THE EVOLUTION AND FUNCTIONAL SIGNIFICANCE OF STIGMA-HEIGHT DIMORPHISM IN FLOWERING PLANTS

by

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The evolution and functional significance of stigma-height dimorphism in flowering plants
Doctor of Philosophy 2000
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ABSTRACT

Genetic polymorphisms have been widely used as model systems for evolutionary studies of adaptation and natural selection. This thesis investigates a distinct plant sexual polymorphism – stigma-height dimorphism (SHD) – and is the first comprehensive analysis of the nature of SHD, its taxonomic distribution, and its evolution and functional significance in animal-pollinated plant populations.

SHD is characterized by discrete variation in stigma height with anthers positioned similarly in flowers of long (L)- and short (S)-styled morphs. The lack of reciprocity between stigmas and anthers in species with SHD distinguishes it from the well-known sexual polymorphism heterostyly. I compared floral morphology, incompatibility type and population morph-ratios of taxa with SHD vs. distyly. The systems typically differ in incompatibility status and morph ratios, yet SHD, like heterostyly, probably functions to increase proficient pollen transfer between floral morphs.

I examined features of SHD in populations of the Mediterranean geophyte *Narcissus*. SHD is widespread in the genus making it valuable for studying questions related to the evolution and function of the dimorphism. Self-incompatible populations of *N. assoanus* had morph ratios ranging from isoplethy (1:1) to L-biased, while populations of self-compatible *N. dubius* were L-biased or monomorphic-L. There were no morph-specific differences in the species in outcrossing rates or female fertility suggesting that differences in pollen transfer and levels of assortative and disassortive mating contribute to the variable morph ratios. A computer mating model provided evidence supporting this hypothesis.

To investigate the role of SHD in the evolution of distyly, pollen transfer and male fertility were examined in experimental populations of the annual herb *Eichhornia paniculata*. Pollen transfer between morphs was typically greater than among individuals of the same morph. Mating patterns partially supported the validity of the Lloyd & Webb (1992) model of the evolution of distyly with SHD as an intermediate stage. The conditions of the model were satisfied when outcross transfers were considered but were met less often when selfing was included. While SHD seems likely to have been an intermediate step in the evolution of heterostyly in some groups, its occurrence in non-heterostylous families indicates that this is not always true.

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LIST OF ABBREVIATIONS AND SYMBOLS

1:1	- morph ratio of population is isoplethic or 1L:1S
A	- approach herkogamous floral phenotype
Aat	- aspartate aminotransferase (also known as *Got*)
Acp	- acid phosphatase
Adh	- alcohol dehydrogenase
aL	- assortative mating in the long-styled morph (for mating model – Ch. 4)
aS	- assortative mating in the short-styled morph (for mating model – Ch. 4)
CV	- coefficient of variation
D	- indicates the presence of distyly in family
dL	- disassortative mating in the long-styled morph (for mating model – Ch. 4)
dS	- disassortative mating in the short-styled morph (for mating model – Ch. 4)
Gdh	- glutamate dehydrogenase
Got	- glutamate oxaloacetate transaminase
HP	- hand-pollinated
Idh	- isocitrate dehydrogenase
L	- the long-styled floral morph of species with stigma-height dimorphism or tristyly
L-anther	- the lower anther whorl in species with two anther whorls in a flower
M	- the mid-styled floral morph of tristylous species
MLTR	- program for maximum likelihood estimation of outcrossing rate by Kermit Ritland
N/A	- not available
OP	- open-pollinated
Pgi	- phosphoglucoisomerase
Pgm	- phosphoglucomutase
q_{ij}	- quantity of pollen transferred from morph i to morph j
n_{ij}	- total number of seeds produced by an individual of morph i that are sired by morph j
R	- reverse herkogamous floral phenotype
S	- the short-styled floral morph of species with stigma-height dimorphism or tristyly

T - the short-styled floral morph of species with distyly

$\hat{s}$ - estimated selfing rate

ss - homozygous recessive genotype

Ss - heterozygous genotype

SS - homozygous dominant genotype

SHD - stigma-height dimorphism

S. W. - south-west

S/O - seed : ovule ratio

$\hat{t}$ - estimated outcrossing rate

T - indicates the presence of tristyly in family

Tpi - triose-phosphate isomerase

U-anther - the upper anther whorl in species with two anther whorls in a flower

CHAPTER 1

GENERAL INTRODUCTION

When one examines a natural population, it is immediately obvious that most characters vary at least to some extent. This variation is usually continuous or quantitative (e.g. small differences in flower size among individuals) but in some cases is discrete or qualitative (e.g. blue versus white flowers). Polymorphism refers to this latter situation in which "two or more discretely different phenotypes are fairly common within a population" (Futuyma 1986). In most cases, morphological polymorphisms are under simple genetic control, usually governed by the segregation of alleles at one or two gene loci, and the different phenotypes are easily recognizable in natural populations. Studies of polymorphisms have addressed questions regarding the role that genetics and natural selection play in maintaining variation in populations. Examples include studies of industrial melanism in insects (Bishop & Cook 1980; Lees & Dent 1983), colouration patterns of snails (Goodhart 1987), the maintenance of floral colour polymorphisms (Stanton *et al.* 1989; Jones 1996), foraging behaviour of fruit fly larvae and adults (Sokolowski *et al.* 1986; Pereira & Sokolowski 1993), and mating in heterostylous plant populations (reviewed in Ganders 1979a; Barrett 1992a; Richards 1986). Polymorphic systems can thus provide excellent opportunities for addressing numerous questions in evolutionary biology.

Understanding the evolution and maintenance of morphological polymorphisms involves a thorough examination of the traits associated with the polymorphism, the genetic basis of the morphology, and the role that environmental or demographic factors may play in the manifestation of the polymorphism. The primary step in examining sex-organ polymorphisms in flowering plants is to characterize the morphology of the syndrome and should thus include information about the manner in which sex organs are deployed, the nature of variation present (i.e. among individuals and populations), the compatibility status and mating patterns of the morphs, and studies of the ecological genetics and reproductive consequences of the sexual system in natural populations. Examining the reproductive ecology of the morphs among many populations is particularly important when examining polymorphisms of the sexual organs in animal-pollinated plants, since plant reproductive success is highly susceptible to the uncertainty of visitation patterns by pollinators (Schemske *et al.* 1978; Wilson & Thomson 1991; Harder & Barrett 1996). Once this information has been collected from a number of taxa or populations, hypotheses regarding the functional significance and maintenance of the polymorphism can be tested. These hypotheses inevitably involve detailed examination of mating patterns and it is often logistically difficult to quantify these parameters in natural populations since plant fertility and fitness must be measured. While female fertility is relatively easy to quantify, measuring male fertility in natural populations is problematic, although progress has been made with the advent of more powerful genetic markers and new computational methods of determining paternity (reviewed in Snow & Lewis 1993; Smouse & Meagher 1994; Cruzan 1998). In lieu of examining male fitness in natural populations, experimental populations composed of genetically marked plants allow for the measurement of both male and female fertility with relative ease (e.g. Devlin & Ellstrand 1990; Kohn & Barrett 1992; Fenster 1995; Harder & Barrett 1995; Karron *et al.* 1997), and reproductive characters in these populations can be manipulated in order to assess their influence on mating patterns.

In my thesis, I investigate a plant sexual polymorphism, stigma-height dimorphism, of which little is known regarding its functional significance or evolutionary relations to other sex-organ polymorphisms. In this introduction, I begin by briefly describing the types of stylar polymorphisms found in flowering plants. Next, I discuss functional aspects related to the study of the evolution of floral design, particularly the arrangement of the sexual organs of a flower. I then review theoretical models that incorporate stigma-height dimorphism as an intermediate stage in the evolution of distyly. Since one of these models involves pollen limitation of seed set, I briefly discuss pollen limitation of fertility in plant populations. Finally, I outline the specific research objectives of this thesis. My thesis represents the first comprehensive analysis of the evolution and ecology of stigma-height polymorphism in plants.

Stylar polymorphisms

Although most plant species show continuous variation in sex-organ position and are therefore monomorphic (e.g. approach and reverse herkogamy; flowers with stigma positioned above or below the stamens, respectively), populations of some species exhibit discrete variation in the length or position of the stigma (Webb & Lloyd 1986; Barrett *et al.* 2000a,b). The most common stylar polymorphisms are the heterostylous conditions, distyly and tristyly, and these polymorphisms have been the focus of a great deal of research (reviewed in Barrett 1992a; Richards 1986, 1997) beginning with Darwin's (1877) work well over a century ago. Heterostyly is a genetic polymorphism in which a population is comprised of two (distyly) or three (tristyly) floral morphs which differ reciprocally in the placement of stigmas and anthers (Fig. 1-1a,b) and has evolved independently in at least 28 plant families (reviewed in Barrett *et al.* 2000b). The polymorphism is typically associated with a heteromorphic incompatibility system in which self- and intramorph pollinations produce much less seed than cross-pollination between anthers and stigmas of equivalent heights (Fig 1-1).

Heterostylous plants have provided valuable model systems for diverse questions related to genetics, development, and population biology. For example, the common association of heterostyly with an incompatibility system allows for studies of the physiology and mechanism responsible for self-recognition and rejection (reviewed in Ganders 1979a; Dulberger 1992; but see Sage *et al.* 1999). Additionally, in the early part of the twentieth century, heterostyly was used as a model system in the study of Mendelian genetics, linkage and supergenes (e.g. Bateson & Gregory 1905; Mather & De Winton 1941). Distyly and tristyly have been shown to be under simple genetic control in various taxa. Distyly is controlled by a single diallelic locus and tristyly is controlled by two epistatic diallelic loci (reviewed in Lewis & Jones 1992). The simple genetic control of heterostyly has made the polymorphism an ideal system for questions related to population and ecological genetics. Theoretical models have examined morph ratios at equilibrium, the effects of genetic drift, founder effect, and morph-specific variation in reproductive success within populations (e.g. Heuch 1979, 1980; Charlesworth 1979; Morgan and Barrett 1988; Barrett 1993). These theoretical studies provided hypotheses that have been tested empirically in natural populations (reviewed in Barrett 1992b). Finally, studies of pollen transfer and the reproductive biology of heterostylous species have confirmed Darwin's (1877) hypothesis that heterostyly functions to promote effective animal-mediated pollen transfer among the morphs (reviewed in Barrett 1992a; Lloyd & Webb 1992b; Richards 1997).

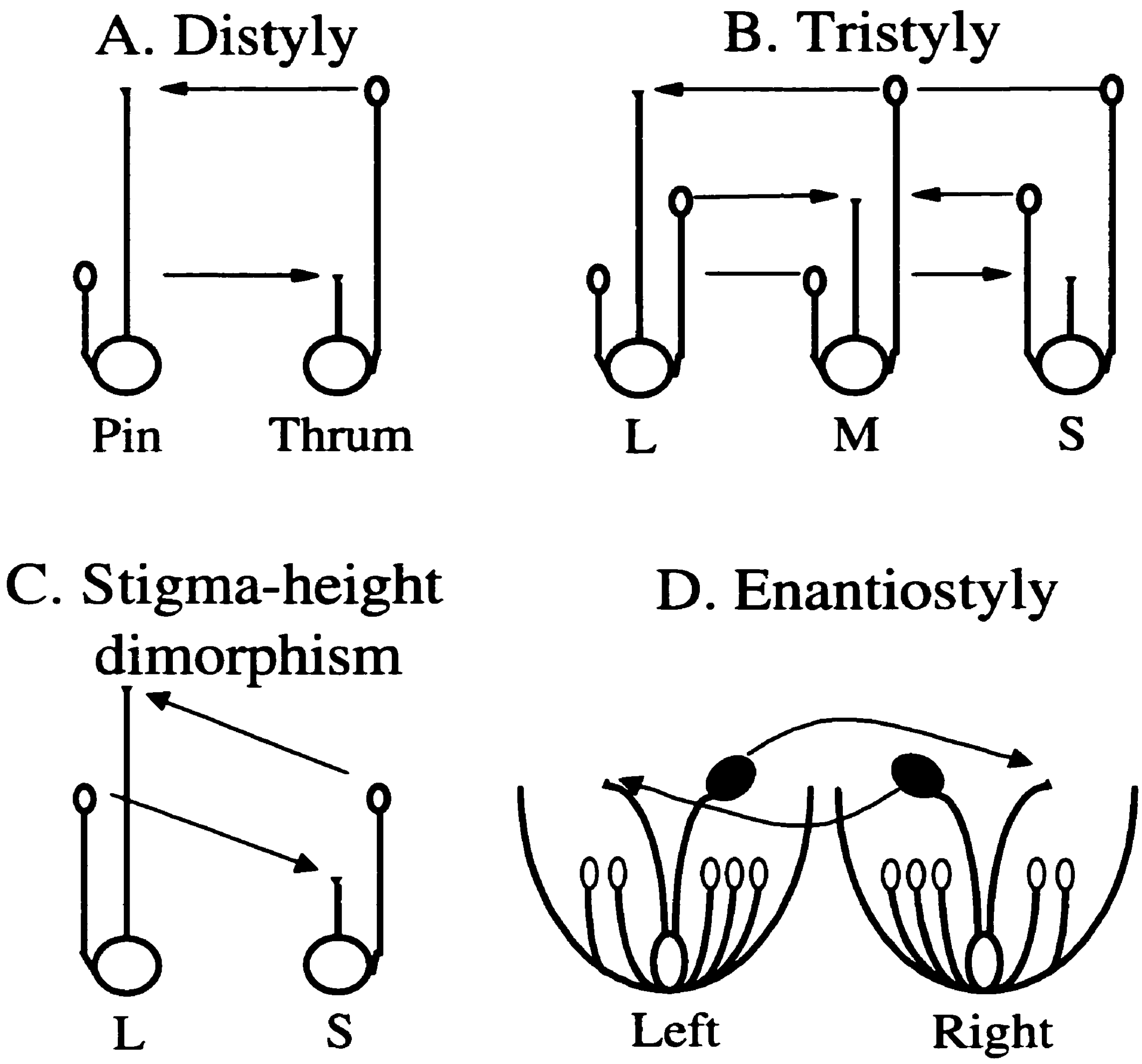


Figure 1-1: The four principal stylar polymorphisms in flowering plants. (A) Distyly; (B) tristyly; (C) stigma-height dimorphism; (D) enantiostyly. Arrows indicate intermorph (disassortative) matings promoted by these floral designs (after Barrett *et al.* 2000b).

On the other hand, very little is known about two other stylar polymorphisms that occur in flowering plants: stigma-height dimorphism and enantiostyly (Fig. 1-1c,d). In my thesis, I undertake a detailed investigation of stigma-height dimorphism, a polymorphism characterized by discrete variation in style length and hence stigma height. Studies on the evolution and function of the other polymorphism, enantiostyly, are the topic of another doctoral dissertation at the University of Toronto by my colleague, Linley Jesson.

Populations of species with stigma-height dimorphism are composed of two floral morphs: long- and short-styled (hereafter L-morph and S-morph, respectively) with anthers positioned similarly between the morphs. These phenotypes have also been referred to as approach and reverse herkogamous forms, respectively (Webb & Lloyd 1986; Stone & Thomson 1994). Like heterostyly, stigma-height dimorphism is a polymorphism in which all of the flowers of an individual plant are of a single floral phenotype, and there is some controversy as to whether the dimorphism merits recognition as a distinct polymorphism from heterostyly (e.g. Barrett & Richards 1990; Dulberger 1992; Richards 1997). Genetic control of stigma-height dimorphism is by a single diallelic locus with the L-morph homozygous recessive (*Narcissus tazetta* – Dulberger 1964; *Anchusa officinalis* – Schou & Philipp 1984). Most commonly, the polymorphism has been described as an intermediate stage in the evolution of distyly (Lloyd and Webb 1992a,b; Richards 1998). Two pieces of evidence have been used to support this hypothesis. First, stigma-height dimorphism has been reported from plant families with distylous representatives and second, the rarity of stigma-height dimorphism in flowering plants suggests that it may be a transient stage that is quickly passed through in the evolution of distyly. Stigma-height dimorphism, like heterostyly, likely functions to increase the proficiency of pollen transfer among the morphs and to minimize self-interference, yet no detailed studies of the functional significance or reproductive consequences of the polymorphism have been conducted. I now briefly discuss general theories regarding the evolution and maintenance of floral design and the application of these ideas to stigma-height dimorphism.

Functional interpretations of floral design

In hermaphroditic plants, male and female reproduction through pollen and ovules governs the total genetic contribution of an individual to the next generation. Although individual plants may vary in their relative success as male or female parents (e.g. Lloyd 1980; Bawa & Webb 1983; Lloyd & Bawa 1984), average male and female fertilities in a population are equivalent since every seed has a mother and father (Charnov 1982; Morgan & Schoen 1997). A major problem faced by hermaphroditic plants is to simultaneously maximize both male and female fitness. When the sex organs are placed at similar positions within a flower, the precision of pollen transfer from anthers to stigmas is optimized since sex organs contact similar parts of the pollinator's body. However, the close proximity of anthers and stigmas may result in self-pollination leading to self-fertilization (e.g. Baker 1964; Piper *et al.* 1984, 1986; Belaoussoff & Shore 1995) and consequently inbreeding depression (Charlesworth & Charlesworth 1987). Traditionally, features of floral design (the architecture of a flower including the deployment of sex organs) and display (the size and number of flowers produced by a plant) have been hypothesized to be "anti-selfing" devices that passively increase an individual's female reproductive success while actively reducing self-pollination, self-fertilization and inbreeding depression (e.g. Todd 1882; Fisher 1949; reviewed in Webb & Lloyd 1986; Barrett 1992a). This female-biased perspective of plant reproduction may be, in part, due to the relative ease of quantifying female fertility (e.g. counting seeds) when compared with male fertility (e.g. determining paternity and siring success; Morgan & Schoen 1997). However, many taxa with these putative anti-selfing devices also possess a physiological self-incompatibility system which functions efficiently to reduce self-fertilization (discussed in Charlesworth & Charlesworth 1979; Webb & Lloyd 1986; Barrett *et al.* 2000b). The anti-selfing and inbreeding depression hypothesis is therefore not a satisfactory explanation of the functional significance of some floral designs in these taxa. Recent interest in the male aspect of plant mating (reviewed in Barrett & Harder 1996; Harder & Barrett 1996; Holsinger 1996; and see above), and the recent ability to measure male fitness in populations has resulted in reinterpretation of the functional significance of many floral characters.

In this thesis, I concentrate on studies of sex-organ position in flowering plants and, in particular, the influence of stigma-height dimorphism on mating patterns and reproduction, although much of this discussion can be extended to other floral traits, such as the temporal separation of male and female function (dichogamy; Lloyd & Webb 1986; Harder *et al.* 2000). These floral features likely function to minimize the interference of male and female function and to ensure efficient pollen transfer among individuals (Webb & Lloyd 1986). The spatial separation of anthers and stigmas (herkogamy) can reduce interference of male and female function (e.g. Darwin 1877; Baker 1964; Ganders 1979a; Lloyd & Yates 1982; Lloyd & Webb 1986). The most obvious consequence of this interference is self-fertilization and inbreeding depression (Charlesworth & Charlesworth 1987), and the resulting loss of pollen to the outcross pollen pool (pollen discounting – Harder & Wilson 1998). However, self-interference may also affect fitness during the prezygotic stages of mating (e.g. physical obstruction of pollen removal by stigmas or pollen receipt by stigmas; stigma or stylar clogging with self pollen or tubes; or ovule discounting – Shore & Barrett 1984; Bertin & Sullivan 1988; Waser & Price 1991; Harder & Barrett 1995; Sage *et al.* 1999). The recognition of paternal contributions to plant mating has therefore led to the suggestion that some floral designs may function to promote more effective pollen dispersal among individuals and the export of pollen from flowers.

Evolutionary relations of stigma-height dimorphism and distyly

Stigma-height dimorphism has most commonly been recognized as an intermediate stage in the evolution of heterostyly rather than an evolutionarily stable plant sexual polymorphism. Models for the evolution of distyly usually consider two independent events: (1) the evolution of the reciprocal arrangement of anthers and stigmas (reciprocal herkogamy), and (2) the evolution of a dimorphic incompatibility system. Early authors have favoured the sequence of events in which the evolution of reciprocal herkogamy is preceded by the establishment of the dimorphic incompatibility system (Baker 1966; Vuilleumier 1967; Ganders 1979a). Three theoretical models have specifically examined the role that stigma-height dimorphism may play in the evolution of distyly. I review these in chronological order.

The Charlesworth and Charlesworth (1979) model

Charlesworth and Charlesworth (1979) performed simulations that suggested that a stigma-height dimorphism was difficult to maintain in the absence of self-incompatibility. The authors assert that it is difficult for a stigma-height dimorphism to become established since a novel style morph will either spread to fixation if the change increases pollen receipt, or be eliminated from the population if too great a loss in female fertility occurs. Because of this finding, they prefer the sequence of events depicted in Fig. 1-2. The ancestral population

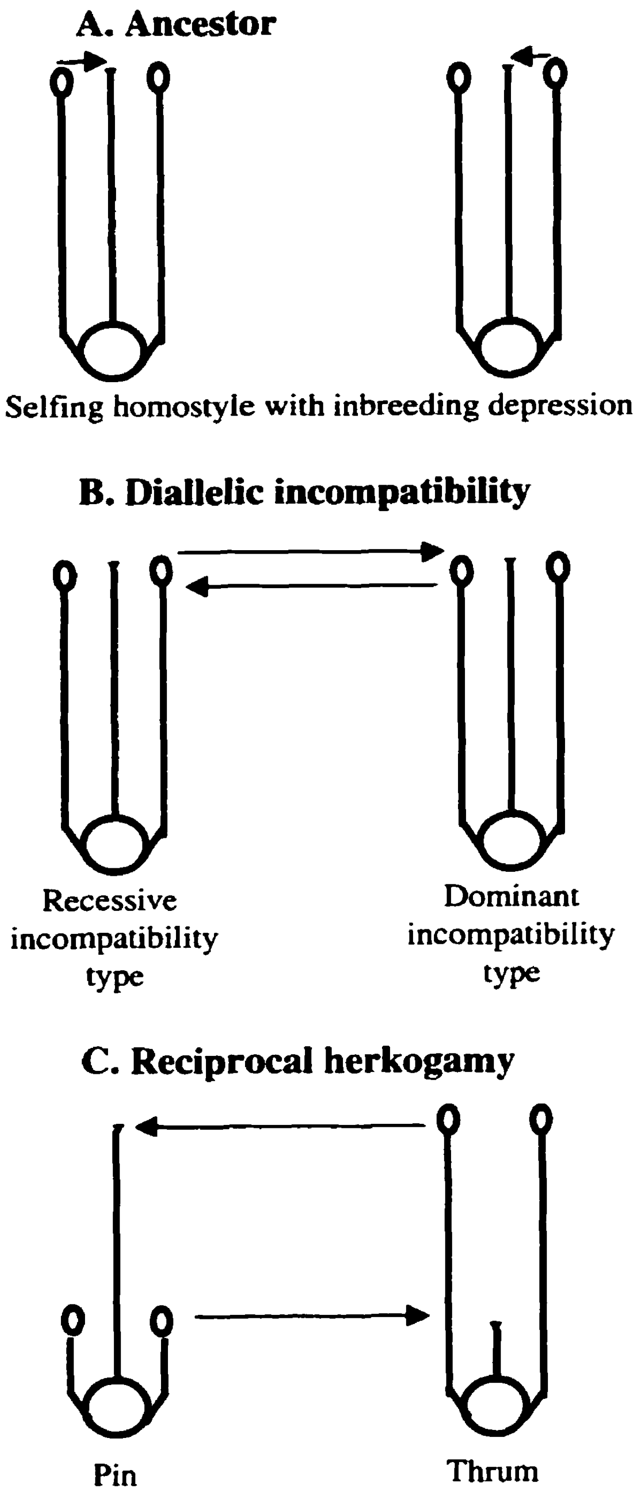
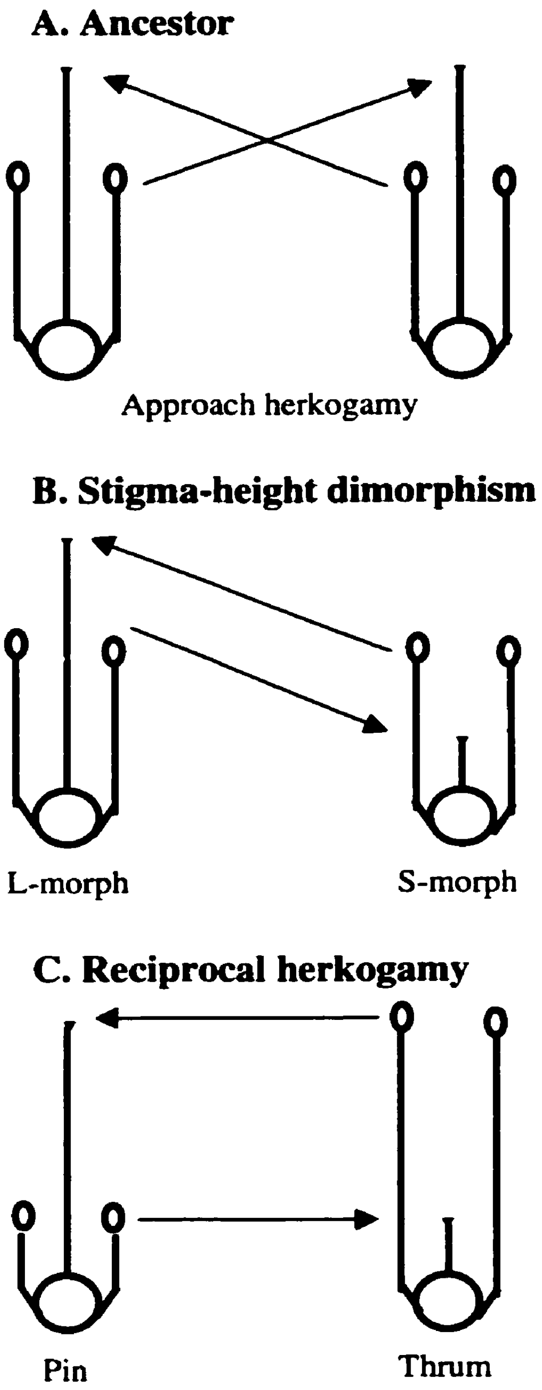


Figure 1-2: The Charlesworth and Charlesworth (1979) model for the evolution of distyly. The Charlesworths conclude that stigma-height dimorphism is difficult to maintain in self-compatible populations (see text for details). Arrows indicate the most proficient pollen transfers.

consists of individuals with anthers and stigmas borne at the same height and consequently experiences high levels of selfing (Fig.1-2A). Diallelic incompatibility is established in two stages; first, a mutant arises with sterile pollen and then a linked mutation for a new stigma type compatible with the pollen type in 1 arises and can spread if inbreeding depression and selfing rates are sufficiently high (Fig 1-2B). Reciprocal herkogamy (Fig. 1-2C) evolves later if it increases disassortative mating between the two incompatibility groups.

The Lloyd and Webb (1992a,b) model

In contrast to Charlesworth and Charlesworth (1979), Lloyd and Webb (1992a,b) incorporate a stage with a stigma-height dimorphism in their model for the evolution of distyly. They assume that an ancestral population of approach herkogamous plants is invaded by a short-styled mutant (Fig. 1-3B). They chose this ancestral phenotype since it is a very common floral design in animal-pollinated species. A stigma-height dimorphism becomes established because of a frequency-dependent fitness advantage of the rare morph and efficient pollen transfer between the morphs. Lloyd and Webb (1992b) derive specific pollen transfer inequalities necessary for the establishment of the stigma-height dimorphism and these conditions depend on whether fertility is pollen- or resource-limited. Basically, pollen transfer between the morphs must be greater than pollen transfer within each morph. The postulated mechanism responsible for pollen transfer among the morphs is related to the "free path" that the proboscis of a pollinator takes when probing a flower. Pollen deposition on the proboscis is segregated because of differences in the angle at which the proboscis enters the L- and S-morph (Lloyd & Webb 1992b, p. 200). Following the establishment of the stigma-height dimorphism, an anther-height dimorphism quickly becomes established since a mutant with anthers in a reciprocal position will donate more pollen to the alternate morph than the morph that lacks reciprocally placed anthers (Fig. 1-3C), or it may have reduced selfing (and inbreeding depression is high). Finally, the dimorphic incompatibility system arises when pollen tubes become co-adapted to the stylar environment that they most commonly grow in because of the prevailing disassortative pollen transfer promoted by reciprocal herkogamy.



A. Ancestor

B. Stigma-height dimorphism

C. Reciprocal herkogamy

Figure 1-3: The principal stages of the evolution of reciprocal herkogamy according to the Lloyd and Webb (1992a,b) model. Arrows indicate the directions of the most proficient pollen transfer.

The Richards (1998) model

Richards (1998) also incorporates a stigma-height dimorphism in his model for the evolution of distyly. He assumes an ancestral population of highly selfed homostyles (Fig. 1-4A) and suggests that a short-style dominant mutant arises which carries recessive lethal genes tightly linked to the gene for stigma-height. This mutant can spread in the population because it mates proficiently with the alternate homostylous morph (Fig. 1-4B). Fitness of the mutant morph is highest when it is rare (negative frequency dependence) and the morph is maintained in the heterozygous form since the most proficient pollen transfer is between this morph and the homostyle. Reciprocal herkogamy is established when there is selection for the lowering of the anthers in the homostylous mutant so that they are placed for proficient pollen transfer to the short-styled morph (Fig. 1-4C).

The Charlesworth and Charlesworth (1979) model has been criticized since it is unlikely that the homostylous plants which they assume are ancestral would have the levels of inbreeding depression required for the evolution of dimorphic incompatibility (Ganders 1979a). Evidence for the lethal linkage groups suggested by Richards (1998) is limited to the Primulaceae (reviewed in Shore & Barrett 1985; Eckert & Barrett 1993; Richards 1998). Lloyd and Webb's (1992b) model differs from the others in that it is primarily non-genetic (Barrett 1990). The authors establish predictions regarding pollen transfer and morph-specific fitness that can be quantified in dimorphic populations (Lloyd & Webb 1992b). It is therefore possible to at least evaluate the plausibility of the Lloyd and Webb (1992a,b) model by examining pollen transfer and morph-specific fitness in populations with stigma-height dimorphism. This approach was used by Stone and Thomson (1994) using experimental arrays of artificial flowers. In this thesis, I use experimental populations of modified *Eichhornia paniculata* to evaluate the plausibility of the Lloyd and Webb (1992a,b) model.

Pollen limitation and pollen transfer models

Plant fertility may be limited by resources and/or pollen availability (reviewed in Charlesworth 1989). Lloyd and Webb (1992b) establish pollen transfer conditions for the evolution of stigma-height dimorphism for both pollen- and resource-limited situations. Here, I briefly review the causes of pollen limitation in animal-pollinated plants.

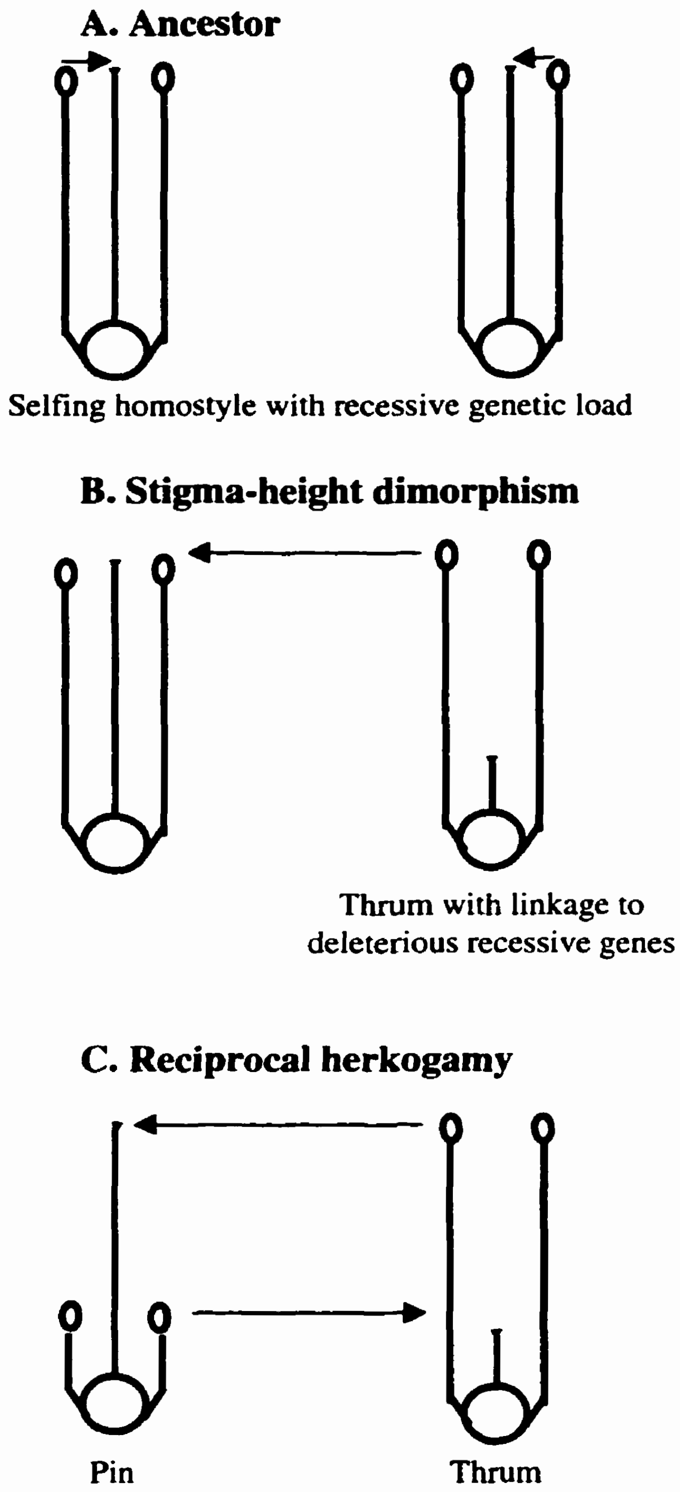


Figure 1-4: The Richards (1998) model for the evolution of distyly with stigma-height dimorphism as an intermediate stage. Arrows indicate the direction of the most proficient pollen transfers.

Pollen limitation of female reproductive success is assessed in populations by comparing the fertility of open- vs. hand-pollinated flowers (Bierzychudek 1981; Rathcke 1983; Johnston 1991a). It has been demonstrated in 62% of the species that have been examined (reviewed in Burd 1994; Larson & Barrett 2000). Pollinators may limit female fertility in two ways. Most commonly, pollen limitation occurs because pollinator visits to flowers are infrequent (McCall & Primack 1992; Johnson & Bond 1997; Kearns & Inouye 1997). Animal-pollinated plants which flower early in the spring are particularly prone to pollen-limitation of fertility since pollinator visitation rates may be adversely influenced by poor weather conditions (e.g. Schemske *et al.* 1978; Motten 1986; Barrett & Helenurm 1987).

Pollen limitation of seed fertility may also occur when poor-quality pollen is deposited on stigmas (Harder & Barrett 1996; Ramsey & Vaughton 2000). For example, self-pollination and self-fertilization may result in seed abortion because of inbreeding depression (reviewed in Charlesworth & Charlesworth 1987). Self-pollination may also limit fertility in self-incompatible plants because of stylar clogging or ovule discounting (e.g. Shore & Barrett 1984; Piper *et al.* 1986; Barrett *et al.* 1996; Sage *et al.* 1999). Variable pollination environments can therefore significantly affect plant mating and fertility in animal-pollinated species (reviewed in Harder & Barrett 1996). Studies of variation of pollen limitation across spatial and temporal scales provide valuable information on the ecology and evolution of plant reproduction (Haig & Westoby 1988; Morgan & Schoen 1997). I examined the extent to which pollen limitation was evident in natural and experimental populations because of its relevance to pollen transfer and the maintenance of stigma-height dimorphism.

Research objectives

The objectives of this thesis are to investigate the evolutionary maintenance of stigma-height dimorphism and to assess the validity of Lloyd and Webb's (1992a,b) pollen transfer model for the evolution of distyly with stigma-height dimorphism as an intermediate stage. The first research chapter of the thesis reviews the current state of knowledge regarding the distribution, morphology, and functional significance of stigma-height dimorphism in the angiosperms. Subsequent chapters involve empirical studies using two different herbaceous plant taxa. Below, I briefly discuss their general features.

Narcissus species (Amaryllidaceae) are perennial geophytes native to Europe and north Africa, particularly the Mediterranean region. The genus consists of approximately 40 species, and the insect-pollinated flowers are characterized by a distinct corona as well as a fused perianth that usually forms a long floral tube. The genus provides a valuable model system for addressing questions related to the evolution and functional significance of stigma-height dimorphism. The genus contains the full range of stylar conditions from monomorphism to heterostyly, including at least a dozen species with stigma-height dimorphism in three sections of the genus (Barrett *et al.* 1996). Of the genera in which stigma-height dimorphism has been reported, its most common occurrence is in the genus *Narcissus*. However, little is known about the floral biology and pollination systems of these spring-flowering perennial bulbs. In Chapters 3 and 4, I focus on the comparative reproductive biology of stigma-height dimorphism in *Narcissus assoanus* (section *Jonquillae*; Fig. 1-5A) and *N. dubius* (section *Tazettae*; Fig. 1-5B), two common species occurring in southern France with contrasting expressions of the polymorphism. In Chapter 5, I investigate pollen limitation of fruit and seed set across a spatial and temporal gradient in populations of *N. assoanus*.

Eichhornia paniculata (Pontederiaceae) is a tristylous, bee-pollinated aquatic native to the Neotropics (Fig. 1-6). The species exhibits considerable genetic diversity at allozyme loci and plants can be raised from seed to flower in a relatively short period of time (3 – 4 months). The species has large flowers which can be hand-pollinated so that plants with morph-specific homozgous allozyme markers can be generated without difficulty. *Eichhornia paniculata* is amenable to studies of inter- and intramorph pollen transfer since the floral morphs are self-compatible, with similar levels of seed set from self- and intramorph crosses (Barrett 1985). Because of these features, the direct effects of sex organ placement on mating patterns can be examined without the confounding effect of heteromorphic incompatibility (see Kohn & Barrett 1992). With partial emasculations of tristylous flowers, it is possible to simulate the floral morphs present in stigma-height dimorphism. Hence *E. paniculata* provides a useful experimental system for manipulative and experimental studies of the function of stigma-height dimorphism. The final research chapter examines the functional significance and role of stigma-height dimorphism in the evolution of distyly using experimental populations of *E. paniculata* plants.

