphism. Male flowers that are twice, or less, as attractive as female flowers are at a low risk of extinction due to pollen limitation. Any sexual dimorphism where males are visited more than twice as often as females may be heavily reliant upon having a large number of pollinators, a fact that could bear on the extinction risk of such species. Being reliant on a large pollinator population may also limit the spread and colonization ability of extremely sexually dimorphic, unisexual plants. More studies on the strength of pollinator preferences and discrimination, as well as on how pollinator frequencies change from year to year, would be useful in determining how important a force pollinators are in (1) promoting the evolution of showy, sexually dimorphic floral displays, and (2) causing higher risks of extinction during periods of pollinator limitation, thereby causing some of the correlations we see between dioecy and frequency of generalist-pollinated, inconspicuous flowers.

TABLE 4.1: Fitness in terms of visitation (V) and fecundity (R). The relative probability of all possible visit sequences and the resulting fitness in terms of seed-set is given when a pollinator visits only two flowers in a patch. Pollinators visit a plain P flower at a rate proportional to its frequency p and visit a showy S flower at frequency $q(1 + V)$, where V is the visitation advantage. (A) shows the relative frequency and fitnesses resulting from pollinators visiting an SI (self-incompatible) patch. Relative seed set in showy flowers is reduced due to resource allocation away from ovules by R compared to plain flowers. (B) shows the relative frequency and fitnesses resulting from pollinators visiting a UNI (unisexual) patch. Male and females are assumed to be in a 1:1 sex ratio. NF stands for "No Fertilization" taking place as fertilization of ovules only takes place when a female is visited after a male.

A.

| Visit #2 | P | S |
|-------------------|-------------|----------------|
| Visit #1 | | |
| P | p^2 | $pq(1 + V)$ |
| S | $pq(1 + V)$ | $[q(1 + V)]^2$ |
| Relative Seed Set | 1 | $1 - R$ |

B.

| Visit #2 | P (male) | P (female) | S (male) | S (female) |
|-------------------|------------|--------------|------------|----------------|
| Visit #1 | | | | |
| P (male) | NF | p^2 | NF | $pq(1 + V)$ |
| P (female) | NF | NF | NF | NF |
| S (male) | NF | $pq(1 + V)$ | NF | $[q(1 + V)]^2$ |
| S (female) | NF | NF | NF | NF |
| Relative Seed Set | - | 1 | - | $1 - R$ |

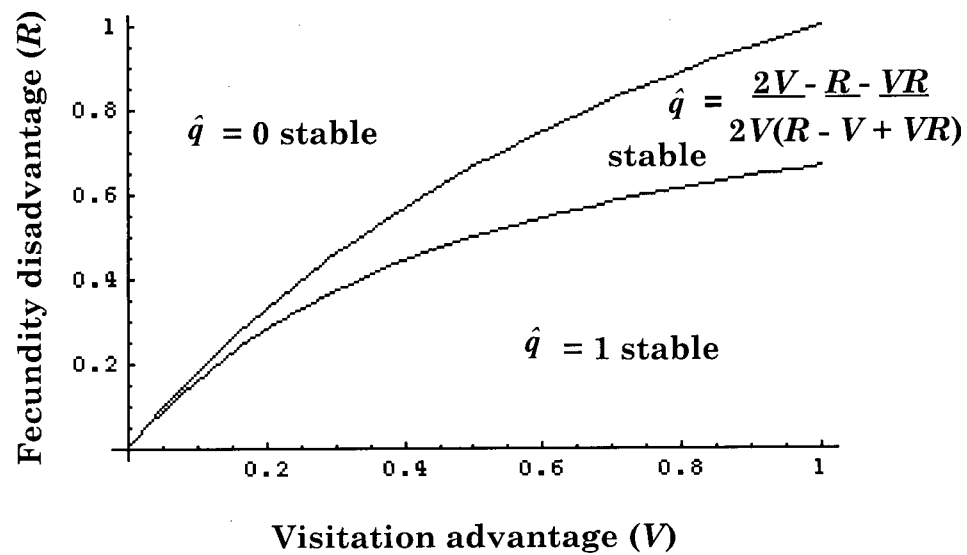


FIGURE 4.1: Three stable equilibria exist for showy alleles. Showy alleles that are not sex-limited in expression can be at equilibrium at: $\hat{q}=1$ (below the lower line), $\hat{q}=(2V-R-VR)/[2V(R-V+VR)]$ (between the two lines), and $\hat{q}=0$ (above the upper line). Only one of these equilibria is stable for a given combination of V (increase in attractiveness) and R (decrease in seed set), as shown.

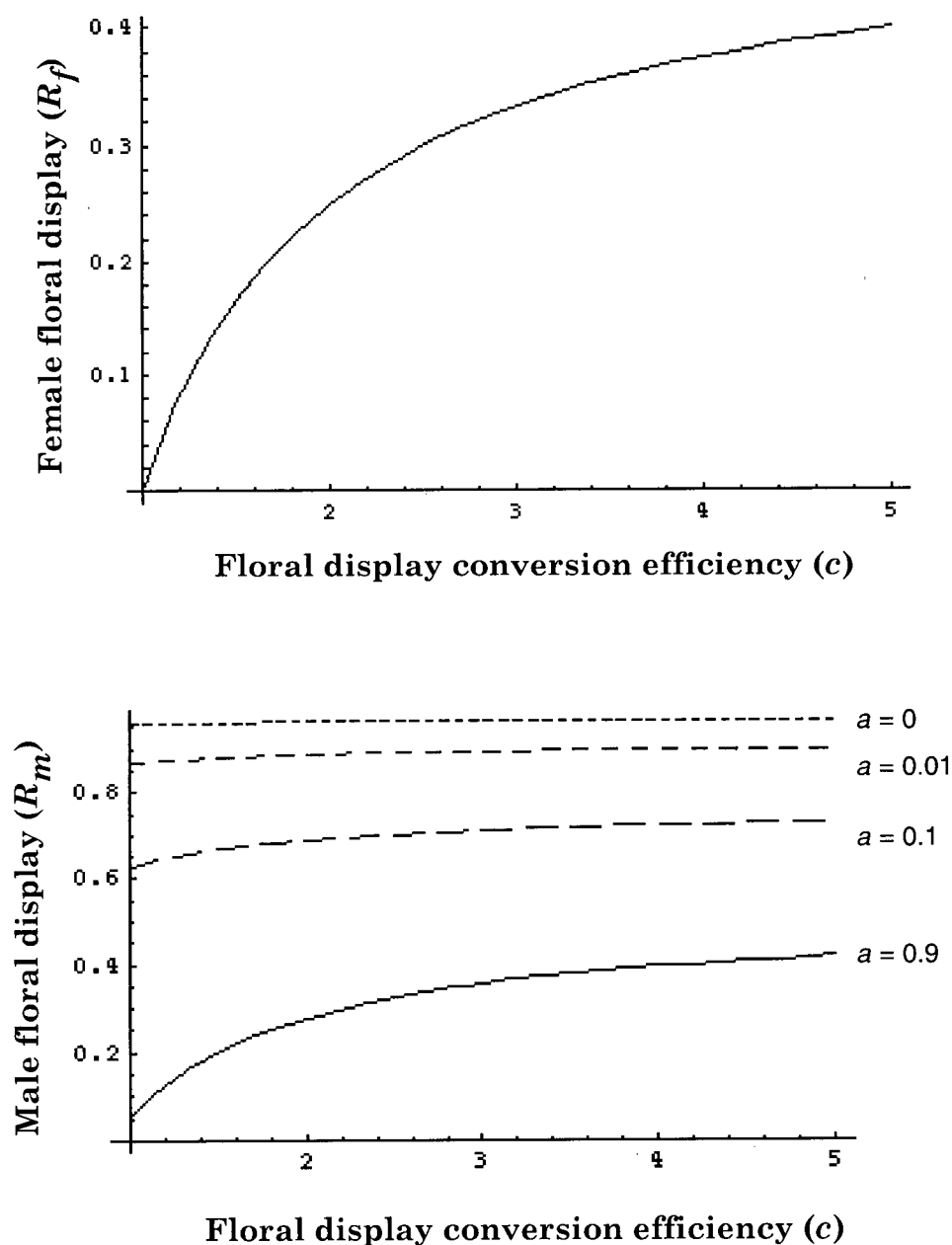


FIGURE 4.2: The extent of investment in floral display at ESS. The values of (A) R_f^* and (B) R_m^* obtained for values of c ranging from 1 to 5 and for $a = 0, 0.01, 0.1$, and 0.9 (for R_m^*). Assuming pollinators are limiting (as $R_f^* = 0$ if pollinators are abundant), these ESS values are found whether female-limited (A) or male-limited (B) mutations are considered separately or their co-evolution is considered.

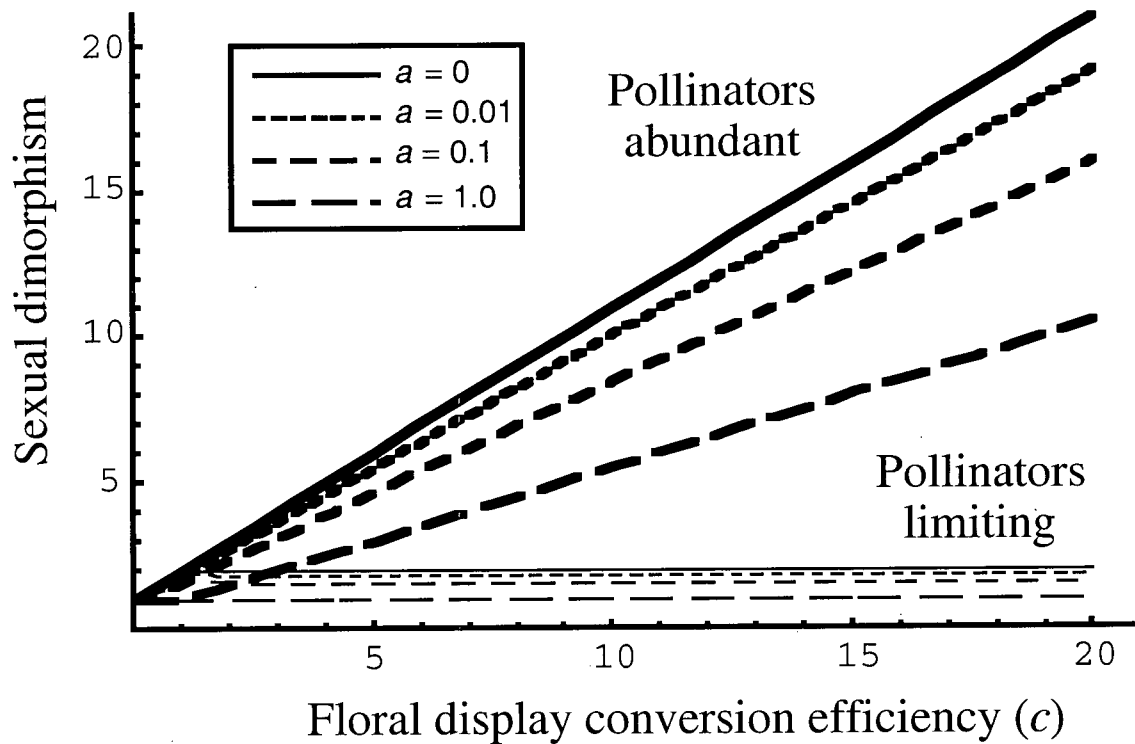


FIGURE 4.3: The amount of sexual dimorphism (D). The population evolves to be much more sexually dimorphic for floral display when pollinators are abundant (bold lines) than when pollinators are scarce (thin lines), when pollen is abundant (a is low), and when it is easy to increase the attractiveness of a flower (c is high).

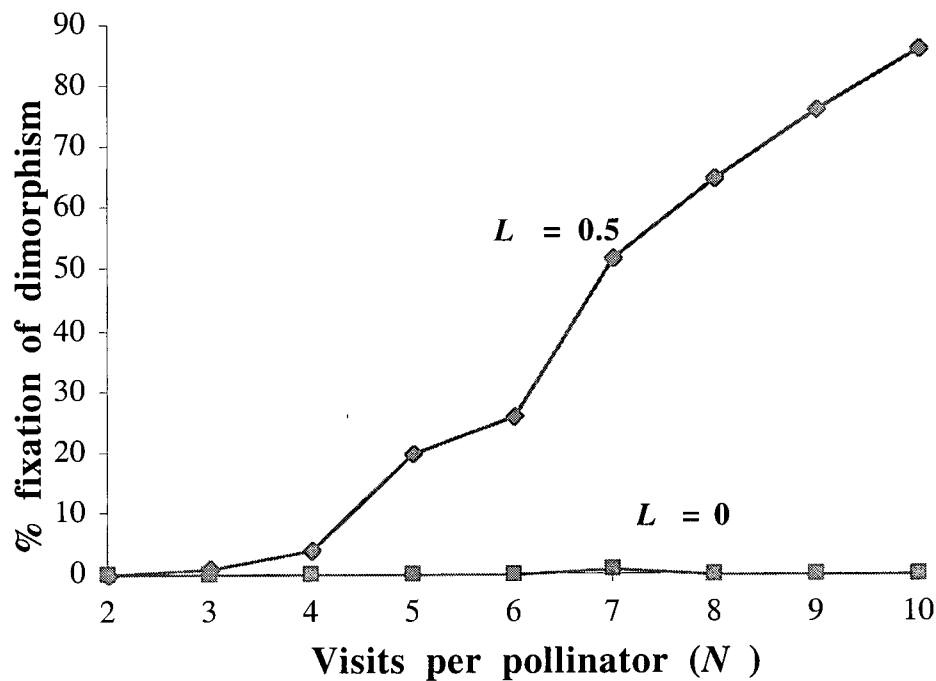


FIGURE 4.4: The effect of pollen carryover. The number of times (out of 100 simulations) that a gene increasing sexually-dimorphism by a factor 10 (males 10× as attractive as females) fixes as a function of the number of visits per pollinator (N). Fixation rarely occurs when there is low pollen carryover ($L = 0$) (Initial parameter: $R_m = R_f = 0.9$; Introduced parameters: $R_m = 0.9$, $R_f = 0$. Constant parameters: $c_m = c_f = 10$, Population size = 2500, $B = 1000$). The initial allele frequency was set to $p = 0.01$, which implies that a neutral allele is expected to fix 1% of the time.