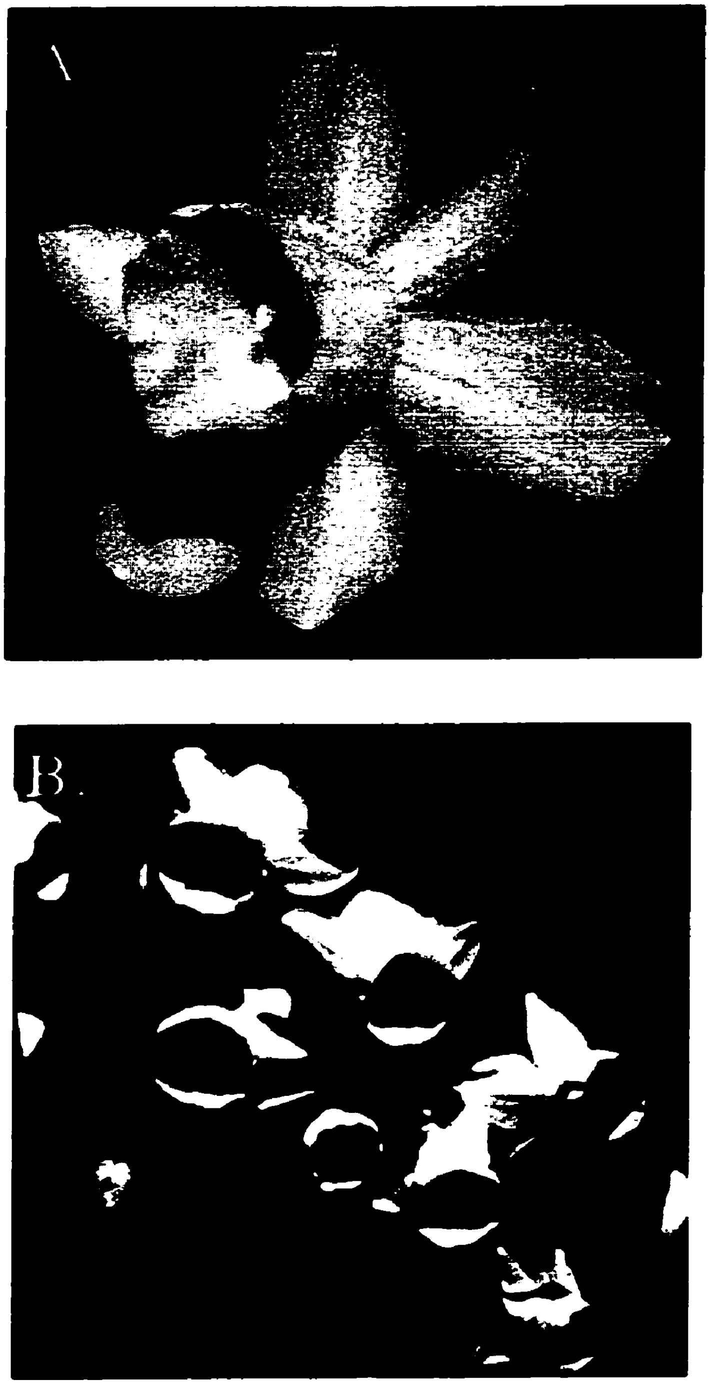


Figure 1-5: Long-styled floral morph of (A) *Narcissus assoanus* and (B) *N. dubius* from southern France.

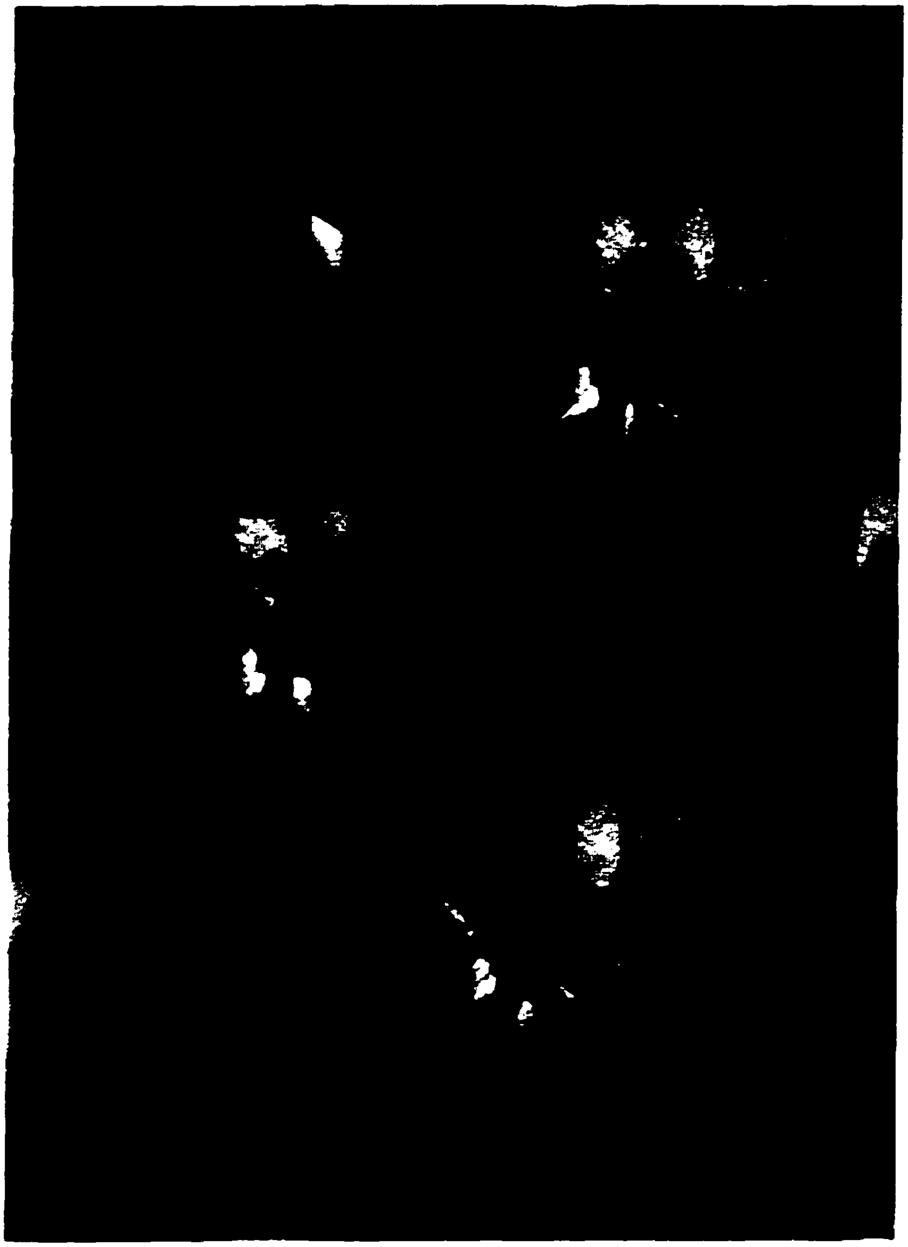


Figure 1-6: Short-styled floral morph of tristylous *Eichhornia paniculata* (Pontederiaceae).

Below, I briefly outline the specific goals of each of the five chapters.

Chapter Two – Stigma-height dimorphism has typically been confused with the more common distylous syndrome (in which both stigmas and anthers exhibit discrete variation in their position). However, stigma-height dimorphism differs from distyly in important ways that merit its recognition as a distinct polymorphism. The objective of this chapter is to review the floral morphology, taxonomic distribution, mating patterns, and population morph ratios of stigma-height dimorphism in the genera in which it has been described.

Chapters Three and Four – The goals of these two chapters were to assess the morphology, reproductive consequences, and functional significance of stigma-height dimorphism in *N. assoanus* and *N. dubius* in southern France. In Chapter 3, I discuss the morphological characters that distinguish stigma-height dimorphism from distyly and the role that differences in stigma and anther position among the morphs play in the maintenance of the polymorphism. I report morph-ratio variation from populations of the two *Narcissus* species and discuss features of floral biology and mating which may be responsible for the observed morph-ratio variation among populations and between the two species. In Chapter 4, I examine the incompatibility status, female fertility, and mating patterns (based on isozyme electrophoresis) in the two *Narcissus* spp. I develop a computer model to examine the effects that variation in levels of assortative and disassortative mating has on morph ratios and compare the results with observed morph ratios presented in Chapter 3.

Chapter Five – As reviewed above, models for the evolution of distyly with stigma-height dimorphism as an intermediate stage have incorporated pollen transfer and pollen limitation of female fertility (Lloyd & Webb 1992b). In this chapter, I examine the degree of pollen limitation of fruit and seed set in *N. assoanus* in southern France over both spatial and temporal scales. Since this species flowers in early spring, it may be especially vulnerable to infrequent pollinator visitation and pollen limitation.

Chapter Six – Here I use experimental populations of manipulated, genetically-marked *Eichhornia paniculata* to examine the functional significance of sex-organ placement in monomorphic populations and in populations dimorphic for stigma-height. Furthermore, I estimate morph-specific fitness and pollen transfer within and among floral morphs to test the validity of Lloyd and Webb's (1992a,b) pollen transfer model.

The following six chapters have been written as self-contained research papers which are either published or will be submitted for publication. As a result there is inevitably repetition in the introductions and discussion in some of the chapters. Chapters three and four have been published in collaboration with John Thompson and Spencer Barrett (Ch. 3 – Baker AM, Thompson JD, & Barrett SCH. 2000. Evolution and maintenance of stigma-height dimorphism in *Narcissus* I. Floral variation and style-morph ratios. *Heredity* **84**:504-515; Ch. 4 – Baker AM, Thompson JD, & Barrett SCH. 2000. Evolution and maintenance of stigma-height dimorphism in *Narcissus* II. Fitness comparisons between style morphs. *Heredity* **84**:516-526). Chapter five is in press in *Oecologia* (Baker AM, Barrett SCH, & Thompson JD. 2000. Variation of pollen limitation in the early flowering Mediterranean geophyte *Narcissus assoanus* (Amaryllidaceae). *Oecologia* – in press).

CHAPTER 2

THE BIOLOGY OF STIGMA-HEIGHT DIMORPHISM: A DISTINCT PLANT SEXUAL POLYMORPHISM

Summary

Stigma-height dimorphism is a plant sexual polymorphism that is reported from at least seven animal-pollinated angiosperm families of both monocotyledons and dicotyledons. The dimorphism has commonly been confused with the more common distylous syndrome, yet it differs from distyly in ways that merit its recognition as a distinct plant sexual polymorphism. Whereas distylous species are typically characterized by reciprocal herkogamy, ancillary polymorphisms of the anthers and stigmas, a heteromorphic incompatibility system, and isoplethic (1:1) morph ratios, these features are rarely associated with stigma-height dimorphism. The fact that many populations with stigma-height dimorphism are biased for the L-morph suggests that assortative mating may be higher in this morph than in the S-morph. However, populations with 1:1 morph ratios are reported and indicate that relatively high levels of intermorph mating can occur, at least under some conditions. No differences in morph-specific female fertility have been found in the species examined to date. Stigma-height dimorphism, like distyly, probably functions to increase the proficiency of animal-mediated pollen transfer between morphs and to reduce lost mating opportunities associated with self-interference. However, the mechanics of intermorph pollen transfer in the absence of reciprocal herkogamy are unknown. Stigma-height dimorphism has often been viewed as a temporary stage in the evolution of distyly, and in some heterostylous groups this may have been the case. However, its occurrence in non-heterostylous families (e.g. Ericaceae, Haemodoraceae, Liliaceae), as well as its widespread distribution in the genus *Narcissus* indicates the polymorphism can be an evolutionarily stable floral strategy.

Introduction

The manner in which male and female sexual organs are deployed in flowering plants is crucial to pollen dispersal, mating patterns, and fertility (reviewed in Barrett *et al.* 1998). Many unrelated plant families exhibit sexual polymorphisms involving populations with discrete variation in the length or position of the style suggesting convergent selective pressures on sex organ arrangement (Webb & Lloyd 1986; Barrett *et al.* 2000a,b). Since the pioneering work of Hildebrand (1866) and Darwin (1877), the plant sexual polymorphism heterostyly has attracted the attention of biologists interested in the evolution and functional significance of plant reproductive systems. Heterostyly is a polymorphism in which a population is composed of two (distyly) or three (tristyly) floral morphs differing reciprocally in anther and stigma position. Another overlooked stylar polymorphism is stigma-height dimorphism. While species with stigma-height dimorphism share some features in common with heterostyly, the dimorphism differs in important ways that merit its recognition as a distinct sexual polymorphism. Most significantly, the dimorphism shows discrete variation in stigma height, but anthers are similarly positioned in the two morphs (Fig. 2-1). In this review, I compare and contrast the morphological features of taxa with stigma-height dimorphism and distyly to show that stigma-height dimorphism is distinct from the more common distylous syndrome. I then outline what is known about the manner in which stigma-height dimorphism is manifested in natural populations, including a review of the compatibility status of various taxa with stigma-height dimorphism and its effects on the morph ratios of populations. I discuss evidence that stigma-height dimorphism, like heterostyly, functions to increase the proficiency of cross-pollination and reduce lost mating opportunities arising from self-interference (the interference of male and female function within a plant). Finally, I review theoretical models examining the establishment of stigma-height dimorphism and its role in the evolution of distyly.

Sex-organ position in species with stigma-height dimorphism vs. distyly

Interest in stigma-height dimorphism arose primarily as a result of reports of "unusual" (Philipp & Schou 1981), "imperfect" (Dulberger 1964; 1970), or "anomalous" (Barrett & Richards 1990) cases of heterostyly. This apparently stemmed from differing views of the variation encompassed by the heterostylous syndrome. Darwin (1877, p. 2) described heterostylous species as those composed of "individuals… which exist under two or three forms,

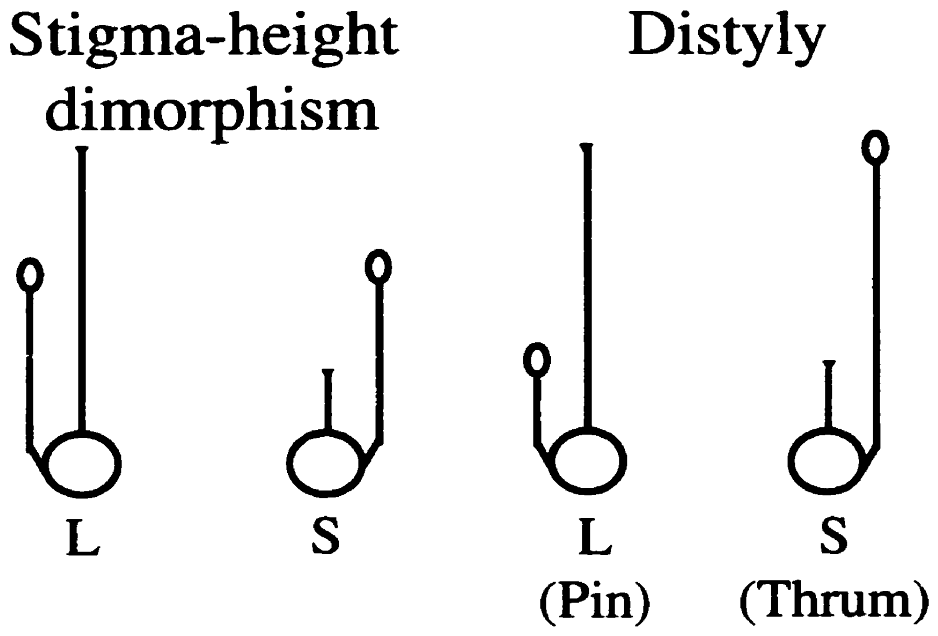


Figure 2-1: Style-length dimorphisms in flowering plants. Species with stigma-height dimorphism exhibit populations composed of long-styled (L) and short-styled (S) morphs that differ in the position of the stigmas but not in the position of the anthers. (B) Distylous populations exhibit reciprocal positioning of male and female sex organs (reciprocal herkogamy). I refer to the long- and short-styled distylous morphs by their traditional "pin" and "thrum" designations throughout the text.

differing in the length of their pistils and stamens, and in other respects." Descriptions of heterostyly have usually stressed the presence of discrete style-length variation in combination with a physiological heteromorphic incompatibility system (Vuilleumier 1967; Philipp & Schou 1981 and refs. contained within). More recently, the morphological component of this definition has been emphasized to define heterostyly as a floral polymorphism in which the morphs produce flowers "that differ reciprocally in style and stamen lengths" (Ganders 1979a, p. 607). This reciprocal herkogamy of the male and female sex organs in alternate morphs is now considered to be the defining feature of the heterostylous syndrome (Lloyd & Webb 1992a; Richards 1986). Classical distylous species such as *Primula vulgaris* (Primulaceae) exhibit discrete variation in both stigma (Fig. 2-2A) and anther position (Fig. 2-2B) and the sex organs are reciprocally positioned among the two style morphs (Fig. 2-2C). I measured the style length, anther height, and flower length of flowers of *P. vulgaris* preserved in 95% ethanol.

The pattern of sex-organ position in species with stigma-height dimorphism is quite different, as exemplified in *Anchusa officinalis* of the Boraginaceae (Fig. 2-3). Data for this species were also collected from flowers preserved in alcohol. The length of the style in the population exhibits a bimodal distribution (Fig. 2-3A), whereas the height of the anthers is unimodally distributed (Fig. 2-3B), so that reciprocal herkogamy between the floral morphs is absent (Fig. 2-3C). In the long-styled morph, stigmas are placed above the anthers while in the short-styled morph the stigma is located below the anthers (Fig. 2-1, 2-3C). The absence of reciprocal herkogamy among the floral morphs is the most important feature distinguishing stigma-height dimorphism from distyly. However distylous species can show considerable variation in the degree of reciprocal herkogamy (e.g. *Guettarda* of the Rubiaceae – Richards & Koptur 1993), and it is possible that there may be a continuum of reciprocal herkogamy among taxa with stigma-height dimorphism and distyly. However, the species with stigma-height dimorphism examined here do not show reciprocal herkogamy (see below). Several authors have struggled with the most appropriate classification of atypical sex-organ polymorphisms (e.g. Baker 1964; Ganders 1979a: Richards 1986; Barrett & Richards 1990; Lloyd & Webb 1992a). Nonetheless, taxa exhibiting stigma-height dimorphism are valuable analogues to typical distylous species and warrant attention (Dulberger 1992).

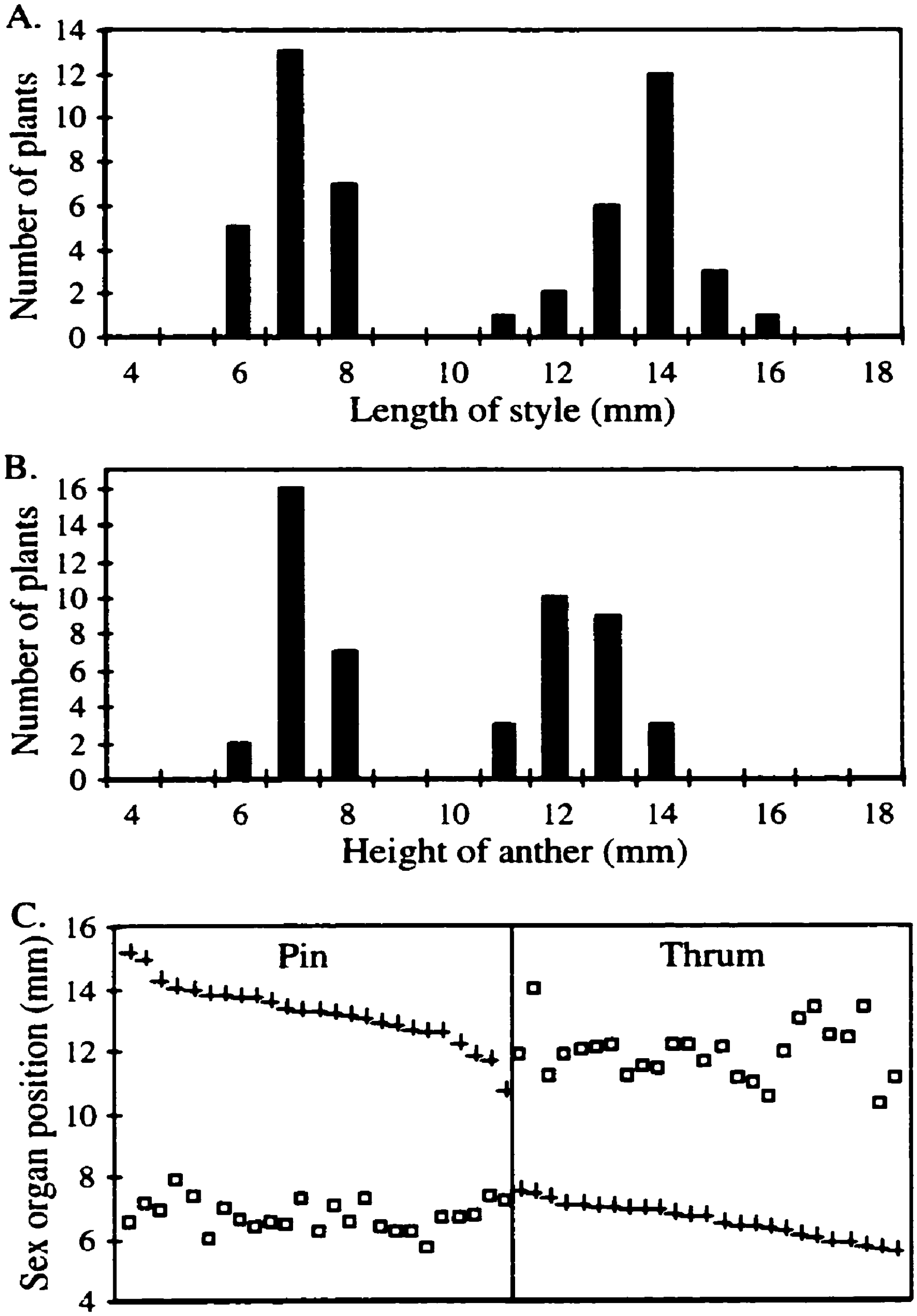


Figure 2-2: Distyly in ***Primula vulgaris*** (Primulaceae). The population was sampled near St. Andrews, Scotland and sex-organ measurements were adjusted to account for the effect of flower length using ANCOVA. There is discrete variation between the two morphs in (A) stigma position, and (B) anther position. (C) Individual flowers ranked by style length illustrate the reciprocal nature of stigma (+) and anther (□) position.

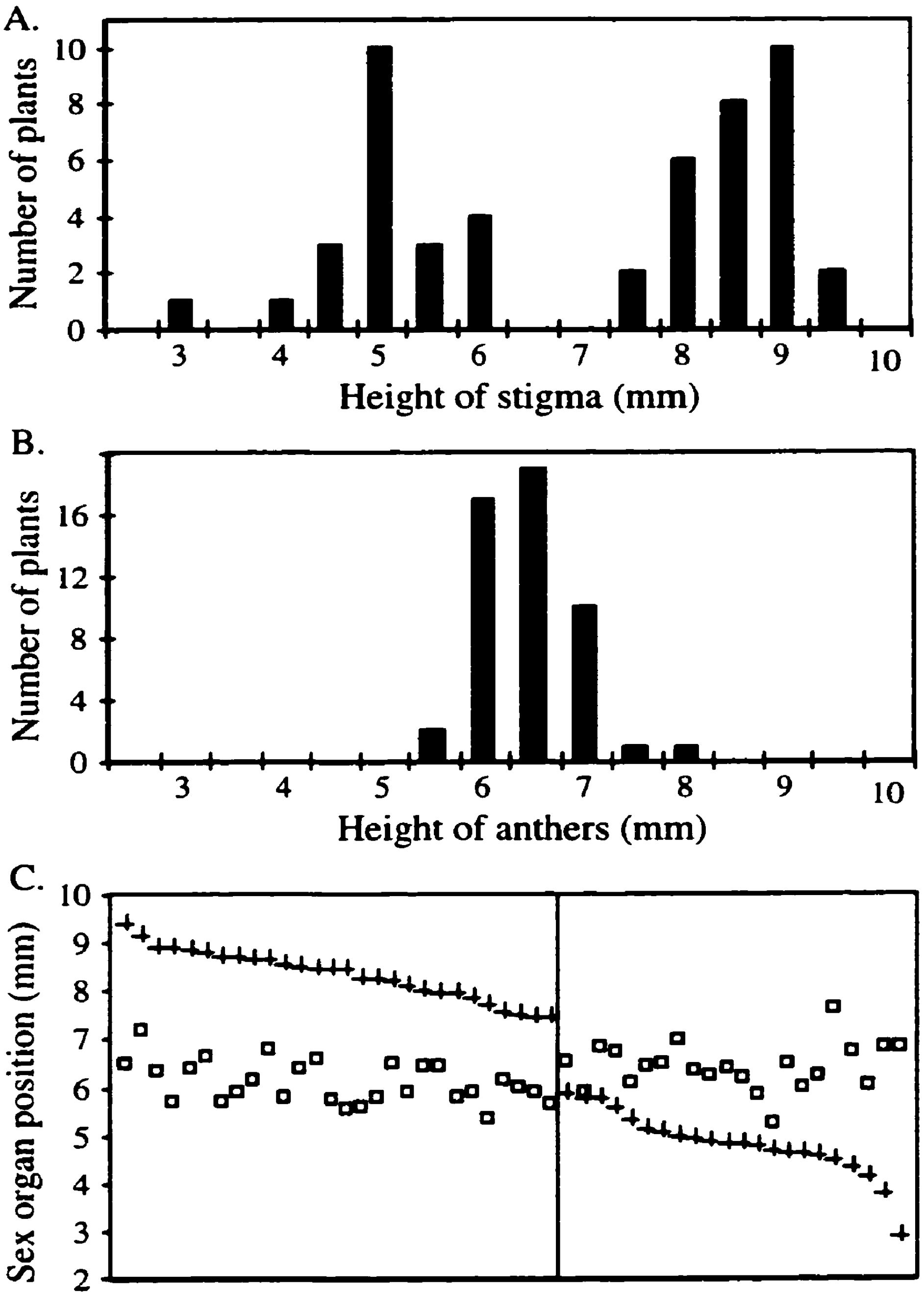


Figure 2-3: Stigma-height dimorphism in *Anchusa officinalis* (Boraginaceae). The population was sampled from Copenhagen, Denmark. There is discrete variation between the two morphs in (A) stigma position, but not in (B) anther position. (C) Individual flowers ranked by style length illustrate the lack of reciprocal herkogamy between the stigmas (+) and anthers (□) of the two morphs. Sex organ measurements have been adjusted to account for the effect of flower length using ANCOVA.

In referring to the floral morphs of stigma-height dimorphic and distylous species subsequently in this review, I identify the long- and short-styled morphs of stigma-height dimorphic taxa as the L- and S-morph. The L- and S-styled morphs have also been referred to as approach herkogamous and reverse herkogamous forms, respectively, (e.g. Webb & Lloyd 1986; Lloyd & Webb 1992a,b). Following Darwin, and to distinguish the respective morphs in the two polymorphisms, I use the traditional 'pin' and 'thrum' designations when referring to the long- and short-styled morphs, respectively, of distylous species.

A comparison of reciprocal herkogamy in stigma-height dimorphic vs. distylous species

Plant species are typically described as possessing a stigma-height polymorphism based on observations of flowers with stigmas positioned above or below the anthers. Classification made on the basis of this sort of casual observation may not be accurate since some species can show continuous rather than discrete variation in sex-organ position. For example, Darwin (1877) reported that *Linum grandiflorum* (Linaceae) exhibited discrete variation in the length of the style only, but recent quantitative studies have shown that there is a much greater difference between the morphs in anther height than previously reported, so that the species legitimately qualifies as distylous (Lloyd & Webb 1992a, p. 166). I compared sex-organ position in species previously identified as possessing anomalous variation and compared this variation with several distylous species to assess the degree of reciprocal herkogamy in these species. In this comparison, the stamen level that most closely corresponds in height to the stigma of the alternate morph was plotted.

I was particularly interested to compare the degree of reciprocal herkogamy in families or genera that contained both stigma-height dimorphic and distylous representatives. Both sexual dimorphisms are found in the genus *Lithodora* (Boraginaceae; Table 2-1). This family also contains stigma-height dimorphic *Anchusa* spp., and distyly also occurs throughout the family in at least seven other genera (Ganders 1979a). I compared the degree of reciprocal herkogamy in species with stigma-height dimorphism vs. distyly in the Boraginaceae (stigma-height dimorphism – *Anchusa hybrida*, *Anchusa officinalis*, *Lithodora fructicosum*, & *Lithodora prostrata* and distylous – *Amsinckia douglasiana*, *Lithodora hispidula*, *Lithospermum cobrense*, *Pulmonaria longifolia*). As Fig. 2-4A indicates, the degree of reciprocal herkogamy is typically

Table 2-1: Reported cases of stigma-height dimorphism in flowering plants. The presence of distyly (D) and tristyly (T) in a family is also indicated. N/A indicates where data is unavailable.

Family and Species	Reference(s)	Floral tube?	Ancillary polymorphisms	Incompatibility status	Population morph ratios
AMARYLLIDACEAE (D, T)					
Narcissus spp. (at least 12 spp.)	Dulberger (1964) Arroyo & Dafni (1995) Barrett *et al.* (1996) Chapter 3 & 4	Yes	Absent	Self-sterile, inter- & intramorph compatible	Variable, mostly L-biased but 1:1 in some species
BORAGINACEAE (D)					
Anchusa spp.	Dulberger (1970) Philipp & Schou (1981) Selvi (1998)	Yes	Weakly developed	Self-sterile, inter- & intramorph compatible	Variable, but strongly L-biased in *A. officinalis*
Lithodora spp.	J.D. Thompson & S.C.H. Barrett (unpubl. data)	Yes	Absent	Self-sterile, inter- & intramorph compatible	Often L-biased
EPACRIDACEAE					
Epacris impressa	O'Brien & Calder (1989)	Yes	N/A	Self-sterile, inter- & intramorph compatible	L-biased
ERICACEAE					
Kalmiopsis leachiana	Marquis (unpubl. data) Barrett *et al.* (2000b)	Poorly developed	Absent	N/A	Variable

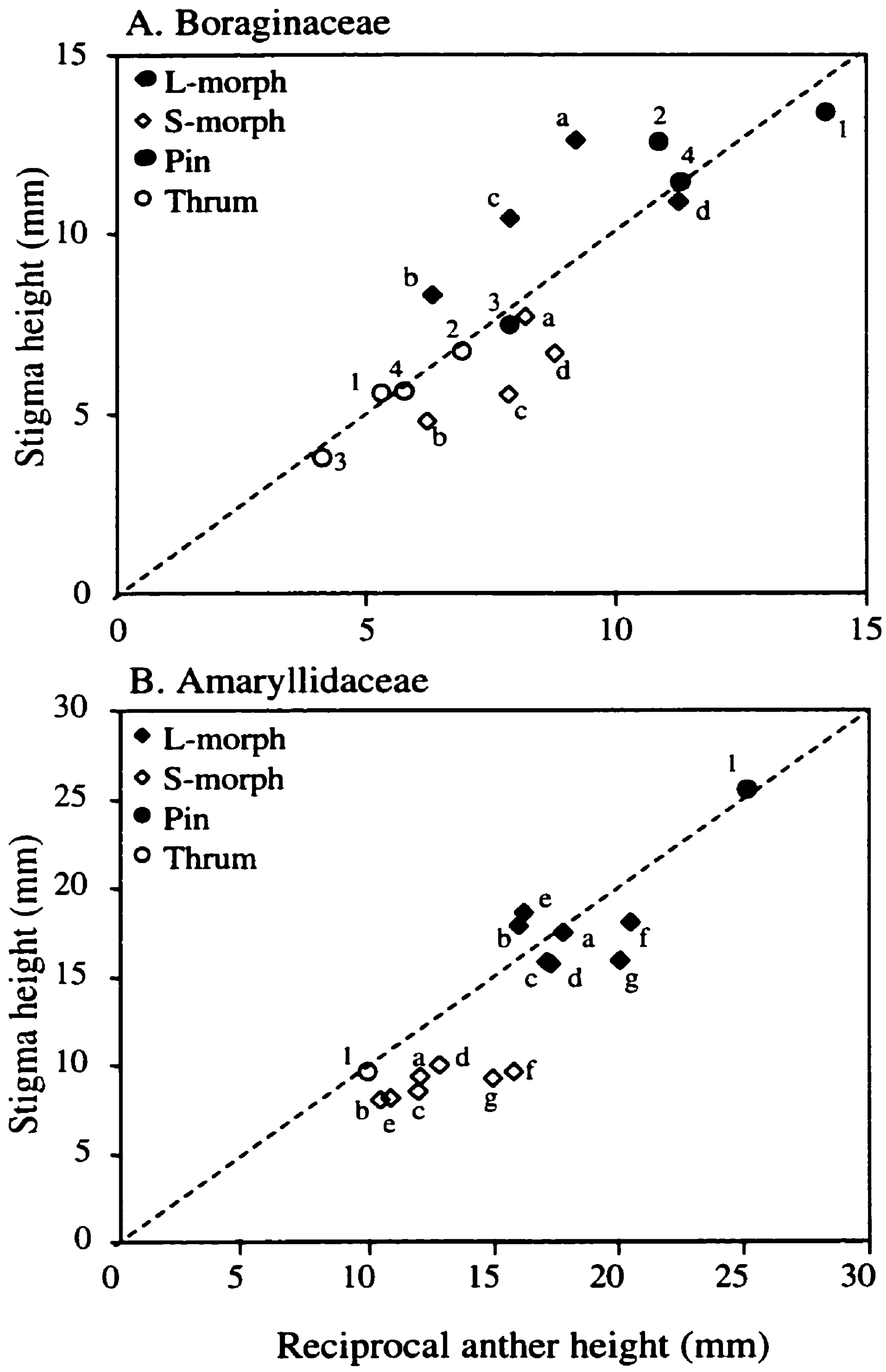
....continued on next page

Table 2-1: (continued from previous page) **Stigma-height dimorphism in flowering plants.**

Family and Species	Reference(s)	Floral tube?	Ancillary polymorphisms	Incompatibility status	Population morph ratios
HAEMODORACEAE					
Anigozanthos humilis	S.D. Hopper (pers.comm)	Yes	N/A	N/A	L-biased (L.D. Harder pers. comm)
LILIACEAE					
Chlorogalum angustifolium	Jernstedt (1982) Barrett *et al.* (2000a)	Poorly developed	Absent	Self-sterility in L not S, Inter- & intramorph compatible	L-biased
PRIMULACEAE (D)					
Primula boveana	Al Wadi & Richards (1993)	Yes	Weakly developed	All crosses fertile	N/A
SCROPHULARIACEAE*					
Penstemon spp.	Keck (1937) Clinebell (1998)	Yes	N/A	N/A	N/A

* The reports from *Penstemon* require further examination. Keck (1937, p. 822) states that there are "three distinct lengths of style, but unlike the usual case of heterostyly, the stamens do not undergo corresponding changes." Clinebell (1998, p. 73) also identifies three floral morphs that differ in the placement of stigmas with respect to anthers. He states that "These observations are the first reported for style-length polymorphism in the genus *Penstemon*." However, neither author presents quantitative data on sex-organ position. Furthermore, the variation described differs from species with stigma-height dimorphism in that there are three floral morphs rather than two.

Figure 2-4 (following page): The degree of sex-organ reciprocity of the floral morphs in species with stigma-height dimorphism and distyly of (A) Boraginaceae and (B) Amaryllidaceae. For each style morph, the corresponding stamen level from the alternate morph is plotted. The letters or numbers refer to species with stigma-height dimorphism or distyly, respectively. In the Boraginaceae (A): a-*Anchusa hybrida*, b-*A. officinalis*; c-*Lithodora fructicosum*, d-*L. prostrata*; and 1-*Amsinckia douglasiana*, 2-*Lithodora hispidula*, 3-*Lithospermum cobrense*, 4-*Pulmonaria longiflora*. Sources are a - Dulberger 1970; c,2,4 – J.D. Thompson (unpubl. data); 1,3 - Ganders 1976, 1979b. Figure 2-4(B) is modified from Arroyo & Barrett 2000 and illustrates species from the genus *Narcissus* (Amaryllidaceae): a-*Narcissus assoanus*, b-*N. calcicola*, c-*N. cuatrecasasii*, d-*N. dubius*, e-N.*gaditanus*, f-*N. papyraceus*, g-N. *rupicola* and 1-*N.albimarginatus*. Sources are a,d - Ch. 3; c,e,f,g - Barrett *et al.* 1996; 1 - Arroyo & Barrett 2000.


A. Boraginaceae
● L-morph
◇ S-morph
● Pin
○ Thrum
Stigma height (mm)
0
5
10
15
0
5
10
15
a
b
c
d
1
2
3
4
B. Amaryllidaceae
◆ L-morph
◇ S-morph
● Pin
○ Thrum
Stigma height (mm)
0
5
10
15
20
25
30
0
5
10
15
20
25
30
a
b
c
d
e
f
g
1
Reciprocal anther height (mm)

much greater in distylous species than stigma-height dimorphic species of the Boraginaceae. (Note that the diagonal line in Fig. 2-4A represents reciprocity of anthers and stigmas).

While both morphs of distylous species typically fall on the diagonal line, the L- and S-morph of stigma-height dimorphic taxa usually fall above or below the line, respectively. For example, *L. fructicosum* and *A. officinalis* do not show reciprocal positioning of anthers and stigmas in either style morph, but there is a greater degree of reciprocal herkogamy in each of the distylous species (*Amsinckia douglasiana*, *Lithodora hispidula*, *Lithospermum cobrense*, and *Pulmonaria longiflora*). There is reciprocity between the stigma of the L-morph and anthers of the S-morph in *Lithodora prostrata* but not between the stigma of the S-morph and anthers of the L-morph. Similarly, *Anchusa hybrida* shows reciprocal positioning in one of the style morphs (stigma of S-morph and anthers of L-morph) but not the other (stigma of L-morph with anthers of S-morph).

Similarly, the degree of reciprocal herkogamy for species with stigma-height dimorphism in the genus *Narcissus* (Amaryllidaceae) is much less than for distylous *N. albimarginatus* (Fig. 2-4B). This genus contains at least 12 stigma-height dimorphic species (Barrett *et al.* 1996; Graham 1997) as well as the single distylous species *N. albimarginatus* (Arroyo & Barrett 2000). Since there are two anther whorls in *Narcissus* flowers that differ in their placement, I plotted the height of the stigma against the height of the closest anther in the opposite morph in order to examine sex-organ reciprocity. Stigma-height dimorphic *Narcissus* species typically exhibit some degree of reciprocity between the L-stigma and upper-anther whorl of the S-morph but not between the S-stigma and the lower-anther whorl of the L-morph. This is because the upper-anther whorl of the S-morph is placed high in the floral tube while the lower-level anthers of the L-morph are not positioned deep within the floral tube. In comparison, distylous *N. albimarginatus* exhibits reciprocal herkogamy (Fig 2-4B; Arroyo & Barrett 2000).

Stigma-height dimorphism is also present in a single species in what is likely the most well known distylous family, the Primulaceae. Al Wadi and Richards (1993) report that *Primula boveana* of the subgenus *Sphondylia* exhibits discrete variation in stigma position but not in anther position. I compared the degree of reciprocal herkogamy in *Primula boveana* with distylous *P. vulgaris* and *P. veris* (Fig. 2-5). The style morphs of each of the distylous *Primula* spp. tend to fall on or close to the line representing reciprocal sex-organ positioning, whereas the morphs of stigma-height dimorphic *P. boveana* do not.

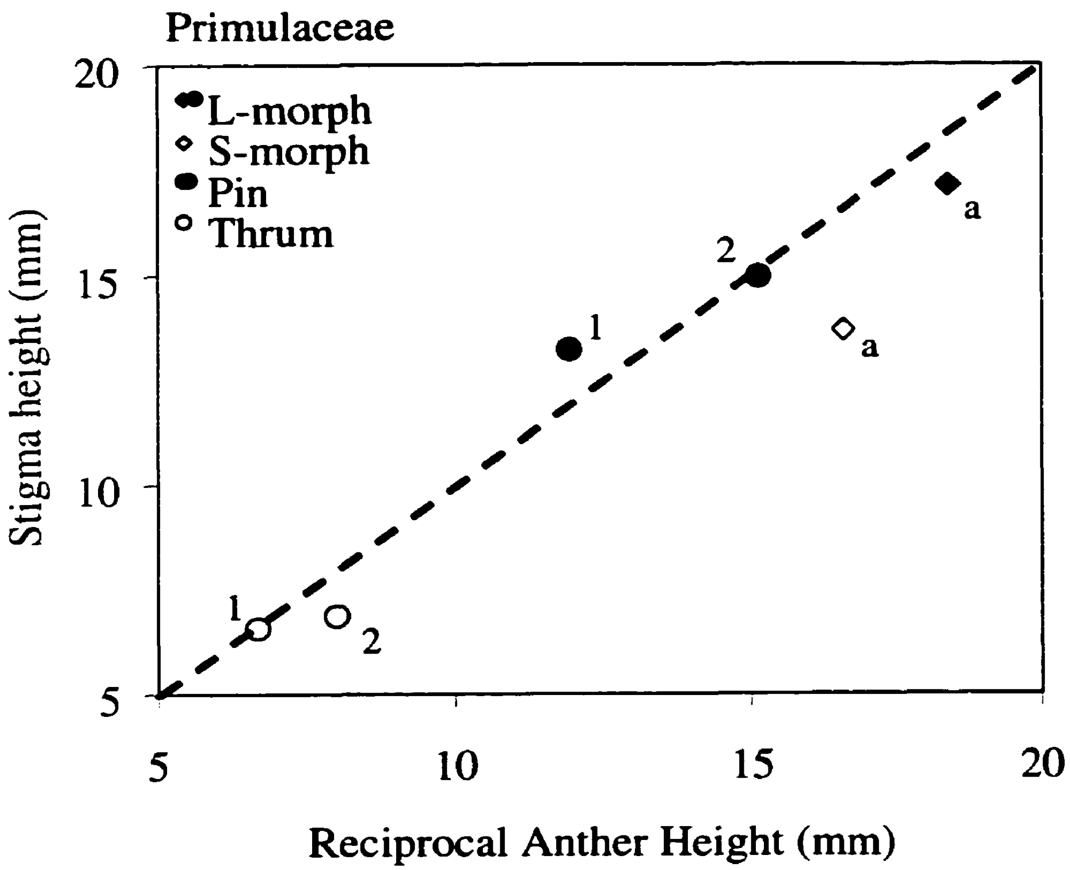


Figure 2-5: The degree of sex-organ reciprocity in a species with stigma-height dimorphism (*Primula boveana*) compared with distyly in the Primulaceae. For each style morph the stamen level from the opposite morph is plotted. One population each of (a) *P. boveana*, (1) *P. vulgaris*, and (2) *P. veris* is shown. Shaded symbols are the L- and pin morphs and open symbols are the S- and thrum morphs, respectively. Sources are a – Al Wadi & Richards 1993; 1,2 – A.M. Baker this thesis.

The absence of reciprocal herkogamy in the stigma-height dimorphic species examined here clearly indicates that the nature of sex-organ placement differs between these taxa and those described as distylous (and see Barrett *et al.* 2000a,b). Rather than having an expanded view of heterostyly that includes species that lack reciprocal herkogamy (e.g. Dulberger 1992; Richards 1997), I prefer the view that the species described here represent a sexual polymorphism that is distinct from heterostyly (and see Ganders 1979a, Charlesworth & Charlesworth 1979; Barrett & Richards 1990; Lloyd & Webb 1992a,b; Barrett *et al.* 2000a,b).

Floral morphology of species with stigma-height dimorphism vs. distyly

Corolla morphology and pollinators

Heterostyly is typically associated with regular, sympetalous flowers (but see Lloyd & Webb 1992a who state that petals are not usually fused), with some development of a corolla tube (Ganders 1979a). This is also true of the floral morphology of most species with stigma-height dimorphism (Table 2-1). Presumably the tubular corolla plays a role in positioning animal pollinators for efficient contact with the sexual organs (Darwin 1877; Baker 1964; Ganders 1979a) so that the proficiency of intermorph pollen transfer is increased. Yet some heterostylous species are bowl shaped with poorly developed corollas (e.g. *Turnera*-Turneraceae and *Fagopyrum*-Polygonaceae; Ganders 1979a). In these species it is unlikely that the shape of the corolla contributes to substantial levels of intermorph pollen transfer especially when compared to species with tubular corollas (Björkman 1995). The corolla shape of some species with stigma-height dimorphism is relatively open as well and is best described as broadly campanulate with only a poorly developed or short corolla tube (Table 2-1). This morphology is present in *Kalmiopsis leachiana* and *Chlorogalum angustifolium*, yet the presence of dimorphic populations of these species indicates that some intermorph pollen transfer must occur even in the absence of a well-developed floral tube. The role of floral morphology in pollen transfer for species with stigma-height dimorphism requires further study.

There have been few detailed field studies examining the pollination biology of species with stigma-height dimorphism, but like heterostylous species, insects (primarily bees but also moths and butterflies) pollinate species with stigma-height dimorphism. Studies of the pollination biology of a number of *Narcissus* species with stigma-height dimorphism have been undertaken (e.g. Arroyo & Dafni 1995; Herrera 1995; Barrett *et al.* 1996; Ch. 5). Members of the genus typically flower early in the spring during unpredictable weather and often experience unreliable pollinator service (reviewed in Ch. 5). Pollinator service can also vary across the species range or among populations. For example, in lowland marshes in Israel, *N. tazetta* is primarily visited by day-flying hawkmoths (*Macroglossum stellarum*), but populations in the hills are more frequently visited by syrphid files (*Eristalis tenax*) and solitary bees (*Anthophora* spp. and *Proxylcopa olivieri*; Arroyo & Dafni 1995). The most common insect visitors to flowers of *N. assoanus* in southern France are butterflies (*Gonepteryx cleopatra*), day-flying hawkmoths, and solitary bees, whereas *N. dubius* is visited primarily by hawkmoths, flies, and solitary bees (Ch. 3). Studies of the pollination biology of other stigma-height dimorphic species have indicated that the most frequent pollinators of *Anchusa officinalis* (Philipp & Schou 1981) and *Kalmiopsis leachiana* (R. J. Marquis unpubl. data) are bumblebees. The flowers of *Chlorogalum angustifolium* are white, strongly scented, open at dusk, and are pollinated by moths (Barrett *et al.* 2000a). Hummingbird pollination is reported from a few heterostylous species (Ganders 1979a) but not for species with stigma-height dimorphism. Bat or wind-pollination is not reported for either plant sexual polymorphism, likely because these pollen vectors are not capable of proficient pollen transfer between anthers and stigmas.

Ancillary polymorphisms

The stigmas of heterostylous morphs can differ in their size, shape and, most frequently, in the size of the stigmatic papillae (reviewed in Dulberger 1992). Differences in size are also the most widespread pollen heteromorphism, although there may also be differences in pollen production, colour, and exine sculpting. While there is some variation among genera with stigma-height dimorphism in the expression of ancillary polymorphisms, they are absent in most species for which data are available (Table 2-1). Philipp and Schou (1981) reported that pollen length and width differed significantly among the two stylar morphs of *Anchusa officinalis*, but it is unlikely that overall pollen volume differed, since pollen of the S-morph was longer while pollen of the L-morph was wider. The number of stigmatic papillae was greater in the S-morph of *A. officinalis* (Philipp & Schou 1981); dimorphic *A. hybrida* exhibits slight differences in stigma shape among the two floral morphs (Dulberger 1970). *Primula boveana* exhibits morph-specific differences in stigmatic papilla length and pollen size but the differences are considerably weaker than in distylous *Primula* spp. (Al Wadi & Richards 1993).

Studies of pollen size and number have not found significant differences among the morphs in *Narcissus assoanus* and *N. dubius* (Ch. 3), *Kalmiopsis leachiana* (R.J. Marquis-unpubl. data cited in Barrett 1992b), or *Chlorogalum angustifolium* (Jernstedt 1982). These taxa encompass most of the genera with stigma-height dimorphism, and although there are no detailed reports of stigmatic morphology, the available data suggests that ancillary polymorphisms are not likely to be strongly associated with stigma-height dimorphism. It has been proposed that ancillary polymorphisms of the pollen and stigmas participate in the heteromorphic incompatibility reactions commonly found in heterostylous plants (Baker 1966; Dulberger 1975, 1992; Barrett & Cruzan 1994). Based on this hypothesis, it seems unlikely that heteromorphic incompatibility is present in groups with stigma-height dimorphism.

Self-sterility and intramorph compatibility

Compatibility status of species with stigma-height dimorphism

While the key feature that defines the heterostylous syndrome is the presence of reciprocal herkogamy, the physiological heteromorphic incompatibility system typically possessed by heterostylous species has also been used to identify the polymorphism (e.g. Darwin 1877; Yeo 1975; Charlesworth & Charlesworth 1979; Barrett 1992b; Lloyd & Webb 1992a). Heteromorphic incompatibility prevents self- and intramorph fertilizations and, as discussed above, it has been hypothesized that pollen and stigma heteromorphisms may play a role in the incompatibility reactions. Heteromorphisms of the pollen and stigma are often absent in stigma-height dimorphic species and, as predicted, all of the taxa which have been examined also lack heteromorphic incompatibility (Table 2-1). Most commonly, species with stigma-height dimorphism are self-sterile but all outcrosses (i.e. both inter- and intramorph) are fully compatible. Notable exceptions include stigma-height dimorphic *Primula boveana* (Al Wadi & Richards 1993) and *Narcissus dubius* (Ch. 4) which are both self-compatible. All other stigma-height dimorphic *Narcissus* species for which compatibility relations have been examined are self-sterile but intramorph compatible (reviewed in Barrett *et al.* 1996; Ch. 4). In *Chlorogalum angustifolium*, the L-morph is self-sterile while the S-morph is not (Jernstedt 1982). The lack of heteromorphic incompatibility in groups with stigma-height dimorphism is another feature distinguishing the dimorphism from heterostyly. Unlike distylous species in which heteromorphic incompatibility results only in intermorph mating, mating in species with stigma-height dimorphism is dependent on the relative success of pollen transfer between and within the two morphs since both intra- and intermorph matings are equally fertile.

Effects of intramorph compatibility on population morph ratios

A consequence of the type of self-sterility system typically found in species with stigma-height dimorphism concerns its effects on mating patterns and style-morph frequencies in populations. Studies of the genetics of *N. tazetta* (Dulberger 1964) and *A. officinalis* (Schou & Philipp 1984) have indicated that the inheritance of the dimorphism for stigma position conforms to the single-locus two allele control common to most distylous species in which the allele for short styles is dominant. With this inheritance and equivalent amounts of disassortative (intermorph) mating among the style morphs, 1:1 (isoplethic) style-morph ratios are expected, as in distylous species. However, unlike most distylous species which possess a heteromorphic incompatibility system, there are apparently no barriers to assortative (intramorph) mating in stigma-height dimorphic species. Thus mating in populations with stigma-height dimorphism can involve, in principle, variable amounts of assortative and disassortative mating and, as a result, style-morph ratios should be more variable than in distylous species (Barrett *et al.* 2000b; Ch. 4). Indeed, the limited empirical data available indicate that morph ratios vary dramatically among populations of stigma-height dimorphic species (Table 2-1).

Most commonly, taxa with stigma-height dimorphism exhibit morph ratios that are biased for the L-morph (*Anchusa officinalis* – Philipp & Schou 1981; *Chlorogalum angustifolium* – Jernstedt 1982, S.C.H. Barrett unpubl. data; *Epacris impressa* – O'Brien & Calder 1989; most *Narcissus* spp. – Barrett *et al.* 1996; *Lithodora fructicosum* – J.D. Thompson unpubl. data; *L. prostrata* – S.C.H. Barrett unpubl. data). Mating models incorporating the proportion of disassortative to assortative mating and the genetic control of stigma-height dimorphism indicate that biased morph ratios occur if one style morph mates assortatively at a higher rate than the other morph (Ch. 4). The morph that shows higher levels of assortative mating will increase in frequency, leading to biased morph ratios. Therefore, in L-biased populations of species with stigma-height dimorphism, it seems likely that assortative mating in the L-morph occurs more frequently than disassortative mating. There are few reports of populations biased for the S-morph, suggesting that pollen transfer among S-styled plants is generally inefficient (and see Webb & Lloyd 1986). The mechanism most likely responsible for greater assortative mating in the L-morph compared to the S-morph is reduced herkogamy in the L-morph (Barrett *et al.* 1996). *Narcissus* spp. exhibit this morph-specific difference in stigma-anther separation (Dulberger 1964; Barrett *et al.* 1996, Ch. 3).

Species with stigma-height dimorphism exhibit wide variation in morph ratios among populations. For example, morph ratios of hill populations of *Narcissus tazetta* are typically L-biased or monomorphic for this morph whereas marsh populations are S-biased (Arroyo & Dafni 1995). This could result from differing pollinator faunas in the two habitats and their influence on mating patterns. In marsh populations, the most frequent pollinators are day-flying hawkmoths capable of contacting the S-stigma. While thrum flowers cannot be considered equivalent to the S-morph of stigma-height dimorphic species, Stone (1996) found that butterflies most efficiently carried out transfer between the thrum plants of *Psychotria suerrenis* (Rubiaceae). Long-tongued pollinators likely play a significant role in transferring pollen to short-level stigmas (and see Richards 1997). The most frequent floral visitors in hill populations of *N. tazetta* were hoverflies that did not contact the S-level organs (Arroyo & Dafni 1995), and therefore transferred pollen efficiently only to the L-stigma. This variation suggests that pollen transfer within and among the morphs is highly dependent on the types of pollinators and their effectiveness in transferring pollen (and see refs. in Stone 1996). Butterflies and hawkmoths or moths, respectively, visit populations of *Narcissus assoanus* (Ch. 5) and *Chlorogalum angustifolium* (Barrett *et al.* 2000a) and likely contact short-level organs. Further studies are required to fully understand the pollination process in species with stigma-height dimorphism, yet the maintenance of dimorphism implies that efficient pollen transfer between the floral morphs is a feature of this floral design.

Function and adaptive significance of stigma-height dimorphism

Similar function of heterostyly and stigma-height dimorphism

Traditionally, herkogamous floral designs were considered to be anti-selfing mechanisms, but this viewpoint is problematic if the traits occur in taxa that already possess a self-sterility system. Instead it has been suggested that these floral designs, including stigma-height dimorphism and heterostyly, function to increase pollen transfer among the morphs and to minimize the interference of male and female function (Lloyd & Webb 1986; Harder & Barrett 1996; Barrett *et al.* 2000b). In plants that deploy male and female sex organs at similar positions, male (pollen removal) and female function (pollen receipt) may interfere with each other, leading to lost mating opportunities due to self-pollination (Darwin 1877; van der Pijl 1978; Lloyd & Yates 1982; Webb & Lloyd 1986). Self-pollination may occur autonomously (within a flower) or geitonogamously (between flowers on a plant; Lloyd & Schoen 1992), and can negatively affect fitness through selfing and inbreeding depression (Charlesworth & Charlesworth 1987) or through pollen discounting (the loss of pollen for outcrossing due to selfing, Harder & Wilson 1998). Self-interference may also affect fitness when the stamens or styles obstruct pollen deposition or removal, respectively; by stigma or stylar clogging with self pollen or tubes; and via ovule discounting (Shore & Barrett 1984; Bertin & Sullivan 1988; Waser & Price 1991; Harder & Barrett 1995; Sage *et al.* 1999). Because of the various ways that self-interference can potentially be expressed, mechanisms that reduce its effects should commonly occur (Barrett *et al.* 2000b). For example, various forms of herkogamy have been interpreted as floral adaptations that reduce stigma-anther interference, particularly when they occur in self-sterile species where the benefits of outcrossing are assured (Webb & Lloyd 1986). Recent studies of the functional significance of herkogamous floral designs, primarily heterostyly, have examined these hypotheses by quantifying pollen transfer and mating patterns in populations.

Two lines of evidence have supported Darwin's (1877) hypothesis that heterostyly actively promotes efficient cross-pollination among style morphs. First, studies of pollen transfer have demonstrated significantly greater intermorph than intramorph pollen transfer (e.g. Ganders 1974; Barrett & Glover 1985; Lloyd & Webb 1992b) and second, studies of mating patterns in experimental garden populations of self-compatible tristylous *Eichhornia paniculata* (Pontederiaceae, Kohn & Barrett 1992) demonstrated effective mating among morphs. Due to the reciprocal placement of anthers and stigmas, intermorph pollen transfers occur more frequently than intramorph transfers because of segregated pollen placement on different pollinator parts (Darwin, 1877; Wolfe & Barrett 1989; Lloyd & Webb 1992b). In heterostylous species with heteromorphic incompatibility, proficient pollen transfer among the morphs reduces pollen loss to incompatible stigmas and thus results in more efficient male function.

Self-interference, proficient pollen transfer and stigma-height dimorphism

Stigma-height dimorphism also likely functions to increase the proficiency of male mating among the morphs and to reduce self-interference (Lloyd & Webb 1992b; Barrett *et al.* 1996; 2000a,b). Initially, S-styled mutants may spread in a population of approach herkogamous plants if the mutant is more proficient at donating pollen to the long-level stigma of approach plants than other approach plants are in donating pollen among themselves. Furthermore, the ability of the mutant to invade a population is increased if it has a higher selfing rate (either through geitonogamy or autogamy) than the L-morph (Lloyd & Webb 1992b). Studies of mating patterns and pollen transfer in manipulated flowers of tristylous *Eichhornia paniculata* (Pontederiaceae) presented in Chapter six indicate that the S-morph does have higher levels of selfing and stronger negative frequency-dependent fitness than the L-morph (Ch. 6). This suggests that it is easier for the S-morph to establish in a population of plants with long styles rather than vice versa. However, manipulated tristylous flowers were used in the study and in *Narcissus* spp. with stigma-height dimorphism the L-morph is more susceptible to self-pollen deposition than the S-morph (Arroyo & Dafni 1995; A.M. Baker unpubl. data). Moreover, no differences in morph-specific female fertility resulting from ovule discounting have been found in populations (Ch. 4). As in my studies of female fertility in *Narcissus*, Schou & Philipp (1981) found no differences in fruit set among the morphs of self-sterile *Anchusa officinalis*. Further studies are required to examine the ecological and evolutionary significance of self-interference in populations with stigma-height dimorphism.

The existence of isoplethic populations of *Narcissus assoanus* in southern France (Ch. 3) and *Anchusa hybrida* in Israel (Dulberger 1970) indicate that, at least under some conditions, high levels of disassortative mating can be achieved even in the absence of reciprocal herkogamy and diallelic incompatibility. In *N. assoanus*, populations occurring in fragmented landscapes near urban centres are often small and typically exhibit L-biased morph ratios, whereas the much larger populations further from urban areas exhibit isoplethic morph ratios. This suggests different patterns of pollen transfer between populations of *N. assoanus* in the two areas sampled. What aspects of the pollination process are involved are not known, nor is it known why population size is related to pollen transfer patterns. From a functional perspective, it is difficult to see how the arrangement of sex organs in stigma-height dimorphic taxa can promote efficient pollen transfer when there is no reciprocal herkogamy among alternate style morphs. It is possible that pollen picked up by long-tongued pollinators is placed on a restricted area of the proboscis and then subsequently redistributed through proboscis coiling (L.D. Harder pers. comm.) or that pollen is deposited over a broader area of the pollinator during feeding (Barrett *et al.* 2000b).

Selection on sex-organ position

Sex-organ position within a flower can have a significant effect on the reproductive success of an individual (reviewed above). I examined the relative amounts of variation (coefficient of variation – CV) of morph-specific sex-organ position in species with distyly or stigma-height dimorphism to determine whether patterns of sex-organ variation were similar between the two sexual systems. The first analysis examined species with stigma-height dimorphism and only one anther level in a flower (*Anchusa officinalis*, *Lithodora fructicosum*, *Kalmiopsis leachiana*, and *Chlorogalum angustifolium*). Preliminary analyses indicated no differences among populations of a species, so I present least squares mean values for each species following a fully crossed ANOVA with species, morph, and sex organ as the main effects. For the distylous species (*Lithodora hispidula*, *Pulmonaria longifolia*, *Lithospermum cobrense*, *Primula veris*, and *P. vulgaris*), I present the pooled least squares mean since ANOVA indicated that there was no significant variation in the CV among species, morph or organ position (Whole model $F_{41,120}$=0.9945, P=0.4918). In the analysis involving species with stigma-height dimorphism, there are morph-specific differences in the CV for anthers and stigmas (Table 2-2). In general, the relative amount of variation in stigma position is greater than in anther placement for the S-morph but not for the L-morph (as indicated by a morph × organ position effect in the ANOVA, $F_{1,44}$=10.24, P<0.01). A similar trend is seen in the distylous species although the whole model test is not significant ($F_{19,4}$=1.84, P=0.2933) nor is the interaction (morph × organ position $F_{1,4}$=7.65, P=0.051).

In a second analysis, I examined the CV in stigma and anther position for *Narcisuss* spp. that possess two anther whorls. As in the analysis of species with one anther whorl, I found that variation in anther position was less than in stigma position for the S-morph but not for the L-morph (Table 2-3; morph-by-organ interaction $F_{2,120}$=9.34, P<0.001). Interestingly, in both analyses of species with stigma-height dimorphism, the CV was largest for the stigma in S-plants. However, there were no significant differences in mean CV among the L-stigma and

Table 2-2: Mean coefficient of variation (CV) and standard error (in brackets) of anther and stigma position in species with stigma-height dimorphism. The CV of *Anchusa officinalis*, *Lithodora fructicosum*, *Kalmiopsis leachiana*, *Chlorogalum angustifolium* is based on 5, 2, 4, and 2 populations, respectively. Sources are *A. officinalis* – this thesis; *L. fructicosum* – J.D. Thompson (unpubl. data); *K. leachiana* – R.J. Marquis (unpubl. data), Barrett *et al.* 2000b; *C. angustifolium* – Barrett *et al.* 2000a, this thesis. I also present the least squares mean CV for distylous species (see text for further details).

	L- or pin morph		S- or thrum morph	
Species	Stigma	Anther	Stigma	Anther
A. officinalis	7.97 (0.72)	8.33 (0.55)	15.38 (3.04)	7.68 (1.15)
L. fructicosum	13.13 (1.49)	11.13 (0.18)	9.44 (0.24)	8.36 (0.34)
K. leachiana	10.65 (2.83)	12.70 (0.99)	14.25 (2.55)	11.72 (1.63)
C. angustifolium	15.53 (2.77)	18.30 (0.93)	25.77 (1.56)	11.63 (2.99)
Least squares mean	11.06 (1.27)	12.15 (1.03)	16.29 (1.85)	9.91 (0.95)
Pooled distylous	10.89 (1.32)	14.32 (1.32)	13.70 (1.32)	9.83 (1.32)

Table 2-3: Mean coefficient of variation and standard error (in brackets) of stigma and upper (U) and lower (L) anther position in stigma-height dimorphic *Narcissus* spp. I include distylous *N. albimarginatus* for comparison. The CV of *N. assoanus* and *N. dubius is* based on 15 and 7 populations, respectively. All other samples are from single population. Sources for the sex-organ position data are given in the caption for Fig. 2-4B.

	L- or pin morph			S- or thrum morph		
	Stigma	U-anther	L-anther	Stigma	U-anther	L-anther
N. assoanus	10.51	8.80	11.36	13.79	8.57	10.18
	(0.53)	(0.33)	(0.48)	(0.62)	(0.41)	(0.59)
N. calcicola	7.92	11.61	15.53	17.66	10.98	11.61
N. cuatrecasasii	8.16	6.59	9.98	15.10	6.26	7.89
N. dubius	8.27	7.58	9.42	12.67	8.23	9.62
	(0.40)	(0.52)	(0.71)	(3.64)	(1.80)	(1.86)
N. gaditanus	8.48	9.45	12.71	19.48	9.28	10.92
N. papyraceus	8.87	8.37	9.43	16.70	8.32	8.86
N. rupicola	7.57	8.60	10.13	13.07	8.83	9.67
Least squares mean	8.54	8.71	11.22	15.50	8.64	9.93
	(1.03)	(1.03)	(1.03)	(1.03)	(1.03)	(1.03)
N. albimarginatus	7.33	8.36	11.37	15.64	8.24	8.91

anthers of either morph ($P>0.22$ and $P>0.07$ in all cases for stigma-height dimorphic species with one anther whorl and *Narcissus*, respectively). In distylous species with one anther whorl, there was no significant difference in the relative variation of anthers and stigmas of the morphs. However, there is a trend for short-level organs (S-stigma and anthers of the L-morph) to exhibit more relative variation than long-level organs (L-stigma and anthers of the S-morph; Table 2-2). Distylous *N. albimarginatus* showed a trend similar to the stigma-height dimorphic populations with the CV being largest for S-stigma.

Why is there greater variation in the position of sex organs (both anthers and stigmas) placed low in the corolla tube, but particularly the S-stigma of species with stigma-height dimorphism (Tables 2-2 & 2-3)? Harder and Barrett (1993) suggested that there may be an evolutionary priority on the selection of anther position since studies have indicated a greater impact of variation in floral characters on pollen removal and dispersal than on pollen receipt (e.g. Lloyd & Yates 1982; Bell 1985; Stanton *et al.* 1986; Cruzan *et al.* 1988; Galen & Stanton 1989; Campbell *et al.* 1991). This hypothesis predicts that there will be a smaller amount of relative variation in the position of anthers than of stigmas. Evidence in support of this hypothesis is the fact that while numerous species show polymorphism in the position of stigmas, there are no species with an anther-height polymorphism in the absence of stigma-height polymorphism. However, in the species examined here, the relative amount of variation for stigmas is greater than for anthers in the S-morph but not for the L-morph.

The observed patterns may be related to the efficiency of pollen removal from anthers and pollen deposition on stigmas placed at different positions in the flower. Studies of the position of sex organs in distylous species have shown that the optimal position for both pollen removal and receipt is at the opening of the perianth mouth (reviewed in Dulberger 1992; Kohn & Barrett 1992; Harder & Barrett 1993). If pollen is more efficiently removed from anthers placed closer to the opening of the floral tube rather than from those at the base, there may be stabilizing selection on this anther position, especially when there is enough herkogamy between stigma and anther position to minimize self-interference. Furthermore, because pollen receipt is also optimized near the opening of the corolla tube, variation in stigma position is likely to be less in the L-morph than in the S-morph. The long-level stigma may be further constrained by the fact that if the style grows too long, pollinators that visit the flowers will not contact the stigma. The positioning of the stigmas and the resultant proficiency of pollen receipt is particularly relevant in species that experience pollen limitation (e.g. Johnston 1991b). These patterns may not be as pronounced in distylous species since the reciprocal positioning of anthers and stigmas likely serves to increase intermorph pollen transfer more efficiently than in species with stigma-height dimorphism. While species with stigma-height dimorphism are also capable of intermorph mating allowing for the maintenance of a dimorphism, the fact that many populations exhibit L-biased morph ratios suggests that assortative mating can be relatively high in this morph. There may be a fitness advantage when stigmas and anthers are placed near the opening of the floral tube. Although data on female fertility has not shown morph-specific fitness differences (Chapter 4), no thorough study of male fitness has been conducted in natural populations with stigma-height dimorphism.

Origin of stigma-height dimorphism

Evolution of stigma-height dimorphism in non-heterostylous families

Among the angiosperms, stigma-height dimorphism is found in at least seven families (excluding Scrophulariaceae – ***Penstemon*** until examined in detail) including monocots and dicots. Like heterostyly, stigma-height dimorphism does not occur in the most primitive families but rather in families with an intermediate level of specialization. Stigma-height dimorphism is polyphyletic and has likely arisen independently in each of the families it occurs in (Fig. 2-6). These include four families in which heterostyly is unknown and three heterostylous families (Table 2-1). Because of the lack of reciprocity in sex-organ positioning among morphs, most workers have assumed that stigma-height dimorphism is an unstable condition representing a transient stage in the evolution of distyly (Lloyd & Webb 1992a,b; Richards 1998). In some cases, the dimorphism may well be a stage in the evolution of distyly (e.g. ***Primula boveana*** of the distylous Primulaceae, Al Wadi & Richards 1993; Richards 1998). However, virtually nothing is known about the reproductive biology of stigma-height dimorphism in non-heterostylous families. These taxa represent an eclectic mix of animal-pollinated species, none of which possess traits typically associated with the heterostylous syndrome (e.g. reciprocal herkogamy, ancillary polymorphisms, heteromorphic incompatibility, isoplethic morph ratios). How stigma-height dimorphism is maintained in these groups is unclear, but its occurrence at

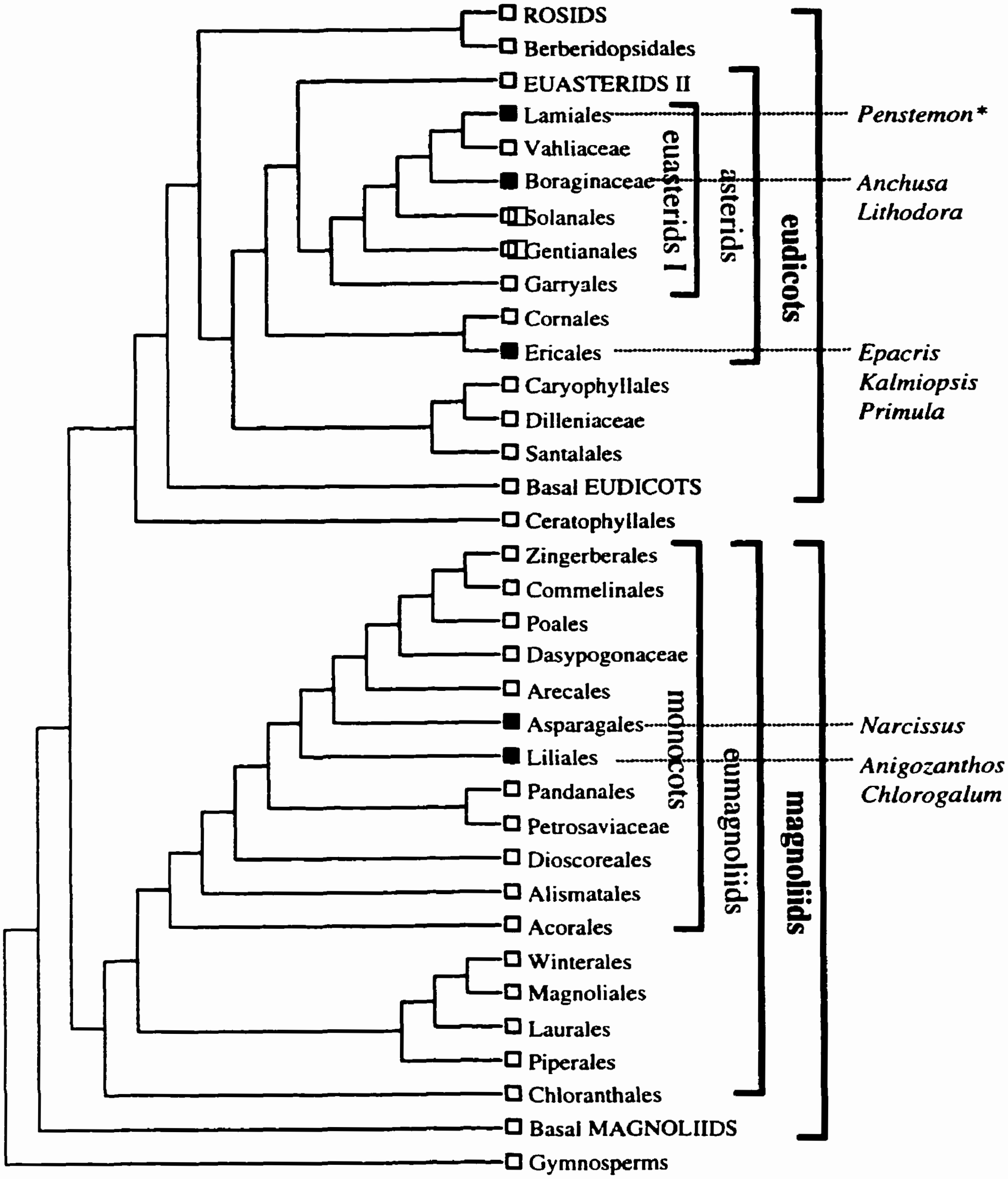


Figure 2-6: Ordinal distribution of stigma-height dimorphism in the angiosperms. I indicate orders in which stigma-height dimorphism is present on the phylogenetic tree from Soltis *et al.* (1999). Note that the Boraginaceae are unplaced to order. *Stigma-height polymorphism in *Penstemon* requires thorough examination.

least demonstrates that the origin of stigma-height dimorphism does not necessarily lead to the evolution of distyly.

Evolutionary relationships of stigma-height dimorphism and distyly

Theoretical models have addressed the role that stigma-height dimorphism may play in the evolution of distyly. Using simulations, Charlesworth and Charlesworth (1979) found that a polymorphism for stigma-height is difficult to maintain in self-compatible populations since a mutant with altered stigma height would either be eliminated (if it caused too great a loss in female fertility) or spread to fixation. Richards (1998) considered the establishment of a stigma-height dimorphism consisting of a long-homostyle and short-styled mutant (Richards 1998). Lloyd and Webb (1992b) also consider an intermediate stigma-height dimorphic stage in their pollen-transfer model of the evolution of distyly. They suggest that stigma-height dimorphism is difficult to maintain since selection of mutations giving rise to anther-height dimorphism will rapidly spread to fixation, resulting in a population with reciprocal herkogamy. In fact, the rarity of stigma-height dimorphism in flowering plants has often been interpreted as evidence that the condition is quickly passed through during the evolution of distyly.

Determining the role of stigma-height dimorphism in the evolution of distyly is fraught with difficulty, although the development of phylogenies for genera or families containing both polymorphisms may help. Graham (1997) was unable to reconstruct the evolution of stigma-height dimorphism in *Narcissus* due to lack of resolution of certain regions of a *ndh*F-based phylogeny of the genus. Arbitrary resolutions of these areas, in combination with weighting schemes for the gain or loss of stigma-height dimorphism, revealed a large degree of uncertainty in the reconstructed number of origins (range=0 to 10 gains depending on weighting scheme, Graham 1997). There is only one origin of distyly (the recently discovered *N. albimarginatus*). The distylous species was not included in the phylogeny although work is currently underway to expand the molecular data set (S.W. Graham, pers. comm). Similarly, there has been some progress in the construction of phylogenies of the Boraginaceae that may be useful in determining the evolutionary relations among stigma-height dimorphism and distyly in this family (H.H. Hilger, pers. comm.). There are at least two independent origins of stigma-height dimorphism in this family (E. Lönn; H.H. Hilger, pers. comm. based on unpublished phylogenies) but a species-level phylogeny is required to examine questions related to the evolutionary relationships among taxa with stigma-height dimorphism and distyly, particularly in the Boraginaceae and Amaryllidaceae.

The Lloyd and Webb (1992b) model for the evolution of distyly with the establishment of a stigma-height dimorphism as an intermediate step can be tested (see Stone & Thomson 1994; Ch. 6) by measuring pollen transfer in experimental populations with stigma-height dimorphism. In the model, a stigma-height dimorphism is established when a novel mutant spreads via a frequency-dependent fitness advantage in pollen transfer. Stone and Thomson (1994) used artificial flowers to show that pollen transfer inequalities (i.e. intermorph pollen transfer must be greater than intramorph) for the establishment of a stigma-height dimorphism can be satisfied although their data did not support the second stage of the model in which an anther-height dimorphism becomes established. Another test of Lloyd and Webb's (1992b) model used genetically-marked, self-compatible *Eichhornia paniculata* (Pontederiaceae) modified to exhibit a stigma-height dimorphism (Ch. 6). I found that the pollen transfer inequalities of the Lloyd and Webb model (1992b) were satisfied when outcross pollen transfer were considered, but were usually not met when both self and outcross pollen transfers were examined. This finding may suggest why most species with stigma-height dimorphism are self-sterile.