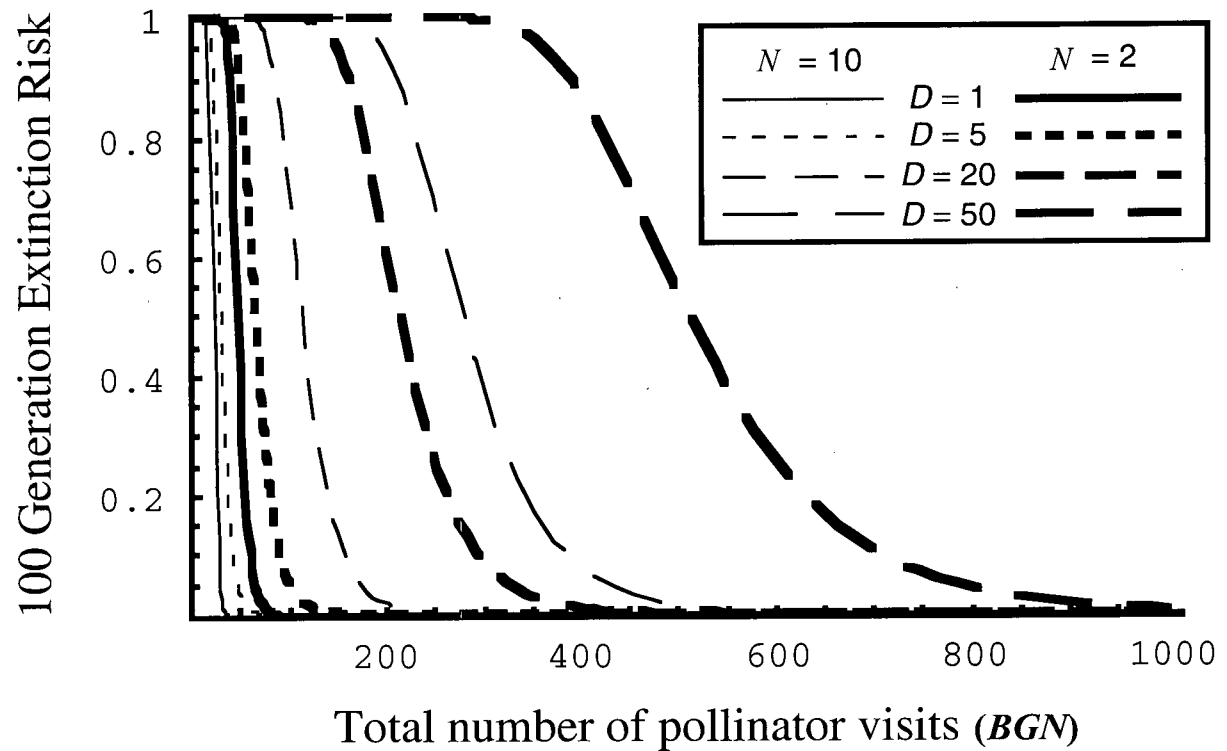


FIGURE 4.5: The risk of extinction in 100 generations for sexually dimorphic plants. The risk is plotted as a function of the total number of pollinator visits over the plant's life span (BGN) when each pollinator visits two flowers (thick curves) or ten flowers (thin curves). The risk of extinction increases as the level of sexual dimorphism increases and as the total number of pollinator visits decreases.

CHAPTER V: INVESTIGATING THE PRESENCE AND CAUSE OF THE ECOLOGICAL CORRELATES OF DIOECY

INTRODUCTION

Determining the presence and cause of ecological correlations in nature is an important prerequisite for understanding the connection between ecology and adaptation. Dioecy, where male and female functions are performed by separate individuals, is a rare breeding system among the angiosperms (Givnish 1980; Bawa 1980; Thomson and Brunet 1990), and is often associated with several ecologically important traits, including fleshy fruits (Muenchow 1980), small, white flowers (Bawa 1980), woody growth form (Fox 1985), and a tropical distribution (Carlquist 1974; Bawa 1980). Such correlations have often been found in studies that were limited by the use of only local floras and that did not control for the relatedness among dioecious taxa. Recent studies have tried to correct for these flaws by looking at worldwide angiosperms (Renner and Ricklefs 1995) or by correcting for phylogeny (Donoghue 1989; Sakai *et al.* 1997; Weiblen *et al.* 2000). However, no studies to date have attempted to look at worldwide angiosperm taxa in a phylogenetic context to ensure that the ecological correlates hold broadly across all the major angiosperm taxa. Although much theory has been proposed regarding the causes of these correlations (e.g., Bawa 1980, Givnish 1980, Charlesworth 1993), studies have just recently begun to examine phylogenies to verify these theories and, to date, have been unable to reveal any distinct mechanisms for the observed associations between dioecy and fleshy fruits (Donoghue 1989) or between dioecy and wind pollination (Sakai *et al.* 1997).

A correlation between two traits could arise in three distinct ways. First, the focal trait (e.g., dioecy) may arise more often when the correlated trait is present. Second, the correlated trait may evolve more often when the focal trait is present. Finally, taxa with both the focal and correlated traits may be more likely to speciate, or less likely to go extinct, than taxa without the correlated traits. Determining the cause of correlations between dioecy and ecologically-important traits is important to assess the relative importance of forces involved in the evolution of dioecy and may have repercussions for understanding the relationships between ecological traits, speciation, and extinction. I will discuss the support of each of these scenarios in turn.

The presence of correlated traits promotes the evolution of dioecy

Many theories have been proposed for why dioecy is correlated with white, inconspicuous flowers (Charlesworth 1993), fleshy fruits (Givnish 1980; Bawa 1980; Lloyd 1982), tropical distribution (Bawa and Opler 1975, Bawa 1993), or woody growth (Renner and Ricklefs 1995),

but the most prevalent theories assert that dioecy evolves more often in lineages with these ecological attributes. A model by Charlesworth and Charlesworth (1987) on the evolution of mating systems in plants lends support to this scenario. They found that females (male-sterile mutants) were more likely to invade a hermaphroditic population if there is low allocation to attraction, thus potentially explaining the correlation between dioecy and small, white flowers. Charlesworth and Charlesworth (1987) also predicted that increasing attraction will often increase the average distance from which pollen is received, so that seeds produced by plants with high allocation to attraction might tend to have lower than average inbreeding coefficients, thereby reducing the amount by which dioecy would be favored in showy lineages. Similarly, the presence of expensive flesh-covered seeds, which are thought to be dispersed farther and more frequently by birds, could select for individuals that devote more energy to female reproduction, thereby leading to increased transition rates to dioecy within clades with fleshy fruit. However, this theory, now known as the Givnish-Bawa Hypothesis (Givnish 1980; Bawa 1980) received little support in a phylogenetic study (Donoghue 1989) in non-angiosperm taxa. Large plants such as trees and shrubs are thought to experience higher geitonogamous (within-plant) selfing than herbs due to the sheer increase in number of flowers, and so selection for outcrossing through dioecy may be higher (see Charlesworth 1993), thereby providing a mechanism for the correlation between tree growth habit and dioecy. Tropical distribution and tree habit also have correlations with white, small flowers and fleshy fruits, even in the absence of dioecy (Renner and Ricklefs 1995), and so may be correlated with dioecy because of the indirect relationship between flower and fruit morphology rather than having a causal relationship.

The presence of dioecy promotes the evolution of correlated traits

The correlations between dioecy and its ecological traits could also be explained if the trait in question is favored in dioecious lineages. This explanation has been less explored (but see references within Charlesworth 1993), yet dioecious lineages having higher transition rates to white flowers may be observed if attractive features (e.g., petals specialized for color or shape) entail costs that are endured by non-dioecious plants in order to ensure outcrossing. Dioecious populations may benefit by investing their resources elsewhere since they have reduced selfing by other means. Fleshy fruits could be favored in dioecious lineages to promote dispersal and reduce the seed-shadow handicap (Lloyd 1982; see Chapter Three). These hypotheses should be disproved before concluding that the evolution of dioecy is favored by the presence of its correlated traits.

Differential success of dioecious species with certain ecological traits

Alternatively, the manner in which dioecy becomes correlated with other traits may be less important than the fate of lineages with correlated traits. A dioecious lineage may have higher success rates if it happens to be associated with certain traits. Consider the correlations between dioecy and fleshy fruits, plain flowers, and perenniality. First, seed dispersal of dioecious plants may be compromised because only females disperse seeds (see Chapter Three), a disadvantage that may be alleviated by a higher dispersal rate in species that happen to have fleshy fruits. As well, Donoghue (1989) found some evidence that lineages with the combination of dioecy and fleshy propagules were more "successful" in an evolutionary sense (i.e., had more species) among non-angiosperms. Second, an analysis of the evolution of showy floral traits (see Chapter Four) shows that dioecious species tend to become sexually dimorphic for floral display, which may result in inefficient pollen delivery and a higher risk of extinction for showy dioecious plants. Third, work on mate assurance predicts that woody (and therefore, perennial) dioecious plants may be at a lower risk of extinction as there is less chance of dying without reproducing (Baker 1954; Carlquist 1974; Bawa 1980; Pannell and Barrett 1998). These "differential evolutionary success" theories have been generally overlooked as a possible cause of the correlations between dioecy and certain ecological traits but could prove important.

A possible reason why the "evolutionary success" theories have not been examined in much detail is that some of the ecological correlates of dioecy are thought to reduce evolutionary success. While dioecy is correlated with woody growth and wind pollination, increased diversification has been observed in plants with an herbaceous growth form and biotic pollination systems (Eriksson and Bremer 1992, Ricklefs and Renner 1994; Dodd *et al.* 1999). Herbs may experience increased diversification rates because their small size allows them to partition habitats more finely (Ricklefs and Renner 1994). Biotic pollination systems may have larger clades than abiotic pollination systems as a result of the co-evolutionary dynamics of plants with their pollinators (West-Eberhard 1983; Kiestner *et al.* 1984). If indeed dioecy is correlated with woody habit and abiotic pollination (Renner and Ricklefs 1995), then one might expect these traits to limit, not enhance, the evolutionary success of dioecious lineages. A recent phylogenetic study shows that dioecious clades do experience higher extinction rates and (or) lower speciation rates (see Chapter Two) than non-dioecious clades. An examination of the "evolutionary success" of dioecious lineages with and without certain correlated traits is necessary to uncover whether the common correlates of dioecy encourage or hinder evolutionary success within dioecious clades.

This study focuses on four particular correlated attributes of dioecy: small, white flowers, fleshy fruits, tropical distribution, and(or) woody habit. The attributes of inconspicuous flowers

and fleshy fruits were chosen due to their inferred relevance to species richness indicated in Chapters Three and Four. Woody habit and tropical distribution were chosen because of the strong correlation of these traits with dioecy reported in other studies (Renner and Ricklefs 1995), as well as the abundance of information available regarding these traits. Using the large scale phylogeny of worldwide angiosperms constructed by Soltis *et al.* (1999), I have mapped the presence or absence of dioecy, inconspicuous flowers, fleshy fruits, tropical distribution and woody habit in order (1) to ensure that the correlations between dioecy and these ecological traits among worldwide angiosperm flora hold when corrected for phylogeny and (2) to obtain insight into the order of acquisition of dioecy and its ecological correlates. This analysis only uses information on the presence of these traits and does not use information on the relative success of dioecy with various ecological attributes. Therefore, to observe whether dioecy experiences higher evolutionary success when it is in combination with certain ecological attributes, the species richness of dioecious genera with one, two, three, and four of the aforementioned ecological correlates were compared to non-dioecious sister-groups using both a quantitative (number of species per genus) and qualitative (having higher or lower species richness compared to a non-dioecious sister-group) test. Each correlate of dioecy was also examined independently to see if dioecious clades with a particular trait experienced higher evolutionary success than those dioecious clades without. The results of this study indicate that the increased success of dioecious clades with certain traits is an important force in causing the observed correlations of dioecy.