Conclusions

Stigma-height dimorphism is a simple polymorphism involving discrete variation in stigma position but anthers positioned similarly between the floral morphs. Features commonly associated with the distylous syndrome are typically not found in taxa with stigma-height dimorphism, and this warrants the identification of stigma-height dimorphism as a sexual polymorphism distinct from heterostyly. Stigma-height dimorphic populations lack reciprocal herkogamy, ancillary polymorphisms of the stigmas and anthers, and heteromorphic incompatibility. The genetic control of stigma-height dimorphism appears to be the same as is common in distylous species and the floral shape associated with both sexual polymorphisms is often tubular. Populations of species with stigma-height dimorphism are typically biased for the L-morph, suggesting that pollen transfer among S-styled plants may be relatively poor in comparison with other pollen transfers. However, the presence of isoplethic populations in some species indicates that symmetrical disassortative mating is possible under certain conditions, even when reciprocal herkogamy and heteromorphic incompatibility are absent. Stigma-height dimorphism likely functions to increase the efficiency of pollen transfer between the morphs and to reduce self-interference because of the spatial separation of anthers and stigmas. How proficient pollen transfer between the morphs is accomplished without reciprocal herkogamy is not yet clear. Finally, stigma-height dimorphism may be an intermediate stage in the evolution of distyly in some groups but its occurrence in families without distyly suggest that this is not always the case. Further work is required to understand the mechanics of pollen transfer in populations with stigma-height dimorphism and to examine its reproductive consequences in non-heterostylous families.

CHAPTER 3

EVOLUTION & MAINTENANCE OF STIGMA-HEIGHT DIMORPHISM IN *NARCISSUS*: FLORAL VARIATION & STYLE-MORPH RATIOS

Summary

An unusual stylar dimorphism occurs in *Narcissus*, a plant genus of insect-pollinated Mediterranean geophytes. To determine the characteristics of the sexual polymorphism, I investigated floral variation in 46 populations of *N. assoanus* (section *Jonquillae*) and 21 populations of *N. dubius* (section *Tazettae*) in south-western France. Flowers possess two stamen levels in each morph that occupy slightly different positions within the floral tube. In long-styled plants (L-morph), the stigma is located within or slightly above the upper-level stamens, whereas in short-styled plants (S-morph) the stigma is placed well below the lower-level stamens. The stigma-height dimorphism is distinct from heterostyly because the reciprocity of stigma and anther positions in the two style morphs is only weakly developed and there are no differences between the style morphs in pollen size or production. In both species, mean stigma-anther separation is much greater in the S-morph than the L-morph. In *N. assoanus*, population style-morph ratios vary from isoplethy (1L:1S) to L-biased, whereas in *N. dubius* they are usually strongly L-biased or occasionally contain only the L-morph. Populations fixed for the S-morph, or with S-biased morph ratios, were not observed. In *N. assoanus*, style-morph ratios were associated with population size: large continuous populations always exhibited 1:1 morph ratios, whereas smaller fragmented populations were often L-biased. This pattern was not evident in *N. dubius*. I argue that biased style-morph ratios largely result from morph-specific differences in assortative mating.

Introduction

Sexual polymorphisms involving discrete variation in the length or position of the style are reported from many unrelated flowering plant families (Webb & Lloyd 1986; Barrett *et al.* 2000a,b). The most common stylar polymorphism is heterostyly which Darwin (1877) first examined in detail and that has been the focus of a great deal of subsequent research (reviewed in Ganders 1979a; Barrett 1992a; Richards 1997). Heterostylous populations have two (distyly) or three (tristyly) floral morphs that differ reciprocally in the placement of stigmas and anthers (Lloyd & Webb 1992a). Another, less well known, stylar polymorphism is characterized by discrete variation in the position of the stigma but little or no reciprocal positioning in anther placement between the two style morphs. Populations exhibiting stigma-height dimorphism consist of two floral morphs, one in which the stigma is at the same level as the stamens or protrudes beyond them (long-styled or L-morph) and the other in which the stigma is located below the stamens (short-styled or S-morph). Some authors recognize stigma-height dimorphism as distinct from heterostyly (Charlesworth & Charlesworth 1979; Ganders 1979a; Jernstedt 1982; O'Brien & Calder 1989; Barrett & Richards 1990; Lloyd & Webb 1992a; Arroyo & Dafni 1995; Barrett *et al.* 1996), whereas others view the polymorphism as part of the variation encompassed within heterostyly (Philipp & Schou 1981; Dulberger 1992; Richards 1997 and pers. comm.). Unfortunately, there have been few detailed studies of species with stigma-height dimorphism to assess the nature of floral variation and the evolutionary and functional relationships between this polymorphism and heterostyly. This is unfortunate because stigma-height dimorphism plays an important role in some models of the evolution of distyly (Lloyd & Webb 1992b).

Stigma-height dimorphism occurs commonly in *Narcissus* (Amaryllidaceae), a genus of approximately 40 species of insect-pollinated geophytes largely native to the Mediterranean basin. Dulberger (1964 and unpubl. data) reported stigma-height dimorphism in *N. tazetta* populations from Israel (and see Arroyo & Dafni 1995). Using controlled crosses, Dulberger demonstrated that self-incompatibility was not of the heteromorphic type, although the inheritance of style length conformed to the single-locus two-allele control common in distylous species, with the allele for short styles dominant. Stigma-height dimorphism has subsequently been documented in several additional species from three sections of the genus (*Apodanthae*, *Jonquillae*, *Tazettae*) by Barrett *et al.* (1996). Despite the widespread distribution of stigma-height dimorphism in *Narcissus*, heterostyly occurs only in distylous *N. albimarginatus* of section *Apodanthae* (Arroyo & Barrett 2000) and tristylous *N. triandrus* of section *Ganymedes* (Barrett *et al.* 1997; Sage *et al.* 1999), and the evolutionary relationships between stigma-height dimorphism, distyly and tristyly are unclear. In their model of the evolution of heterostyly, Lloyd & Webb (1992b) suggested that stigma-height dimorphism is rare in flowering plants because it may be difficult to maintain (and see Charlesworth & Charlesworth 1979). According to this view, selection rapidly favours the evolution of discrete anther-height variation in populations with stigma-height dimorphism and hence the polymorphism represents a transient stage in the evolution of heterostyly (and see O'Brien & Calder 1989). However, in *Narcissus* the contrasting frequencies and phylogenetic distributions of the two polymorphisms are not in accord with this model. Further studies are clearly warranted to explain the evolutionary stability of stigma-height dimorphism in the genus.

In large distylous populations with frequent sexual recruitment, equilibrium morph ratios are generally isoplethic (1L:1S) and result from disassortative mating governed by the heteromorphic incompatibility system typical of most heterostylous plants (reviewed in Ganders 1979a). However, in *N. tazetta* (Dulberger 1964) and *N. triandrus* (Barrett *et al.* 1997) both inter- and intramorph pollinations are fully compatible and morph ratios are governed by the influence of floral morphology on the relative fitness of the morphs as female and male parents (Barrett *et al.* 1996). Intramorph mating in *Narcissus* spp. provides opportunities for variation in levels of assortative mating in the style morphs resulting in populations with biased morph ratios or those containing only a single morph. Unlike heterostylous species with conventional heteromorphic incompatibility, a wide range of style-morph ratios is therefore predicted in natural populations of *Narcissus* spp.

I examine these issues in populations in *N. assoanus* and *N. dubius*, two species from different sections of the genus which occur in south-western (S. W.) France. Preliminary observations suggested that both species exhibit stigma-height dimorphism but that the patterns of sex-organ variation differ in association with several features of their floral biology that might be expected to influence pollination and mating. Here I report the findings of a comparative study in which the goal was to provide insights into the evolution and maintenance of stigma-height dimorphism. In this chapter, I ask the following questions. (1) What are the patterns of sex-organ variation in natural populations of *N. assoanus* and *N. dubius* and do the two species exhibit a true dimorphism for stigma height? (2) Are there morph-specific differences in pollen size and production in either *Narcissus* species? These features are commonly associated with distyly. (3) What style-morph ratios characterize populations of *N. assoanus* and *N. dubius* and what factors might explain any differences observed both within and between species? In the following chapter of my thesis (Ch. 4), I examine fitness components of the style morphs in an effort to understand the selective mechanisms maintaining the polymorphism and the contrasting style-morph ratios in the two species reported in this chapter.

Materials and methods

Natural history of Narcissus assoanus *and* N. dubius

Narcissus assoanus (section *Jonquillae*) is a diminutive species, approximately 10-15 cm in height, that is widespread throughout southern Spain to S. W. France. In S. W. France, it typically occurs in meadows and stony pastures on limestone from sea level to 700 m altitude. Plants produce a single inflorescence with one to three deep yellow flowers with prominent coronas and floral tubes. In S. W. France, the vast majority of plants produce a single flower. Flowering time depends on elevation and usually occurs from late February to April.

Narcissus dubius (section *Tazettae*) is larger (20-25 cm) with inflorescences of one to seven white flowers also with prominent coronas and floral tubes. In S. W. France, flowering begins in mid-February and ends in late March. The species has a more restricted distribution, occurring primarily from the east coast of southern Spain to S. W. France at less than 300m elevation and in some areas is found in sympatry with *N. assoanus*. *Narcissus dubius* occurs almost exclusively in garrigues (dry, rocky, limestone substrate) and on or around cliff faces. *Narcissus assoanus* is visited by butterflies, hawkmoths and solitary bees, whereas *N. dubius* is visited by hawkmoths, flies and solitary bees.

Patterns of sex-organ variation

To establish whether *N. assoanus* and *N. dubius* exhibit a true dimorphism for stigma-height, I sampled populations of both species for floral measurements at peak flowering (late February-March for *N. dubius*; March-April for *N. assoanus*). All sampling was in the Languedoc-Roussillon region of S. W. France in an area bounded by the Rhône River to the east and the city of Perpignan near the France-Spain border to the west. In each population, flowers were sampled randomly from throughout the entire population, with care taken to not sample more than one inflorescence from dense clumps which may have arisen via bulb fragmentation (particularly in *N. dubius*). Floral measurements were made on a single mature flower from at least 45 randomly selected individuals from 15 *N. assoanus* and 10 *N. dubius* populations. These populations were chosen from throughout the range of style-morph ratios revealed from our survey (see below). The following floral traits were measured to 0.01 mm using digital calipers: flower length, stigma height, and the distance to the top and bottom of the upper- and lower-level stamens. For each stamen level, the distance to the middle of the anthers was calculated by taking the mean of the distance to the top and bottom of the anthers within a level. All measurements were made from the top of the ovary (Fig. 3-1).

To account for the influence of flower size on patterns of sex-organ variation I adjusted the measurements of stigma height and upper- and lower-stamen height for flower length. I first performed an analysis of covariance (ANCOVA) using JMP statistical software (SAS 1994) in which population and style morph were treated as main effects and flower length was treated as a covariate. Secondly, two- and three-way interaction terms were eliminated from the model using backward elimination if they contributed less than 5% of the variation in organ height (cf. Sokal & Rohlf 1995). Finally, I adjusted organ position as follows: *adjusted organ position = organ position - b (flower length - mean flower length)* where *b* represents the slope of the line of organ position plotted against flower length. This process accounts for variation in organ position caused by variation in flower size among individuals but does not significantly alter the value of mean organ position. Differences in mean adjusted organ position between the morphs for populations of each species were then assessed using ANOVA with population and style morph as main effects.

Pollen characteristics

All three anthers from a given stamen level were collected prior to anthesis and placed in Eppendorf sample tubes. Anthers were allowed to dry and fully dehisce before the tube was filled with 70% ethanol. Pollen size and production were quantified using an electronic Elzone 282 particle counter with a 190µm aperture. Each sample was vortexed for 15 seconds to dislodge pollen grains from anthers and 0.5mL was removed from the tube and added to a vial containing 24.5mL of saline solution (0.5% NaCl). The particle counter counted the number of particles in 0.25mL samples, assigned them to logarithmic size classes, and calculated the mean geometric

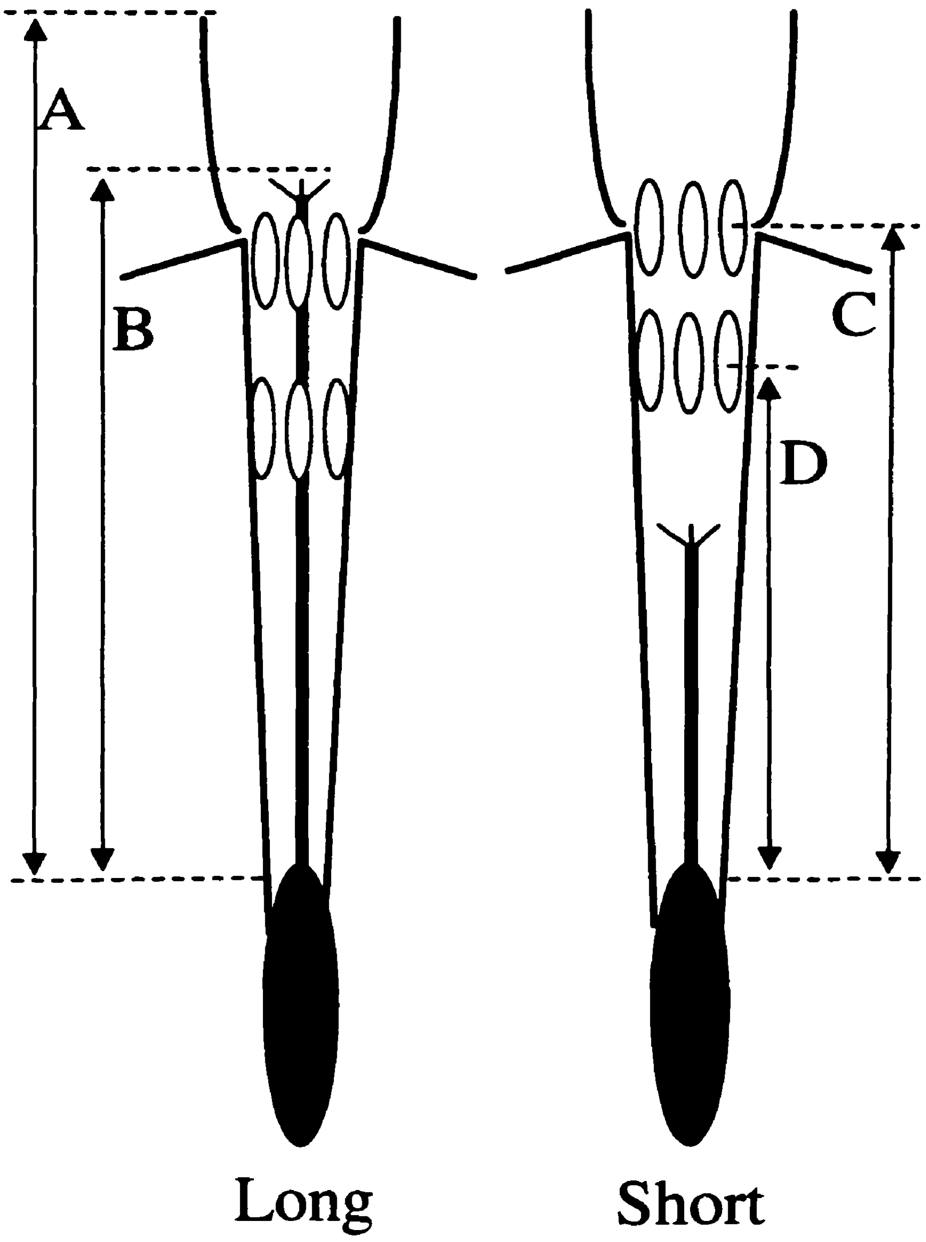


Figure 3-1: Floral measurements on flowers of *Narcissus assoanus* and *N. dubius*. The illustration shows the L- and S-morphs of *N. assoanus* drawn to scale based on average measurements from all populations. Flower length (A) and stigma height (B) were measured directly; the distance to the middle of the upper-level stamens (C) and the middle of the lower-level stamens (D) were calculated (see text for details).

size of pollen grains. For each pollen sample, mean estimates of pollen size and number are based on four 0.25mL subsamples. I counted pollen samples from 12 plants of each style morph in one *N. assoanus* population and 12 and 10 plants of the L- and S-morph, respectively, from a *N. dubius* population. Pollen size and number were analysed separately in each population using a two-way ANOVA with style morph and stamen level as main effects.

Surveys of style-morph ratios

Floral measurements established that populations of both *N. assoanus* and *N. dubius* were dimorphic for stigma height. Plants could be classified as either L- or S-styled based on the placement of the stigma with respect to the two stamen levels. I conducted an extensive survey to determine the relative frequencies of the two style morphs in populations of each species. During spring 1996-1998 I sampled 46 and 21 populations of *N. assoanus* and *N. dubius*, respectively, for style-morph ratios. Pooled goodness of fit *G*-tests were calculated to determine if pooled morph ratios differed significantly from 1L:1S. I also calculated $G_{\text{heterogeneity}}$ statistics to test for heterogeneous morph ratios among populations. For each population, two or three independent estimates of population size were made and the average value was taken. Data on style-morph ratios and individual sizes for populations in the survey are listed in Appendix 1.

Results

Variation in sex-organ position in natural populations

Populations of *N. assoanus* and *N. dubius* both exhibit a stigma-height dimorphism despite considerable variation in the relative positions of stigmas and anthers among individuals within each population (Fig. 3-2). With the exception of monomorphic populations of *N. dubius* fixed for the L-morph, there is a clear discontinuity in style length between individuals of the L- and S-morph. The data presented in Fig. 3-2 were not adjusted to account for flower size and therefore some of the variation undoubtedly results from developmental or environmental influences. However, despite these sources of variability, a fundamental dimorphism in stigma height is evident. A two-way ANOVA on the data obtained from all populations with style morph and population as main effects indicated that mean stigma height (mm) was significantly different between the style morphs in both species (*N. assoanus*: L-morph=17.43, s.e.=0.082; S-morph= 8.94, s.e.=0.079; $F_{1,692}=3956.66$, $P< 0.001$; *N. dubius*: L-morph=15.61, s.e.=0.095; S-

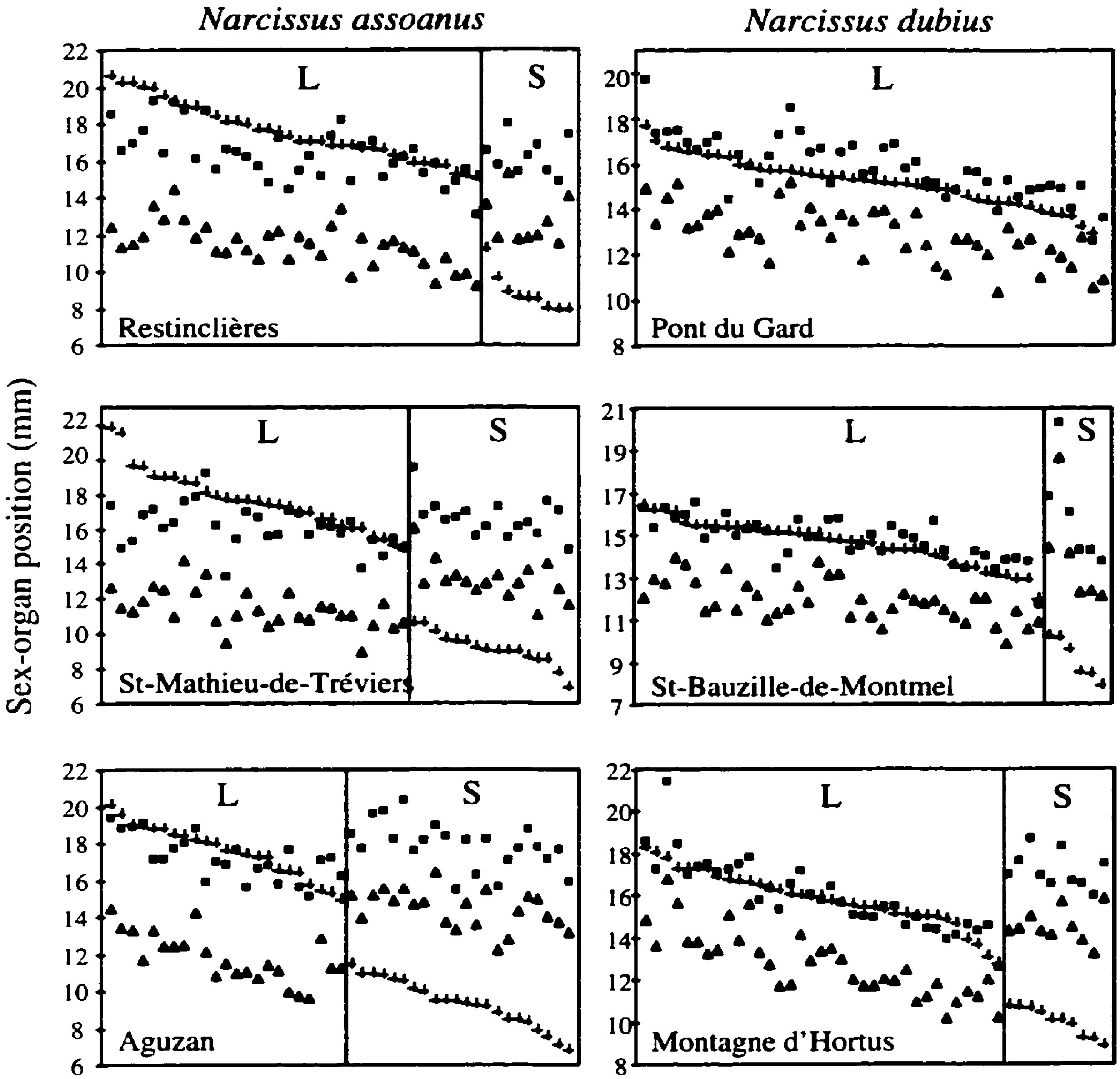


Figure 3-2: The range of variation in stigma height (+), height of upper-level stamens (□) and lower-level stamens (Δ) among long- (L) and short-styled (S) individuals from three populations of *Narcissus assoanus* and *N. dubius* from S. W. France. Individual flowers are ranked by stigma height. Sex-organ position was not adjusted to account for flower size.

morph=9.49, s.e.=0.215; $F_{1,314}=295.07$, $P<0.001$). When sex-organ position is adjusted to account for differences in flower size, the ANOVA results were not qualitatively different (Table 3-1). In all analyses of variation in sex-organ position, there were no significant differences in the results obtained between the adjusted or raw data. Consequently, all subsequent *F*-values I present are those using data that were adjusted to account for flower size.

Although mean stigma height was strongly differentiated between the style morphs in both species, the corresponding positions of the two stamen levels differed much less, although consistent differences were evident when data were examined across populations. In a two-way ANOVA with morph and population as main effects, mean positions of upper- and lower-level stamens (mm) were significantly lower in the floral tube of the L-morph compared to the S-morph in both species (Table 3-1). In both species, mean stamen position differs significantly among populations and the interaction between population and style morph was significant in *N. dubius* but not in *N. assoanus* (Table 3-2). The two stamen levels in the L-morph were more strongly differentiated from one another than the corresponding stamen levels of the S-morph, especially in *N. assoanus*. This can be seen by inspection of Fig. 3-1 which is drawn to scale using mean values obtained from *N. assoanus* populations.

In both species, flowers of the L-morph typically have stigmas positioned within or slightly above the upper-level stamens. In contrast, stigmas are located well below the lower-level stamens in the S-morph (Fig. 3-2). These contrasts in the relative position of stigmas and anthers result in striking differences in the degree of herkogamy exhibited by the style morphs (Fig. 3-3). Over the 15 sampled populations of *N. assoanus*, mean stigma-anther separation in the S-morph was 5.4 times greater than in the L-morph. The same pattern was also observed in *N. dubius*, where the degree of stigma-anther separation between the style morphs was even greater. Mean stigma-anther separation in *N. dubius* was 33 times greater in the S-morph than in the L-morph averaged over the seven dimorphic populations sampled. In *N. dubius* most flowers of the L-morph have their stigmas positioned within the upper-level stamens (Fig. 3-2).

Variation in pollen size and number

I found no evidence for morph-specific or stamen-level differences in pollen size in either of the species under study (*N. assoanus*: L-morph=21.86μm, s.e.=0.081; S-morph=21.70μm,

Table 3-1: The position of sexual organs in 15 and seven dimorphic populations, respectively, of *Narcissus assoanus* and *N. dubius*. Mean values, in mm, are given for stigma height, upper-stamen height and lower-stamen height. Sample sizes were 450 L- and 272 S-styled flowers for *N. assoanus* and 275 L- and 53 S-styled flowers for *N. dubius*. Sex-organ position was adjusted to account for differences in flower size and significant differences were assessed using ANOVA (see text for details). There are statistically significant differences between the style morphs in stigma height, upper stamen height, and lower stamen height in both species.

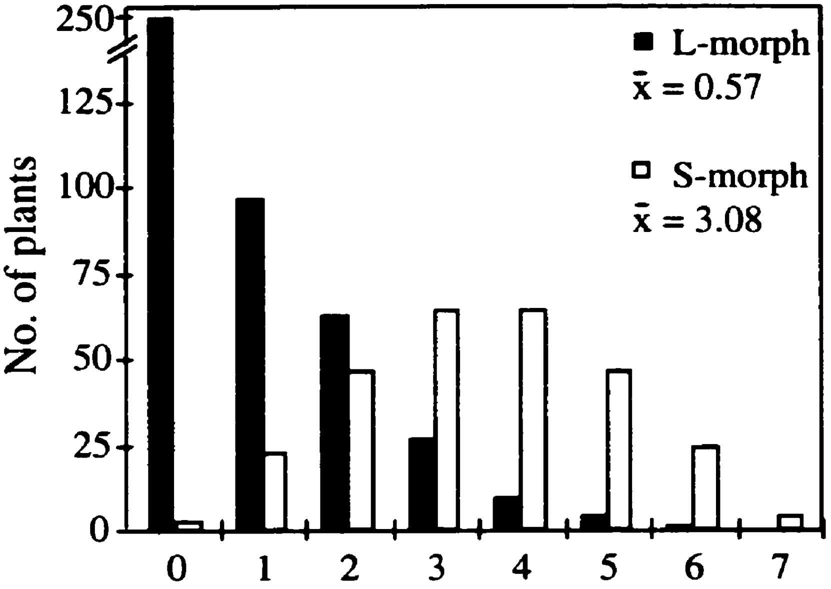
	Narcissus assoanus			*Narcissus dubius*		
	Long		Short	Long		Short
Stigma height	17.43	***	8.94	15.62	***	9.38
Standard error	0.080		0.071	0.081		0.180
Range	11.29 - 22.20		6.54 - 12.50	12.04 - 20.01		3.11 - 12.38
Upper stamen height	16.34	***	16.85	15.42	*	15.79
Standard error	0.053		0.061	0.058		0.171
Range	12.34 - 20.40		13.38 - 19.63	13.15 - 17.96		13.10 - 18.19
Lower stamen height	11.62	***	13.34	12.20	**	13.28
Standard error	0.053		0.067	0.064		0.184
Range	7.51 -15.72		9.61 - 15.76	9.25 - 15.46		10.67 - 16.44

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3-2: Two-way ANOVA of stigma height, and position of upper and lower stamens in 15 *Narcissus assoanus* and seven *N. dubius* populations. Sex organ position was adjusted to account for differences in flower length (see text for details).

	Narcissus assoanus				*Narcissus dubius*			
Source	d.f.	MS	*F*	*P*	d.f.	MS	*F*	*P*
Stigma height								
Morph	1	9999.99	3627.22	0.0001	1	1290.07	411.50	0.0001
Population	14	14.93	5.30	0.0018	6	7.01	1.96	0.2161
Morph X population	14	2.85	1.48	0.1146	6	3.57	2.78	0.0119
Upper stamen height								
Morph	1	47.84	37.51	0.0001	1	15.09	7.08	0.0288
Population	14	5.57	4.31	0.0049	6	14.69	6.43	0.0197
Morph X population	14	1.29	1.25	0.2345	6	2.28	1.53	0.1673
Lower stamen height								
Morph	1	490.05	298.81	0.0001	1	60.44	19.34	0.0035
Population	14	6.09	3.63	0.0108	6	16.00	4.38	0.0478
Morph X population	14	1.68	1.51	0.1011	6	3.66	4.25	0.0004

(a) *Narcissus assoanus*



(b) *Narcissus dubius*

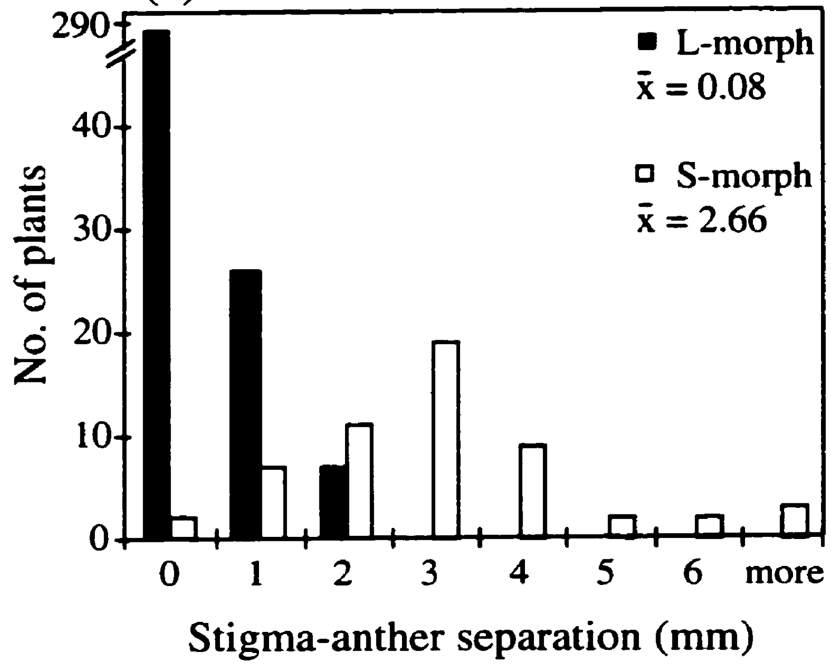


Figure 3-3. Stigma-anther separation (herkogamy) in dimorphic populations of *Narcissus assoanus* and *N. dubius*. Stigma-anther separation was calculated for 450 and 262 flowers of the L- and S-styled morphs, respectively, from 15 *N. assoanus* populations and 315 and 55 flowers of the L- and S-morphs, respectively, from eight *N. dubius* populations. Stigma-anther separation was calculated by determining the difference between stigma height and the nearest anther. Stigma-anther separation is 0 when the stigma is located within a stamen level.

s.e.=0.126; $F_{3,44}$=0.7225, P=0.5440; *N. dubius*: L-morph= 25.89µm, s.e.=0.100; S-morph=25.33µm, s.e=0.475; $F_{3,40}$=0.5230, P=0.6690). Also, there were no significant differences between style morphs or stamen levels in the number of pollen grains produced in either species (*N. assoanus*: L-morph= 64 912 pollen grains per flower, s.e.=2260; S-morph=64 875, s.e.=3743; $F_{3,44}$=0.3926, P=0.7589; *N. dubius*: L-morph=59 113, s.e.=2733; S-morph=50 355, s.e.=3609; $F_{3,40}$=1.3810, P=0.2625).

Style-morph ratios in natural populations

All 46 populations of *N. assoanus* sampled were dimorphic for style length. Style-morph ratios varied from 1L: 1S (N=25 populations) to L-biased (N=21 populations). There was no relation between style-morph ratios (frequency of S-morph) and the degree of S-level organ reciprocity (mean difference in height of lower anthers of L-morph and stigmas of S-morph) among the 15 populations for which detailed floral measurements were undertaken (r=0.20, P=0.476). No populations with a statistically significant excess of the S-morph were observed. A significant excess of the L-morph was evident when the morph-ratio data were pooled across all populations (L-morph=0.62, S-morph=0.38; G_{pooled}=204.56, d.f.=1, P<0.001) and there was significant heterogeneity among population morph ratios (G_{het}=44 603.18, d.f.=45, P<0.001). Of the 21 populations of *N. dubius* sampled for morph ratios, 15 were dimorphic and six were monomorphic, containing only the L-morph. Dimorphic populations were strongly L-biased and, as in *N. assoanus*, there was also a significant excess of the L-morph when data were pooled across populations (L-morph=0.93, S-morph=0.07; G_{pooled}=1208.23, d.f.=1, P<0.001). Significant heterogeneity among population morph ratios was also evident (G_{het}=52.45, d.f.=20, P<0.001).

In *N. assoanus*, the observed morph-ratio variation among populations was non-randomly distributed over the geographical region sampled (Fig. 3-4). On the other hand, in *N. dubius*, with the exception of a small concentration of four monomorphic populations located along the Gardon river valley north of Nîmes, there was no obvious geographical pattern to morph-ratio variation (Fig. 3-5). Populations of *N. assoanus* closest to the coast occurring in the garrigue landscape surrounding Montpellier were smaller and more isolated from one another. All exhibited strongly L-biased morph ratios. In contrast, populations further inland and away from Montpellier were more likely to have isoplethic morph ratios. This geographical pattern was

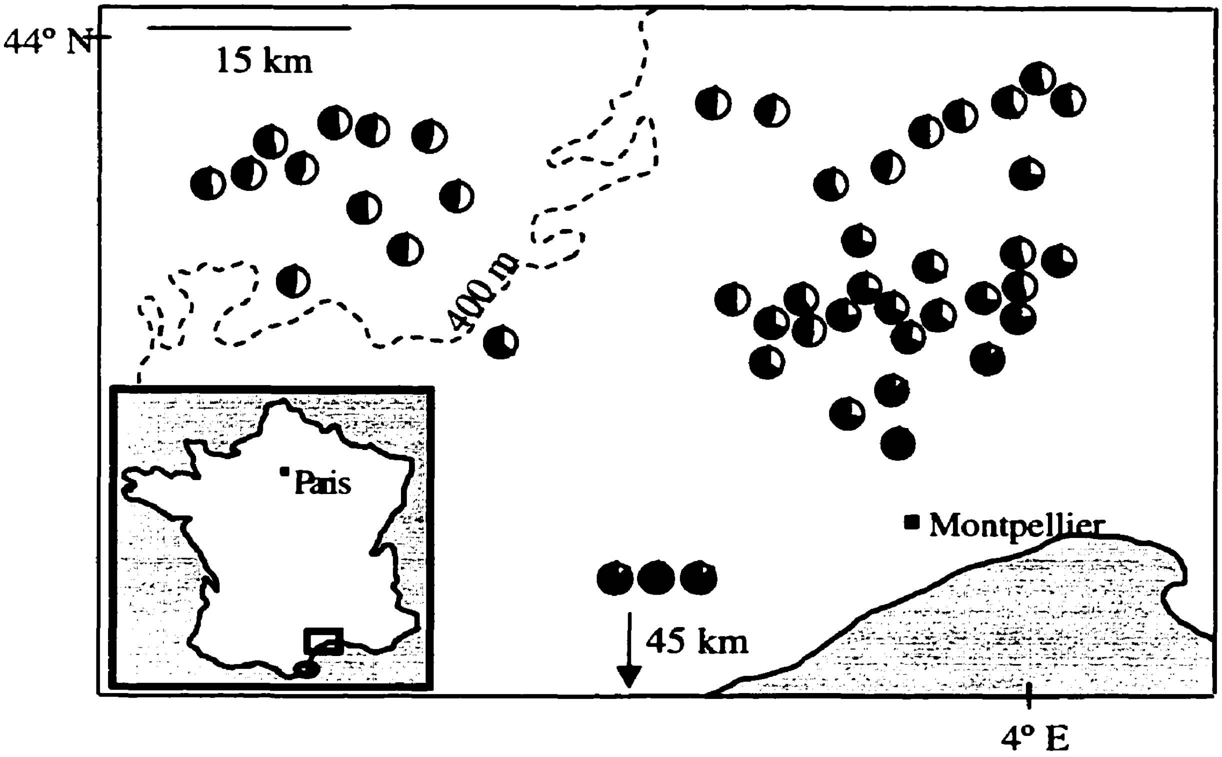


Figure 3-4. Geographical distribution of style-morph ratios in 46 populations of *Narcissus assoanus* in S. W. France. The frequency of the L- (black) and S-morphs (white) are indicated for each population. Smaller populations occurring in areas disturbed by urbanization and agriculture tended to have L-biased morph ratios, whereas larger populations further from Montpellier were always isoplethic. The dashed line represents the 400 m contour line.

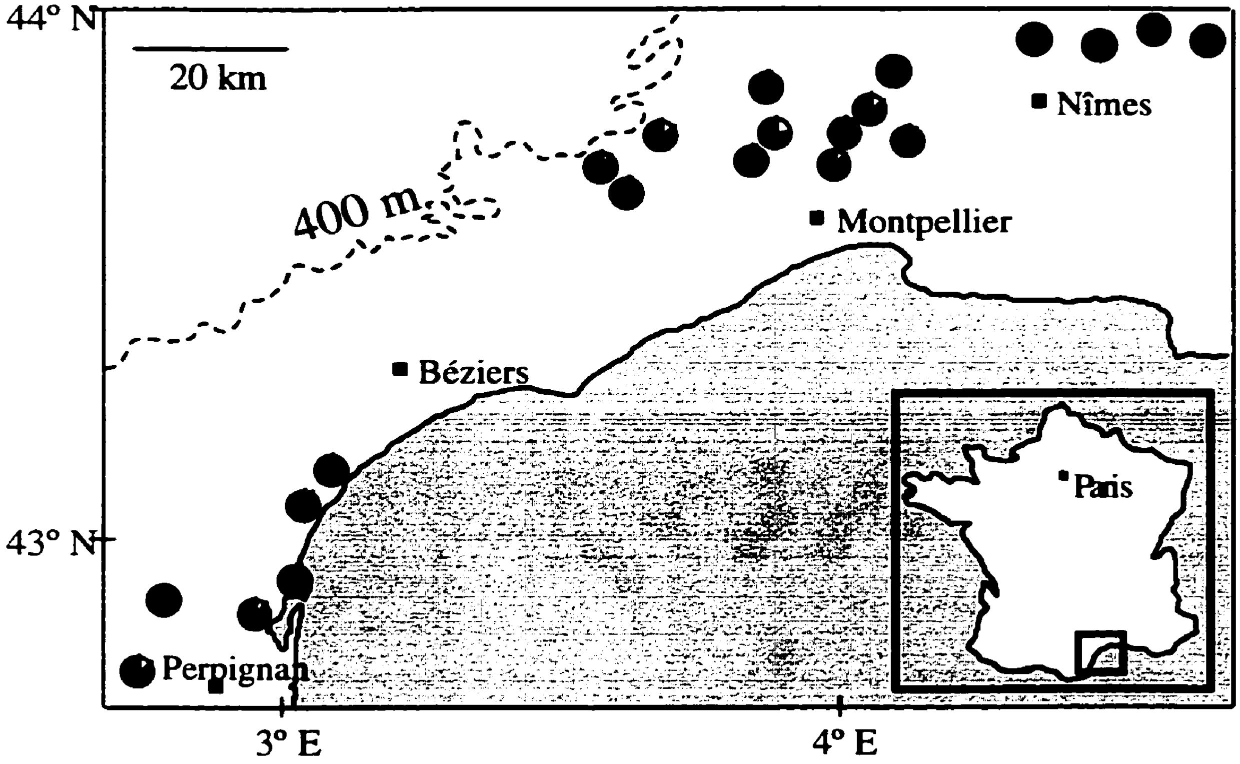


Figure 3-5. Geographical distribution of style-morph ratios in 21 populations of *Narcissus dubius* in S. W. France. The frequency of the L- (black) and S-morphs (white) are indicated for each population. The dashed line represents the 400m contour line.

associated with habitat fragmentation and population size. Inland populations tended to be much larger than those closer to Montpellier where suitable habitats for the species are more restricted in area. In particular, populations in the N. W. portion of the region sampled on the "Le Causse de Blandas" and "Le Causse du Larzac" limestone plateaus were very large in size and always exhibited isoplethic morph ratios. Figure 3-6a illustrates the relation between population size and morph ratio in *N. assoanus*. Whereas smaller populations display variable morph ratios, very large populations were always isoplethic. In *N. dubius* there was no association between population size and morph ratio (Fig. 3-6b). However, populations of this species never attain the size of the larger *N. assoanus* populations.

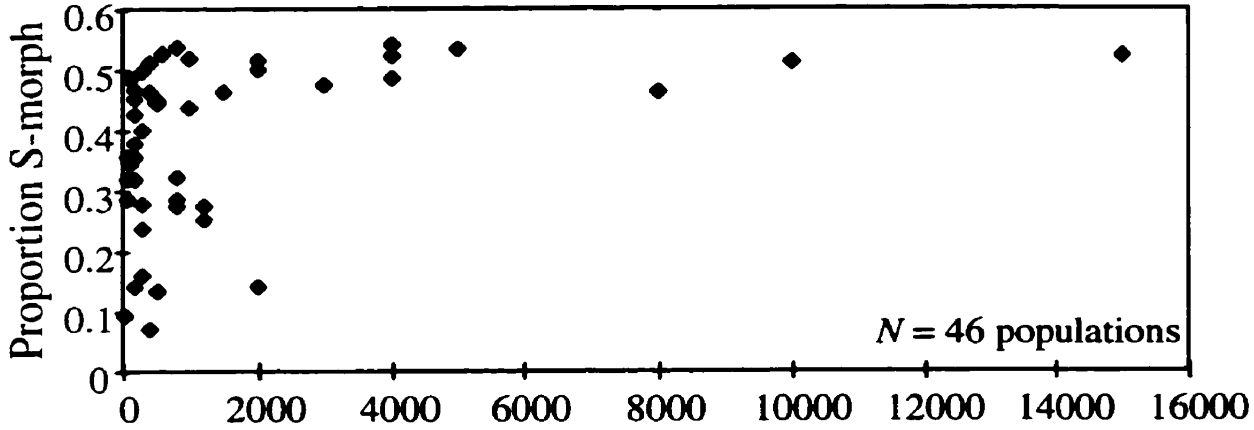
Discussion

The major findings of this study are that populations of *N. assoanus* and *N. dubius* possess a sexual polymorphism involving discrete variation in style length. Minor differences in the positions of the two stamen levels within a flower also occur. Population surveys revealed different patterns of variation in style-morph ratios in the two species, although L-biased morph ratios commonly occur in both. Here, I begin by reviewing what is known about stigma-height dimorphism in plants and address the question of whether the two *Narcissus* species should be considered heterostylous. I then discuss functional aspects of the dimorphism and consider the selective mechanisms that may account for the contrasting patterns of morph-ratio variation revealed in the surveys.

Stigma-height dimorphism and its relationship to heterostyly

Reports of stigma-height dimorphism are infrequent in other genera of flowering plants. They include *Anchusa* and *Lithodora* spp. in the Boraginaceae, *Chlorogalum angustifolium* in the Liliaceae, *Epacris impressa* in the Epacridaceae, *Kalmiopsis leachiana* in the Ericaceae, and *Anigozanthus humilis* in the Haemodoraceae (reviewed in Ch. 2). In common with dimorphic *Narcissus* spp., populations of these taxa are generally characterized by a bimodal distribution of style length but little or no differentiation in stamen position in the two style morphs. Several genera with stigma-height dimorphism occur in families in which heterostyly is common (e.g. Boraginaceae) raising the question of whether the polymorphism represents a transitional stage in the evolution of distyly (and see Lloyd & Webb 1992a,b). However, in other cases (e.g. Liliaceae and Ericaceae), heterostyly is absent from the families and the evolution of stigma-height

(a) *Narcissus assoanus*



(b) *Narcissus dubius*

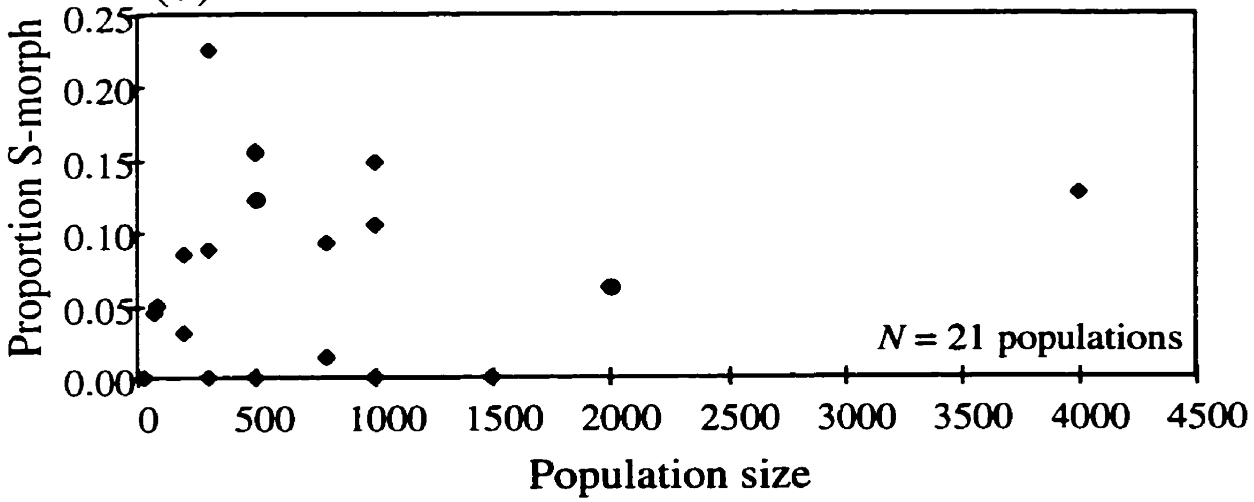


Figure 3-6. The relation between the proportion of short-styled plants in a population and its size in (a) *Narcissus assoanus*, and (b) *N. dubius*.

dimorphism appears to involve isolated events of unknown adaptive significance.

Heterostyly has been defined as "a genetically determined polymorphism in which the morphs differ in the sequence of heights at which the anthers and stigmas are presented within their flowers" (Lloyd & Webb 1992a, p. 152). The morphological features of *N. assoanus* and *N. dubius* flowers match this description well. However, Lloyd & Webb (1992a) elaborated this definition further to emphasize that the position of stigmas and anthers in the floral morphs must differ in a reciprocal manner. Indeed this reciprocal herkogamy (Richards 1986, p. 262) was considered by Lloyd & Webb (1992a) to be a defining feature of heterostyly and on this basis they distinguished distyly from stigma-height dimorphism using *Narcissus* as an example of the latter (also see Ganders 1979a, p. 608). I agree with this perspective which differs from the view held by Dulberger (1992) and Richards (1997 and pers. comm.) who advocate a broader view of heterostyly which includes species without reciprocal herkogamy. This more restricted definition of heterostyly excludes species that lack a clear reciprocal correspondence between stigmas and anthers. Additionally, although pollen size and production commonly differ between the style morphs in heterostylous species, there is no evidence of morph-specific differences in pollen characters of *N. assoanus* or *N. dubius*. Based on these findings, I prefer to classify *N. assoanus* and *N. dubius* as non-heterostylous. As discussed further in Ch. 4, other features of the reproductive systems of *N. assoanus* and *N. dubius* are atypical for heterostylous plants. These distinguishing features merit the recognition of stigma-height dimorphism as a distinct yet functionally similar plant sexual polymorphism to heterostyly (and see Ch. 2).

Functional consequences of sex-organ deployment

Casual observations of the two style morphs in *N. assoanus* and *N. dubius* had indicated that stamen levels were located at similar positions within the floral tube. However, detailed measurements revealed subtle differences between the style morphs in both species. Are these differences of functional significance for pollination and mating? This seems unlikely in the case of the upper-level stamens, where there is less than 0.5 mm difference between the morphs in their position in both species. In fact, within most populations there was no significant difference between the height of the upper-level stamens in the two morphs (nine of 15 populations for *N. assoanus*; four of seven for *N. dubius* following *t*-tests, results not shown). It is difficult to imagine that this small difference in mean height of upper-level stamens has significant morph-specific influences on pollen dispersal, given the large amount of variation among individual plants in their position (Fig. 3-2).

The difference between style morphs in the position of lower-level stamens was much greater than for upper-level stamens and hence may have functional significance. This difference was evident in both species but was more pronounced in *N. assoanus* [mean difference (mm): 1.7 in *N. assoanus* and 1.0 in *N. dubius*]. Lower-level stamens in the L-morph were always positioned significantly lower in the floral tube relative to the corresponding stamen level of the S-morph. Interestingly, this difference was a consistent feature of all populations sampled in both species, suggesting that the positioning may influence pollen dispersal. It is possible that the lower-level stamens are under disruptive selection in the two style morphs. Selection to reduce the height of these stamens in the L-morph may increase male fertility because of more proficient pollen dispersal to stigmas of the S-morph. In contrast, selection to increase the height of lower-level stamens in the S-morph may reduce self-pollination and self-interference and also increase the effectiveness of pollen dispersal to stigmas of the L-morph. Because upper-level stamens in both morphs probably transfer most of their pollen to stigmas of the L-morph, selection would be more likely to maintain them at a similar position corresponding to the height of stigmas in the L-morph.

The most striking morphological difference between the style morphs of both species involves the degree of herkogamy that they exhibit. Stigmas and anthers are in close proximity in flowers of the L-morph, whereas there is considerable spatial separation in the S-morph. There is substantial evidence that the degree of herkogamy has important functional consequences for mating patterns, especially selfing rates (see Belaoussoff & Shore 1995 and references therein). Plants with weakly developed herkogamy usually experience more self-pollination than those with well separated sex organs. Indeed, studies of self-pollen deposition in caged individuals of *N. assoanus* demonstrated that flowers of the L-morph often have upwards of 200 self pollen grains deposited on stigmas through autonomous self-pollination whereas flowers of the S-morph always have fewer than 15 (A.M. Baker unpubl. data). Similar results were also obtained in *N. tazetta* by Arroyo & Dafni (1995), a species that exhibits the same morph-specific differences in herkogamy.

What are the likely functional consequences of different rates of autonomous self-pollination in the style morphs? As yet there is no clear answer to this question. Experimental studies have demonstrated that *N. assoanus* is moderately self-sterile whereas *N. dubius* is highly self-compatible. These differences between the two species are reflected in contrasting selfing rates in natural populations (Ch. 4). Yet comparative data on the mating systems and female fertility of the style morphs of both species failed to detect significant morph-specific influences on maternal selfing rates or seed-set (Ch. 4). This suggests that although the quantity and composition (self vs. outcrossed) of pollen deposited on stigmas of the style morphs are likely to be different, postpollination mechanisms may act to filter heterogeneous pollen loads resulting in the similar observed maternal fitness components.

Factors influencing morph-ratio variation

The surveys of *N. assoanus* and *N. dubius* in S. W. France revealed a wide range of style-morph ratios ranging from isoplethy to populations fixed for the L-morph. This finding is consistent with the results of earlier studies of *N. tazetta* (Arroyo & Dafni 1995) in Israel and *N. papyraceus* in S. Spain (Barrett *et al.* 1996). In these surveys, similar patterns of variation were reported with many populations exhibiting biased morph ratios and monomorphic populations fixed only for the L-morph. A survey of nine populations of five dimorphic *Narcissus* species, including two populations of *N. assoanus* from S. Spain, also revealed L-biased morph ratios in all populations (Barrett *et al.* 1996). Collectively, these results raise the question of why *Narcissus* populations often exhibit L-biased morph ratios?

Barrett *et al.* (1996) and I (Ch. 4) developed pollination and mating models, respectively, that can explain biased style-morph ratios in *Narcissus* populations with a stigma-height dimorphism. The models indicate that L-biased morph ratios will occur if there are higher levels of assortative mating in this morph compared to the S-morph. Morph-specific differences in sex-organ deployment in *N. assoanus* and *N. dubius* suggest that this is likely to occur. Although upper-level anthers of the L-morph are well positioned to transfer pollen to stigmas of other plants of this morph, the high degree of herkogamy in the S-morph reduces the likelihood of proficient assortative pollen transfer. Most pollen transferred to S-stigmas probably originates from the lower-level anthers of the L-morph which are positioned below the corresponding anther level in the S-morph. Morph-specific differences in the ratio of assortative to disassortative mating resulting from asymmetric pollen transfer within and between the style-morphs can explain the L-biased morph ratios observed in *Narcissus* spp. (Ch. 4).

Why are monomorphic populations of *Narcissus* spp. almost always fixed for the L-morph? If offspring from plants of the S-morph are largely the products of disassortative mating, they will mostly be heterozygous (*Ss*) at the locus controlling style length, assuming that inheritance is of the single-locus control demonstrated in *N. tazetta* (Dulberger 1964) and most heterostylous species (Ganders 1979a). Heterozygosity at the *S*-locus is also promoted in populations with L-biased morph ratios. This is because plants of the S-morph are more likely to mate with individuals of the L-morph due to the high numerical abundance of the latter. Hence, homozygous short-styled plants (*SS*) should occur rather infrequently in most populations of *Narcissus* spp. because of low levels of assortative mating in this morph and L-biased morph ratios. Interestingly, studies on the inheritance of style-length in *N. tazetta* (Dulberger 1964) found no homozygous short-styled plants despite the absence of intrinsic barriers to intramorph mating. A similar finding was reported by Schou & Philipp (1984) in their genetic studies of *Anchusa officinalis*, a species that exhibits stigma-height dimorphism, intramorph compatibility and strongly L-biased morph ratios.

The apparent rarity of homozygous plants of the S-morph restricts opportunities for S-monomorphy. This is because heterozygous plants of the S-morph that found new populations will always segregate both morphs thus guaranteeing establishment of stylar dimorphism. In contrast, L-morph (*ss*) founders will only produce long-styled offspring giving rise to populations monomorphic for this morph. Thus, according to this hypothesis, the contrasting occurrence of L- versus S-morph monomorphism arises because of morph-specific differences in mating and the dominance relationships at the *S*-locus governing stylar dimorphism.

However, two alternative hypotheses could also explain the absence of S-monomorphy in *Narcissus* spp. Strong selection against homozygous (*SS*) plants because of inbreeding depression could prevent the establishment of monomorphic populations composed of the S-morph. In this regard, Richards (1997, p. 284) has argued that S-morph-linked recessive lethality is "a pervasive feature of heterostylous plants" (and see Richards 1998), but unfortunately the experimental evidence for this phenomenon is only mixed at best (Shore & Barrett 1985; Eckert & Barrett 1993). Nevertheless it would be worth investigating this possibility through controlled crosses of short-styled plants. Finally, it is possible that plants of the S-morph are poor at establishing populations because their concealed stigmas are only accessible to long-tongued pollinators which may be less inclined to visit isolated plants. According to this hypothesis, fertility selection favours the L-morph as a founding morph because its sex organs are more accessible to short-tongued generalist pollinators that might be more likely to visit small, newly established populations. I have not been able to detect morph-specific differences in female fertility in established populations of *N. assoanus* or *N. dubius*, but it would certainly be worth comparing patterns of seed-set in experimental monomorphic populations composed of the L- versus the S-morph to test this hypothesis.

Populations with equal frequencies of the two style morphs were commonly observed in *N. assoanus*, indicating that stigma-height dimorphism can result in equivalent levels of disassortative mating. This finding is significant because it is an assumption for Lloyd and Webb's (1992a,b) model for the evolution of distyly from stigma-height dimorphism. The floral morphologies of isoplethic populations were similar to those exhibiting biased morph ratios and there was no evidence of a greater reciprocal correspondence in anther and stigma positions that might favour symmetrical disassortative mating. Isoplethic populations tended to occur in the less disturbed landscapes and particularly on the upland limestone plateaus north-west of Montpellier where populations were all very large. It is possible that the frequency and composition of pollinators visiting isoplethic vs. nonisoplethic populations differ in ways that influence pollen transfer and mating patterns. Observations of pollinator visits to *N. assoanus* indicate that the primary visitors are butterflies (*Gonepteryx cleopatra*), solitary bees (*Anthophora* spp.) and day-flying hawkmoths (*Macroglossum stellatarum*). As yet I have not been able to determine whether visitation patterns differ between populations with contrasting style-morph ratios. Elsewhere it has been argued that differences in the types of pollinators visiting populations of *N. tazetta* (Arroyo & Dafni 1995), *N. papyraceus* and *N. triandrus* (Barrett *et al.* 1996) may explain the striking patterns of morph-ratio variation that these species also exhibit. To distinguish how different pollinators might influence pollination and mating, measurements of pollen transfer and male fertility are required.

Isoplethic style-morph ratios were not observed in *N. dubius* and the S-morph was generally either rare or absent from populations. Strongly biased morph ratios in this species probably reflect the mating consequences of weak disassortative mating compared with *N. assoanus*. In common with many plant species that flower in very early spring (Schemske *et al.* 1978), *N. dubius* is highly self-compatible. Not unexpectedly, populations of this species exhibit higher selfing rates than the self-sterile *N. assoanus* (Ch. 4). Higher selfing in *N. dubius* is also probably influenced by opportunities for geitonogamy because of the multiflowered inflorescences of this species. It is possible that weak disassortative mating may be destabilizing the stigma-height dimorphism in *N. dubius* resulting in the gradual elimination of the S-morph from many populations. Selection against the S-morph may also be occurring in hill populations of *N. tazetta* (Arroyo & Dafni 1995) and *N. papyraceus* (Barrett *et al.* 1996), two other members of section Tazettae.

In conclusion, stigma-height dimorphism represents a curious and perplexing floral design. It seems probable that the polymorphism functions to promote more proficient pollen transfer among plants in a manner similar to heterostyly. However, how disassortative pollen transfer is achieved, at least in some populations, without well-developed sex-organ reciprocity is unclear. The wide range of style-morph ratios that characterize populations of *Narcissus* spp. suggests that the patterns of pollen transfer within and between style morphs vary considerably. Detailed studies of the pollination biology of populations with contrasting style-morph ratios should provide insights into how this occurs.

CHAPTER 4

EVOLUTION AND MAINTENANCE OF STIGMA-HEIGHT DIMORPHISM IN *NARCISSUS*: FITNESS COMPARISONS BETWEEN STYLE MORPHS

Summary

Populations of the insect-pollinated geophytes *Narcissus assoanus* and *N. dubius* (Amaryllidaceae) are commonly dimorphic for stigma height. An extensive survey of populations of the two species in S. W. France revealed a wide range of style-morph frequencies, particularly populations with significantly more long-styled than short-styled plants (Ch. 3). Here I employ experimental and theoretical approaches to investigate potential selective mechanisms governing the variation in style-morph frequencies. Controlled pollinations of both species demonstrated that *N. assoanus* is moderately self-sterile whereas *N. dubius* is self-compatible. Both intra- and inter-morph crosses of *N. assoanus* were equally fertile, indicating that the species does not exhibit heteromorphic incompatibility. Estimates of female fertility (fruit- and seed-set) and multilocus estimates of selfing using allozyme markers provided no evidence of morph-specific differences in maternal components of reproductive success in natural populations of the two species. This result suggested that differences between the morphs in male fertility might be largely responsible for the observed morph-ratio variation. To investigate this hypothesis, I developed a mating model that incorporates the genetics of stigma-height dimorphism and contrasting rates of assortative and disassortative mating in the style morphs. Simulation results demonstrated that stigma-height dimorphism will always be maintained when levels of disassortative mating are greater than assortative mating, and that the observed L-biased populations in *Narcissus* spp. probably result from greater levels of assortative mating in this morph in comparison with the S-morph.

Introduction

In the previous chapter, I described an unusual plant sexual polymorphism in which populations of *Narcissus* spp. (Amaryllidaceae), a genus of insect-pollinated geophytes native to the Mediterranean Basin, are dimorphic for style length (Ch. 3). Most populations of *N. assoanus* and *N. dubius* in S. W. France consist of two floral morphs, one in which the stigma is at the same level as the stamens or protrudes beyond them (long-styled or L-morph) and the other in which the stigma is located well below the stamens (short-styled or S-morph). The polymorphism occurs in at least a dozen *Narcissus* species distributed across three sections of the genus (Barrett *et al.* 1996). Stigma-height dimorphism is distinct from the well-known heterstylous condition distyly (Darwin 1877; Barrett *et al.* 2000a,b), because stamen levels in the two floral morphs are positioned similarly, rather than showing a reciprocal correspondence with the two stigma heights. In addition, unlike most heterostylous species, the two floral morphs in *Narcissus* spp. do not differ in pollen size or pollen production.

Although *N. assoanus* and *N. dubius* show similar patterns of sex-organ deployment within flowers, population surveys in S. W. France revealed that the two species differ in the frequencies of style morphs that occur within populations (Ch. 3). In *N. assoanus*, style-morph ratios ranged from isoplethy (1L: 1S) to L-biased depending on population size. Large populations were always isoplethic, whereas smaller populations exhibited variable style-morph frequencies, particularly L-biased ratios. In contrast, populations of *N. dubius* were either strongly L-biased or monomorphic for the L-morph. Of the 67 populations of the two species that were surveyed, none was S-biased or contained only this morph. Similar patterns of style-morph frequency variation have also been reported in other dimorphic species of *Narcissus* with L-biased populations and L-monomorphy most commonly occurring (Arroyo & Dafni 1995; Barrett *et al.* 1996).

Here I consider the maintenance of stigma-height dimorphism in *Narcissus* and investigate likely selective mechanisms that might account for the unusual patterns of morph-frequency variation observed in *N. assoanus* and *N. dubius*. I begin by determining the compatibility status of the two species through controlled pollinations and then examine the possibility that morph-specific differences in maternal fitness of the style morphs could potentially lead to biased morph ratios. I compare estimates of the female fertility of style-morphs in natural populations by examining patterns of fruit and seed set. Style-morph ratios can also be influenced by morph-specific differences in mating patterns, and an extensive literature on this topic is available for heterostylous plants (e.g. Crosby 1949; Bodmer 1960; Ganders 1975; Charlesworth & Charlesworth 1979; Barrett *et al*, 1987, 1989). I measure outcrossing rates in natural populations to determine whether morph-specific differences in floral morphology within each species might be associated with different patterns of mating. Finally, I develop a mating model to investigate how variation in levels of assortative and disassortative mating between the style morphs might influence their frequency within populations. In particular, I was interested in exploring the possibility that differences in assortative mating between style morphs caused by their differing sex-organ positions could explain the L-biased morph ratios that are typical of many *Narcissus* populations.

Materials and methods

All experimental studies described below were conducted from 1996-8 on populations of *N. assoanus* and *N. dubius* occurring within 50 km of Montpellier, S.W. France. See Figs. 3-4 and 3-5 in Chapter three for the general location of populations.

Compatibility status

I transplanted plants of the L- and S-morph of each *Narcissus* species to a pollinator-free glasshouse at CNRS, Montpellier for hand-pollinations to determine their compatibility status. It was particularly important to determine whether the species were self-sterile, and, if so, whether stylar dimorphism was associated with an intramorph incompatibility system typical of distylous plants. In *N. assoanus*, I conducted crosses over two years using three populations (St-Mathieu-de-Tréviers in 1996, and Bancel and Ceyrac in 1997). In *N. dubius*, it was difficult to find a substantial number of plants of the S-morph even in large dimorphic populations. Thus, in *N. dubius*, I performed the crossing programme in only one population in 1998.

In both species, the treatments were: (1) intramorph pollination, (2) intermorph pollination, (3) self-pollination, and (4) no hand-pollination to detect the ability for autonomous selfing. All cross-pollinations involved a single, randomly chosen pollen donor. In both style morphs, I removed anthers from flowers prior to dehiscence and examined stigmas using a 16X hand lens to ensure that no self pollen was present before hand-pollination. It was necessary to slit open part of the floral tube to pollinate the S-morph, so flowers of both morphs were opened in the same manner. I collected mature fruits six to eight weeks after pollination, counted the number of plump, filled seeds and flake-like ovules, and estimated total ovule number of flowers.

I analysed seed-set data from experimental pollinations by analysis of covariance (ANCOVA) in JMP (SAS 1994) using total ovule number as a covariate. Only capsules that produced seed were included in the analysis. In *N. assoanus*, I examined the effects of style morph, pollination type (self, intermorph or intramorph), population, and their interactions. Three-way interaction terms were backwards eliminated from the analyses if they did not have a significant effect on the mean number of seeds set (i.e. $P>0.05$; cf. Sokal & Rohlf 1995). I performed contrasts to compare self vs. outcrossed and intramorph vs. intermorph seed-set; and logistic regression to examine the effects of the above variables on mean fruit-set. I employed a similar approach in analysing seed- and fruit-set data in *N. dubius*. I used ANCOVA to examine the effects of style morph, pollination type and their interaction on mean seed-set. I performed contrasts as above and logistic regression to analyse mean proportion of fruit set.

Female fertility in natural populations

I measured seed-set of tagged L- and S-styled plants in six dimorphic *N. assoanus* populations in 1996 [Bancel (Ban), Col Hortus (Col), Hortus Mountain (Hmt), Pont sur Brestalou (Psb), Rigalderie (Rig) and St-Bauzille-de-Montmel (StB)], and both seed- and fruit-set in four populations in 1997 [Col, Psb, StB and Aguzan (Agu)]. Seed-set was measured in *N. dubius* for 1996 and 1998. In 1996, I sampled three populations exhibiting stigma-height dimorphism [La Clause (Clau), Hortus Mountain (Hmt), and St-Bauzille-de-Montmel (StB)] and two populations monomorphic for the L-morph [La Baune (Bau) and Pont du Gard (PdG)]. In 1998, I measured both fruit- and seed-set in a subset of these populations: Clau, StB and PdG.

In *N. assoanus*, I analysed seed-set data for both years using a mixed-model ANCOVA with total ovule number as the covariate. Random effects included year sampled and population nested within year. Style morph was treated as a fixed effect. Interaction terms in the model included morph-by-year and morph-by-population (nested within year). I analysed fruit-set data for 1997 using logistic regression. The two-way model examined the effects of morph, population, and their interaction on fruit-set. In *N. dubius*, I analysed seed-set over the two years using a nested design. Effects included sampling year, population nested within year, and morph nested within population and year. Total ovule number was used as a covariate. I analysed the

1998 fruit-set data using logistic regression and examined the effects of population and morph nested within population on proportion of fruit set.

Mating patterns

I performed horizontal starch gel electrophoresis on open-pollinated seeds from six populations of *N. assoanus* (Ban, Col, Hmt, Psb, Rig, StB) and three populations of *N. dubius* (Clau, Hmt, StB), following the methods of Shore & Barrett (1987) and Wendel & Weeden (1989). All populations were dimorphic for style length. Electrophoresis was also performed on seed families from two monomorphic *N. dubius* populations (Bau, PdG). I initially screened all populations for 18 enzyme systems: from these, six polymorphic enzyme systems (*Adh*, *Gdh*, *Idh*, *Got*, *Pgi*, *Tpi*) were reliably resolved in *N. assoanus* and two (*Acp*, *Pgm*) in *N. dubius*. I used Ritland's MLTR program (1990) to estimate morph-specific outcrossing rates. Outcrossing rates ($\hat{t}$) were based on approximately 20 seed families per morph (range=15 to 21) for a given population. Standard deviations of the estimates were based on 1000 bootstrap values. For each seed family, eight individual seeds were genotyped.

Ritland's MLTR program assumes that allele frequencies do not differ significantly between groups, implying that mating is random with respect to morph (i.e. no disassortative mating between morphs). However, this seems highly unlikely given the 1:1 morph ratios of populations which imply significant disassortative mating (Ch. 3). If this assumption is violated, there will be a downward bias in $\hat{t}$ when a common pollen pool is used to estimate the outcrossing rate rather than a separate pollen pool. Because trial runs indicated that there was a significant difference in allele frequencies between the morphs at some loci in all populations, outcrossing rates were estimated using separate pollen pools (and see Eckert & Barrett 1994).

Differences in outcrossing rate between the style morphs in dimorphic populations were assessed by pairwise comparisons of bootstrap estimates following the method of Eckert & Barrett (1994). With this approach, morphs were considered to differ significantly if at least $100(1-\alpha_{PC}/2)$ per cent of the differences between paired bootstrap values were either all greater than zero or all less than zero (note that α_{PC} represents the type-I error rate per contrast). Differences in $\hat{t}$ between populations were evaluated in a similar manner: 1000 average estimates of outcrossing were calculated for each population by calculating the average of the L- and S-morph $\hat{t}$ for a given bootstrap and two populations were considered to differ significantly if, as above, $100(1-\alpha_{PC}/2)$ per cent of the differences between paired bootstraps were either all greater than zero or all less than zero.

A genetic mating model

I developed a computer model to calculate equilibrium genotype and phenotype frequencies given varying levels of morph-specific assortative and disassortative mating. The explicit inclusion of assortative mating distinguishes this model from earlier mating models of heterostylous populations (e.g. Charlesworth 1979; Heuch 1979). Table 4-1 is a matrix of which the elements are the expected proportions of progeny genotypes from all matings for each maternal genotype. In my model, I assumed that the genetic control of stigma-height dimorphism is a single diallelic locus with the L-morph homozygous recessive (*ss*) and the S-morph *Ss* or *SS*, as reported in *Narcissus tazetta* (Dulberger 1964 and unpubl. data). In each morph, matings were either assortative (within-morph) or disassortative (between-morph). For example in Table 4-1, *aS* represents the proportion of matings between S-plants (assortative) and *dS* represents the proportion of matings in which a maternal S-plant is pollinated by pollen from a plant of the L-morph (disassortative). I do not explicitly incorporate the rate of self-fertilization in my model because selfing can be considered genetically equivalent to assortative mating (i.e. the genotypes of selfed progeny are the same as progeny resulting from assortative mating).

In the model, I assumed initial genotype frequencies of 1*ss* (long): 1*Ss* (short). Previous studies have not found short-styled *SS* individuals in natural populations of species with a stigma-height dimorphism (see discussion in Ch. 3). In addition, large, presumably equilibrium populations of *N. assoanus* have equal frequencies of the two style morphs so I was interested to examine how variable mating patterns might change morph frequencies from this equilibrium. Calculations were run in which mating varied from complete disassortative mating ($aS/dS=aL/dL=0$) to relatively high assortative mating ($aS/dS=aL/dL=2$) and genotype and phenotype frequencies were followed. Each set of calculations was run until an equilibrium in genotype frequency was reached (i.e. the per generation change in progeny genotype frequency was less than 0.0001). Results of the calculations were plotted in three-dimensions to illustrate the relationship between morph-specific mating in the L- and S-morphs and genotype frequency at equilibrium.

Table 4-1: Matrix of probabilities of genotypic proportions of progeny for each maternal genotype. All matings may be classified as either assortative (selfing and within-morph mating) or disassortative (between-morph mating). The terms aL and aS represent assortative matings whereas dL and dS are the proficiencies of matings that are disassortative in the L- and S-morphs, respectively. Within the matrix, G_1, G_2, and G_3 represent the frequencies of the L-morph, S-heterozygote and S-homozygote, respectively, in the population. The sum of each column is the frequency of each genotype in subsequent generations.

Genotype in next generation

$$\begin{array}{cc}
 & \begin{array}{ccc} \text{L-morph } (ss) & \text{S-morph } (Ss) & \text{S-morph } (SS) \end{array} \\
\text{Maternal genotype} \begin{array}{c} ss \\ Ss \\ SS \end{array} & \begin{bmatrix} aLG_1^2 + 0.5dLG_1G_2 & dLG_1G_3 + 0.5dLG_1G_2 & 0 \\ 0.5dSG_1G_2 + 0.25aSG_2^2 & 0.5aSG_2^2 + 0.5aSG_2G_3 + 0.5dSG_1G_2 & 0.5aSG_2G_3 + 0.25aSG_2^2 \\ 0 & dSG_1G_3 + 0.5aSG_2G_3 & aSG_3^2 + 0.5aSG_2G_3 \end{bmatrix}
\end{array}$$

Results

Compatibility relationships

Controlled self- and cross-pollinations indicate that *Narcissus assoanus* is moderately self-sterile whereas *N. dubius* is highly self-compatible (Fig. 4-1). The similar levels of seed-set obtained following intra- and intermorph crosses in *N. assoanus* demonstrate that this species does not possess a heteromorphic incompatibility system. In *N. assoanus*, self-pollination resulted in significantly fewer seed than intra- and intermorph cross-pollination in each of the three populations examined (grand mean seed set: self=8.32, SE=0.82, n=57; intramorph=21.36, SE=1.32, n=74; intermorph=18.18, SE=1.32, n=74). The results of paired contrasts indicated a significant difference in seed-set between self vs. outcross pollinations, but not between the seed-set of intramorph vs. intermorph crosses (Table 4-2). Fruit-set in *N. assoanus* showed a similar pattern to the results obtained for seed-set. Mean fruit-set differed significantly between pollination treatments (χ^2_2=16.89, P<0.001) with higher fruit-set in outcrossed pollinations (mean=77.9%, SE= 4.3, n=95 for both intra- and intermorph pollinations) compared with self-pollinations (mean=55.9%, SE=4.9, n=102). Plants of neither morph set seed autonomously (n=8 for each morph in the St-Mathieu-de-Tréviers population) and this treatment was not included in the ANCOVA. An interesting feature of the compatibility data in *N. assoanus* is the significant effect of style morph on the number of seeds produced (Fig. 4-1; Table 4-2). Long-styled plants had significantly lower seed-set than S-plants and this pattern was consistent across all populations examined. Fruit-set was also reduced in the L-morph (mean=57.9%, SE=4.0, n=152) when compared to the S-morph (mean=83.6%, SE=3.1, n=140; χ^2_1=17.51, P<0.001).

There were no significant differences in seed-set between self-, intramorph and intermorph pollinations of *N. dubius* (self=14.29, SE=1.48, n=38; intramorph=18.37 SE=1.74, n=38; intermorph=17.07, SE=1.38, n=41; Fig. 4-1, Table 4-3) and mean fruit-set was consistently high across all pollination types (self=84.4%, SE=5.5, n=45; intermorph=93.2%, SE=3.8, n=44; intramorph=88.4%; SE=4.9, n=43). There were no significant effects of pollination type, morph, or their interaction on fruit-set (χ^2_5=3.64, P=0.6022). Unlike *N. assoanus*, there was no significant difference in seed-set between the style morphs in response to the pollination treatments (Fig. 4-1, Table 4-2). In spite of the lack of self-sterility, plants of *N. dubius* do not set fruit autonomously (n=17 and 4, respectively, for L- and S-styled plants).

Table 4-2. ANCOVA of seed-set data from controlled pollinations in three populations of *Narcissus assoanus*. Nonsignificant two-way interactions are not shown. Total ovule number was used as a covariate (see text for details).

Source	d.f.	MS	*F*	*P*
Main effects				
Morph	1	2623.47	18.45	0.0365
Pollination type	2	2082.41	32.76	0.0008
Population	2	366.48	2.41	0.3114
Significant interactions				
Morph X pollination type	2	229.21	3.39	0.0359
Covariate				
Total ovule number	1	4254.52	62.96	0.0001
Contrasts				
Self vs. outcross	1	3898.8	8.76	0.0001
Intermorph vs. intramorph	1	143.25	1.68	0.1441

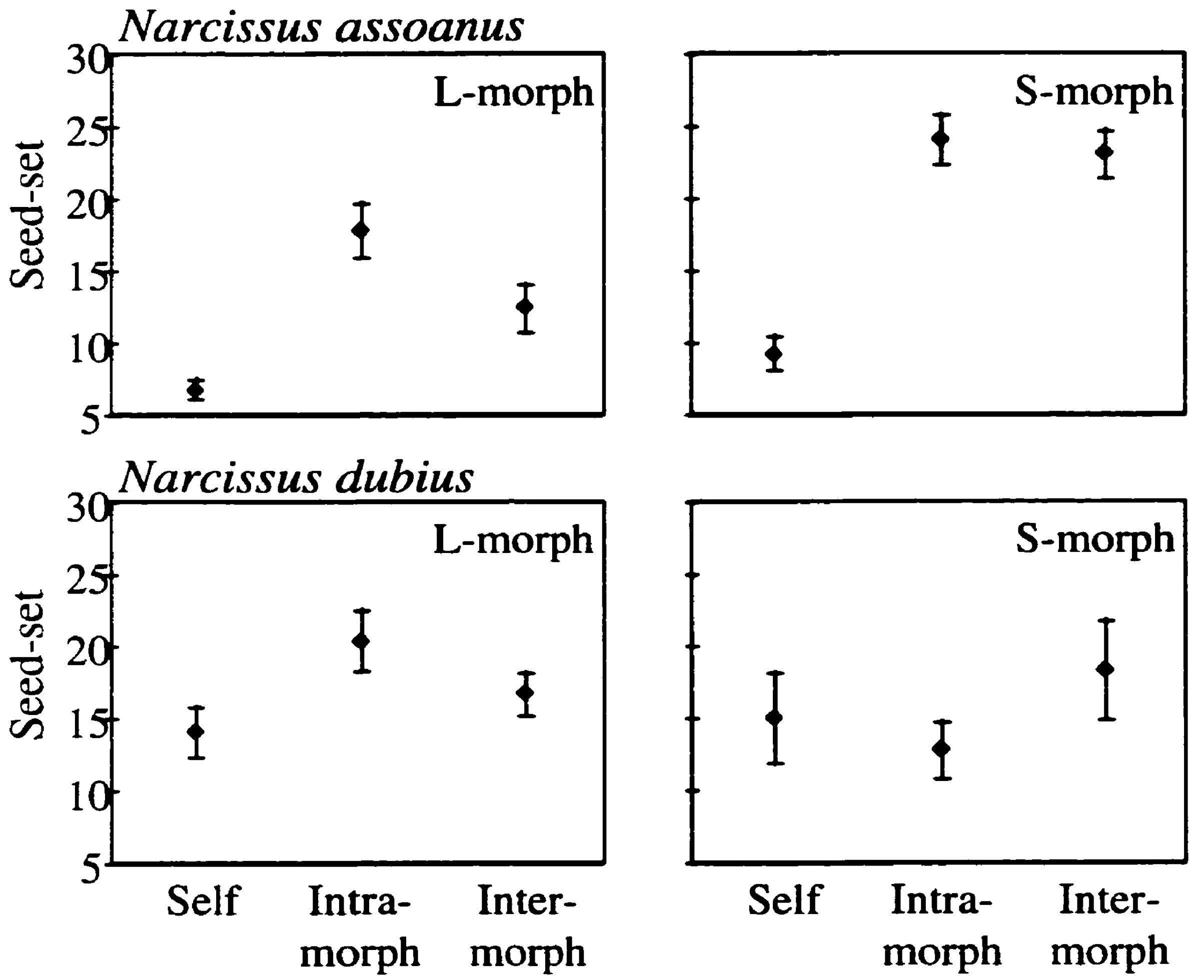


Figure 4-1. Mean seed-set of the L- and S-morphs in three populations of *Narcissus assoanus* and one population of *N. dubius* following intra-, intermorph, and self-pollinations in the glasshouse. *Narcissus assoanus* exhibits moderate self-sterility whereas *N. dubius* is self-compatible. There was no significant difference in seed-set between inter- and intramorph pollinations in either species.