METHODS

Ordering the correlates of dioecy

Using DISCRETE, a maximum-likelihood based program (Pagel 1994), I tested for correlated change between discrete characters. Breeding system (dioecious or non-dioecious), flower type (showy or inconspicuous), fruit type (fleshy or dry), distribution (tropical or temperate), and growth habit (woody or herbaceous) were mapped onto the genera included on the shortest tree of the angiosperm phylogeny of Soltis *et al.* (1999). The three major monophyletic taxa of the angiosperms (asterids, rosids, and eumagnoliids), together making up 493 of the 560 species on the phylogeny (see Figure 5.1), were analyzed separately due to memory constraints of the program. All traits were kept binary in the DISCRETE program and therefore the genera were coded according to the state of the trait in the majority ($\geq 50\%$) of its members. Breeding system status was determined from Tahkajan (1997), Mabberly (1997), and the database, generously provided by Suzanne Renner, used in Renner and Ricklefs (1995). Based on these sources, 56 out of 506 genera (approx. 10%) have a majority ($\geq 50\%$) of species that are dioecious. This proportion of dioecy is similar to estimates of dioecy in worldwide angiosperm flora (7-10%; Yampolsky and Yampolsky 1922; Renner and Ricklefs 1995; Weiblen *et al.* 2000). Information

regarding flower type, fruit type, distribution, and habit for each genus was obtained from literature sources including Bentham and Hooker (1880), Hutchinson (1967), Tahktajan (1997), Mabberley (1997), various sites on the worldwide web (list of sources available from the author), or by contacting experts on a particular genus (see Acknowledgments). When sources did not report the presence of either fleshy or dry fruits, then fruit types such as drupes and berries were coded as fleshy fruits, while fruit types such as capsules and samaras were coded as dry. Genera with tropical or sub-tropical distributions were coded as tropical, and trees, shrubs and lianas as woody. By far, the most subjective of the traits was flower type. When information regarding showiness of flower could not be found, then the status of the trait was gained by examining pictures and drawings of representatives of the genus. Flowers judged to be white or yellow-green and smaller than 10 mm long were scored as inconspicuous, even though these inconspicuous flowers may group together into showy inflorescences. Genera reported to have showy bracts were coded as showy.

Using the DISCRETE program (Pagel 1994), the probability of observing specified character states for breeding system (X) and the correlated trait (Y) at the tips of a phylogeny is estimated given transition rates (q) for each of the traits (Table 5.1). To determine if dioecy is correlated with a trait, it is necessary to compare the likelihood estimate obtained when it is assumed that the evolution of the two traits (dioecy and showy flowers, for example) occurs independently, $L(I)$, to the likelihood estimate obtained when the transition rates of the traits are allowed to be non-independent, $L(D)$. That is, $L(I)$ is obtained by assuming $q_{12} = q_{34}$, $q_{13} = q_{24}$, $q_{42} = q_{31}$, and $q_{43} = q_{21}$, while $L(D)$ is obtained without these constraints. Likelihood ratios, which approximately follow a chi-square distribution (Pagel 1994), indicate correlated evolution if $L(D)$ is significantly greater than $L(I)$ tested by comparing $-2(L(D) - L(I))$ to a χ^2 with four degrees of freedom. This model can also be used to test specific hypotheses about the nature of the correlated changes in X and Y to distinguish if there exists a more frequent order of acquisition of the traits. For instance, using flower type as an example, the four scenarios that could cause a relationship between inconspicuous flowers and dioecy are that (1) plain-flowered lineages evolve dioecy at rates higher than expected, (2) showy flowers evolve dioecy at rates lower than expected, (3) dioecious angiosperms evolve plain flowers at rates higher than expected, or (4) dioecious angiosperms evolve showy flowers at rates lower than expected. Distinguishing these possibilities may be accomplished by comparing the transition rate estimates between the character states (see Table 5.1). Likelihood estimates obtained with the unrestricted model ($L(D)$) can be compared to the likelihood estimates obtained with a model that restricts two of the rates (e.g., restricting the rate that dioecious and non-dioecious clades evolve showy flowers to be equal ($q_{12} = q_{34}$); see Table 5.1b). A likelihood ratio, which is asymptotically distributed as a chi-square with one

degree of freedom, is then calculated between the restricted maximum likelihood estimate and the unrestricted maximum likelihood estimate. The model was run to test scenarios 1-4 above for each of the four traits of interest separately (see Table 5.1a). For all traits, transition rates were obtained within the three major clades of angiosperms (rosids, asterids, and eumagnoliids) to see if similar patterns hold in each group. Because of a large ratio between largest to smallest branch lengths in the Soltis *et al.* (1999) phylogeny, the scaling parameter of DISCRETE was employed, as suggested by Pagel (1994). This scaling parameter reduces the ratio of the largest to shortest branches and makes the ML estimates more stable. Three likelihood estimates were obtained for every scenario, L(D), L(I), and restricted likelihoods, with the largest ML estimate taken in all cases.

Species richness within genera

The DISCRETE analysis does not include information on species richness within genera. If the correlation between breeding system and flower and fruit morphology, distribution, and habit are caused by the differential success rates of dioecious taxa with these traits, a pattern should be apparent between the number of species within each dioecious genus and the number of correlations it has with flower, fruit, distribution and habit. Although genera are commonly considered arbitrary constructs (Dodd *et al.* 1999), there is no reason to believe that dioecious genera with some combinations of traits should be arbitrarily larger than dioecious genus with other combinations. Using the same genera included in Soltis *et al.* (1999), the number of species within each dioecious genus was plotted against the number of correlated traits characteristic of the genus. A similar test was also performed when the genera were corrected for phylogeny by combining closely related dioecious genera that appear as sister-groups on the phylogeny. This made little difference to the analysis as few of the dioecious genera formed clades on Soltis *et al.* (1999); 15 genera can be condensed into seven clades; reducing the number of independent points from 62 to 54. This procedure was then repeated for a random sample of non-dioecious genera of equal size. Number of species was then ln-transformed and tested for normality. A non-parametric test of regression called the ordering test (Sokal and Rohlf 1995), which is equivalent to Kendall's Rank correlation coefficient, was used to determine if the number of species in a genus varies according to the numbers of correlated attributes. A test of homogeneity was performed between the correlation coefficients obtained to determine if the relationship between species per genus versus number of correlates is different between the dioecious and non-dioecious groups.

Finally, I re-analyzed the sister-group comparisons of Chapter Two with respect to the number of correlates of the focal (dioecious) group. Four of the focal groups had to be removed from the analysis because no consistent information could be found regarding the status of their

flowers, fruit, habit or growth form, reducing the sample size to 62. A logistic regression was performed to see if having higher numbers of correlated attributes results in a greater chance of the dioecious group having higher species richness than its sister-group. To see whether any of the correlates were associated with higher evolutionary success independently of the others, I performed a contingency test to see whether the presence/absence of each correlate had an effect on the number of clades that had higher species richness versus the number of clades with lower species richness.

RESULTS

Transition rate analysis

The results of the maximum likelihood analysis (summarized in Table 5.2) show that, when phylogenetic relationships are taken into account, dioecy remains correlated with white, inconspicuous flowers and tropical distribution in all three major groups of angiosperms ($p < 0.005$ in each). Dioecy is also correlated with fleshy fruits and woody habit in eumagnoliids ($p = 0.003$ and $p < 0.001$, respectively) and asterids ($p = 0.015$ and $p < 0.001$, respectively). Combining the probabilities obtained in the three major taxa resulted in highly significant correlations for all four ecologically-important attributes ($p \leq 0.001$; Fisher's combined probability test).

In analyzing the evidence for differences in transition rates, 48 tests were performed (four traits \times three groups \times four transition rate tests). Employing a Bonferroni correction requires that any one comparison have $p \leq 0.001$ to avoid type I errors. None of the comparison were significant at this level (Table 5.2). Four comparisons had p -values below 0.05, but this is not significantly more than the 2.4 expected ($= 0.05 \times 48$; $p = 0.218$, binomial test). While there is no convincing evidence for the significance of any one comparison or any one pattern (none of the four significant comparisons with $p < 0.05$ revealed common patterns in all three taxonomic groups), some of the trends with $p < 0.05$ may be biologically relevant. Here I consider the four most significant results as potentially important trends. In the eumagnoliids, the correlations with fleshy fruits and tropical distribution seem to be caused because dioecious groups are more likely to gain fleshy fruits and a tropical distribution than non-dioecious groups ($q_{12} < q_{34}$; $p = 0.040$ and $p = 0.023$, respectively). In the asterids, the correlation with fleshy fruits and tropical distribution seems to have the exact opposite cause, that is, dioecy evolves more commonly in tropical clades and clades with fleshy fruits ($q_{13} < q_{24}$; $p = 0.025$ and $p = 0.028$, respectively). Combined probability tests were not appropriate for finding general patterns as many of the transition rates have relationships that would cause negative correlations between dioecy and its correlates (see Table 5.3).

Species Richness within genera

Dioecious genera having more of the correlates have significantly greater species-richness ($p = 0.005$; non-parametric regression ; Fig 5.2). When 15 genera are condensed into seven clades, reducing the number of independent points from 62 to 54, the effect is still significant ($p = 0.010$). The trend for species richness is specific to dioecious genera as a similar analysis done on a random selection of non-dioecious genera revealed no difference in species number per genus as a function of these same correlates either when 62 or 54 random genera were chosen ($p = 0.423$ and $p = 0.759$, respectively). The correlation coefficients between species richness and number of correlates were significantly different between dioecious and non-dioecious groups when the dioecious groups were not corrected for phylogeny ($p = 0.040$; test of homogeneity) but not when the phylogenetic correction was used ($p = 0.102$). This analysis indicates that a significant force in causing the correlations between dioecy and inconspicuous flowers, fleshy fruits, tropical distribution, and woody habit is that dioecious taxa with these traits are more likely to avoid extinction or more likely to speciate.

On the other hand, the proportion of clades having higher species richness than their sister-groups did not significantly increase with the number of common correlates in the clades ($p = 0.509$; Table 5.4). Furthermore, when each of the four correlates is examined with a separate contingency test (Table 5.4), none of the correlates make dioecious groups more likely to have higher species richness than its sister-group. The lack of significant findings might simply reflect that sample size became too low after dividing the 63 clades from Chapter Two into different subsets, and reducing species richness information to a binary category of "bigger" or "smaller" than its sister-group. The results may also indicate that, although dioecious groups with more correlates may have elevated success in comparison to dioecious groups without the correlates (Fig. 5.2), they still do not have elevated success compared to their non-dioecious sister-groups. In other words, a sister-species analysis lacks the power to detect differences in species richness that exist but are not large enough to affect whether a clade is smaller or larger than its sister clade.

DISCUSSION

The phylogenetic evidence in this study agrees with the evidence gained in other studies indicating that dioecy is indeed correlated with white, inconspicuous flowers, fleshy fruits, tropical distribution, and woody habit. By splitting all angiosperms into three groups, it becomes evident that the correlations are stronger among the eumagnoliids than in the other groups, particularly for the correlations between dioecy and woody growth form and tropical habitat. There are a variety of reasons that may explain the correlation between dioecy and each of its correlates. If the

correlation is caused by an increase in some transition rate(s) then a correlation (between dioecy and white flowers, for instance) could be caused by: (1) white-flowered lineages evolving dioecy at rates higher than expected, (2) non-white flowers evolving dioecy at rates lower than expected (3) dioecious angiosperms evolving white flowers at rates higher than expected, or (4) dioecious angiosperms evolving non-white flowers at rates lower than expected. However, in this analysis, none of these four scenarios stood out from the others as a more common mechanism behind the correlation between small, white flowers and dioecy. A similar lack of significant findings was found among the other correlations tested although four trends were identified as being potentially significant. Dioecious lineages were significantly more likely to evolve fleshy fruits and a tropical distribution in the eumagnoliids, and dioecy was more likely to evolve in tropical lineages and lineages with fleshy fruits in the asterids. These results should be reanalyzed on phylogenies within families to ensure that the use of the phylogeny of Soltis *et al.* (1999) has not obscured major patterns by only including a few representatives of each family. Incomplete phylogenies may place a showy, cosexual genus as the sister genus to a plain-flowered dioecious taxon. When there is no intermediate genus with only one of the traits, the order of acquisition of each trait is hard to surmise. The results of this study may, however, reflect true patterns, which would indicate there is not always additional selection for dioecy within taxa with certain ecological characters or additional selection for these correlated traits in dioecious lineages. Rather, these results indicate that either a combination of different transition rates is responsible for creating the correlations with dioecy or that an altogether different mechanism is at work.

The transition rate analysis only takes into account the occurrence of dioecy within a genus and does not take into account the number of species in each genus. The overall correlations could also be caused if dioecious groups with a certain correlate or combination of correlates are apt to speciate more or go extinct less than dioecious groups without the correlate(s) -- i.e., it could be a species-richness phenomenon and not a transition rate (q_{ij}) phenomenon. When the numbers of species within genera that have one, two, three, or four of the correlates are compared, those genera having more of the common correlates of dioecy are seen to have higher numbers of species (Figure 5.2). Hence, this analysis seems to indicate that the correlated attributes of dioecy have encouraged rather than curtailed the evolutionary success of dioecious lineages. The observation that genera with more correlates are more successful in terms of speciosity agrees with several other studies on dioecy. Showy, dioecious flowers may develop extreme sexual dimorphism, which can lead to higher extinction rates when pollinators become rare and neglect to visit the females (Chapter Four). Fleshy fruits are often dispersed by birds, which are thought by some to disperse fruit farther (Givnish 1980; Bawa 1980). Greater seed dispersal could be an important determinant of success of dioecious species that have twice the amount of sib-competition as seeds

disperse around only the females in a population (Chapter Three). The correlation with woody habit could be because long-lived dioecious species have less likelihood of dying without finding a mate (Pannell and Barrett 1998).

This increase in species richness per genus did not suffice to place dioecious clades on equal footing with their non-dioecious sister-clades, however, as dioecious clades with higher numbers of correlates were no more likely to be more species-rich than their sister clades than those with lower numbers of correlates. Sample sizes were very low in this analysis, however, especially for clades with showy-flowers, temperate distribution, or herbaceous growth form. Low sample sizes in these three categories appears to be specific to dioecious groups as indicated in the analysis of transition rates (Table 1a). The low sample sizes in these categories could be caused because of lower transition rates towards dioecy in groups with these attributes (or vice versa, lower transition rates towards these traits in dioecious clades) but little support for this idea was detected in this study. Alternatively, the lower sample sizes of these groups could be caused by higher extinction rates of these groups. If extinction is indeed causing these patterns, then the sister-group comparison may be a rather invalid approach as extinction rates may be so high in groups that have showy flowers, temperate distribution or herbaceous growth as to make the entire clade disappear, in which case we would be missing these sister-group comparisons from our analysis. As well, differential extinction rates could decrease the power of the transition rate analysis to detect patterns. Fossil studies would be required to tell whether the low sample sizes seen in the sister-group comparisons are caused because of extinction rate differences or different transition rates that have not been detected in this study.

Phylogenetic studies such as the ones performed by Ricklefs and Renner (1994) and Dodd *et al.* (1999) have indicated that herbaceousness and biotic pollination (of which showy flowers are a subset) are associated with an increase in diversification. The data of this study, however, would predict that the opposite is true for herbaceous, showy, dioecious angiosperms (although a direct comparison with biotic pollination cannot be made). Opposite trends in diversification between non-dioecious and dioecious angiosperms could be caused by the different dynamics that arise due to spatially-separated sexes for seed dispersal (Chapter Three), the evolution of sexually-dimorphic floral display and pollinator limitation (Chapter Four) and mate assurance (Pannell and Barrett 1998). If differential evolutionary success is the cause of the correlations between dioecy and inconspicuous flowers, fleshy fruits, woody growth and tropical distribution, this would explain why the transition rate analysis was able to confirm the presence of the correlations but was not able to rule out any of the theories of the most common order of acquisition of traits that form the correlations of dioecy. Similar analyses to the ones used in this study with more in-depth

examination within smaller clades or with the inclusion of extinct clades should clarify the patterns between dioecy, evolutionary success, and ecology.

TABLE 5.1: Codes used for transition rates (q). (A) For each trait analyzed, each genus has two features of interest, its breeding system (dioecious and non-dioecious) and one other trait of interest (described below), both of which may be in one of two states (a,b). The four possible combinations of states are coded as follows: 1 = [a,a], 2 = [a,b], 3 = [b,a], 4 = [b,b]. Also shown is the number of genera in each combination represented in the three clades (R= rosids; A= asterids; E=eumagnoliids) in the DISCRETE analysis. (B) shows an example, using the trait of inconspicuous flowers, of how certain inequalities observed in comparisons among transition rates can be interpreted in terms of the order in which the traits tend to evolve.

A .

| | <u>State a</u> | <u>State b</u> |
|----------------------------------|--|---|
| Primary trait | | |
| breeding system | non-dioecious | dioecious |
| Secondary trait: | | |
| flowers | showy ND: R = 78; A = 98; E = 65 D: R = 0; A = 2; E = 1 | inconspicuous ND: R = 79; A = 65; E = 55 D: R = 17; A = 16; E = 17 |
| fruit | dry ND: R = 102; A = 110; E = 69 D: R = 9; A = 6; E = 5 | fleshy ND: R = 53; A = 53; E = 51 D: R = 8; A = 12; E = 13 |
| geographical distribution | temperate ND: R = 47; A = 48; E = 30 D: R = 5; A = 2; E = 2 | tropical ND: R = 110; A = 115; E = 90 D: R = 12; A = 16; E = 16 |
| habit | herb ND: R = 32; A = 73; E = 80 D: R = 2; A = 1; E = 5 | shrub/liana/tree ND: R = 125; A = 83; E = 40 D: R = 15; A = 17; E = 13 |

B .

| <u>Comparison</u> | <u>Verbal Translation</u> |
|-------------------|--|
| $q_{12} < q_{34}$ | showiness is lost more often in dioecious lineages than in non-dioecious lineages (i.e, the transition rate from state 3 to state 4 is faster than the transition rate from state 1 to state 2). |
| $q_{13} < q_{24}$ | dioecy evolves more often in inconspicuous lineages than in showy lineages |
| $q_{42} < q_{31}$ | dioecy is lost more often in showy lineages than in inconspicuous lineages |
| $q_{43} < q_{21}$ | non-dioecious lineages evolve showy features more than dioecious lineages |

TABLE 5.2: Likelihood and p -values. The likelihood and p -values obtained using the methods in Pagel (1994). p -values (in parentheses) are obtained by comparing the likelihood in question to the likelihood obtained when all rates are unrestricted, $L(D)$. Likelihoods, $L()$, are given in the form of negative log-likelihoods. The test statistic, $[-2*(L(D) - L(I))]$, has an approximate chi-square distribution with four degrees of freedom, while all the other test statistics (e.g. $-2(L(D) - L(q_{12}=q_{34}))$) have an approximate chi-square distribution with one degree of freedom.

| | $L(D)$ | $L(I)$ | $L(q_{12}=q_{34})$ | $L(q_{13}=q_{24})$ | $L(q_{42}=q_{31})$ | $L(q_{43}=q_{21})$ |
|---------------------|--------|----------------------|--------------------|--------------------|--------------------|--------------------|
| <u>Flower Type</u> | | | | | | |
| Rosids | 140.7 | 159.2 | 141.6 | 142.0 | 140.5 | 141.1 |
| | | (<0.001) | (0.180) | (0.107) | (1) | (0.371) |
| Asterids | 156.1 | 164.1 | 157.5 | 157.3 | 157.5 | 156.6 |
| | | (0.003) | (0.094) | (0.121) | (0.094) | (0.317) |
| Eumagnoliids | 104.5 | 143.6 | 104.5 | 104.7 | 106.3 | 104.6 |
| | | (<0.001) | (1) | (0.527) | (0.058) | (0.655) |
| Combined | | <0.001 | | | | |
| p -value | | | | | | |
| <u>Fruit Type</u> | | | | | | |
| Rosids | 155.8 | 157.0 | 157.4 | 155.4 | 156.0 | 156.5 |
| | | (0.6) | (0.074) | (1) | (0.527) | (0.237) |
| Asterids | 156.7 | 162.9 | 156.3 | 159.1 | 156.8 | 156.9 |
| | | (0.015) | (1) | (0.028) | (0.655) | (0.527) |
| Eumagnoliids | 108.2 | 116.1 | 110.3 | 108.1 | 108.6 | 108.1 |
| | | (0.003) | (0.040) | (1) | (0.655) | (1) |
| Combined | | <0.001 | | | | |
| p -value | | | | | | |
| <u>Distribution</u> | | | | | | |
| Rosids | 149.8 | 158.6 | 151.2 | 148.9 | 149.6 | 150.8 |
| | | (0.001) | (0.09) | (1) | (1) | (0.157) |
| Asterids | 156.5 | 163.9 | 156.9 | 159.0 | 157.6 | 156.7 |
| | | (0.005) | (0.371) | (0.025) | (0.138) | (0.527) |
| Eumagnoliids | 114.3 | 126.8 | 116.9 | 114.9 | 115.8 | 114.7 |
| | | (<0.001) | (0.023) | (0.273) | (0.083) | (0.371) |
| Combined | | <0.001 | | | | |

p-valueGrowth Habit

| | | | | | | |
|--------------|-------|----------|---------|---------|---------|---------|
| Rosids | 127.2 | 127.2 | 127.7 | 127.0 | 127.7 | 127.6 |
| | | (1) | (0.317) | (1) | (0.317) | (0.371) |
| Asterids | 151.5 | 161.1 | 152.2 | 153.2 | 151.6 | 151.4 |
| | | (<0.001) | (0.237) | (0.065) | (0.655) | (1) |
| Eumagnoliids | 101.4 | 143.9 | 102.5 | 101.5 | 102.3 | 100.8 |
| | | (<0.001) | (0.138) | (0.655) | (0.180) | (1) |
| Combined | | <0.001 | | | | |

p-value

TABLE 5.3: Transition rate estimates. Phylogenetic analysis of the correlates of dioecy with small, inconspicuous flowers (*Fl.*), fleshy fruits (*Fr.*), tropical distribution (*D*), and woody habit (*H*). q values indicate the rate of change between the four combinations of traits, (1 = [a, a]; 2 = [a,b]; 3 = [b,a]; 4 = [b,b] see Table 5.1a). Boldface denotes combinations of transition rates whose difference is consistent with the sign of the correlation between dioecy and the specified ecological trait (e.g. $q_{12} > q_{34}$ is consistent with the positive correlation between dioecy and plain flowers).

| | Rosids | | | | Asterids | | | | Eumagnoliids | | | |
|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|-------------|-------------|-------------|
| | <i>Fl.</i> | <i>Fr.</i> | <i>D</i> | <i>H</i> | <i>Fl.</i> | <i>Fr.</i> | <i>D</i> | <i>H</i> | <i>Fl.</i> | <i>Fr.</i> | <i>D</i> | <i>H</i> |
| q_{12} | 0.06 | 0.08 | 0.18 | 0.02 | 0.05 | 0.02 | 0.34 | 0.15 | 0.03 | 0.00 | 0.01 | 0.03 |
| q_{34} | 1.22 | 0.85 | 1.06 | 0.01 | 0.00 | 0.00 | 0.55 | 1.69 | 0.00 | 2.49 | 1.22 | 0.13 |
| q_{13} | 0.00 | 0.05 | 0.07 | 0.01 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.16 |
| q_{24} | 0.04 | 0.00 | 0.00 | 0.13 | 0.06 | 0.04 | 0.16 | 0.15 | 0.01 | 0.00 | 0.00 | 0.07 |
| q_{42} | 0.18 | 0.24 | 1.25 | 1.94 | 0.27 | 1.29 | 1.29 | 0.75 | 0.00 | 0.12 | 0.02 | 0.22 |
| q_{31} | 3.07 | 0.40 | 0.06 | 0.07 | 1.15 | 0.00 | 0.00 | 0.00 | 0.12 | 0.00 | 0.06 | 2.49 |
| q_{43} | 0.00 | 0.77 | 1.36 | 0.00 | 0.00 | 1.06 | 0.09 | 0.08 | 2.49 | 0.69 | 0.17 | 0.000 |
| q_{21} | 0.06 | 0.18 | 0.62 | 0.06 | 0.10 | 0.18 | 0.15 | 0.17 | 0.00 | 0.03 | 0.00 | 0.000 |

TABLE 5.4: Sister-group comparisons delineated by ecological correlates. The sister-group comparisons (taken from Chapter Two) were divided according to the presence or absence of inconspicuous flowers, fleshy fruits, tropical distribution and woody habit. Whether the dioecious focal clade had higher (or equal) (+/=) or lower (-) species-richness than its sister-group was seen not to depend on the number of correlates that the focal group had (tested with logistic regression) or on the presence of a particular correlate (tested with contingency tests).

| Dioecious Focal Clade | | +/= | - |
|---------------------------------|---------------|-----|----|
| <u># of correlates</u> | | | |
| | 1 | 2 | 4 |
| | 2 | 3 | 8 |
| | 3 | 7 | 17 |
| | 4 | 9 | 12 |
| Logistic regression $p = 0.509$ | | | |
| <u>Flowers</u> | Inconspicuous | 19 | 37 |
| | Showy | 2 | 4 |
| contingency test $p = 0.977$ | | | |
| <u>Fruit</u> | Fleshy | 13 | 19 |
| | Dry | 8 | 22 |
| contingency test $p = 0.244$ | | | |
| <u>Distribution</u> | Tropical | 16 | 32 |
| | Temperate | 5 | 9 |
| contingency test $p = 0.869$ | | | |
| <u>Growth habit</u> | Woody | 17 | 33 |
| | Herbaceous | 4 | 8 |
| contingency test $p = 0.965$ | | | |