Table 4-3. ANCOVA of seed-set data from controlled pollinations in a single population of *Narcissus dubius*. Total ovule number was used as a covariate (see text for details).

Source	d.f.	MS	*F*	*P*
Effects				
Morph	1	11.22	0.13	0.7208
Pollination type	2	227.09	1.30	0.2770
Morph X pollination type	2	429.96	2.46	0.0902
Covariate				
Total ovule number	1	446.47	5.11	0.0258
Contrasts				
Self vs. outcross	1	23.31	0.52	0.6066
Intermorph vs. intramorph	1	204.46	1.53	0.1290

Natural fertility

Comparisons of fruit- and seed-set of the L- and S-morphs in populations of *N. assoanus* and *N. dubius* failed to detect any general morph-specific effects. In only one of the 18 paired comparisons (*N. dubius*-StB-1996) was there a significant difference in the fertility of the style morphs (Fig. 4-2). In *N. assoanus*, mean seed set varied significantly between years (1996=23.38, SE=0.65, n=458; 1997=13.92, SE=1.17, n=184; $F_{1,555}$=34.62, P=0.0138), but style morph and population (nested within year) had no effect on mean seed set (P>0.13 in both cases). Fruit-set data were available only for populations sampled in 1997. Analysis of these data indicated no effect of style morph, population or their interaction (χ^2_7=9.09, P=0.2463). In *N. dubius* populations, style morph (nested within population and year) had a significant influence on seed-set ($F_{5,310}$=3.25, P=0.0071). This effect was largely caused by the results from population StB in 1996 (L-morph=27.78, SE=1.96, n=35; S-morph=16.52, SE=4.32, n=7; contrast t_5=3.93, P=0.0001). However, when I performed contrasts to compare seed-set between the style morphs in each dimorphic population, no other significant differences were evident (Clau in 1996 and 1998, Hmt in 1996, and StB in 1998; P>0.56 for all contrasts). Population nested within year also had a significant effect on mean seed-set ($F_{6,310}$=3.03, P=0.0067) although there was no significant difference in mean seed-set between years ($F_{1,310}$=0.03, P=0.8659). Fruit-set data were only available for populations sampled in 1998. There was no evidence for morph-specific differences in mean fruit-set (L-morph=87.02%, SE=3.00, n=75; S-morph=79.29, SE=6.71%, n=19) or of differences in fruit-set between populations (χ^2_4=6.25, P=0.1809).

Mating patterns of style morphs in natural populations

Comparisons of outcrossing rate in *N. assoanus* and *N. dubius* populations failed to detect significant differences between the L- and S-morphs (Fig. 4-3). In no case were all pairwise comparisons of morph-specific bootstrap values above or below zero. Populations of *N. assoanus* and *N. dubius* exhibited contrasting mating systems. *Narcissus assoanus* is highly outcrossing (mean $\hat{t}$: L-morph=0.997, SD=0.0038; S-morph=0.999, SD=0.0014), whereas *N. dubius* exhibits a mixed mating system (mean $\hat{t}$ in dimorphic populations: L-morph=0.579, SD=0.071; S-morph=0.653, SD=0.099). The larger variances in $\hat{t}$ values obtained for populations of *N. dubius*

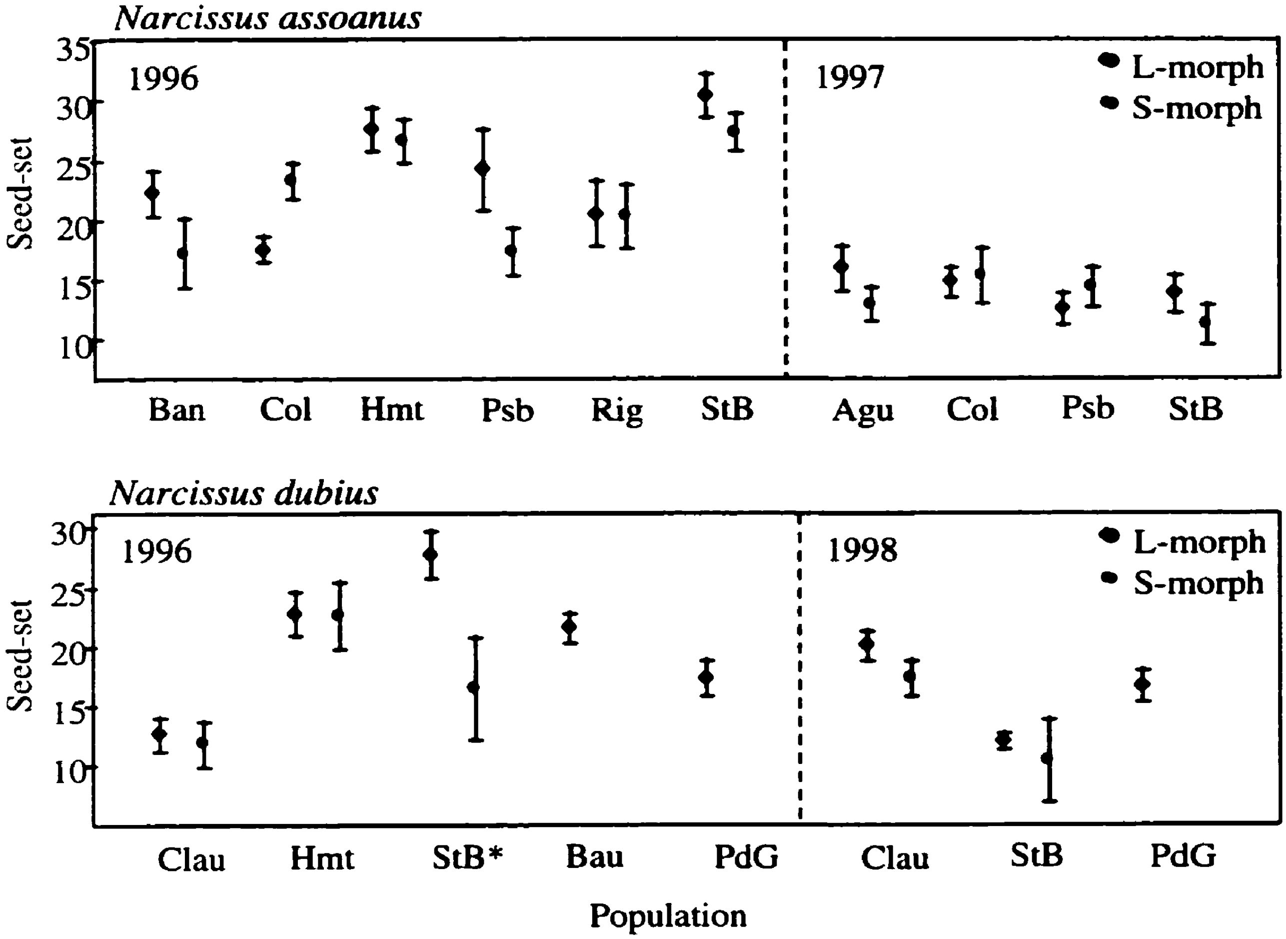


Figure 4-2. Natural seed-set of the L- and S-morph in dimorphic populations of *Narcissus assoanus* and dimorphic and monomorphic populations of *N. dubius* over two flowering seasons. An asterisk indicates the population in which a morph-specific difference in seed-set was detected following contrasts (see text for details). Error bars indicate the standard error of the mean.

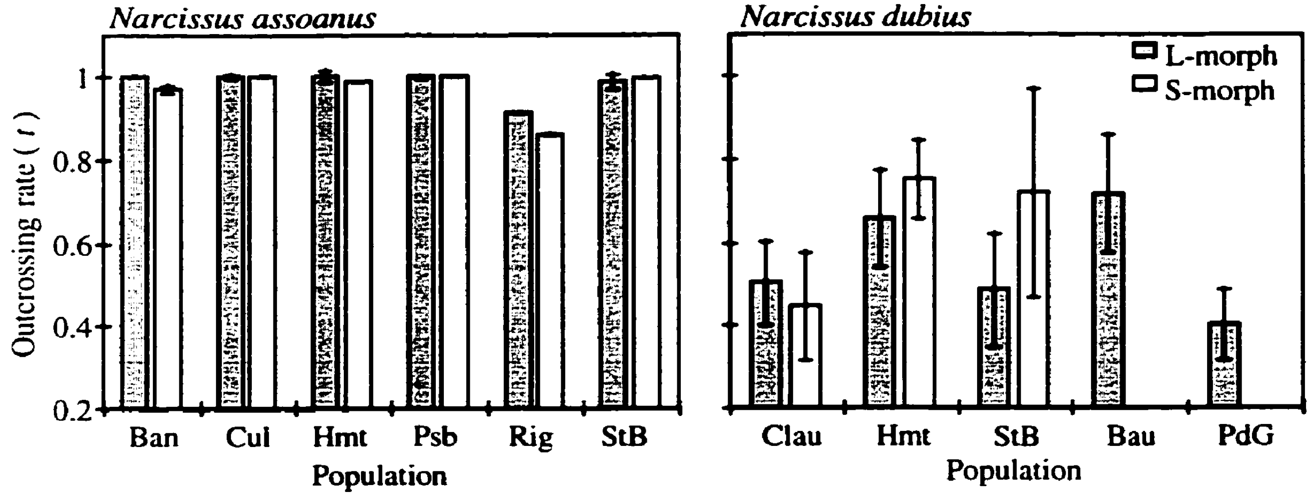


Figure 4-3. Estimated multilocus outcrossing rates ($\hat{t}$) of the L- and S-morph in populations of *Narcissus assoanus* and *N. dubius*. Morph-specific estimates were obtained using separate pollen pools in Ritland's (1990) MLTR program and error bars are standard deviations based on 1000 bootstrap estimates. Within each dimorphic population there were no morph-specific differences in mating system in either species.

compared with *N. assoanus* (Fig. 4-3) undoubtedly result, in part, from the small number of allozyme loci available for these estimates (two loci in *N. dubius* and six loci in *N. assoanus*). Comparisons of $\hat{t}$ among *N. assoanus* populations indicated no significant differences. Only one of the pairwise comparisons among *N. dubius* populations was significant: $\hat{t}$ in the monomorphic PdG population was significantly lower than in the dimorphic Hmt population (PDG mean $\hat{t}$ =0.396, SD=0.085; Hmt mean $\hat{t}$ =0.735, SD=0.077).

Effects of assortative and disassortative mating on style-morph frequencies

The results of computer calculations on the effects of assortative and disassortative mating on style-morph frequencies are illustrated in Fig. 4-4. With complete disassortative mating ($aS/dS=aL/dL=0$) equilibrium morph ratios in a population were 1L:1S (Fig. 4-4a,b) and all individuals of the S-morph were heterozygous at the locus controlling style length. Stylar dimorphism was maintained when disassortative mating occurred more frequently than assortative mating and the rate of disassortative mating was equivalent among the morphs (aL/dL and $aS/dS < 1$; Fig. 4-4a). When these conditions were met, morph ratios did not differ from 1L:1S and the genotype of the S-morph was primarily *Ss* (Fig. 4-4b), although the frequency of the *SS* genotype increased as assortative mating in plants of the S-morph (aS) increased (Fig. 4-4c).

The computer calculations indicated when L-biased morph ratios will occur. When assortative mating was low in the S-morph ($aS/dS<0.8$) but intermediate in frequency in the L-morph ($aL/dL=0.8$ to 1.2), equilibrium morph ratios were biased towards the L-morph and populations became fixed for this morph if the proportion of assortative matings in the L-morph increased (Fig. 4-4a). At very high levels of aS or aL, populations became fixed for the S- or L-morph, respectively. This is because assortative mating in the S-morph results in the segregation of the *SS* genotype and assortative mating in the L-morph results only in L-styled offspring. S-monomorphy occurred when assortative mating was high in the S-morph ($aS/dS>1.7$) but low in the L-morph (Fig. 4-4a). Under this particular scenario, the genotypes of S-individuals were primarily *SS* (Fig. 4-4b,c).

Discussion

This study provides additional insight into the nature and maintenance of stigma-height dimorphism in *Narcissus*. The polymorphism in *N. assoanus* and *N. dubius* differs from distyly

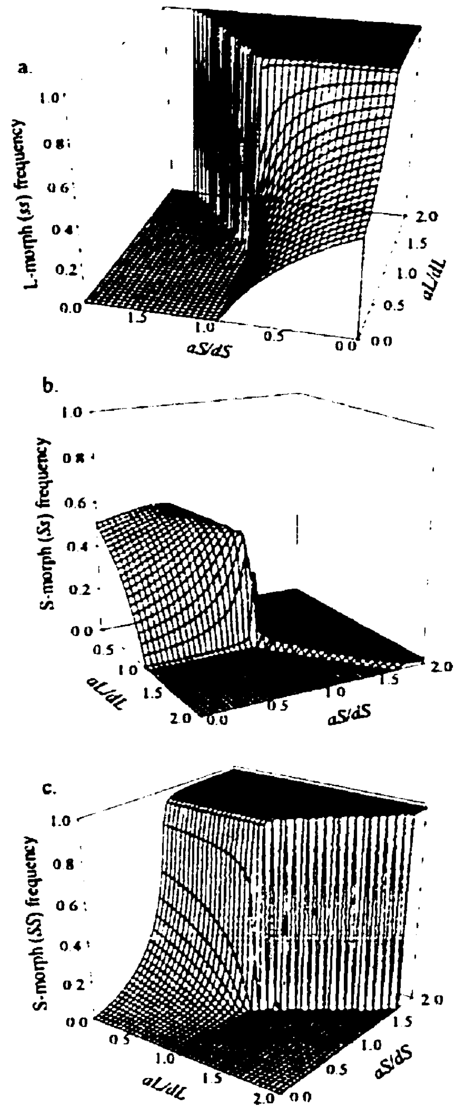


Figure 4-4. Equilibrium genotype frequencies with varying amounts of assortative and disassortative mating in the style morphs: (a) frequency of L-morph (*ss*); (b) frequency of the S-morph (*Ss*); and (c) frequency of the S-morph (*SS*). *aL/dL* and *aS/dS* represent the ratio of assortative to disassortative mating in the maternal parent such that *aL/dL* and *aS/dS*> 1 is high assortative mating in both morphs and *aL/dL*=*aS/dS*=0 is complete disassortative mating. Note that the orientation of the *x* (*aL/dL*)- & *y* (*aS/dS*)-axes differs between graphs for optimal viewing of the surface representing genotype frequency.

because neither species possesses reciprocal herkogamy, pollen heteromorphisms or heteromorphic incompatibility. These facts support the argument that the dimorphism is fundamentally distinct from heterostyly (Ch. 2, Ch. 3). Yet the stigma-height dimorphism is manifested quite differently in the two *Narcissus* species because of features of their floral biology that influence the mating systems of populations. Here I discuss how contrasting physiological and morphological traits in *N. assoanus* and *N. dubius* affect mating and fertility and result in the patterns of morph-frequency variation observed in populations of the two species in S.W. France.

Compatibility status of Narcissus assoanus *and* N. dubius.

The controlled glasshouse pollinations indicated that whereas *N. assoanus* is moderately self-sterile, *N. dubius* is thoroughly self-compatible. Most *Narcissus* species that have been examined experimentally have proved to be self-incompatible (Bateman 1954; Dulberger 1964; Barrett *et al.* 1996, 1997; Sage *et al.* 1999), although several fully self-compatible species have been reported (Herrera 1995; Barrett *et al.* 1996). Despite the presence of stylar polymorphisms in *Narcissus*, there is no evidence that any species in the genus possesses heteromorphic incompatibility. However, the class of incompatibility (gametophytic vs. sporophytic) and its genetic basis (single-locus vs. multilocus) remain unclear. Studies on dimorphic *N. tazetta* and trimorphic *N. triandrus* indicate a late-acting self-incompatibility system in which self and cross pollen tubes grow at equivalent rates and self rejection occurs in the ovary (Bateman 1954; Dulberger 1964; Barrett *et al.* 1997; Sage *et al.* 1999). It is possible that *N. assoanus* also possesses late-acting incompatibility because in common with these species, preliminary crosses have indicated that prior self-pollination of flowers 48h before outcrossing results in a significant reduction in fertility (A.M. Baker unpubl. data). However, the reduced seed-set upon selfing could also arise from early-acting inbreeding depression (Seavey & Bawa 1986; Husband & Schemske 1996). Detailed investigations of pollen-pistil interactions and ovule and seed development would be required to confirm this (for example, see Sage *et al.* 1999).

All three populations of *N. assoanus* responded similarly to the pollination treatments. There was considerable variation among plants in their response to self-pollination with some strongly self-sterile and others in which self-sterility was only partially expressed. A few individuals were detected that set equivalent amounts of seed from both self- and cross-pollinations. There was no evidence that the style morphs differed in the overall strength of self-sterility that might influence selfing rates. Data on mating patterns in natural populations were consistent with this finding because no differences in outcrossing rate between style morphs were observed (see below). In *N. dubius*, most individuals set abundant seed on self-pollination. Self-compatibility in this species may have arisen through selection of individuals in ancestral populations with weak expression of self-sterility similar to those observed in *N. assoanus*. Uncertain pollinator service could explain the self-compatible status of this early spring-flowering species. *Narcissus longispathus*, another early flowering species, is also highly self-compatible (Herrera 1995), and early flowering has often been invoked to explain the evolution of increased levels of self-fertilization (Lloyd 1965; Jain 1976).

An unexpected feature of the crossing data for *N. assoanus* was the significantly lower fertility of the L-morph in comparison with the S-morph (Fig. 4-1) in hand pollinations. On average, plants of the L-morph set 33% less seed and 31% fewer fruit from cross-pollinations than those of the S-morph. This pattern was evident in all three populations used in the crossing programme (data not shown) in two separate years. Yet my measurements of the natural fertility of six *N. assoanus* populations, including one of the three populations used in glasshouse pollinations, failed to detect significant morph-specific differences in female fertility. It seems unlikely that this result is caused by sampling effects associated with the particular plants of the L-morph used in the glasshouse studies, as sample sizes were generally large. Also, the contrasting patterns of fertility cannot result from ovule discounting because of the influence of different degrees of herkogamy on autonomous self-pollination (see Barrett *et al.* 1996; Ch. 3). All flowers used in hand-pollinations were emasculated prior to being cross-pollinated. It is possible that environmental conditions in the glasshouse influenced the fertility of the style morphs differently. For example, if temperature and humidity conditions were suboptimal for pollen-pistil interactions, fewer pollen tubes may have reached receptive ovules in the L-morph in comparison with the S-morph because of differences between the morphs in style length. Of relevance to this interpretation is the finding that the time taken for pollen tubes to reach ovules is longest in the L-morph and shortest in the S-morph in tristylous *N. triandrus* (Sage *et al.* 1999).

Maternal fitness components in natural populations

Style-morph ratios are likely to be more sensitive to morph-specific differences in fitness in populations of *Narcissus* species than in species in which disassortative mating is guaranteed because of heteromorphic incompatibility. I therefore sought to establish whether differences in maternal fitness components of the style morphs could explain the wide range of population morph frequencies that occur in *N. assoanus* and *N. dubius*. However, the measurements of female fertility and outcrossing rate in natural populations failed to detect differences between the style morphs. Thus, it is unlikely that these features of the reproductive biology are of any significance in accounting for the patterns of morph-ratio variation previously documented (Ch. 3). Although occasional differences between morphs within particular populations were sometimes detected, there was no evidence of any clear trends that could account for the L-biased ratios that commonly occur.

Within populations of *N. assoanus*, levels of fruit- and seed-set of the style morphs were similar despite striking variation among years. Outcrossing rates of the morphs were near 1.0 in all populations, indicating that despite the potential for high levels of autonomous self-pollination in the L-morph self-sterility apparently prevents both increased ovule discounting and selfing in this morph. How this is achieved is by no means clear. Previous studies demonstrated that prior selfing can reduce fertility in this and other *Narcissus* species (Dulberger 1964; Barrett *et al.* 1997; A.M. Baker unpubl. data), leading us to expect that autonomous self-pollination might result in reduced female fertility in the L-morph in comparison with the S-morph. This could occur if autonomous self-pollination renders ovules unreceptive prior to the arrival of outcrossed pollen. This expectation was not borne out, suggesting that either sufficient outcross pollen is delivered to L-stigmas by pollinators before autodeposition occurs, or that post-pollination mechanisms act to favour outcross fertilizations and hence maintain fertility.

Although the sampling of *N. dubius* was more limited, there were no consistent fertility differences between the style morphs that seem likely to account for the strongly L-biased morph ratios observed in this species. In one population (StB) in 1996, the L-morph set 68% more seed than the S-morph. If this difference occurred regularly it could influence the dynamics of morph frequencies in this population. However, in 1998, comparisons of the fertility of style morphs in this population failed to detect the pattern observed in 1996, so it is not clear how often such an effect occurs. Estimates of outcrossing in the three dimorphic populations that were examined also failed to reveal significant style morph effects although the power to detect small differences in mating was low because of the limited number of allozyme loci (two) available for the estimates. Overall, *N. dubius* exhibited a mixed-mating system with an average of 42% of seed arising from self-fertilization. Higher selfing in *N. dubius*, compared to *N. assoanus*, was not unexpected given the self-compatible status and the possession of multiflowered inflorescences. Even the relatively small floral displays found in *N. dubius* (one to seven flowers per inflorescence) provide opportunities for geitonogamy that are not possible in most *N. assoanus* plants which are largely solitary-flowered in S.W. France. The occurrence of several inflorescences per plant in *N. dubius* because of bulb fragmentation also increases the likelihood of geitonogamous selfing.

Pollen dispersal and its influence on mating patterns

The failure to detect differences in maternal fitness components between the style morphs of *N. assoanus* and *N. dubius* motivated me to consider the influence of pollen dispersal on mating patterns. Unfortunately, measurement of the key parameters required to understand the relationships between floral morphology, pollen dispersal and mating was hampered by the absence of pollen-size heteromorphism in *Narcissus* (Ch. 3), and a lack of suitable genetic markers for estimating pollen transfer and levels of assortative and disassortative mating. Because of these difficulties, I adopted a theoretical approach by developing a simple genetic model that explored the relationships between mating patterns and style-morph ratios. The model demonstrated that stigma-height dimorphism will always be maintained when levels of disassortative mating are greater than assortative mating, and that morph-specific differences in these mating parameters give rise to populations with biased style-morph ratios. These findings imply that the commonly observed L-biased morph ratios in *Narcissus* spp. result from higher levels of assortative mating in the L- vs. the S-morph. How likely is this to occur?

Several lines of evidence support the view that assortative mating will be more frequent in the L-morph in comparison to the S-morph. Because most monomorphic species of *Narcissus* have sex organs in a similar position to the L-morph it seems reasonable to assume that effective pollen transfer between plants with this morphology is generally adaptive. The common occurrence of populations monomorphic for the L-morph, but not the S-morph, among dimorphic *Narcissus* species is consistent with this view (Barrett *et al.* 1996; Ch. 3). Thus, in dimorphic populations, matings between plants of the L-morph should be common. However, although stigmas of the L-morph correspond in height to upper-level anthers of both morphs, stigmas of the S-morph are situated well below the lower-level anthers, particularly those of the S-morph (Ch. 3). The disparity in organ height may reduce the precision of intramorph pollination in the

S-morph. Indeed, experimental studies of heterostylous plants have demonstrated that pollen transfer between anthers and stigmas of equivalent height occurs much more often than between sex organs of dissimilar position (Ganders 1974; Barrett & Glover 1985; Kohn & Barrett 1992; Lloyd & Webb 1992b). Therefore, it seems likely that the high degree of herkogamy in the S-morph, compared to the L-morph, reduces the likelihood of assortative mating in this morph. According to this interpretation, most matings in the S-morph are disassortative whereas a mixture of assortative and disassortative mating is likely to occur in the L-morph. As my simulations demonstrate, this mating pattern results in populations with L-biased morph ratios or populations fixed for the L-morph. This mating pattern also predicts that plants of the S-morph will largely be of the *Ss* genotype, a situation consistent with genetic data obtained from *N. tazetta* (Dulberger 1964).

In conclusion, populations of *N. assoanus* and *N. dubius* exhibit a continuum of style-morph frequencies from isoplethy (1:1) to populations fixed for the L-morph. *Narcissus assoanus* populations are always dimorphic and some occur at the isoplethic end of the continuum, whereas populations of *N. dubius* are strongly L-biased and occasionally fixed for this morph. I interpret this variation as resulting from differences between the two species in mating patterns, particularly levels of assortative and disassortative mating. Self-sterility in *N. assoanus* favours the maintenance of stigma-height dimorphism because the vast majority of matings arise from outcrossing, and in many populations, a sizeable fraction must arise from crosses between the style morphs. In contrast, in *N. dubius*, disassortative mating appears to be more difficult to achieve and self-compatibility may have aided secondary reversion to stylar monomorphism in some populations through founder events. These contrasting influences on style-morph ratios in *Narcissus* spp. serve to illustrate the complex dynamics that can occur when morphological and physiological traits are not genetically associated as in most heterostylous species. Because of this, stigma-height polymorphisms provide excellent opportunities to investigate natural selection on simply inherited floral traits without the confounding effects of heteromorphic incompatibility.

CHAPTER 5

VARIATION OF POLLEN LIMITATION IN THE EARLY FLOWERING MEDITERRANEAN GEOPHYTE *NARCISSUS ASSOANUS* (AMARYLLIDACEAE)

Summary

A recent literature review indicates that pollen limitation of female fertility is a common feature of flowering plants (Burd 1994). Despite the ecological and evolutionary significance of pollen limitation, most studies have examined fertility in only a single population at one time. Here I investigate pollen limitation of fruit and seed set in five populations of *Narcissus assoanus*, a moderately self-sterile, insect-pollinated geophyte over two to three years in southern France. In common with many early-spring flowering plants, pollinator visitation to *N. assoanus* is infrequent. Supplemental hand-pollination of flowers with outcross pollen significantly increased overall fruit and seed set by 11% and 19%, respectively. Four of the five populations experienced some pollen limitation during the study. For a given year, there was significant variation in pollen limitation among populations. Two of the populations were pollen limited in one year but not in other years in which they were studied. Seed:ovule ratios for open- and hand-pollinated flowers averaged 0.29 and 0.33, respectively. While hand pollination significantly increased seed:ovule ratio, the low value obtained indicates that the majority of ovules in flowers do not mature seeds despite hand pollination. The roles of genetic and environmental factors governing low seed:ovule ratios in *N. assoanus* are discussed.

Introduction

Plant fertility may be limited by resources (Bell 1985; McCall & Primack 1987) and/or pollen availability (reviewed in Burd 1994; Wilson *et al.* 1994). Although these factors are not mutually exclusive (e.g. Haig & Westoby 1988; Campbell & Halama 1993), it has often been assumed, following Bateman's principle [i.e. female reproduction is typically limited by resources whereas male reproductive success is limited by access to females (Bateman 1948)], that pollen limitation of female reproductive success rarely occurs (see refs. in Burd 1994). However, a recent literature review demonstrated that pollen limitation was evident in 62% of the species that have been examined (Burd 1994). Pollen limitation can be assessed in populations through comparisons of the fertility of open- vs. hand-pollinated flowers (Bierzychudek 1981; Rathcke 1983). While the intensity of pollen limitation seems likely to vary with environmental conditions, few studies have examined the effects of supplemental hand-pollination at both spatial and temporal scales (but see Dieringer 1992; Alexandersson & Ågren 1996; Dudash & Fenster 1997). Of the 258 species included in Burd's review (1994), only 15% involved data from more than one site *or* year and only 4% were examined in multiple sites *and* years. Variable pollination environments are often invoked as an evolutionary force influencing mating and fertility, particularly in animal-pollinated species (reviewed in Harder & Barrett 1996). Hence information about spatial and temporal variation in pollen limitation may provide valuable information on the ecology and evolution of plant reproduction (Haig & Westoby 1988; Johnston 1991a; Johnson & Bond 1997; Morgan & Schoen 1997).

When flowers receive insufficient pollen and not all ovules are fertilized, the ratio of seeds to ovules (hereafter S/O) is less than one. In addition to pollen limitation, several other factors can influence variation in S/O ratios including resource limitation and genetic load (reviewed in Charlesworth 1989). A survey of S/O ratios in flowering plants indicated that outcrossing perennials exhibited significantly lower ratios than selfing annuals (Wiens 1984). Wiens interpreted this pattern as resulting from differences between the two life histories in genetic load. However, Wiens' survey of S/O ratios involved data only from open-pollinated flowers and therefore it is possible that the low S/O ratios reported in perennial plants also resulted from pollen limitation of seed set (and see Burd 1994). Comparisons of the S/O ratios of open- vs. hand-pollinated flowers could provide insights into the relative importance of pollen limitation and genetic load as mechanisms responsible for reduced female fertility in flowering plants.

In most temperate regions, pollinator abundance generally increases during the growing season as a result of warmer temperatures in summer than early spring. Animal-pollinated species that flower in early spring therefore often experience pollen limitation owing to unreliable pollinator service (Schemske *et al.* 1978; Motten 1986; Barrett & Helenurm 1987; McCall & Primack 1992). One of the most conspicuous early-spring flowering genera native to the Mediterranean is *Narcissus* (Amaryllidaceae). Pollinator observations in populations of *Narcissus* species have often indicated that visitation rates are low (e.g. Fernandes 1965; Barrett *et al.* 1996). For example, in self-compatible *N. longispathus*, unsuitable weather during February-April often limits pollinator activity and supplemental pollinations demonstrated that fruit set was pollen limited (Herrera 1995). Unlike *N. longispathus*, most *Narcissus* species are self-sterile (reviewed in Barrett *et al.* 1996; Ch. 4) and are therefore likely to be especially vulnerable to unreliable pollinator service. Here, I investigate pollen limitation in moderately self-sterile *N. assoanus* Léon-Dufour (section *Jonquillae*), a diminutive insect-pollinated species that flowers from mid-February to late-April in southern France and Spain.

Narcissus assoanus is particularly suitable for studies of pollen limitation since populations are abundant and flowers are easily hand pollinated. In addition, the modal display size of individual plants in southern France where I conducted this study is a single flower. Therefore, problems of interpretation associated with resource allocation among multiple flowers are not an issue (reviewed in Burd 1994). In this chapter, I report on the extent of pollen limitation of fruit and seed set in five natural populations occurring in the Languedoc-Roussillon region of southern France over a three-year period. I was particularly interested in determining if there were differences among populations in pollen limitation for a given year and if the degree of pollen limitation in a population varied among years. I also examined whether supplemental hand pollination increased S/O ratios in comparison with open-pollinated flowers.

Materials and Methods

Study organism and populations

Narcissus assoanus is a perennial geophyte widespread in southern France and eastern Spain (Blanchard 1990). It typically occurs in meadows and stony pastures on limestone from sea level to at least 1900 m. In southern France, the solitary yellow flowers remain open for approximately three weeks (range 15 to 28 days), have prominent coronas, long floral tubes containing nectar, and are visited primarily by butterflies (*Gonepteryx cleopatra*), day-flying hawkmoths (*Macroglossum stellatarum*) and solitary bees (*Anthophora* spp.). *Narcissus assoanus* is moderately self-sterile and all outcross pollinations are compatible (Ch. 4).

The five populations in which I investigated pollen limitation were separated by a minimum distance of eight km. Localities and information on altitude, flowering periods and population size are presented in Table 5-1. Preliminary observations of pollinator activity in the five populations indicated that visitation rates were often low. During most visits to populations, no pollinators were seen because of inclement weather. However, I was able to quantify pollinator abundance on fine sunny days by recording the number of flower visitors entering 1-2 m^2 quadrats with an average density of 25 flowering plants per m^2 at 15 minute intervals. The total number of observation periods per population ranged from ten to 30 during the three-year study. All populations except for the upland population at Rigalderie were located on stony, well-drained limestone substrate with south to south-east exposure. The Rigalderie population was located in an open pasture. At peak flowering, density was similar at all lowland sites (Aguzan, Col Hortus, Pont sur Brestalou, and St-Bauzille) with 15 – 20 flowering individuals per m^2 on average. The density of flowering individuals was higher at Rigalderie (approx. 50 per m^2). In all populations, flowering periods lasted for approximately four to six weeks.

Measurement of pollen limitation of fruit and seed set

I investigated spatial and temporal components of pollen limitation of fruit and seed set in the five populations in 1997–1999. Aguzan, Col Hortus, and St-Bauzille were studied in every year. Pont sur Brestalou and Rigalderie were each examined only in two years because of insect herbivory and poor weather conditions, respectively. I began pollination treatments one to three weeks after flowering began in each population. By three weeks, all populations were in peak

Table 5-1: Populations of ***Narcissus assoanus*** in southern France examined for pollen limitation of fruit and seed set. Pollinator abundance was measured as the proportion of all observation periods in which pollinators were seen.

	Population				
	Aguzan	Col Hortus	P. sur Brest.	Rigalderie	St-Bauzille
Longitude	03°53´10"E	03°51´20"E	03°58´50"E	03°30´20"E	03°57´30"E
Latitude	43°56´20"N	43°48´55"N	43°50´45"N	43°55´45"N	43°46´20"N
Elevation	200 m	240 m	150 m	680 m	220 m
Peak flowering	mid March	mid March	mid March	mid April	mid March
Pollinator abundance	0.67	0.37	0.46	0.20	0.82
Population size	1200	1200	400	10 000	600

flower. I set up a series of five to eight 1-2m^2 plots in each population. Within these plots flowering density was approximately 25 per m^2 and I used flowers in these plots for my study. Flowers were chosen one to two days after opening and supplemental outcross pollen from a single pollen donor was brushed on to the stigmas of the flower using fine-tipped forceps. Pollen donors were chosen from one to five metres away. Because of the high density of flowering plants, this ensured that crosses were not between near neighbours. Other flowering individuals in the plots were marked with white tape at the base of the flowering stem and left as open-pollinated controls. *Narcissus assoanus* possesses a stigma-height dimorphism (Ch. 3, 4). Plants of the long- and short-styled morph were both chosen for pollination treatments in each population. To facilitate pollination of the short-styled morph, the perianth was slit with forceps. This procedure was also applied to the long-styled morph. Flowers were left undisturbed after pollination, allowed to senesce, and seed capsules were collected when mature (four to five weeks after anthesis). Seed number and the number of ovules per flower were counted using a dissecting microscope. An earlier study of morph-specific fertility in these populations indicated that there was no significant difference in fruit or seed set between the style-morphs (Ch. 4). Consequently, in all analyses here I used pooled data and did not consider the effects of style morph on fruit or seed set.

Statistical analyses

Percent fruit set was analyzed with logistic regression (PROC GENMOD, SAS 6.12, 1997) with population nested within year, year, and pollination treatment as main categorical effects. The interaction of year-by-pollination treatment examined the intensity of pollen limitation among years, whereas the term pollination treatment-by-population nested within year investigated the degree of pollen limitation among populations for a given year. This analysis did not allow me to assess whether or not the intensity of pollen limitation differed among years for a given population. To address this question, I performed *G*-tests of independence (Sokal & Rohlf, 1995) for each of the population-by-year combinations. I used the Dunn-Sidák correction to adjust the experimentwise error rate to account for multiple comparisons (Sokal & Rohlf 1995). This correction is conservative and lowers the chance of making type I errors in the entire series of tests. Two approaches were used to examine the effects of population, year, and pollination treatment on seed set. In an analysis similar to the one above, I used a mixed-model ANCOVA

(PROC GLM, SAS 6.12, 1997) to examine the effects of the above variables on the square-root of seed set per fruit. This transformation increased the normality of the data. In this analysis, year was treated as a random variable. To assess pollen limitation among years for a given population, I performed orthogonal contrasts (Student's *t*-test; Kirk, 1995) to compare seed set in open- vs. hand-pollinated capsules in the 13 population-by-year combinations. I back-transformed least-square means for presentation in figures. In a second analysis, I used a fully factorial mixed model ANCOVA and the Type IV sum of squares (PROC GLM, SAS 6.12, 1997). This analysis accounted for the missing population-by-year combination allowing us to compare the results of this factorial design with the nested design described previously. I calculated S/O ratios by dividing the number of seeds in a capsule by the total ovule number (seeds + ovules). The distributions of S/O ratios for open- vs. hand-pollination treatments were compared using a Kolmogorov-Smirnov two sample test (Sokal &Rohlf 1995).