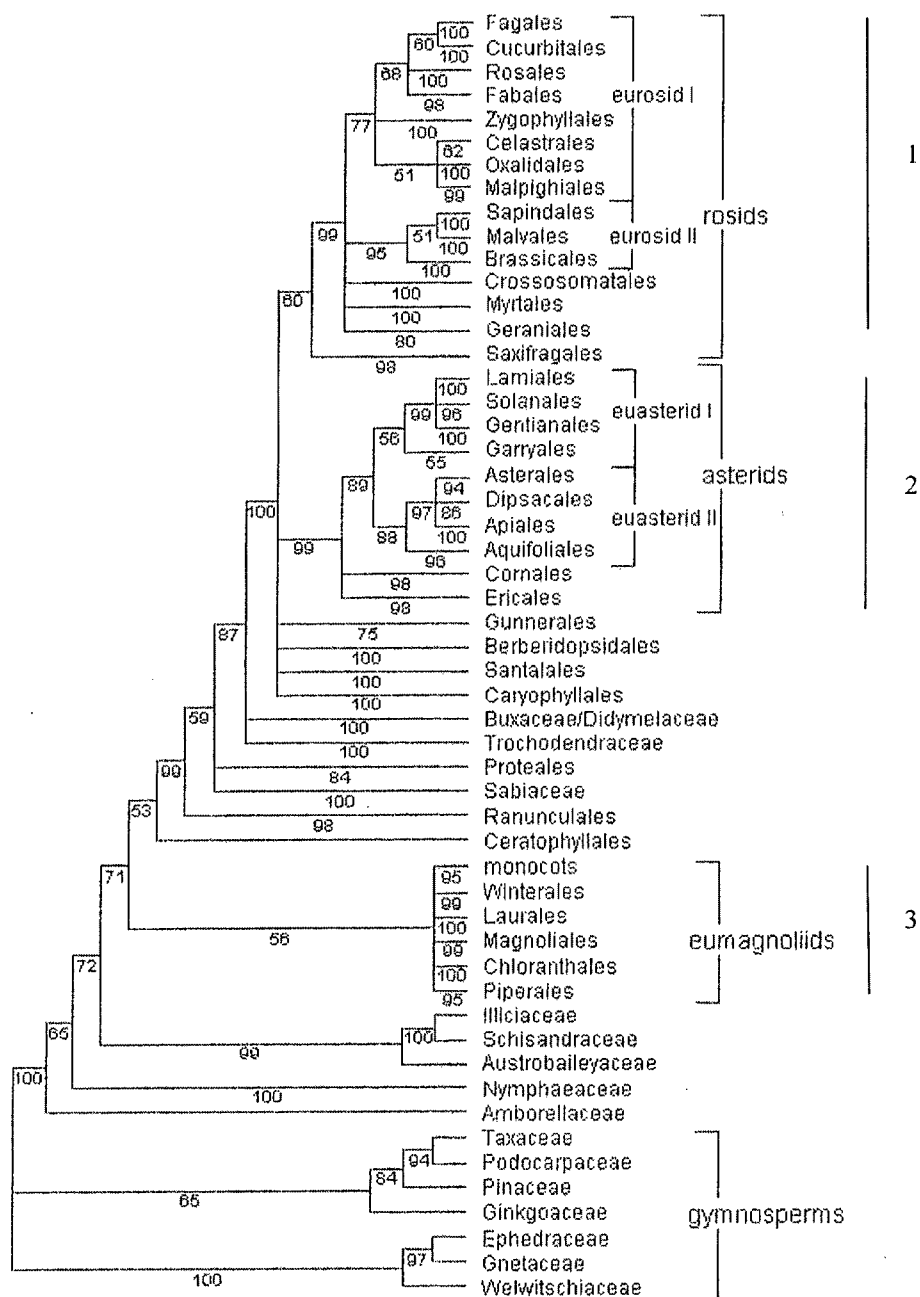


FIGURE 5.1: Phylogeny reproduced from Soltis *et al.* (1999)[®]. The figure above portrays the large-scale relationships of 560 angiosperm species. The three main groups used in this study are: (1) rosids (due to memory constraints of the DISCRETE program, the analysis of this clade excluded Saxifragales), (2) asterids, and (3) eumagnoliids, as indicated in the figure. [Numbers below lines indicate jackknife support.]

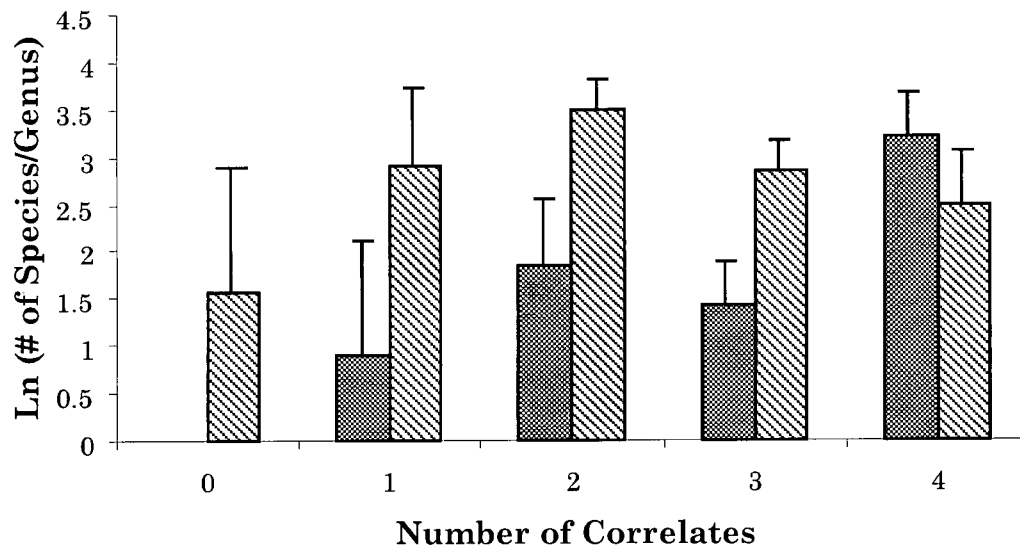


FIGURE 5.2: Relationship between species richness and the number of correlated traits of interest. The mean (+ SE) of ln-transformed species richness for genera having 0, 1, 2, 3, or 4 of the common correlates of dioecy (inconspicuous flowers, fleshy fruits, tropical distribution, woody growth form) is shown for dioecious genera (solid bars) and non-dioecious genera (striped bars). Non-parametric regression reveals that the species richness of genera that have more correlates is significantly higher for dioecious genera ($p = 0.005$) but not for non-dioecious genera ($p = 0.423$). These distributions are significantly different when all 62 genera are used ($p = 0.041$; test of homogeneity).

CHAPTER VI: SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

Summary

Chapter Two reports on the evolutionary success of worldwide dioecious flora utilizing recent phylogenetic work by performing sister-group comparisons of species richness between clades of angiosperms with different breeding systems. Whether this analysis is performed at the family or genus level, species richness is generally far lower in dioecious taxa when compared to their hermaphroditic or monoecious sister taxa. Despite the advantages of avoiding inbreeding depression and of allocating resources separately to male and female function, dioecy in angiosperms does not appear to be a key innovation promoting evolutionary radiation. A potential explanation for the low representation of dioecious lineages is that dioecious plants may have lower colonization rates. Baker's Law states that self-compatible lineages will have higher rates of successful long-range dispersal since they do not require a mate; consequently, self-compatible lineages may have higher rates of allopatric speciation. However, an identical analysis performed with hermaphroditic self-incompatible angiosperms did not produce similar results suggesting that Baker's Law is not the reason for the poor representation of dioecy among angiosperm species.

Chapter Three and Chapter Four elucidate two of the possible reasons for the decrease in speciation rates or increase in extinction rates. As asserted in Chapter Three, dioecious species suffer a competitive disadvantage with cosexuals because only half of the individuals in a dioecious population are seed-bearing. When only females produce seed, offspring will be more spatially clumped and will experience more local resource competition than when every individual produces seed. We examined two spatially-explicit models to determine the effect of reduced seed dispersal on the invasibility and persistence of dioecious populations. Even though dioecious females were allowed to produce twice as many seeds as cosexuals, the results show that a reduction in number of seed dispersers causes a decrease in the ability of dioecious progeny to find uninhabited sites, thus reducing persistence times. These results suggest that the maintenance of dioecy in the presence of hermaphroditic competitors requires a substantial increase in relative fitness or a large dispersal advantage of dioecious seeds.

Chapter Four attempts to investigate the correlation between unisexuality (separate male and female flowers) and white, inconspicuous flowers. Proposed theories for why this correlation exists include that unisexuality evolves more often in lineages with a low allocation to attraction and that white flowers evolve more often in unisexual lineages. This chapter introduces and explores the possibilities of yet another cause for the correlation. I develop a new model that explores whether (1) unisexuality, when they arise, may be prevented from evolving attractive

structures, or (2) unisexually flowered forms that evolve showiness may suffer an increased extinction risk. The results of analytical and computer simulation models suggest that unisexual lineages can evolve attractive structures, especially if genes for attractiveness are sex-limited. However, showiness may evolve more readily among male flowers than among female flowers, a possibility which may have dire consequences in terms of pollinator limitation. Depending on the pollinator abundance, males may become more attractive to pollinators than females, even though this decreases the amount of pollen delivered to females. This decrease in pollination efficiency could lead to a higher extinction risk of showy unisexual plants, should the pollinator abundance change, thus providing a possible explanation of the correlation between unisexuality and plain flowers among extant angiosperms.

Chapter Three and Chapter Four suggest that an important connection exists between ecological traits, breeding system and diversification. There has been much work recently on documenting the correlates of dioecy and many theories proposed to explain why dioecy is correlated with certain ecological factors. Dioecy has been correlated with small, white flowers, fleshy fruits, tropical distribution, and woody habit. Prevalent theories propose that dioecy can more easily evolve, or is more likely to persist, in lineages with these features. An alternative suggestion is that the correlation between dioecy and these traits results from species with the correlated traits being less prone to extinction than dioecious species without correlated traits. To verify that the correlations are caused by independent evolutionary events, I have analyzed the evolutionary relationships between breeding system, flower and fruit morphology, geographical distribution and growth habit using a maximum likelihood approach and a large-scale phylogeny with representatives from all major extant angiosperm taxa (Chapter Five). These same genera are then analyzed in a simple comparative test to see if dioecious genera with correlated traits such as inconspicuous flowers, fleshy fruits, tropical distribution and woody habit are more speciose than ones without these traits. The results suggest that, although differences occur among major taxa, the increased evolutionary success experienced by dioecious groups that bear these commonly correlated traits is likely to be a significant force causing the correlations between dioecy and certain ecological attributes.

Conclusions and Future Directions

Dioecious lineages tend to be smaller in size than their non-dioecious relatives. This does not seem to be caused by the higher outbreeding rates that occur in dioecious clades as non-dioecious outbreeders do not show the same pattern. Higher extinction rates could result from different seed dispersal abilities or different pollination dynamics between dioecious and non-dioecious populations. Indeed, the correlations of dioecy with white, inconspicuous flowers and

fleshy fruit, as well as tropical distribution and woody habit, seem to play a part in enhancing the evolutionary success of dioecious lineages (Figure 5.2), while dioecious groups without these correlations may suffer. In short, my work suggests that to truly understand the distribution of breeding systems among angiosperms, a change in focus is necessary from investigating the factors that can lead to dioecy evolving to what happens once it has evolved. More detailed examinations should be pursued (1) within genera and families displaying variation in breeding systems, and (2) within groups other than the angiosperms, as information on the specific taxa where, and scale at which, these patterns can be observed will allow fuller understanding of how breeding systems affect the processes of speciation and extinction and provide insight into how the remarkable diversity of angiosperms is maintained.

Chapters Three, Four, and Five all indicate that the factors creating the pattern of lower species richness in dioecious clades are numerous and complex. The connection between dioecy and both fleshy fruit and small, white flowers should, and surely can, be tested empirically by comparing the dispersal rates and pollination success of extant dioecious and non-dioecious taxa. Yet still more phylogenetic comparative work could prove rewarding. Recent studies have performed comparative tests much like the ones described here and found that diversification in angiosperms is correlated with increased genetic change and increased morphological change (Barraclough and Savolainen 2001). How genetic change is correlated with breeding system would be a fruitful line of investigation. Further work uncovering whether the lowered representation of dioecious angiosperms is caused by lowered speciation rates or higher extinction rates could be directly inferred from fossils and phylogenies. Recent papers (Harvey *et al.* 1994; Nee *et al.* 1994; Nee 2001) describe ways to estimate speciation and extinction rates within a lineage using only its present-day phylogeny, and these methods are becoming increasingly more reliable. In addition, the incidence of dioecious fossil finds through time could be compared to that of non-dioecious plants. Studies like this have been performed with insects (Labandeira and Sepkoski 1993) and have revealed striking patterns regarding extinction rates over time.

The extensive diversity of the angiosperms (~250,000 angiosperms in existence by recent estimates), combined with the abundance of information we have on their interspecific relationships and morphology, make the flowering plants excellent candidates for many different types of evolutionary investigation. The question of how the reproductive system of an organism affects the ability of a population to diverge and (or) avoid extinction, however, is certainly best addressed in groups with variable breeding systems, such as angiosperms. This thesis gives evidence that theoretical and comparative studies can reveal patterns of differing speciation rates and extinction rates among groups with differing mating systems. Such patterns suggest

appropriate models or field studies that could further elucidate the connection between mating system and diversification. In short, the angiosperms are ripe for vast inquiry; further scrutiny of the links between ecology, mating system, and diversification will prove to be a challengingly complex but fruitful avenue of research.

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APPENDIX 1

Genus Level Differences in Species Diversity. Breeding systems other than true dioecy or monoecy are indicated in superscript (abbreviations: d=dioecy, pgd=polygamodioecy, gd=gynodioecy, ad=androdioecy, sd=subdioecy, am=andromonecy, gm=gynomonoecy). For the monoecious focal groups, comparisons with dioecious sister-groups are indicated. Where the outgroup conflicted on different trees the symbol +/- is used and both the + and - signs are used as separate data points. The symbol = indicates a that an equal number of species is presence in both the focal and sister-group. Phylogenetic references: (1) Manos and Steele 1997; (2) Swensen *et al.* 1998; (3) van Welzen *et al.* 1998; (4) Warwick and Black 1997; (5) Conti *et al.* 1997 (6) Plunkett *et al.* 1996; (7) Xiang *et al.* 1993; (8) Gadek *et al.* 1996; (9) Anzizar *et al.* 1998; (10) Hahn *et al.* 1995; (11) Watson *et al.* 1991; (12) Jansen *et al.* 1991; (13) Mason-Gamer *et al.* 1998; (14) Mathews and Sharrock 1996; (15) Soreng and Davis 1996; (16) Davis and Soreng 1993; (17) Linder *et al.* 1996; (18) Romero 1990; (19) Campbell *et al.* 1995; (20) Graham and Barrett 1995; (21) Simpson 1995; (22) Chase *et al.* 1995; (23) Alverson *et al.* 1998; (24) Soltis *et al.* 1997; (25) Hoot *et al.* 1999; (26) Uhl *et al.* 1995; (27) Esser *et al.* 1997; (28) Rodman *et al.* 1998; (29) Soltis *et al.* 1996; (30) Knapp *et al.* 1997; (31) Bogler and Simpson 1996; (32) Downie and Palmer 1994; (33) Lledo *et al.* 1998; (34) Downie *et al.* 1998; (35) Stahl 1996; (36) Anderberg and Stahl 1995; (37) Rudall and Cutler 1995; (38) Bayer *et al.* 1999; (39) French *et al.* 1995; (40) Crayn *et al.* 1995; (41) Kron 1997; (42) Alverson *et al.* 1999; (43) Kim and Jansen 1998.

| <u>Dioecious Genus Level Sister-Group Comparison</u> | | | | | |
|--|---|----------|--|----------|--------------------|
| <u>Order</u> | <u>Dioecious Genera</u> | <u>#</u> | <u>Sister-Group</u> | <u>#</u> | <u>+ - Ref</u> |
| Apiales | <i>Aciphylla</i> (39)+ | 54 | <i>Ligusticum</i> (45) + | 53 | + 34 |
| | <i>Anisotome</i> (15) | | <i>Lecokia</i> (1) + <i>Smyrniium</i> (7) | | |
| | <i>Meryta</i> | 30 | <i>Munroidendron</i> (1) + <i>Tetraplasandra</i> (6) | 7 | + 6 |
| Arecales | <i>Chamaerops</i> | 1 | <i>Cocos</i> | 1 | = 9 |
| | <i>Hyphaene</i> (10) + | 28 | <i>Nannorrhops</i> | 1 | + 26 |
| | <i>Bismarkia</i> (1) + | | | | |
| | <i>Latania</i> (3) + <i>Lodoicea</i> (1) + <i>Borassodendron</i> (2) + <i>Borassus</i> (11) | | | | |

| | | | | | | |
|-------------|---|-----|---|-----|---|----|
| | <i>Kerridoxa</i> | 1 | <i>Chuniophoenix</i> | 3 | - | 26 |
| | <i>Mauritiella</i> | 14 | <i>Nypa</i> | 1 | + | 9 |
| | <i>Phoenix</i> | 17 | <i>Sabal</i> (16) + <i>Chelyocarpus</i> (4) + <i>Itaya</i> (1) + <i>Schippia</i> (1) + <i>Coccothrinax</i> (47) + <i>Zombia</i> (1) | 70 | - | 26 |
| | <i>Phytelephas</i> (4) + <i>Ammandra</i> (2) + <i>Ravenea</i> (17) + <i>Oraniopsis</i> (1) + <i>Ceroxylon</i> (15) + <i>Juania</i> (1) | 40 | <i>Wallichia</i> (7) + <i>Iriarte</i> (1) + <i>Manicaria</i> (4) + <i>Butia</i> (8) + <i>Allagoptera</i> (5) + <i>Euterpe</i> (30) + <i>Kentiopsis</i> (1) + <i>Podococcus</i> (1) + <i>Synechanthus</i> (2) + <i>Gaussia</i> (4) + <i>Hyophorbe</i> (5) OR <i>Colpothrinax</i> (2) + <i>Pritchardia</i> (25) + <i>Copernicia</i> (25) + <i>Livistona</i> (28) + <i>Pholidocarpus</i> (6) + <i>Johannesteijsmannia</i> (4) + <i>Licuala</i> (108) + <i>Brahea</i> (12) + <i>Washingtonia</i> (2) + <i>Acoelorrhaphe</i> (1) + <i>Corypha</i> (6) + <i>Chuniophoenix</i> (3) | ≥68 | - | 26 |
| | <i>Plectocomia</i> (16) + <i>Mauritia</i> (3) | 19 | outgroup to all other Arecaceae on this phylogeny | ≥68 | - | 26 |
| | <i>Trachycarpus</i> | 4 | <i>Trithrinax</i> | 5 | - | 9 |
| | <i>Wendlandiella</i> (3) + <i>Chamaedorea</i> (100) | 103 | <i>Hyophorbe</i> (5) OR <i>Gaussia</i> (4) OR <i>Synechanthus</i> (2) | ≤5 | + | 26 |
| Asparagales | <i>Lomandra</i> | 50 | <i>Sowerbaea</i> | 5 | + | 22 |

| | | | | | | |
|--------------------------|--|----|---------------------------------------|-----|---|----|
| | <i>Nolina</i> (30) ^{d,pgd} + | 47 | <i>Dracaena</i> (60)+ | 263 | - | 31 |
| | <i>Calibanus</i> (1)+ | | <i>Sansevieria</i> (100)+ | | | |
| | <i>Dasyilirion</i> (15) + | | <i>Liriope</i> (5)+ | | | |
| | <i>Beaucarnea</i> (1) ^{d,pgd} | | <i>Maianthemum</i> (27) + | | | |
| | | | <i>Aspidistra</i> (16)+ | | | |
| | | | <i>Polygonatum</i> (55) | | | |
| | <i>Ruscus</i> | 6 | <i>Asparagus</i> | 135 | - | 37 |
| Asterales | <i>Antennaria</i> | 71 | <i>Gnaphalium</i> ^{am} | 50 | + | 11 |
| Begoniales | <i>Datisca</i> ^{ad} | 2 | <i>Symbegonia</i> (14) + | 915 | - | 2 |
| | | | <i>Begonia</i> (900) + | | | |
| | | | <i>Hillebrandia</i> (1) | | | |
| Butomales | <i>Vallisneria</i> | 6 | <i>Butomus</i> | 1 | + | 22 |
| Buxales | <i>Didymeles</i> | 2 | <i>Buxus</i> (50) + | 53 | - | 25 |
| | | | <i>Pachysandra</i> (3) | | | |
| Capparales | <i>Carica</i> (23) + <i>Batis</i> (2) | 25 | <i>Floerkea</i> (1)+ | 355 | - | 43 |
| | | | <i>Limnanthes</i> (7)+ <i>Tovaria</i> | | | |
| | | | (2) + <i>Capparis</i> (250) + | | | |
| | | | <i>Reseda</i> (60) + <i>Brassica</i> | | | |
| | | | (35) | | | |
| | <i>Hirschfeldia</i> ^{gd} | 2 | <i>Erucastrum virgatum</i> | 1 | + | 4 |
| Caryophyllales | <i>Didierea</i> (2) + | 8 | <i>Anredera</i> (12) OR | ≥12 | - | 32 |
| | <i>Alluaudia</i> (6) | | <i>Claytonia</i> (24) | | | |
| | <i>Spinacia</i> | 4 | <i>Chenopodium</i> | 100 | - | 32 |
| Cercidiphyllales | <i>Cercidiphyllum</i> | 2 | <i>Heuchera</i> | 55 | - | 6 |
| Corylales | <i>Ticodendron</i> ^{d,pgd} | 1 | <i>Betula</i> (35) + <i>Corylus</i> | 45 | - | 1 |
| | | | (10) | | | |
| Cornales/ Eucommiales | <i>Aralidium</i> | 1 | <i>Melanophylla</i> * | 8 | - | 6 |
| | <i>Garrya</i> (13)+ <i>Aucuba</i> | 28 | <i>Nicotiana</i> (67)+ <i>Borago</i> | 465 | - | 6 |
| | (14)+ <i>Eucommia</i> (1) | | (3) + <i>Antirrhinum</i> (20) + | | | |
| | | | <i>Gentiana</i> (361) + | | | |
| | | | <i>Apocynum</i> (12) + | | | |
| | | | <i>Gelsemium</i> (2) | | | |
| | <i>Nyssa</i> ^{pgd} | 8 | <i>Davidia</i> ^{am} | 1 | + | 6 |
| Cucurbitales | <i>Abobra</i> | 1 | <i>Corallocarpus</i> (13) OR | ≥6 | - | 2 |
| | | | <i>Luffa</i> (6) OR <i>Marah</i> (7) | | | |

| | | | | | | |
|----------------|---|-----|---|-----|---|----|
| Dioscoreales | <i>Dioscorea</i> | 850 | <i>Tacca</i> | 10 | + | 22 |
| Ericales | <i>Diospyros</i> | 475 | <i>Symplocos</i> | 250 | + | 6 |
| Euphorbiales | <i>Cheilosa</i> (1) + <i>Pimelodendron</i> (7) | 8 | <i>Falconeria</i> (1) + <i>Stillingia</i> (30) + <i>Sapium</i> (100) + <i>Gymnanthes</i> (15) + <i>Shirakia</i> (8) + <i>Triadica</i> (2) + <i>Excoecaria</i> (40) + <i>Sebastiania</i> (100) + <i>Microstacys</i> (17) + <i>Omаланthus</i> (35) | 348 | - | 27 |
| | <i>Wetria</i> (1) + <i>Homonoia</i> (2) | 3 | <i>Lasiococca</i> (3) + <i>Spathiostemon</i> (3) | 6 | - | 3 |
| Griselinales | <i>Griselinia</i> | 7 | <i>Pittosporum</i> | 150 | - | 7 |
| Juncals | <i>Distichia</i> (3) + <i>Oxychloe</i> (7) | 10 | <i>Marsippospermum</i> (3) + <i>Rostkovia</i> (2) | 5 | + | 21 |
| Lardizabalales | <i>Sargentodoxa</i> | 1 | <i>Akebia</i> | 2 | - | 24 |
| Liliales | <i>Chamaelirium</i> | 1 | <i>Heloniopsis</i> | 4 | - | 22 |
| | <i>Collospermum</i> | 2 | <i>Blandfordia</i> (4) + <i>Milligania</i> (5) | 9 | - | 22 |
| | <i>Smilax</i> | 300 | <i>Ripogonum</i> | 8 | + | 22 |
| Malvales | <i>Byttneria</i> | 132 | <i>Kleinhovia</i> | 1 | + | 42 |
| | <i>Carpodiptera</i> | 8 | <i>Berrya</i> | 4 | + | 42 |
| | <i>Christiana</i> | 2 | <i>Berrya</i> | 4 | - | 38 |
| | <i>Heliocarpus</i> | 10 | <i>Triumfetta</i> | 70 | - | 42 |
| Myrothamnales | <i>Myrothamnus</i> | 2 | <i>Gunnera</i> | 40 | - | 25 |
| Nepenthales | <i>Nepenthes</i> | 82 | <i>Dionaea</i> (1) + <i>Drosera</i> (110) + <i>Drosophyllum</i> (1) + <i>Triphyophyllum</i> (1) + <i>Ancistrocladus</i> (12) | 125 | - | 33 |
| Physenales | <i>Physena</i> | 2 | <i>Krameria</i> (15) + <i>Guaiacum</i> (6) + <i>Tribulus</i> (25) | 46 | - | 40 |
| Poales | <i>Distichlis</i> | 5 | <i>Eragrostis</i> | 300 | - | 15 |
| | <i>Gynerium</i> | 1 | <i>Pennisetum</i> ^{gm} (130) + <i>Cenchrus</i> (30) + <i>Neurachne</i> (6) + <i>Zea</i> (4) + | 271 | - | 17 |