Results

Pollen limitation of fruit set

Overall, supplemental hand-pollination of *N. assoanus* flowers significantly increased percent fruit set by 11% in comparison with open-pollinated flowers [grand mean open-pollinated (OP)=66.1, s.e.=1.6, *n*=821; hand-pollinated (HP)=73.7, s.e.=1.9, *n*=585; Table 5-2]. The intensity of pollination limitation of fruit set did not differ among years or among populations within a given year (Table 5-2). Although no significant interactions between pollination treatment and year or population were found, *G*-tests of independence revealed significant pollen limitation of fruit set at Rigalderie in 1998 and 1999. However, in the other four populations (Aguzan, Col Hortus, Pont sur Brestalou, and St-Bauzille), hand pollination did not significantly increase fruit set in any year (Fig. 5-1). Rigalderie also exhibited significantly lower fruit set than the four lowland populations.

Pollen limitation of seed set

The results of the fully factorial design did not differ qualitatively from the nested design (data not shown). Here I present the results of the nested ANCOVA to maintain symmetry of interpretation with the results of the fruit set analysis. Hand-pollinated flowers of *N. assoanus*

Table 5-2: Logistic regression analysis of the effects of year, population, pollination treatment, and their interactions on percent fruit set in populations of *Narcissus assoanus*.

Source	d.f.	χ^2	*P*
Year	2	4.692	0.0958
Population [year]	10	182.91	0.0001
Treatment	1	12.11	0.0005
Year × treatment	2	3.940	0.1394
Trt × population [year]	10	14.21	0.1638
Error	1380		

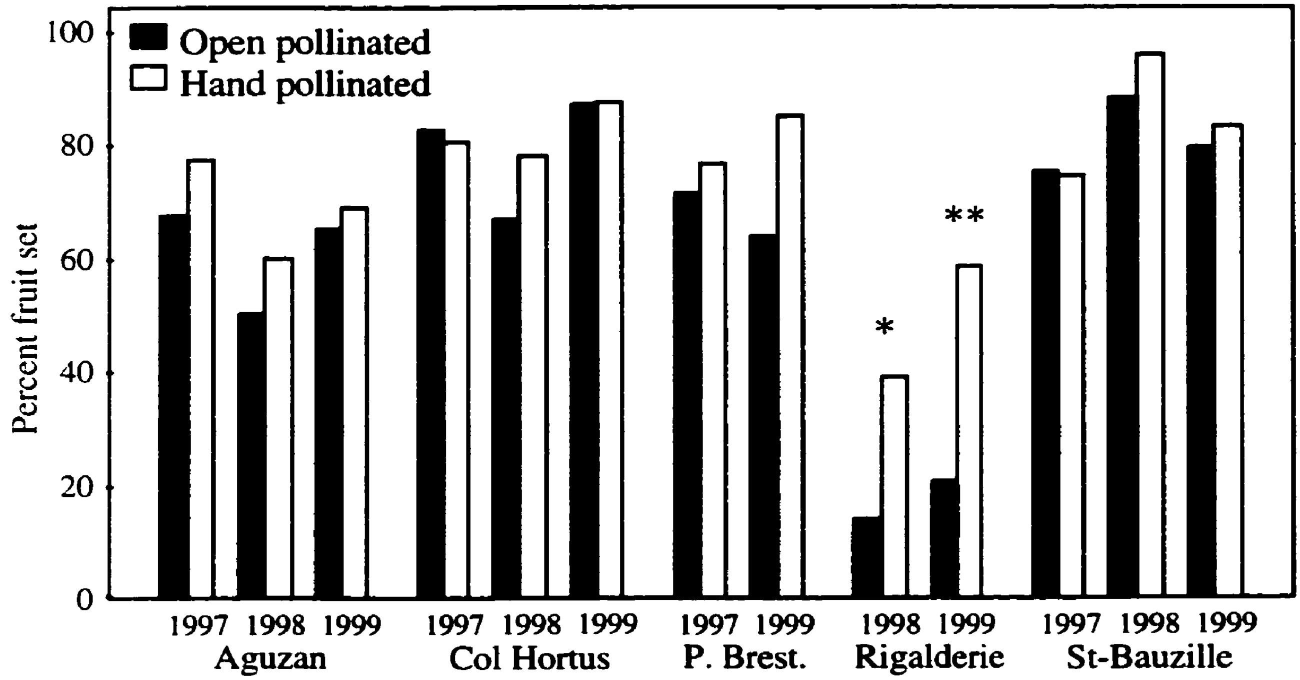


Figure 5-1. Percent fruit set in open-pollinated (OP) vs. hand-pollinated (HP) plants in five populations of *Narcissus assoanus*. Aguzan, Col Hortus, and St-Bauzille were studied in 1997-1999. Pont sur Brestalou and Rigalderie were studied in two years. Overall, there is a statistically significant difference in percent fruit set between OP and HP plants. Significant increases in fruit set with hand-pollination are indicated by asterisks (**P<0.01, *P<0.05; following Dunn-Sidák's correction for multiple comparisons) and are based on G-tests of independence. Mean sample sizes are 63.4 (range 14 – 97) and 45.0 (range 10 – 79) plants for OP and HP, respectively.

produced significantly more seeds (19%) than open-pollinated flowers (OP=12.94, upper s.e.=0.429, lower s.e.=0.422, n=532; HP=15.92, u.s.e.=0.480, l.s.e.=0.473, n=399; Table 5-3). The intensity of pollen limitation differed among populations within a given year, as indicated by the marginally significant interaction of pollination treatment-by-population nested within year (Table 5-3), but did not differ among years (as indicated by the non-significant F-value for the year-by-pollination treatment interaction, Table 5-3). The results of orthogonal contrasts (t-tests) to examine seed set in OP vs. HP plants for each population-by-year combination indicated that there was variation in pollen limitation; seed set was pollen limited in only one year at Aguzan and Pont sur Brestalou but was never pollen limited at Rigalderie or St-Bauzille (Fig. 5-2). Seed set was pollen limited in every year at Col Hortus (Fig. 5-2), although in 1997 the difference between the treatments was only marginally significant.

Seed to ovule ratios

Mean S/O ratio pooled across all populations and years was significantly higher in hand-pollinated plants than in open-pollinated plants (OP=0.287, s.e.=0.007; HP=0.332, s.e.=0.008; t_{929}=4.495, P<0.001; 5- 3). Nevertheless, on average two-thirds of the ovules in hand-pollinated flowers failed to mature seed. I pooled S/O ratios from the five populations because there was no significant population-by-pollination treatment interaction following ANOVA ($F_{4,920}$=1.54, P=0.1882). Although distributions of S/O ratio for the OP and HP treatments were significantly different (D=0.144, P<0.001; Fig. 5-3), both exhibited a wide range of values from 0.1-0.9.

Discussion

The major finding of this study is that populations of *N. assoanus* in southern France exhibit significant spatial variation in pollen limitation. Supplemental hand pollinations increased overall fruit and seed set by 11% and 19%, respectively. Four of the five populations experienced pollen limitation of fertility at some time. Of these, two populations were pollen-limited in one year but not in the other years in which they were sampled. The occurrence of this variation cautions against generalizations about pollen limitation based on limited sampling of a single population. A second significant and unexpected result from my study was the finding that S/O ratios in all populations following hand pollination were surprisingly low with only a third of ovules, on average, producing seeds. Below I discuss some of the factors influencing female fertility in *N.*

Table 5-3: ANCOVA of the effects of year, population, treatment (open- vs. hand-pollinated) and their interactions on mean seed set per flower in populations of *Narcissus assoanus*. Total ovule number was used as a covariate.

Source	d.f.	MS	*F*	*P*
Year	2	0.772	0.80	0.4484
Population [year]	10	3.536	3.68	0.0001
Treatment	1	21.090	21.93	0.0001
Year × treatment	2	1.224	1.27	0.2860
Treatment × pop [year]	10	1.703	1.77	0.0619
Total ovule number	1	134.15	139.48	0.0001
Error	904	0.962		

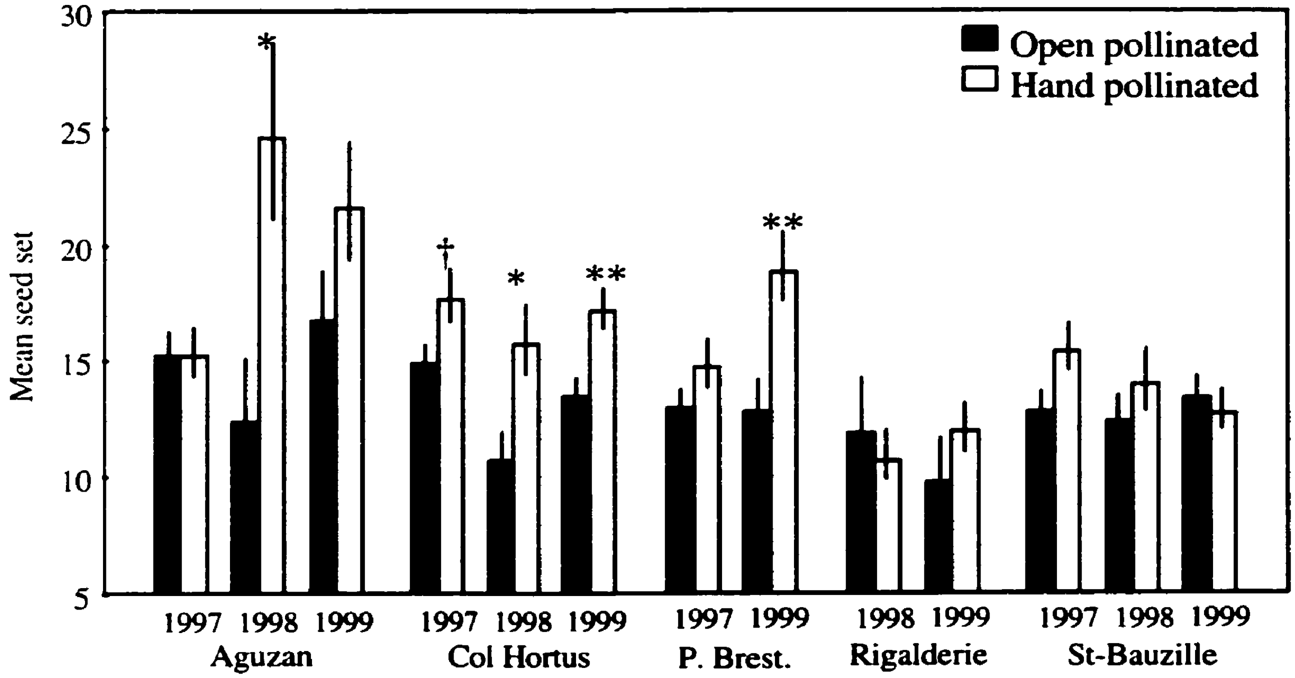


Figure 5-2. Mean seed set in open- (OP) vs. hand-pollinated (HP) plants in five populations of *Narcissus assoanus*. Aguzan, Col Hortus, and St-Bauzille were studied in 1997-1999. Pont sur Brestalou and Rigalderie were studied in two years. Overall, there is a statistically significant difference in mean seed set between OP and HP plants. Population-by-year combinations with significantly higher mean seed set in HP flowers are indicated (**$P<0.01$, *$P<0.05$, † $P<0.10$); following Dunn-Sidák's correction for multiple comparisons) and are based on orthogonal contrasts. Mean sample sizes are 40.9 (range 7 – 80) and 30.7 (range 6 – 60) plants for the OP and HP treatments, respectively. Error bars are equivalent to ±1 SE.

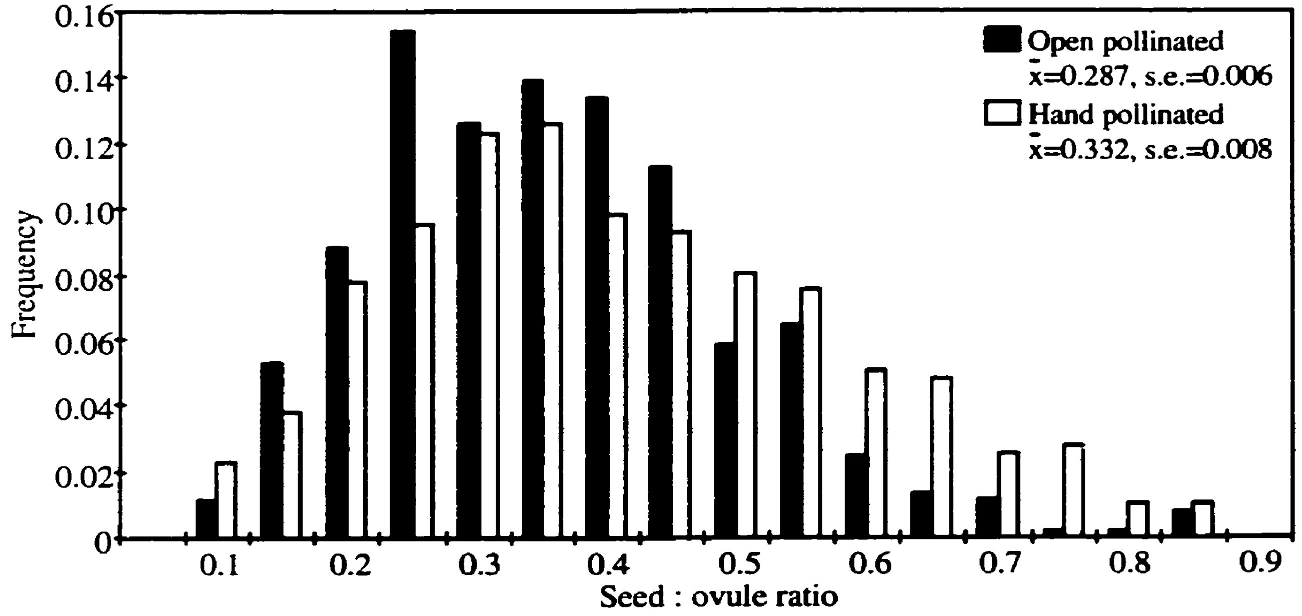


Figure 5-3. Distributions of seed:ovule ratio in open- (OP) vs. hand-pollinated (HP) plants of *Narcissus assoanus*. Data were pooled from five populations over three years. The two distributions are significantly different following a two-sample Kolmogorov-Smirnov test (D=0.144, P<0.001). Sample sizes are OP=532 fruits, HP=398 fruits.

assoanus, discuss their ecological and evolutionary implications, and conclude by reviewing some of the mechanisms influencing S/O ratios in flowering plants

Causes of pollen limitation

Among the five populations of *N. assoanus* that were sampled, 66% of open-pollinated flowers set fruit. Supplemental hand-pollination increased fruit set to 74%. Despite this overall increase, in only one population, Rigalderie, was fruit set significantly pollen limited. This indicates that flowers of *N. assoanus* are visited by pollinators in most populations, despite the low activity during parts of the flowering period. Flowers of *N. assoanus* have long floral longevities, ranging from 15-28d depending on temperature and whether they have been pollinated. This extended floral lifetime undoubtedly increases the probability of visitation and accounts for the absence of significant pollen limitation of fruit set in most populations. Elsewhere, Primack (1985) and Ashman and Schoen (1996) provide empirical and theoretical evidence that extended floral longevities are a common adaptive feature of animal-pollinated species with infrequent pollinator service. Long floral longevities occur in most *Narcissus* species, particularly those that flower at times of the year when pollinator densities are low (Arroyo & Dafni 1995; Herrera 1995; Barrett *et al.* 1996).

The detection of pollen-limited fruit set in both years at Rigalderie likely stems from two primary causes. First, this population is located on an upland plateau at higher altitude and flowers one month later than the remaining populations. During flowering, this location often experienced cool windy conditions that limited pollinator activity during the study period. Poor weather, including low temperatures, nighttime frost, and snow, may also adversely influence fruit set (in open- and hand-pollinated plants) when compared to lowland sites. At this site, flowering occurred during cooler weather in both years of the study. Second, this population is considerably larger in size than the other populations investigated, containing at least 10 000 flowering individuals (Table 5-1). Low fruit set at Rigalderie may also be associated with of an excess of flowers relative to the number of pollinators visiting the population. Recent studies that focus on the proximate ecological mechanisms governing plant reproductive success have tended to emphasize the negative effects of small population size on fertility because of the effects of reduced floral displays on pollinator visitation rates (e.g. Jennersten 1988; Ågren 1996; Alexandersson & Ågren 1996). However, the opposite pattern could also occur in very large populations where mean fertility may be reduced in comparison with smaller populations because of a surplus of floral resources (Fritz & Nilsson 1994; Larson & Barrett 1999). Such effects may be especially likely during unpredictable weather in early spring when pollinator densities may not track rapid changes in flowering density.

Pollen limitation of seed set most often arises because an insufficient number of outcross pollen grains are deposited on stigmas by pollinators. Seed set was pollen limited in four of the five populations of *N. assoanus* but did not occur in every year. Interestingly, while three of the four populations exhibited pollen limitation of seed set, at no time was fruit set in these populations limited by pollen delivery. This indicates that, in general, sufficient pollen was transferred to stigmas to enable fruit maturation but not to ensure full seed set. Unlike many perennial plants that commonly abort fruit with small numbers of developing seeds (reviewed in Stephenson 1981), plants of *N. assoanus* maintain developing fruits even when they contain very few seeds (<5). This feature of the reproductive biology of *N. assoanus* may reflect the low flowering capacity of individual plants, which at these sites produce only a single flower each season. This contrasts with many mass-flowering trees and shrubs that typically exhibit high rates of fruit abortion. Although most flowers of *N. assoanus* matured fruit regardless of seed number, a significant proportion (26%) did not, even when they were provided with abundant pollen through hand cross-pollination. As discussed in more detail below in connection with the low S/O ratios, the causes of this fruit failure are not known but could involve both genetic and environmental factors.

Because of the generally low and unpredictable nature of pollinator service to *N. assoanus* populations, any attempt to correlate pollinator abundance to variation in pollen limitation is fraught with difficulty. This problem was also encountered by Larson and Barrett (1999) in their studies of pollen limitation in *Rhexia virginica*, a buzz-pollinated species that experiences low and unpredictable pollinator service at the geographic margins of its range. The efforts at measuring pollinator visitation in *N. assoanus* were crude at best and provided only qualitative information on the relative abundance of pollinators at the five sites. The problem of quantifying pollinators is a general problem for animal-pollinated species with extended floral longevities and low visitation rates since it requires considerable time and effort, particularly when population comparisons are involved. Assays of pollen loads on stigmas and pollen removal from anthers may, however, provide indirect information on the activities of pollinators and enable inferences to be made on the roles of variation in pollinator abundance in causing pollen limitation (Ackerman 1989; Kearns & Inouye 1993; Johnson & Bond 1997; Larson & Barrett 1999).

Why are seed:ovule ratios so low?

Flowers of *N. assoanus* that were hand pollinated with outcross pollen matured only 33% of their ovules. This value is considerably lower than the mean S/O ratio (0.50) reported from a survey of open-pollinated perennial plants by Wiens (1984) raising the obvious question of why S/O ratios in *N. assoanus* are so low. Although I presented pooled data on S/O ratios in Fig. 5-3, all five populations exhibited similar distributions with mean values for hand-pollinated flowers consistently low (range of mean S/O values 0.27 - 0.37). This result suggests that the low S/O ratios I report are a general feature of this species. Next I consider potential factors that might explain this pattern.

Limited resources could contribute towards the low percent of ovules that mature into seed and also to the submaximal fruit set I recorded in hand-pollinated plants. The habitats that *N. assoanus* populations occupy are often dry, stony and are frequently subject to drought during fruit maturation. I have some evidence that resource limitation plays a role in the low fertility of this species. In 1997 plants from several natural populations in this region were transplanted just prior to flowering to glasshouses at Montpellier for controlled pollination studies reported elsewhere (Ch. 4). These plants received regular watering and nutrients and were used to perform within-population hand cross-pollinations. Mean S/O ratios were significantly higher in these experimental plants (S/O ratio=0.42, s.e.=0.021, n=151) than the values obtained in this study for the same year (S/O ratio of HP plants in the field=0.34, s.e.=0.014, n=100; $F_{1,245}$=12.157, P=0.0006), although they were still well below the average values for most perennial plants (see Wiens 1984). This suggests that while resource limitation plays some role in regulating fruit and seed set in *N. assoanus* under field conditions other factors are also involved.

Several aspects of the hand-pollinations of *N. assoanus* could also have contributed towards reduced fertility. Both maternal and paternal genotype and their interaction play a role in governing the fertility of cross pollinations (Lyons *et al.* 1989). My crosses involved single pollen donors occurring within 1-5 m of the maternal parent. It is possible that despite efforts to avoid near neighbours, the crosses still involved related individuals, and the low mean fertility I obtained reflects a component due to inbreeding depression (Charlesworth & Charlesworth 1987). This is certainly a distinct possibility since *N. assoanus* populations are highly structured and seed dispersal is limited because of the diminutive status of plants. The inbreeding hypothesis could be tested by investigating the fertility of crosses at different spatial scales (Waser 1993). It is also possible that by increasing the number of male parents used in crosses fertility could have been increased (Marshall 1991). Another intriguing possibility concerns ovule maturation schedules within flowers of *Narcissus* species. In *N. triandrus*, an early-flowering species with extended floral longevity, ovules mature asynchronously so that on a given day early in anthesis not all ovules are receptive (Sage *et al.* 1999). If this occurs in *N. assoanus*, then hand pollinations conducted on a single day may not have been sufficient to fertilize most ovules within an ovary. This hypothesis predicts that serial pollinations over the lifetime of a flower should increase S/O ratios in *N. assoanus*.

Wiens (1984) explained the low fertility of outcrossing perennials as resulting largely from genetic load. According to this hypothesis many fertilization products are subsequently aborted during early development because of deleterious allelic combinations. This hypothesis is difficult to evaluate since as in many investigations (see Nakamura & Stanton 1987) I was unable to determine whether ovules in fruits of *N. assoanus* had been fertilized and then aborted very early in development or whether they had remained unfertilized. Charlesworth (1989) suggested that genetic load could provide a partial explanation for low S/O ratios, although she doubted that load alone could account for the low fertility observed in some plants. To critically evaluate genetic hypotheses that account for low fertility in outcrossing species it is necessary to determine both fertilization rates and the viability of female and male gametes. In particular, the assumption that most ovules are viable needs to be verified by careful cytological examination.

CHAPTER 6

STIGMA-HEIGHT DIMORPHISM AND THE EVOLUTION OF DISTYLY: POLLEN TRANSFER IN EXPERIMENTAL POPULATIONS

Summary

A recent model for the evolution of distyly (Lloyd & Webb 1992a,b) established pollen transfer inequalities required for the invasion of an ancestral herkogamous population by a novel morph, creating a stigma-height dimorphic population consisting of individuals with discrete variation in stigma but not anther position. This condition has been hypothesized to be an intermediate stage in the evolution of distyly. I used floral manipulations and genetic markers in garden populations of tristylous *Eichhornia paniculata* to assess the influence of sex-organ position on fertility and mating patterns. These data were used to quantify pollen transfer within and among L- and S-styled morphs and to assess the functional significance of stigma-height dimorphism. I estimated pollen transfer from the allozyme genotypes of seeds produced from open pollination mediated by bumble bees. The arrangement of sex organs in a flower significantly influenced fertility and outcrossing rates, and intermorph mating was typically greater than intramorph mating. Stigma-height dimorphism increased outcrossing rates in both morphs, and female fertility and pollen transfer to the L-morph, when compared with monomorphic populations. This finding partially supports the hypothesis that the dimorphism increases proficient intermorph pollen transfer. Estimates of outcross pollen transfer within and between the floral morphs supported both stages of the Lloyd and Webb model involving the establishment of stigma-height dimorphism and anther-height dimorphism. However, when selfing events were considered in the evaluation of pollen transfer probabilities, the establishment of stigma-height dimorphism was more difficult to achieve. The fitness of morphs in experimental arrays was negatively frequency-dependent. The greater strength of negative frequency-dependence in the S-morph suggests that it may be easier for this morph to invade a population of approach herkogamous plants than vice versa.

Introduction

Distyly is a genetically controlled polymorphism in which plant populations are composed of two floral morphs that differ reciprocally in the position of stigmas and anthers. The dimorphism has evolved independently in at least 28 animal-pollinated plant families (Darwin 1877; Ganders 1979a; Barrett 1992a,b; Richards 1997) and is typically accompanied by a suite of associated characters. These include ancillary polymorphisms of the stigmas and pollen and a diallelic self-incompatibility system that prevents self- and intramorph fertilization (reviewed in Ganders 1979a; Barrett 1992a; Richards 1997). Theoretical models of the evolution of distyly have considered an intermediate stage involving a stigma-height dimorphism (Charlesworth & Charlesworth 1979; Lloyd & Webb 1992a,b; Richards 1998). Stigma-height dimorphic populations consist of individuals which show discrete variation in style length but little variation in anther position. Stigma-height dimorphism is relatively uncommon in flowering plants and little is known about its functional significance (Webb & Lloyd 1986; Barrett *et al.* 2000a,b; Ch. 2, 3, 4). The primary goal of this study was to investigate the reproductive consequences of stigma-height dimorphism and to assess its role in the evolution of distyly using experimental populations.

Stigma-height dimorphism is a key feature of the Lloyd and Webb (1992a,b) model of the evolution of distyly. Lloyd and Webb (1992a) assume that the ancestral population exhibits approach herkogamy (Fig. 1-3A). Approach herkogamous flowers, in which stigmas are exserted beyond anthers, is the most common type of floral herkogamy (Webb & Lloyd 1986). In the first stage of Lloyd and Webb's (1992b) model, a mutant short-styled morph becomes established in a population because of a frequency-dependent advantage in fitness. Lloyd and Webb (1992b) derive specific pollen transfer inequalities for the establishment of a dimorphism depending on whether seed set is pollen- or resource-limited. Simply stated, intermorph pollen transfer must exceed intramorph transfer for the establishment of stigma-height dimorphism. In the second stage of the Lloyd and Webb (1992b) model, there is subsequent selection of an anther-height polymorphism giving rise to the polymorphic system – reciprocal herkogamy. Selection on anther position is based primarily on pollen transfer among the morphs. Once reciprocal herkogamy is established (Fig. 1-3C), associated dimorphic incompatibility and ancillary polymorphisms are thought to evolve gradually over time.

Although Lloyd and Webb's (1992b) model involves the establishment of a population with stigma-height dimorphism due to an increase in male mating success, the model developed by Charlesworth and Charlesworth (1979) considers the effects of a change in stigma position on female fitness. Charlesworth and Charlesworth (1979) show that it would be difficult for a stigma-height dimorphism to establish since a mutant style morph would either spread to fixation if the change in stigma height increased pollen receipt, or be eliminated if too great a loss in female fertility occurred. Since their simulations indicate that a stigma-height dimorphism is difficult to maintain in self-compatible populations, they suggest that dimorphic incompatibility is established prior to reciprocal herkogamy (Charlesworth & Charlesworth 1979).

In a different scenario for the evolution of distyly, Richards (1998) suggested that an ancestral population of self-compatible homostyles is invaded by a short-styled mutant which carries deleterious recessive genes tightly linked to the locus controlling style length, thus creating a stigma-height dimorphic population. Because of more proficient pollen transfer between the morphs, the short-styled dominant mutant should exist in the heterozygous form. Reciprocal herkogamy is established after a subsequent lowering of anthers in the long homostyle for efficient pollen transfer to short-level stigmas.

Unfortunately, the lack of detailed phylogenies of families with stigma-height dimorphism and distyly makes it difficult to determine whether stigma-height dimorphism is involved in the evolution of distyly. Evidence in favour of stigma-height dimorphism as a stage in the evolution of distyly would be the co-occurrence of stigma-height dimorphism and distyly in closely related species (Arroyo & Barrett 2000). Unfortunately, stigma-height dimorphism is relatively rare in flowering plants and this has been interpreted as evidence that the condition is ephemeral and rapidly passed through in the evolution of distyly (Lloyd & Webb 1992a). As a result only three genera are known in which both stylar polymorphisms occur (*Primula* – Al Wadi & Richards 1993; *Narcissus* – Barrett *et al.* 1996, Arroyo & Barrett 2000; *Lithodora* – J.D. Thompson and S.C.H. Barrett unpubl. data).

Darwin's (1877) hypothesis that heterostyly functions to increase proficient pollen transfer among morphs since pollen is placed in discrete positions on a pollinator's body has been investigated using experimental approaches (reviewed in Ganders 1979a; Barrett 1992a; Lloyd & Webb 1992b). Differences in pollen size among the morphs of heterostylous species have enabled the measurement of pollen transfer patterns in natural populations. Results from these studies generally support Darwin's (1877) hypothesis by showing that intermorph pollinations are more proficient than intramorph (e.g. Ganders 1974; Barrett & Glover 1985; Wolfe & Barrett 1989; Lloyd & Webb 1992b). In addition, marker gene studies of experimental populations of *Eichhornia paniculata* (Pontederiaceae) have also yielded results supporting the Darwinian hypothesis (Kohn & Barrett 1992). Unfortunately, naturally occurring stigma-height dimorphic populations provide limited opportunities for testing hypotheses regarding pollen transfer within and among morphs since these taxa do not exhibit pollen heteromorphisms (Ch. 2) or contain the necessary floral variation (Fig. 1-3) required to test Lloyd and Webb's (1992a,b) model. Stone and Thomson (1994) circumvented these problems by constructing artificial flowers of appropriate morphology and using dyed pollen grains to examine pollen transfer. Pollen transfer probabilities were quantified after bumble bees were allowed to visit artificial flowers. Their data supported Lloyd and Webb's (1992b) conditions for the establishment of a stigma-height dimorphism although not for the establishment of a subsequent anther-height dimorphism. Stone and Thomson (1994) also showed that a simple change in stigma height affected bee feeding posture in a way that promoted intermorph pollen transfer even in the absence of an anther-height dimorphism.

Tristylous *Eichhornia paniculata* is amenable to studies of inter- and intramorph pollen transfer, since the floral morphs are self-compatible with similar seed set for self-, and intramorph crosses (Barrett 1985; Kohn & Barrett 1992). Therefore the direct effects of sex-organ placement on mating patterns can be examined without the confounding effects of heteromorphic self-incompatibility (Ganders 1975; Barrett *et al.* 1987). Such a system is valuable for testing hypotheses regarding pollen transfer and mating patterns within populations. Moreover, with partial emasculation of tristylous flowers it is possible to create the floral morphs required to examine the functional significance of stigma-height dimorphism and to evaluate the Lloyd and Webb (1992b) model of the evolution of distyly.

Before evaluating the conditions of the Lloyd and Webb (1992b) model for the evolution of distyly, I first examined the influence of sex-organ position on mating patterns and fecundity in monomorphic populations. This was achieved by comparing pollen deposition, female fertility, and outcrossing rate in monomorphic populations consisting of approach or reverse herkogamous plants (stigmas placed above or below the anthers, respectively). I then assessed the reproductive consequences of stylar monomorphism versus dimorphism to test the hypothesis that stigma-height dimorphism functions to increase pollen transfer between the morphs. Next, I examined whether outcrossing rates were higher in dimorphic than monomorphic populations, and how the morph composition of a population may influence fruit and seed set. Having established how stigma-height dimorphism influences mating patterns and fertility, I then addressed three specific questions related to the Lloyd and Webb (1992b) model: (1) In isoplethic (1:1) stigma-height dimorphic populations, are each of the style morphs more proficient at cross-pollination with the other morph than with their own kind? (2) Are morph-specific differences in mating patterns or female fertility evident in experimental populations? Furthermore, is the total fitness of a style morph related to its frequency in the population? (3) Are the pollen transfer conditions of the first stage of the Lloyd and Webb (1992a,b) model, the invasion of a population by a rare style morph, satisfied?

Materials and Methods

Floral manipulations and morphology of experimental plants

Several different floral phenotypes are involved in the evaluation of the functional significance of stigma-height dimorphism and the Lloyd and Webb (1992b) model of the evolution of distyly. To avoid any terminological confusion, I first describe these phenotypes. Starting material involved the long- and short-styled morphs of tristylous *Eichhornia paniculata*. Flowers possess a single stigma position (long- or short-styled) and two anther positions (Fig. 6-1). I removed anthers from specific levels to produce three floral phenotypes (long- and short-styled with anthers of the mid-level, and short-styled with anthers at the long-level), that were used in different experimental populations. Monomorphic populations consisted of long-styled plants with anthers at the mid-level or short-styled plants with anthers at the mid-level, simulating approach and reverse herkogamous phenotypes, respectively, (Fig. 6-1). Throughout, I refer to these phenotypes as A and R. Approach herkogamy represents the potential ancestral condition in the evolution of distyly (Lloyd and Webb 1992a,b). Dimorphic populations consisting of the A and R floral phenotypes were established to simulate a stigma-height dimorphic population. In these dimorphic populations, I refer to the floral phenotypes as L- and S-styled morphs since A and R are technically not morphs since they usually exist in a monomorphic state. To test the second stage of the Lloyd and Webb (1992b) model, I created

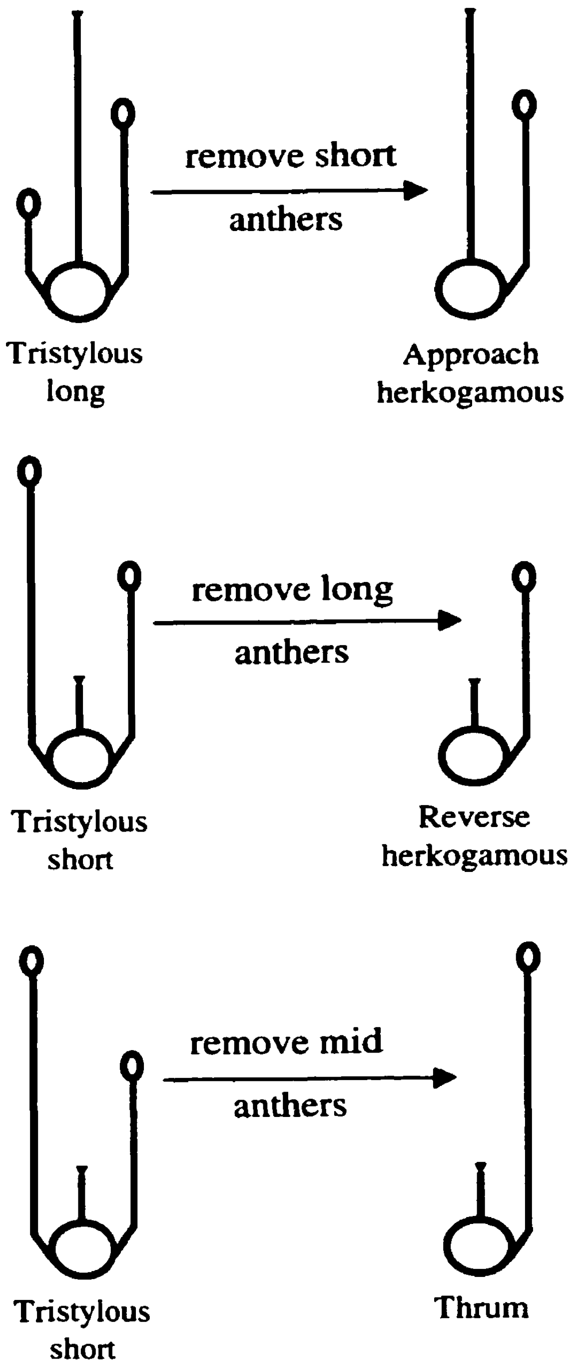


Figure 6-1: Floral manipulations of tristylous *Eichhornia paniculata* flowers to create style-morph phenotypes used in experimental populations.

thrum (T) morphs by removing the mid-level anthers from short-styled tristylous plants (Fig. 6-1). Although not used in my experiments, distylous long-styled morphs are known as pin plants.

Eichhornia paniculata plants used in these experiments were derived from a trimorphic population (B46) from the state of Ceará, Brazil (Husband & Barrett 1992). In 1995, plants were grown from seed generated by crossing among approximately 200 plants originating from open-pollinated bulk seed families. I performed crosses to generate individuals homozygous for alternate alleles at two allozyme loci (AAT-3 and PGI-2) and assayed plants electrophoretically (see Glover & Barrett 1986, 1987; Kohn & Barrett 1992) at flowering. Of 1120 plants screened, I identified 286 as homozygous for both loci. Of these, 172 L- and 72 S-styled plants were used in the experimental populations. I performed additional crosses to generate homozygous individuals of the S-morph for 1996. I used a total of 240 plants (150 L- and 90 S-styled) in these populations.

Experimental populations

Experimental populations consisted of 36 plants placed approximately 30 cm apart in a 6 × 6 square grid in a garden in Etobicoke, southern Ontario in August 1995 and 1996. On a given day, I set up two populations separated by a distance of 30 m. Gene flow between the two populations was low (mean 2.5%, s.e.=0.6). Before flower buds opened in the morning, I pruned inflorescences to 7 flowers (occasionally 6 or 8) and partially emasculated flowers to generate the required morphs (Fig. 6-1). Over the two years of arrays, I performed eight different treatments which were replicated a minimum of three times each (range 3-6). These included isoplethic (1L:1S), moderately biased (2L:1S & 1L:2S), strongly biased (5L:1S & 1L:5S), and monomorphic (A & R) populations. To assess the plausibility of the second stage of the Lloyd and Webb (1992b) model, the evolution of an anther-height dimorphism, I performed a treatment with L-, S-styled, and T plants in a ratio of 2:1:1, respectively. As differences in siring ability could bias the results for this treatment, I performed pollen competition experiments to examine the relative success of legitimate (pollen from long-level anthers) and illegitimate pollen (from mid-level anthers) in fertilizing ovules of the L-morph. Four flowers on ten L-morph plants were pollinated with a mixture of long-and mid-level pollen of two different S-morph plants homozygous for alternate allozyme alleles. Each of the ten L-morph plants received pollen from different S-morph individuals. The plants were placed in a pollinator exclosure near the garden populations. When fruit matured, ten seeds from each capsule were assayed electrophoretically to determine the siring ability of mid- vs. long-level pollen. I used *G*-tests to compare the siring success of mid- and long-level pollen.