| | | | | | | |
|--------------|--|-----|--|-----|---|----|
| | | | <i>Sorghum</i> (24) + | | | |
| | | | <i>Hyparrhenia</i> (55) + | | | |
| | | | <i>Tristachya</i> (22) | | | |
| Polygonales | <i>Triplaris</i> | 18 | <i>Eriogonum</i> | 240 | - | 33 |
| Primulales | <i>Clavija</i> ^{pgd,gd,ad} | 50 | <i>Theophrasta</i> | 2 | + | 36 |
| | <i>Embelia</i> (100) + | 110 | <i>Conomorpha</i> (124) + | 135 | - | 35 |
| | <i>Grenacheria</i> (10) | | <i>Grammadenia</i> *(11) | | | |
| | <i>Myrsine</i> (5) + <i>Rapanea</i> | 150 | <i>Pleiomeris</i> | 1 | + | 35 |
| | (136) + <i>Suttonia</i> (9) | | | | | |
| | <i>Wallenia</i> (25) + | 85 | <i>Ardisia</i> (250) + <i>Parathesis</i> | 347 | - | 35 |
| | <i>Stylogyne</i> (60) | | (84) + <i>Labisia</i> (6) + | | | |
| | | | <i>Tapeinosperma</i> (4) + | | | |
| | | | <i>Conandrium</i> (2) + <i>Solonia</i> | | | |
| | | | (1) | | | |
| Rosales | <i>Osteomeles</i> | 3 | <i>Cotoneaster</i> (261) + | 829 | - | 19 |
| | | | <i>Pyrus</i> (25) + <i>Malus</i> (55) | | | |
| | | | + <i>Heteromeles</i> (1) + | | | |
| | | | <i>Photinia</i> (65) + | | | |
| | | | <i>Chaenomeles</i> (4) + | | | |
| | | | <i>Sorbus</i> (193) + <i>Mespilus</i> | | | |
| | | | (2) + <i>Crataegus</i> (186) + | | | |
| | | | <i>Malacomeles</i> (3) + | | | |
| | | | <i>Peraphyllum</i> (1) + | | | |
| | | | <i>Amelanchier</i> (33) | | | |
| Salvadorales | <i>Azima</i> | 4 | <i>Salvadora</i> | 5 | - | 28 |
| Santalales | <i>Viscum</i> (65) + <i>Osyris</i> | 72 | <i>Opilia</i> | 2 | + | 24 |
| | (7) | | | | | |
| Sapindales | <i>Bursera</i> ^{pgd} | 50 | <i>Commiphora</i> | 190 | - | 8 |
| | <i>Dodonaea</i> (68) ^{ad,pgd} + | 179 | <i>Aesculus</i> | 7 | + | 8 |
| | <i>Acer</i> (111) ^{ad,pgd} | | | | | |
| | <i>Guarea</i> | 40 | <i>Trichilia</i> (84) + <i>Cipadessa</i> | 85 | - | 8 |
| | | | (1) | | | |
| | <i>Leitneria</i> | 1 | <i>Simaba</i> | 14 | - | 8 |
| | <i>Ptaeroxylon</i> ^{pgd} | 1 | <i>Cneorum</i> | 2 | - | 8 |
| | <i>Simarouba</i> | 6 | <i>Quassia</i> (40) + <i>Brucea</i> (8) | 48 | - | 8 |
| | <i>Toxicodendron</i> (30) + | 60 | <i>Mangifera</i> ^{pgm} (30) | 30 | + | 8 |

| | | | | | | |
|--|--|----------|--|----------|-----------------------|------------|
| | <i>Schinus</i> (30) | | | | | |
| | <i>Zanthoxylum</i> | 250 | <i>Flindersia</i> (15) + <i>Acronychia</i> (43) | 58 | + | 8 |
| Saxifragales | <i>Ribes</i> ^{d, sd} | 150 | <i>Saxifraga</i> (440) + <i>Peltoboykinia</i> (1)+ <i>Chrysosplenium</i> (60) + <i>Mitella</i> (20)+ <i>Elmera</i> (1) + <i>Heuchera</i> (55) + <i>Tolmiea</i> (1) + <i>Lithophragma</i> (9) + <i>Bensoniella</i> (1) + <i>Orestitrophe</i> (1) + <i>Mukdenia</i> (2) + <i>Bergenia</i> (7) + <i>Rodgersia</i> (6) + <i>Darmera</i> (1)+ <i>Astilboides</i> (1) + <i>Sullivantia</i> (6) + <i>Suksdorfia</i> (3) + <i>Boykinia</i> (8) + <i>Bolandra</i> (2) + <i>Astilbe</i> (12) | 637 | - | 29 |
| | <i>Tanakaea</i> | 1 | <i>Leptarrhena</i> | 1 | = | 29 |
| Simmondsiales | <i>Simmondsia</i> | 1 | <i>Dianthus</i> (300) + <i>Stegnospermum</i> (4) + <i>Phytolacca</i> (25) + <i>Basella</i> (5) + <i>Schlumbergera</i> (6) | 340 | - | 33 |
| Trochodendrales | <i>Trochodendron</i> ^{ad} (1)+ <i>Tetracentron</i> ^{ad} (1) | 2 | <i>Hydrangea</i> (23) + <i>Berzelia</i> (12) + <i>Hedera</i> (8) + <i>Coriaria</i> (5) + <i>Eucryphia</i> (6) + <i>Francoa</i> (1) + <i>Geranium</i> (300) + <i>Hibbertia</i> (115) + <i>Dillenia</i> (60) + <i>Schumacheria</i> (3) | 533 | - | 25 |
| Winterales | <i>Tasmania</i> | 5 | <i>Drimys</i> | 6 | - | 25 |
| <u>Self-Incompatible Genus Level Sister-Group Comparison</u> | | | | | | |
| <u>Order</u> | <u>Self-</u> <u>Incompatible</u> <u>Genera</u> | <u>#</u> | <u>Sister-Group</u> | <u>#</u> | <u>+</u> <u>/-</u> | <u>Ref</u> |
| Apiales | <i>Daucus</i> | 22 | <i>Pseudorlaya</i> | 2 | + | 34 |

| | | | | | | |
|----------------|----------------------|-----|---|-----------------|---|------------|
| Arales | <i>Symplocarpus</i> | 1 | <i>Lysichiton</i> | 1 | = | 39 |
| Asparagales | <i>Agave</i> | 100 | <i>Bravoa</i> | 2 | + | 22 |
| | <i>Allium</i> | 690 | <i>Ipheton</i> | 20 | + | 22 |
| | <i>Aloe</i> | 365 | <i>Bulbine</i> | 50 | + | 22 |
| | <i>Asparagus</i> | 135 | <i>Hemiphyllacus</i> | 1 | + | 22 |
| | <i>Curculigo</i> | 10 | <i>Spiloxene</i> | 30 | - | 22 |
| | <i>Cordyline</i> | 15 | <i>Chamaescilla</i> | 2 | + | 22 |
| | <i>Hemerocallis</i> | 15 | <i>Dianella</i> | 20 | - | 22 |
| | <i>Hypoxis</i> | 150 | <i>Rhodohypoxis</i> | 6 | + | 22 |
| | <i>Iris</i> | 210 | <i>Orthrosanthus</i> | 9 | + | 22 |
| | <i>Liatris</i> | 43 | <i>Chromolaena</i> | 165 | - | 12 |
| Asterales | <i>Helianthus</i> | 50 | <i>Wyethia</i> | 14 | + | 12 |
| | <i>Solidago</i> | 80 | <i>Bellis</i> | 8 | + | 11 |
| | <i>Sonchus</i> | 62 | <i>Lactuca</i> | 75 | - | 11 |
| | <i>Austrobaileya</i> | 1 | <i>Illicium</i> (42) + <i>Schisandra</i> (25) OR outgroup to all basal eudicots on the phylogeny (>2000 spp) | ≥67 | - | 25 |
| Berberidales | <i>Epimedium</i> | 44 | <i>Vancouveria</i> | 3 | + | 43 |
| | <i>Podophyllum</i> | 5 | <i>Dysosma</i> | 7 | - | 43 |
| Butomales | <i>Butomus</i> | 1 | <i>Vallisneria</i> | 6 | - | 22 |
| Capparales | <i>Brassica</i> | 35 | <i>Cleome</i> (150) OR <i>Arabidopsis</i> (18) | 150 OR 18 | + | 28 / 24 |
| | <i>Crambe</i> | 20 | <i>Muricaria</i> (1) + <i>Coincya</i> (6) + <i>Erucastrum</i> (20) + <i>Hemicrambe</i> (2) + <i>Sinapis</i> (7) + <i>Raffenaldia</i> (2) + <i>Rapistrum</i> (2) + <i>Ceratocnemum</i> (1) + <i>Guiraoa</i> (1) + <i>Otocarpus</i> (1) + <i>Cordylocarpus</i> (1) + <i>Kremeriella</i> (1) | 45 | - | 4 |
| Caryophyllales | <i>Beta</i> | 12 | <i>Chenopodium</i> | 100 | - | 32 |
| Cistales | <i>Cistus</i> | 18 | <i>Helianthemum</i> | 110 | - | 23 |

| | | | | | | |
|---------------|---|-----|---|------|---|----|
| Cornales | <i>Cornus</i> | 65 | <i>Alangium</i> | 21 | + | 7 |
| Ericales | <i>Kalmia</i> | 7 | <i>Leiophyllum</i> | 1 | + | 41 |
| Geraniales | <i>Pelargonium</i> | 280 | <i>Geranium</i> (300) + <i>Monsonia</i> (25) | 325 | - | 8 |
| Liliales | <i>Colchicum</i> | 65 | <i>Androcymbium</i> | 12 | + | 22 |
| | <i>Lilium</i> | 100 | <i>Fritillaria</i> (100) + <i>Nomocharis</i> (7) | 107 | - | 22 |
| Malvales | <i>Abutilon</i> | 100 | <i>Malope</i> (5) OR <i>Gossypium</i> (39) + <i>Hampea</i> (21) + <i>Thespesia</i> (17) | ≤77 | + | 42 |
| | <i>Waltheria</i> | 40 | <i>Hannafordia</i> | 4 | + | 42 |
| Myrtales | <i>Cuphea</i> | 260 | <i>Duabanga</i> (2) + <i>Lawsonia</i> (1) + <i>Nesaea</i> (56) | 59 | + | 5 |
| | <i>Lythrum</i> | 36 | <i>Trapa</i> | 15 | + | 5 |
| | <i>Oenothera</i> | 124 | <i>Clarkia</i> | 41 | + | 5 |
| Oleales | <i>Jasminum</i> | 200 | <i>Ligustrum</i> | 40 | + | 7 |
| Plumbaginales | <i>Acantholimon</i> | 165 | <i>Dictyolimon</i> | 4 | + | 33 |
| | <i>Armeria</i> | 100 | <i>Psylliostachys</i> | 10 | + | 33 |
| Poales | <i>Agropyron</i> | 15 | <i>Eremopyrum</i> | 4 | + | 13 |
| | <i>Calamagrostis</i> | 250 | <i>Ammophila</i> (2) OR <i>Arrhenatherum</i> (6) | ≤6 | + | 15 |
| | <i>Cymbopogon</i> | 56 | <i>Andropogon</i> (100) + <i>Schizachyrium</i> (60) + <i>Hyparrhenia</i> (55) + <i>Zea</i> (4) | 219 | - | 13 |
| | <i>Dactylis</i> | 3 | <i>Poa</i> (200) + <i>Sesleria</i> (27) | 227 | - | 16 |
| | <i>Ehrharta</i> | 35 | outgroup to all other Poaceae on the phylogeny (totaling 41 applicable genera) | 2072 | - | 15 |
| | <i>Molinia</i> | 3 | <i>Phragmites</i> | 3 | = | 17 |
| | <i>Pennisetum</i> | 130 | <i>Panicum</i> | 500 | - | 15 |
| | <i>Phalaris</i> (20) + <i>Agrostis</i> (220) | 258 | <i>Arrhenatherum</i> | 6 | + | 15 |

| | | | | | | | |
|---------------|--|------------------------|-----|--|-----|---|----|
| | | + | | | | | |
| | | <i>Anthoxanthum</i> | | | | | |
| | | (18) | / | | | | |
| | | <i>Psathyrostachys</i> | 8 | <i>Hordeum</i> (20) + | 21 | - | 13 |
| | | | | <i>Peridictyon</i> (1) | | | |
| | | <i>Pseudoroegneria</i> | 16 | <i>Dasypryrum</i> (2) + | 60 | - | 13 |
| | | | | <i>Australopyrum</i> (3) + | | | |
| | | | | <i>Heteranthelium</i> (1) + | | | |
| | | | | <i>Henrardia</i> (2) + | | | |
| | | | | <i>Thinopyrum</i> (20) + <i>Secale</i> | | | |
| | | | | (3) + <i>Aegilops</i> (21) + | | | |
| | | | | <i>Triticum</i> (4) | | | |
| | | <i>Sorghastrum</i> | 17 | <i>Dichanthium</i> (20) + | 190 | - | 13 |
| | | | | <i>Capillipedium</i> (14) + | | | |
| | | | | <i>Bothriochloa</i> (35) + | | | |
| | | | | <i>Ischaemum</i> (65) + | | | |
| | | | | <i>Chrysopogon</i> (26) + | | | |
| | | | | <i>Heteropogon</i> (6) + | | | |
| | | | | <i>Sorghum</i> (24) | | | |
| | | <i>Zoysia</i> | 10 | <i>Spartina</i> | 17 | - | 15 |
| Polygonales | | <i>Rheum</i> | 30 | <i>Polygonum</i> | 20 | + | 33 |
| Pontederiales | | <i>Ananas</i> | 8 | <i>Aechmea</i> | 85 | - | 20 |
| | | <i>Hedychium</i> | 50 | <i>Riedelea</i> | 60 | - | 20 |
| | | <i>Pontederia</i> | 5 | <i>Monochoria</i> | 6 | - | 20 |
| | | <i>Zingiber</i> | 60 | <i>Globba</i> | 35 | + | 20 |
| Primulales | | <i>Cyclamen</i> | 19 | <i>Dodecatheon</i> | 13 | + | 36 |
| Rosales | | <i>Eriobotrya</i> | 26 | <i>Rhaphiolepis</i> (9) + | 12 | + | 19 |
| | | | | <i>Vauquelinia</i> (3) | | | |
| | | <i>Geum</i> | 40 | <i>Spiraea</i> (90) + <i>Photinia</i> | 155 | - | 40 |
| | | | | (65) | | | |
| | | <i>Pyrus</i> | 25 | <i>Heteromeles</i> (1) + <i>Malus</i> | 95 | - | 19 |
| | | | | (55) + <i>Photinia</i> (65) + | | | |
| | | | | <i>Chaenomeles</i> (4) | | | |
| Solanales | | <i>Atropa</i> (4) + | 104 | <i>Nicandra</i> (1) OR | ≤45 | + | 30 |
| | | <i>Lycium</i> (100) | | <i>Juanulloa</i> (8) + <i>Markea</i> | | | |
| | | | | (18) + <i>Solandra</i> (10) + | | | |

Schultesianthus (5) +
Trianaea (4)

Monoecious Genus Level Sister-Group Comparison

| <u>Order</u> | <u>Monoecious</u> | <u>#</u> | <u>Sister-Group</u> | <u>#</u> | <u>+</u> | <u>Ref</u> |
|--------------|------------------------------------|----------|---------------------------------------|----------|-----------|------------|
| | <u>Genera</u> | | | | <u>/-</u> | |
| Apiales | <i>Aralia</i> | 36 | <i>Scheffera</i> | 650 | - | 6 |
| | <i>Centella</i> (40) + | 42 | <i>Delarbrea</i> | 6 | + | 6 |
| | <i>Micropleura</i> (2) | | | | | |
| | <i>Myrrhis</i> ^{am} (1) + | 75 | <i>Daucus</i> (22)+ | 63 | + | 34 |
| | <i>Osmorhiza</i> (10) | | <i>Pseudorlaya</i> (2) + | | | |
| | + <i>Anthriscus</i> | | <i>Laserpitium</i> (35) + | | | |
| | (11) + <i>Scandix</i> | | <i>Cuminum</i> (4) | | | |
| | (18) + | | | | | |
| | <i>Chaerophyllum</i> | | | | | |
| | (35) | | | | | |
| Arales | Philodendroide | 119 | <i>Calla</i> | 1 | + | 39 |
| | ae (600) + | 0 | | | | |
| | Aroideae (590) | | | | | |
| Arecales | <i>Archontophoen</i> | 5 | <i>Oraniopsis</i> ^d | 1 | + | 9 |
| | <i>ix</i> (3) + <i>Howea</i> | | | | | |
| | (2) | | | | | |
| | Arecinae (238) | 129 | Hyophorbeae (11) + | 15 | + | 10 |
| | + Cocoeae | 5 | Cyclospatheae (4) | | | |
| | (966) + | | | | | |
| | Geonomeae | | | | | |
| | (91) | | | | | |
| | <i>Bactris</i> (239) + | 277 | <i>Phoenix</i> ^d | 17 | + | 9 |
| | <i>Aiphanes</i> (38) | | | | | |
| | <i>Cocos</i> | 1 | <i>Chamaerops</i> ^d | 1 | = | 9 |
| | <i>Nypa</i> (1) + | 5 | <i>Salacca</i> ^d (20) + | 132 | - | 9 |
| | <i>Pseudophoenix</i> | | <i>Chamaedorea</i> ^d (100) | | | |
| | (4) | | | | | |
| | <i>Serenoa</i> | 1 | <i>Phoenix reticula</i> ^d | 1 | = | 9 |
| | <i>Thrinax</i> | 7 | <i>Trithrinax</i> (5) + | 9 | - | 9 |
| | | | <i>Trachycarpus</i> ^d (4) | | | |
| Asterales | <i>Achillea</i> ^{gm} | 115 | <i>Santolina</i> | 18 | + | 11 |

| | | | | | | |
|------------|-------------------------------------|-----|--|-----|---|----|
| | <i>Ainsliaea</i> | 40 | <i>Stiffia</i> | 5 | + | 11 |
| | <i>Cacosmia</i> ^{gm} (3) | 41 | <i>Vernonia</i> (500) + <i>Stokesia</i> | 572 | - | 11 |
| | + <i>Liabum</i> ^{gm} | | (1) + <i>Piptocarpha</i> (45) + | | | |
| | (38) | | <i>Lychnophora</i> (26) | | | |
| | <i>Chromolaena</i> ^{gm} | 165 | <i>Liatris</i> | 43 | + | 11 |
| | <i>Cotula</i> ^{gm} | 55 | <i>Ursinia</i> | 38 | + | 11 |
| | <i>Galinsoga</i> ^{gm} | 13 | <i>Stevia</i> (235) + | 280 | - | 11 |
| | | | <i>Eupatorium</i> (45) | | | |
| | <i>Gnaphalium</i> ^{gm} | 50 | <i>Antennaria</i> ^d | 71 | - | 11 |
| | <i>Haplocarpha</i> ^{gm} | 58 | <i>Gazania</i> | 17 | + | 11 |
| | (8) + <i>Arctotis</i> ^{gm} | | | | | |
| | (50) | | | | | |
| | <i>Mutisia</i> ^{gm} | 59 | <i>Gerbera</i> | 35 | + | 12 |
| | <i>Palafoxia</i> ^{gm} | 25 | <i>Marshallia</i> | 7 | + | 11 |
| | (12) + <i>Bahia</i> ^{gm} | | | | | |
| | (13) | | | | | |
| | <i>Perityle</i> ^{gm} | 64 | <i>Geraea</i> | 2 | + | 11 |
| | <i>Wyethia</i> ^{gm} | 14 | <i>Helianthus</i> | 50 | - | 11 |
| Begoniales | <i>Symbegonia</i> | 915 | <i>Datisca</i> ^{ad} (2) + <i>Octomeles</i> ^d | 4 | + | 2 |
| | (14) + <i>Begonia</i> | | (1) + <i>Tetrameles</i> ^d (1) | | | |
| | (900) + | | | | | |
| | <i>Hillebrandia</i> | | | | | |
| | (1) | | | | | |
| Buxales | <i>Buxus</i> (50) + | 58 | <i>Didymeles</i> ^d | 2 | + | 25 |
| | <i>Pachysandra</i> | | | | | |
| | (3) + | | | | | |
| | <i>Styloceras</i> (5) | | | | | |
| Cornales | <i>Curtisia</i> | 1 | <i>Alangium</i> (21) + <i>Cornus</i> | 86 | - | 7 |
| | | | (65) | | | |
| | <i>Davidia</i> | 1 | <i>Nyssa</i> ^{pgd} | 8 | - | 6 |
| Corylales | <i>Betula</i> (35) | 70 | <i>Ticodendron</i> ^{pgd} | 1 | + | 1 |
| | + <i>Corylus</i> (15) | | | | | |
| Cyperales | <i>Kyllinga</i> ^{am} | 40 | <i>Cyperus</i> | 300 | - | 21 |
| | <i>Mapania</i> (73) + | 236 | <i>Rhynchospora</i> | 250 | + | 21 |
| | <i>Hypolytrum</i> | 7 | | | | |
| | (40) + <i>Scleria</i> | | | | | |

| | | | | | |
|--------------|-----------------------------------|-----|--|-----|------|
| | (200) + <i>Carex</i> | | | | |
| | (2000) + | | | | |
| | <i>Uncinia</i> (54) | | | | |
| Euphorbiales | <i>Lasiococca</i> (3) | 6 | <i>Wetria</i> ^d (1) + <i>Homonoia</i> ^d | 3 | + 3 |
| | + | | (2) | | |
| | <i>Spathiostemon</i> | | | | |
| | (3) | | | | |
| Fagales | <i>Fagus</i> (10) + | 425 | <i>Davidsonia</i> | 2 | + 1 |
| | <i>Castanea</i> (10) | | | | |
| | + <i>Chrysolepis</i> | | | | |
| | (2) + <i>Quercus</i> | | | | |
| | (400) + | | | | |
| | <i>Trigonobalanus</i> | | | | |
| | (3) | | | | |
| Gunnerales | <i>Gunnera</i> | 40 | <i>Myrothamnus</i> ^d | 2 | + 25 |
| Juglandales | <i>Carya</i> (18) + | 46 | <i>Rhoiptelea</i> | 1 | + 1 |
| | <i>Juglans</i> (21) + | | | | |
| | <i>Alfaroa</i> (7) | | | | |
| Juncales | <i>Marsippospermum</i> (3) + | 5 | <i>Distichia</i> ^d (3) + <i>Oxychloe</i> ^d | 10 | - 21 |
| | <i>Rostkovia</i> (2) | | (7) | | |
| Malvales | <i>Akebia</i> | 2 | <i>Sargentodoxa</i> ^d | 1 | + 24 |
| | <i>Lactoris</i> ^{gm} | 1 | <i>Saruma</i> (1) + <i>Asarum</i> | 191 | - 24 |
| | | | (70) + <i>Aristolochia</i> (120) | | |
| | <i>Sterculia</i> | 150 | <i>Fremontodendron</i> (3) + | 117 | + 23 |
| | | | <i>Ochroma</i> (1) + <i>Quararibea</i> | | |
| | | | (35) + <i>Bombax</i> (20) + | | |
| | | | <i>Camptostemon</i> (2) + | | |
| | | | <i>Thespesia</i> (17) + | | |
| | | | <i>Gossypium</i> (39) | | |
| Musales | <i>Musa</i> | 35 | <i>Orchidantha</i> | 7 | + 20 |
| Poales | <i>Arrhenatherum</i> ^a | 6 | <i>Anthoxanthum</i> (18) + | 258 | - 15 |
| | ^m | | <i>Phalaris</i> (20) + <i>Agrostis</i> | | |
| | | | (220) | | |
| | <i>Coix</i> | 6 | <i>Bothriochloa</i> (35) + | 86 | - 13 |
| | | | <i>Capillipedium</i> (14) + | | |

| | | | | | | |
|------------|--|-----|--|------------|--------|------------|
| | | | <i>Dichanthium</i> (20) + <i>Sorghastrum</i> (17) | | | |
| | <i>Hordeum</i> ^{am} | 20 | <i>Secale</i> (3) + <i>Thinopyrum</i> (20) + <i>Agropyron</i> (15) + <i>Eremopyrum</i> (4) + <i>Henrardia</i> (2) + <i>Australopyrum</i> (3) + <i>Dasypyrum</i> (2) + <i>Pseudoroegneria</i> (150) + <i>Peridicyon</i> (1) | 200 | - | 13 |
| | <i>Ischaemum</i> ^{am} | 65 | <i>Cymbopogon</i> (56) + <i>Sorghum</i> (24) + <i>Heteropogon</i> (6) + <i>Chrysopogon</i> (26) | 112 | - | 13 |
| | <i>Lithachne</i> (4) + <i>Olyra</i> (23) | 27 | <i>Chusquea</i> (120) + <i>Otatea</i> (2) + <i>Bambusa</i> (120) | 242 | - | 15 |
| | <i>Pharus</i> | 8 | <i>Anomochloa</i> (1) + <i>Hakonechloa</i> (1) + <i>Danthoniopsis</i> (20) OR <i>Nardus</i> (1) | 22 OR 1 | - / | 13 / 16 |
| | <i>Phragmites</i> ^{am} | 3 | <i>Aristida</i> | 330 | - | 14 |
| | <i>Triticum</i> ^{am} | 4 | <i>Aegilops</i> | 21 | - | 13 |
| | <i>Zea</i> | 4 | <i>Hyparrhenia</i> (55) + <i>Schizachyrium</i> (60) + <i>Andropogon</i> (100) | 215 | - | 13 |
| | <i>Zizania</i> | 3 | <i>Oryza</i> (18) + <i>Bambusa</i> (120) | 138 | - | 17 |
| Orchidales | <i>Catasetum</i> | 100 | <i>Clowesia</i> | 6 | + | 18 |
| | <i>Mormodes</i> (60) + <i>Cycnoches</i> (23) | 83 | <i>Dressleria</i> | 5 | + | 18 |
| Sapindales | <i>Kirkia</i> | 5 | <i>Swietenia</i> (3) + <i>Schmardaea</i> (1) + <i>Trichilia</i> (84) + <i>Cipadessa</i> (1) + <i>Nymanian</i> (1) + <i>Guarea</i> (40) + <i>Melia</i> (3) + | 485 | - | 8 |

| | | | | | |
|----------|--|----|---|----|------|
| | | | <i>Quassia</i> (40) + <i>Brucea</i> (8) + <i>Leitneria</i> ^d (1)+ <i>Harrisonia</i> (4) + <i>Cneorum</i> (2) + <i>Ptaeroxylon</i> (1) + <i>Acronychia</i> (43) + <i>Flindersia</i> (16) + <i>Murraya</i> (4) + <i>Ruta</i> (7) + <i>Buchanania</i> (25) + <i>Toxicodendron</i> ^d (30) + <i>Schinus</i> ^d (30) + <i>Mangifera</i> ^{pgm} (30) + <i>Acer</i> ^d pg ^d (111) | | |
| Typhales | <i>Typha</i> (11) + <i>Sparganium</i> (14) | 25 | <i>Prionium</i> (1) + <i>Tradescantia</i> (70) + <i>Pontederia</i> (5) + <i>Eichhornia</i> (7) + <i>Heteranthera</i> (12) | 95 | - 20 |