Measurement of fertility, mating patterns, and pollinator visitation

All experimental populations were exposed to pollinators until the flowers began to wilt (approx. six hrs.). At the end of each day, I painted the calyx of all flowers with acrylic paint to identify the day of the experiment. After 12-13 days (seed maturity), I recorded the numbers of fruit set and collected up to five fruits per plant. I counted seeds from a random sample of 1/3 of the plants in each experimental population, and three seeds from each fruit per each individual (or a total of 15 seeds per plant if fewer than 5 fruit) were assayed electrophoretically to quantify pollen transfer probabilities (see below) and estimate outcrossing rates. I used Ritland's (1990) maximum likelihood outcrossing rate (MLTR) program to estimate a morph-specific female outcrossing rate ($\hat{t}$) and standard error (based on 250 bootstraps) for all populations. The values for $\hat{t}$ were based on the PGI-2 locus, since within a floral morph, plants were chosen so that alternate homozygous genotypes were equally represented at this locus. The number of seeds scored varied among replicate populations (range=409 to 540) due to faint bands and reduced fruit set in some replicates.

To quantify pollen deposition on stigmas, I collected stigmas from one replicate of each of the A, R, and 1L:1S populations and preserved one stigma from each plant in the array in 70% ethanol. Stigmas were subsequently stained with 0.1% aniline blue in 0.1N K_3PO_4 and I counted the number of pollen grains adhered to the stigma using a compound microscope equipped with a fluorescent light.

To determine levels of pollinator activity among experimental populations, I recorded the length of time that bees visited a population during two to three hourly, 15-min observation periods. A focal bee was followed and I recorded the number of inflorescences visited and the number of flowers probed on each inflorescence. I also recorded the amount of time spent foraging on inflorescences in the A, R, and 1L:1S populations to determine if bees forage for different lengths of time on the floral phenotypes. To determine if seed set in experimental populations was limited by pollen receipt, I added pollen to one flower on 12 plants in each population by brushing a dehisced anther across the stigma using forceps. Flowers receiving the supplemental hand-pollination treatment were chosen in a ratio consistent with the morph ratio of the population.

I used replicated goodness of fit *G*-tests (Sokal & Rohlf, 1995) to determine if bees preferentially visited inflorescences of one morph in dimorphic populations and analysis of variance (ANOVA; Proc GLM, SAS 7.0, 1998) to examine if pollinator abundance differed among years, days, or location of the population. I also used ANOVA to determine if there were morph-specific differences in the mean number of flowers visited, mean per flower foraging time, and pollen limitation of seed set. In the pollen limitation analysis, I used a fully crossed design and the Type IV sum of squares since only one phenotype is represented in the A or R populations. I used paired contrasts to compare mean seed set of open- vs. supplemental pollinated flowers for each morph ratio treatment and adjusted the experimentwise error rate for multiple comparisons (Dunn-Sidák correction; Sokal & Rohlf 1995).

Mating patterns & female fertility in monomorphic vs. dimorphic populations

To examine whether sex-organ position in monomorphic populations influenced mating patterns and female fertility, I compared pollen loads, fruit and seed set, and outcrossing rates in A vs. R populations. To compare the number of pollen grains deposited on stigmas of A vs. R plants, I used a paired *t*-test. I used nested ANOVA to compare fruit and seed set and to determine if there was variation among replicates of the A or R treatments. I used a weighted one-way ANOVA to compare $\hat{t}$ in A vs. R populations. In all analyses of $\hat{t}$, I weighted the effect of each observation by the inverse of its squared standard error to account for variation in the estimates (Sokal & Rohlf 1995).

I was also interested in examining if there were differences in these components of reproductive success for the floral phenotypes in monomorphic vs. dimorphic populations. Similar analyses were employed: I used a paired *t*-test to examine differences in pollen loads on stigmas; nested ANOVA to compare fruit and seed set, and a weighted one-way ANOVA to compare $\hat{t}$ for each morph in monomorphic vs. dimorphic populations. Response variables were usually transformed (square-root or arc-sine) to improve the normality of the data, and means and standard errors were back-transformed for presentation.

Pollen transfer probabilities and conditions for the Lloyd and Webb model

The first stage of Lloyd and Webb's (1992b) model for the evolution of distyly involves the establishment of a stigma-height dimorphism. A specific set of pollen transfer conditions must be met. These are defined as q_{ij} and represent the quantity of pollen transferred from morph i to morph j. Pollen transfer requirements for the establishment of stigma-height dimorphism depend on whether seed set is pollen- or resource-limited. If seed set is resource-limited, intermorph pollen transfer must be greater than intramorph (i.e. $q_{sl} > q_{ll}$ for the invasion of the S-morph, and $q_{ls} > q_{ss}$ for the invasion of the L-morph where l and s represent the L- and S-morphs, respectively). If seed set is pollen-limited, the sum of pollen transfer between flowers of different morphs must, on average, be greater than the amount of intramorph transfer (i.e. $q_{sl} + q_{ls} > 2q_{ll}$ for the invasion of the S-morph, and $q_{sl} + q_{ls} > 2q_{ss}$ for the L-morph to invade).

To determine if pollen transfer patterns in experimental population supported these conditions, I quantified pollen transfer probabilities as the percentage of seeds assayed resulting from each of the four transfers. In dimorphic populations, plants of each morph were homozygous for one of two AAT-3 alleles. Thus seeds heterozygous at AAT-3 were the result of mating between the style morphs (q_{sl} and q_{ls}) while homozygous seeds were the result of selfing or intramorph mating (q_{ll} and q_{ss}). I calculated the proportion of mating events due to selfing vs. intramorph mating based on estimated selfing rates ($\hat{s}$, where $1\text{-}\hat{t} = \hat{s}$ and $\hat{t}$ was calculated as described above; and see Kohn & Barrett 1992) for each morph. Lloyd and Webb (1992b) assumed that selfing rates are equivalent among the L- and S-morph (see p. 191, Lloyd & Webb 1992b). I examined this assumption in each dimorphic population by pairwise comparisons of the 250 bootstrap estimates (for additional detail see Eckert & Barrett 1994; Ch. 4).

In the second stage of the Lloyd and Webb (1992a) model of the evolution of distyly, a T-plant replaces the S-morph (see Fig. 6-1 for phenotypes). Two pollen transfer conditions must be met for the evolution of this anther-height polymorphism ($q_{ss} > q_{ts}$ and $q_{tl} > q_{sl}$). To examine the validity of this stage of the Lloyd and Webb model (1992b), I calculated pollen transfer among the morphs as above. I used a G-test to compare pollen transfer probabilities with data from competitive pollinations to determine if differences in siring ability influenced pollen transfer probabilities in the experimental populations.

Calculation of morph fitness in dimorphic arrays

The Lloyd and Webb (1992b) model assumes that there is negative frequency-dependent fitness of the morphs. I calculated morph-specific total fitness as the number of seeds sired and produced by an individual plant using the following equations (modified from eq. 4 of Lloyd & Webb 1992b):

$$(1)\quad w_{female(i)} = freq_i{*}n_{ii} + freq_j{*}n_{ji}$$

$$(2)\quad w_{male(i)} = freq_i{*}n_{ii} + freq_j{*}n_{ij}$$

$$(3)\quad w_{total(i)} = 2freq_i{*}n_{ii} + freq_j(n_{ji}+n_{ij})$$

Equations 1 and 2 represent the female and male fitness, respectively, of morph i and eq. 3 is the total fitness (sum of eqs. 1 and 2); $freq_i$ is the frequency of the morph in the population and n_{ij} represents the total number of seeds produced by an individual of morph j that are sired by morph i. n_{ij} is calculated as the product of the pollen transfer probabilities (q_{ij}) and average seed production per individual of morph j. I used regression analysis (Proc REG, SAS 7.0, 1998) to examine the relation between the total fitness of each morph and the frequency of that morph in the population.

Results

Pollinator visits to experimental populations

The vast majority of pollinator visits (>95%) to the experimental populations were by *Bombus fervidus* and *B. vagans*. All analyses of bee behaviour included visits by these species only. Individual bee visits were dropped from the analysis if a bee visited less than five inflorescences in the population. Bee abundance, measured as average bee minutes per 15-min observation period, did not differ among years, days, or location of array (overall mean=2.25 min., upper s.e.=0.42, lower s.e.= 0.47; whole model ANOVA $F_{32,73}$=0.56, P=0.9512).

Pollinators did not discriminate between manipulated inflorescences or flowers of the style morphs. The total number of inflorescences visited of each morph did not differ significantly from the population morph ratio (p>0.0902 in all cases based on replicated G-tests). Nor did I observe a significant difference in the mean number of flowers visited per inflorescence of each style morph (nested within day, year, and treatment; overall mean=2.88, u.s.e.=0.044, l.s.e.=0.043, $F_{10,1260}$=1.03, P =0.4199) although the mean number of flowers visited differed among days (nested within year; $F_{11,1260}=3.33$, $P<0.001$). In two replicates of the monomorphic treatment, bees foraged significantly longer on R flowers than on A flowers (based on paired contrasts; A-mean=2.84s, s.e.=0.12, and 2.11s, s.e.=0.11, and R-mean=3.64s, s.e.=0.23, and 2.54s, s.e.=0.11) while there was no significant difference in the other monomorphic replicate (A-mean=2.75s, s.e.=0.14, R-mean=2.33s, s.e.=0.18, $P=0.06$), or in the dimorphic population (L-mean=2.75s, s.e.=0.19, S-mean=3.16s, s.e.=0.24, $P=0.17$).

Despite equivalent pollinator visits to flowers of each morph, seed set was pollen-limited in the experimental populations and the degree of pollen limitation differed among morphs and populations (Table 6-1, Fig. 6-2). The L-morph was pollen limited among all morph-ratio treatments except for those that were strongly S-biased whereas seed set in the S-morph was pollen-limited in this treatment only (Fig. 6-2).

Mating & female fertility in monomorphic and dimorphic populations

Sex-organ arrangement within a flower greatly influenced reproductive parameters as indicated by striking differences among A and R plants in monomorphic populations. Stigmas from R flowers had approx. four times as many pollen grains deposited on them as A stigmas (A=27.63, u.s.e=4.15, l.s.e.=3.86; R=116.0, u.s.e.=8.60, l.s.e.=8.29 and Fig. 6-3). Although there was significant heterogeneity in the replicate populations with each treatment, fruit and seed set were always greater in R than A plants (Table 6-2a, Fig. 6-3-seed set only). This may have been associated with the fact that R populations showed significantly higher levels of self-fertilization than A populations (A-$\hat{t}=0.790$, s.e.=0.011, and R-$\hat{t}=0.394$, s.e.=0.008; $F_{1,5}=806.93$, $P<0.001$; Fig. 6-3).

If stigma-height dimorphism functions to increase proficient pollen transfer, the morphs should differ in pollen loads, fecundity and mating patterns in monomorphic vs. dimorphic populations. Pollen loads increased significantly in the L-morph in dimorphic populations (mean$_{\mathrm{di}}$=46.62, u.s.e.=7.87, l.s.e.=7.27; mean$_{\mathrm{mono}}$=see above; $t_{52}=2.36$, $P<0.05$, Fig. 6-3), as did fruit set (mean$_{\mathrm{di}}$=0.752, s.e.=0.023, mean$_{\mathrm{mono}}$=0.640, s.e.=0.020; Table 6-2b, Fig. 6-3) and outcrossing rate (mean$_{\mathrm{di}}$ $\hat{t}=0.911$, s.e.=0.034, mean$_{\mathrm{mono}}$ $\hat{t}=0.790$, s.e.=0.011; $F_{1,7}=8.91$, $P<0.01$, Fig. 6-3). However, seed set was unchanged (mean$_{\mathrm{di}}$=37.42, u.s.e.=2.11, l.s.e.=2.04; mean$_{\mathrm{mono}}$=33.64, u.s.e.=1.72, l.s.e=1.68; Table 6-2b, Fig. 6-3). A different pattern emerged in the S-morph: there was no difference in pollen loads between monomorphic vs. dimorphic

Table 6-1: Pollen limitation of seed set in experimental arrays of modified *Eichhornia paniculata*. Experimental populations varied in morph ratio (see text for further details). Pollination treatment was open- vs. supplemental hand-pollination.

Source	d.f.	MS	*F*	*P*
Morph	1	1.29	0.33	0.5661
Morph ratio	6	32.48	8.31	<0.001
Morph × morph ratio	4	8.37	2.14	0.0734
Pollination treatment	1	726.75	185.87	<0.001
Morph × poll. trt.	1	171.31	43.81	<0.001
Morph ratio × poll. trt.	6	11.09	2.84	0.0094
3-way interaction	4	20.35	5.20	<0.001

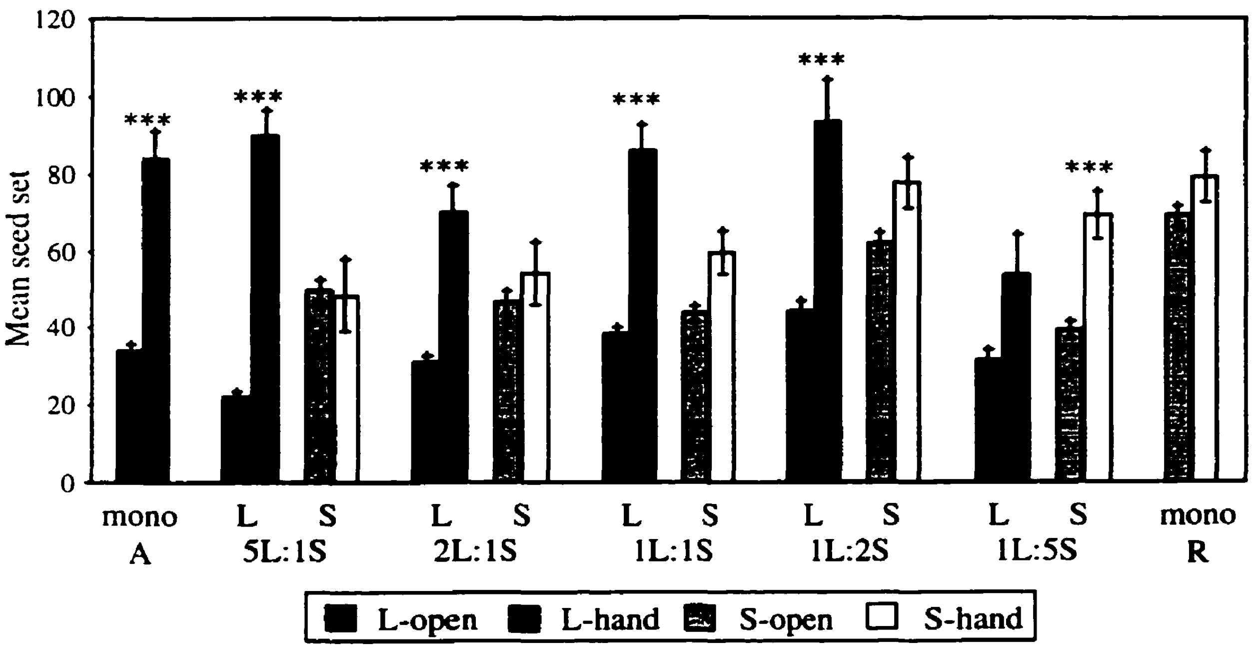


Figure 6-2: Pollen limitation of seed set in experimental populations of *Eichhornia paniculata* modified to exhibit a stigma-height dimorphism. Pollen limitation of seed set typically occurs in L-styled plants but rarely in S-styled plants. Significant increases in seed set with supplemental hand-pollination are indicated using asterisks (***P<0.001; following Dunn-Sidák's correction for multiple comparisons). Error bars are ± 1SE.

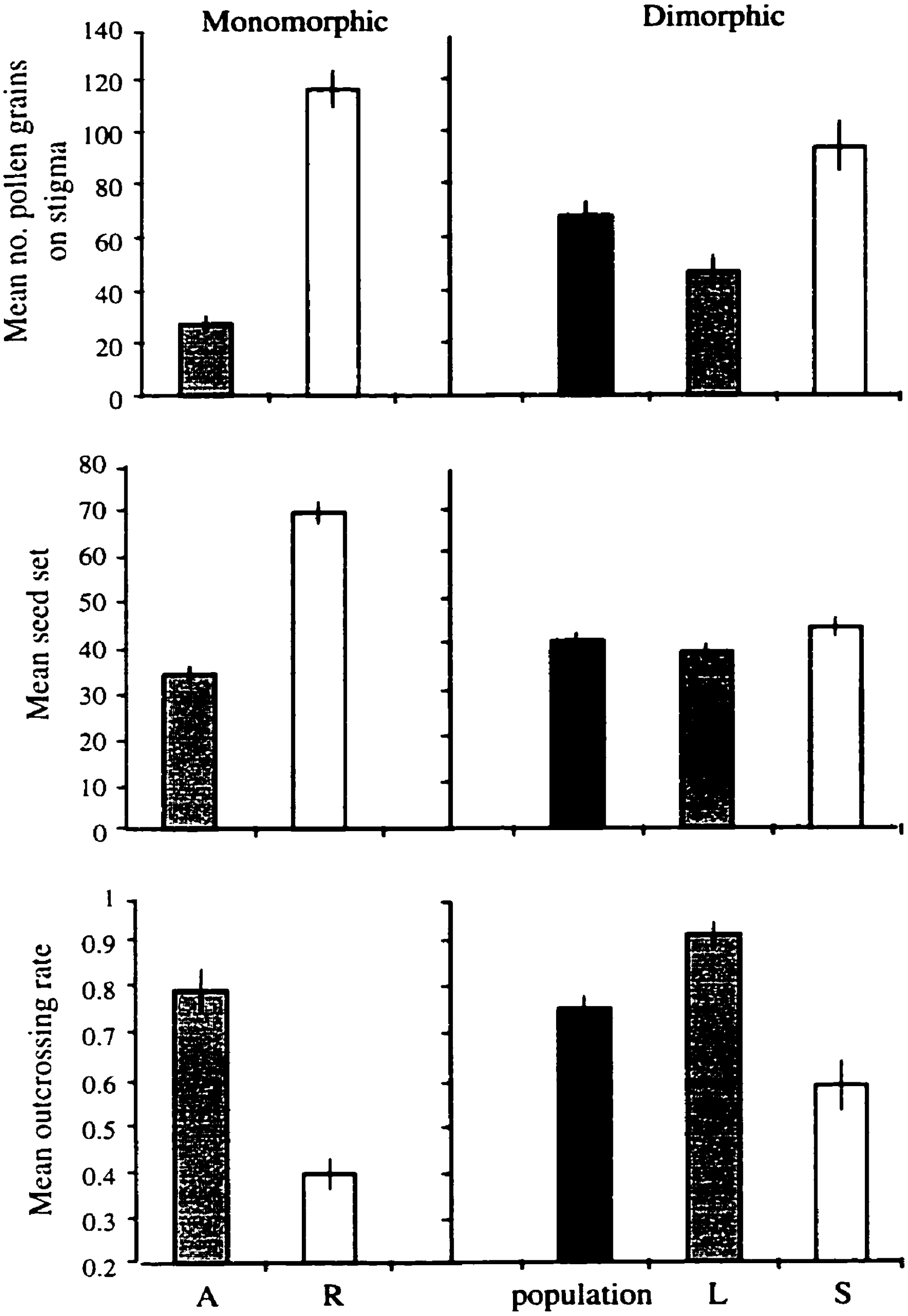


Figure 6-3: Morph-specific stigmatic pollen load, seed set, and outcrossing rate in monomorphic vs. dimorphic populations of *Eichhornia paniculata*. Error bars are ± 1SE.

Table 6-2: Fruit and seed set in experimental populations of modified *Eichhornia paniculata*. Nested analysis of variance of fruit set and seeds per fruit in (a) monomorphic populations and (b) each morph in monomorphic versus dimorphic populations (type).

Response: Fruit set					Response: Seed set			
(a) Monomorphic populations								
Source	df	MS	*F*	*P*	df	MS	*F*	*P*
Morph	1	2.19	55.96	<0.001	1	447.4	151.7	<0.001
Pop (Morph)	4	0.22	5.51	<0.001	4	11.7	3.97	0.0037
Error	195	0.04			297	3.0		

(b) Monomorphic vs dimorphic populations

Response: Fruit set

		L-morph				S-morph		
Source	df	MS	*F*	*P*	df	MS	*F*	*P*
Type	1	26.12	9.82	0.0020	1	26.97	17.29	<0.001
Pop (Type)	7	11.01	4.14	<0.001	7	23.18	14.86	<0.001
Error	194	2.66			195	1.56		

Response: Seed set

		L-morph				S-morph		
Source	df	MS	*F*	*P*	df	MS	*F*	*P*
Type	1	7.54	1.60	0.2065	1	236.5	83.88	<0.001
Pop (Type)	4	47.76	10.14	<0.001	4	43.43	15.40	<0.001
Error	300	4.71			325	2.82		

populations (mean$_{di}$=93.31, u.s.e.=11.02, l.s.e.=10.40; t_{50}=1.63, P=0.1052, Fig. 6-3); and fruit and seed set of the S-morph decreased significantly in dimorphic populations (fruit set: mean$_{di}$=0.74, s.e.=0.028; mean$_{mono}$=0.850, s.e.=0.020; seed set: mean$_{di}$=43.06, u.s.e.=1.72, l.s.e.=1.68, mean$_{mono}$=68.25, u.s.e.=2.27, l.s.e.=2.23 and Table 6-2b, Fig. 6-3). Similar to the L-morph, the S-morph had a significantly higher outcrossing rate in dimorphic than monomorphic populations (dimorphic $\hat{t}$=0.582, s.e.=0.045, $F_{1,7}$=12.69, P<0.001; Fig. 6-3).

Pollen transfer and the maintenance of stigma-height dimorphism

Lloyd and Webb (1992b) suggest that for a stigma-height dimorphism to be maintained, each morph must be more proficient at cross-pollination with the opposite morph than with itself. I examined the relative proficiency of inter- to intramorph outcrossing by comparing estimates of q_{sl} and q_{ls} to q_{ll} and q_{ss}, respectively (Table 6-3). The data indicate that intermorph outcrossing is greater than intramorph outcrossing in most cases. In fact, the S-morph is more proficient at cross-pollination with the L-morph than with other S-plants for all six replicates, while intermorph is more proficient than intramorph mating in only three of the six cases for the L-morph (Table 6-3). Overall, this indicates that under most conditions, a stigma-height dimorphism would be maintained. Intermorph pollen transfer is generally greater from the S- to the L-morph than from the L- to the S-morph.

Morph-specific mating patterns and negative frequency-dependent fitness

Mating patterns and fertility in populations with biased morph ratios were similar to those obtained in monomorphic and isoplethic populations. Overall, the selfing rate of the S-morph is greater than the L-morph in all morph ratio treatments (L=0.233, s.e.=0.030, and S=0.469, s.e.=0.03; $F_{1,17}$=27.29, P<0.001; see Fig. 6-4). This occurred in 12 of the 22 experimental populations. In nine populations morph-specific selfing rates were equivalent and in one population, the L-morph had a greater selfing rate than the S-morph (based on paired comparisons of bootstrap values). The S-morph consistently had higher fertility than the L-morph ($F_{1,743}$=33.40, P<0.001 for fruit set, and $F_{1,1134}$=385.10, P<0.0001 for seed set; Fig. 6-5). Mean fruit and seed set varied among morph ratio treatments ($F_{4,743}$=7.26, P<0.001 for fruit set, and $F_{4,1134}$=18.19, P<0.001 for seed set) and among replicate populations for each morph ratio treatment ($F_{17,743}$=6.85, P<0.001 for fruit set, and $F_{17,1134}$=7.33, P<0.001 for seed set).

Table 6-3: Proficiency of inter- and intramorph pollen transfers in isoplethic populations of *Eichhornia paniculata* modified to exhibit a stigma-height dimorphism. The notation q_{ij} is adopted from Lloyd and Webb (1992b) and represents pollen transfer probabilities from morph i to j. I estimated q_{ij} from the genotypes of seeds produced.

Rep	q_{ll}	q_{sl}	q_{ls}	q_{ss}	$q_{sl}>q_{ll}$	$q_{ls}>q_{ss}$
1	31.13	21.62	32.32	14.92	no	yes
2	24.84	36.06	27.24	11.86	yes	yes
3	32.44	24.98	28.00	14.59	no	yes
4	30.15	28.64	24.49	16.73	no	yes
5	30.61	35.01	18.55	15.83	yes	yes
6	22.74	32.85	24.75	19.66	yes	yes
mean	28.65	29.86	25.89	15.60		
s.e.	1.59	2.36	1.87	1.05		

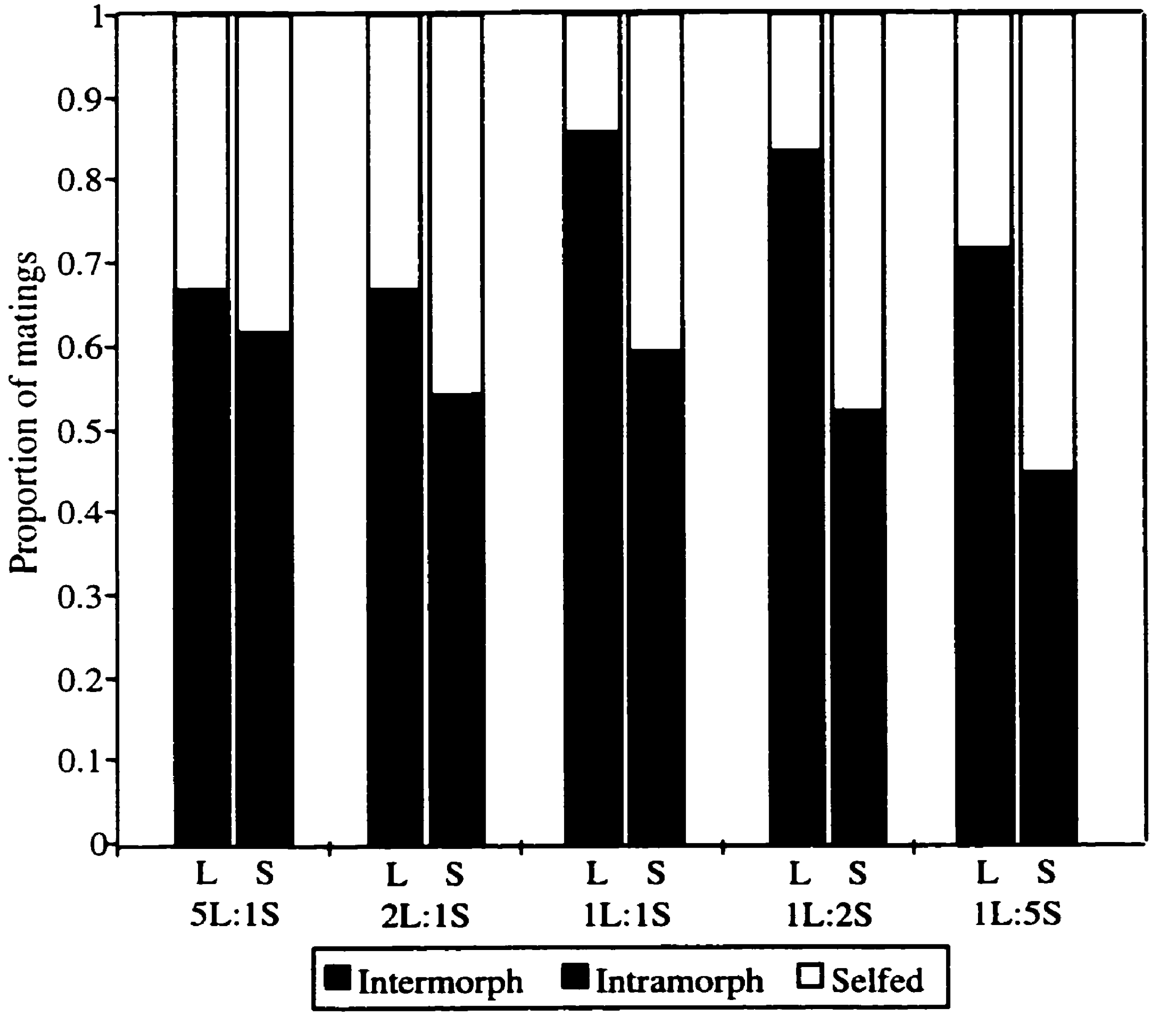


Figure 6-4: Proportion of seeds produced from self, inter- and intramorph mating for each morph of *Eichhornia paniculata* modified to exhibit a stigma-height dimorphism.

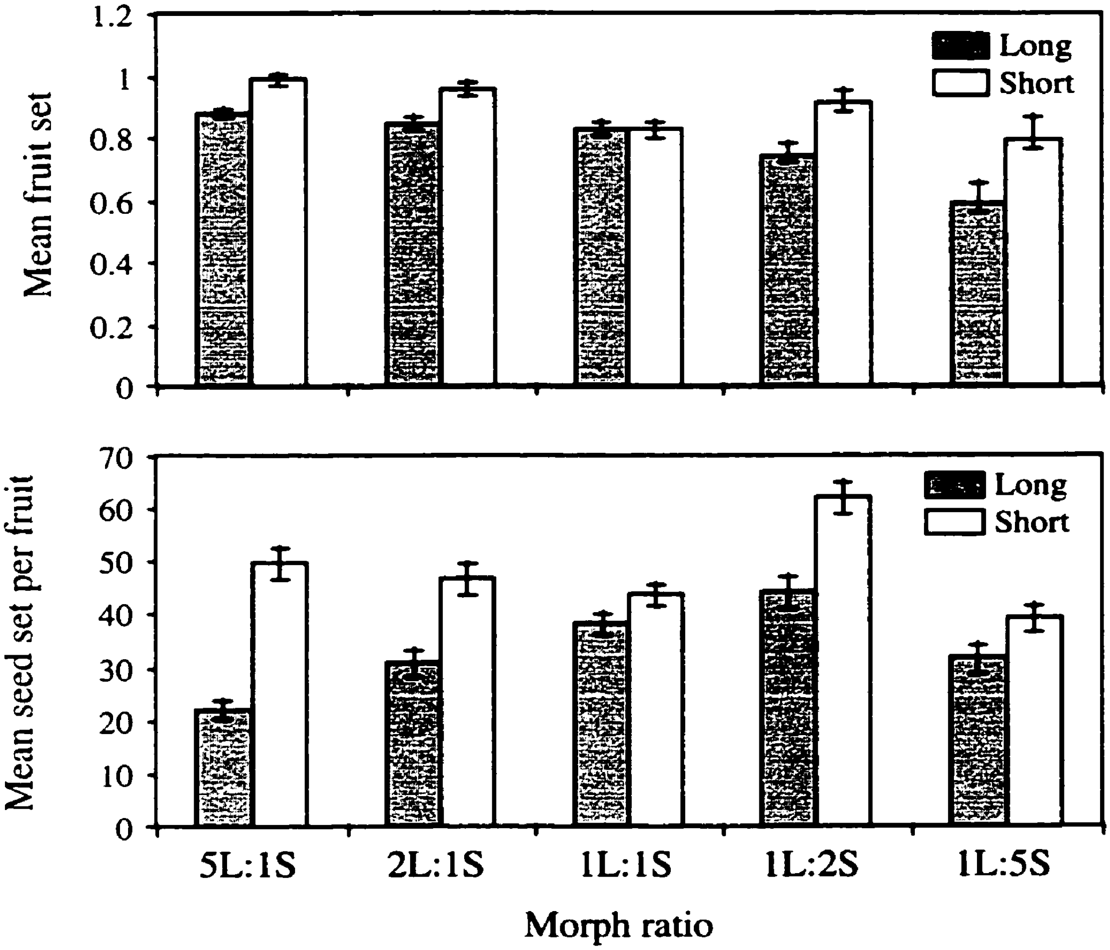


Figure 6-5: Morph-specific female fertility in populations of *Eichhornia paniculata* modified to exhibit a stigma-height dimorphism. Error bars are ± 1SE.

The variation in female fertility complicated the measurement of total fitness since the S-morph consistently produced more fruit than the L-morph. I adjusted absolute fitness measures to account for the morph-specific differences in female fertility using analysis of covariance (ANCOVA; Kirk 1995). I present the total fitness of an individual of each morph from outcrossing only, as well as from selfing and outcrossing combined. Figure 6-6 shows the relation between the average total fitness of an individual of each morph and the frequency of the morph in the population. In all cases, the slope of the line representing the relation is significantly less than zero ($P<0.001$) indicating that there is negative frequency-dependence of fitness in each of the morphs. I compared the slopes of the lines for fitness among the morphs to determine if the strength of negative frequency-dependence differed. When all fertilizations were considered, the negative frequency-dependence of fitness was stronger in the S-morph than the L-morph (outcross+self fitness: $F_{1,40}=23.03$, $P<0.001$ following ANCOVA). There was no difference when fitness from outcrossing only was examined ($F_{1,40}=2.07$, $P=0.158$).

Pollen transfer and the selection of a stigma-height dimorphism

In examining the likelihood that a mutant morph could invade a population, I also considered the pollen transfer inequalities based both on outcrossing events only, and on all fertilizations (i.e. outcrossed and selfed). The estimates of pollen transfer probabilities based only on outcrossing support the plausibility of the Lloyd and Webb (1992b) model (Table 6-4; and see Appendix B for data for all replicates). In populations which simulated the invasion of the S-morph (5L:1S & 2L:1S), the conditions for the successful invasion were satisfied in all cases under pollen limitation and in seven of the eight trials under resource limitation (Table 6-4a). Similarly, pollen transfer inequalities in populations simulating the invasion of an L-morph (1L:5S & 1L:2S), supported the invasion in all cases under pollen limitation and in six of the eight replicates under resource limitation (Table 6-4b).

Table 6-5 (see Appendix B for data from all replicates) presents mean estimated pollen transfer probabilities with self pollen transfer included (in q_{ll} and q_{ss} for the L- and S-morphs, respectively). In only five of the eight replicates would an S-morph successfully invade a population of the L-morph for the pollen-limited case. Under resource limitation, successful invasion occurred in only one of the eight replicates (Table 6-5a). The pollen transfer inequalities for the invasion of an S-morph population by an L-morph are only met six and zero times out of

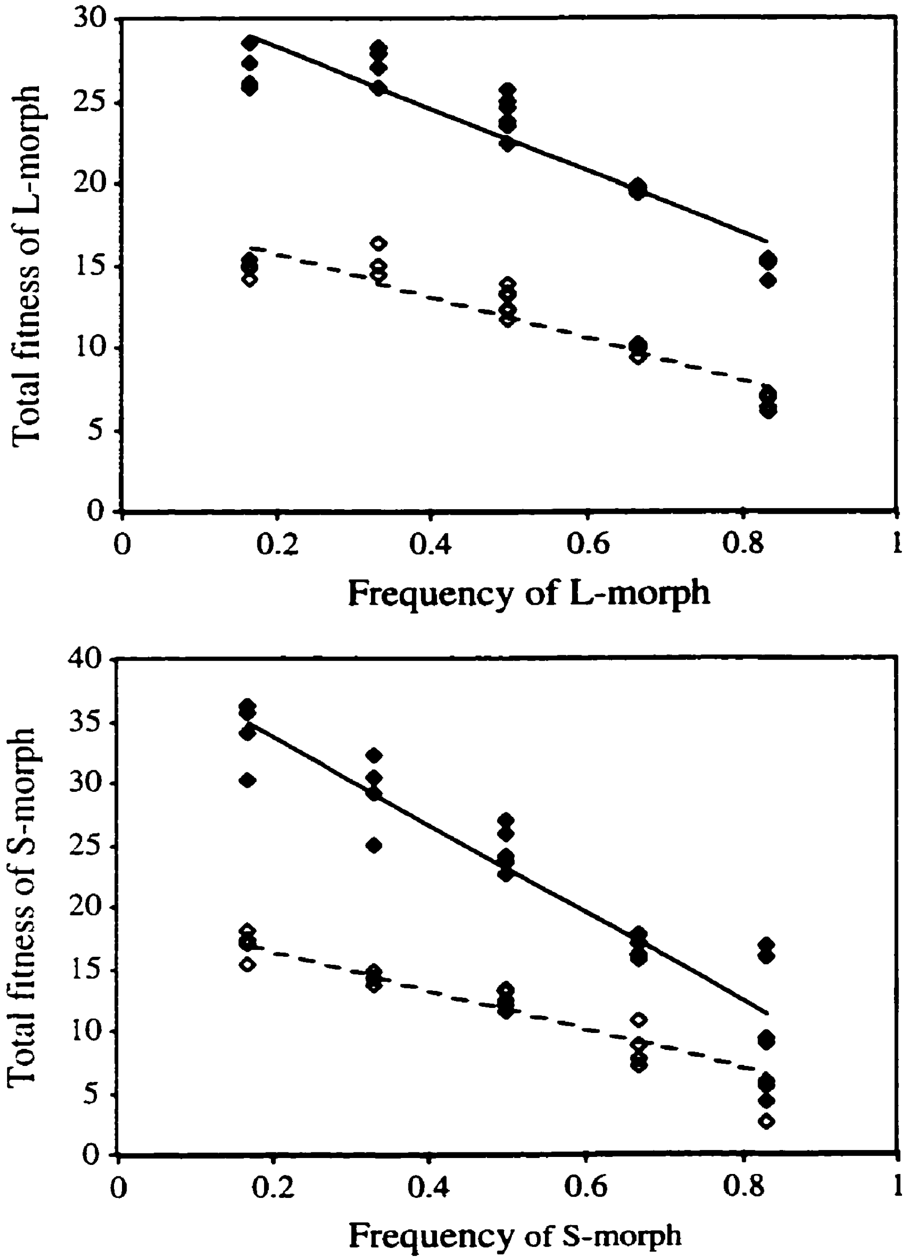


Figure 6-6: Negative frequency dependence of fitness resulting from outcrossing (◊) and outcrossing and selfing combined (◆) in the (a) L-morph and (b) S-morph in modified *Eichhornia paniculata*. The regression equations, their significance level, and the proportion of variance in fitness explained by the model are as follows: L-morph$_{\text{outx}}$: y=18.37-13.01x, P<.0001, r^2=0.87; L-morph$_{\text{outx+self}}$: y=32.14-18.95x, P<.0001, r^2=0.86; S-morph$_{\text{outx}}$: y=19.60-15.69x, P<.0001, r^2=0.85; S-morph$_{\text{outx+self}}$: y=40.90-35.66x, P<.0001, r^2=0.87.

Table 6-4: Mean pollen transfer probabilities based on outcrossing events only in (a) L-biased and (b) S-biased populations of *Eichhornia paniculata* modified to exhibit a stigma-height dimorphism. *qij* represents outcross pollen transfer probabilities from morph *i* to morph *j* and was inferred from the genotypes of seeds. I compare my results with the conditions for the invasion of a mutant morph outlined in Lloyd and Webb (1992b) and indicate the fraction of replicates in which Lloyd and Webb's pollen transfer conditions were met. Data for each replicate is presented in Appendix B.

(a)						Conditions for invasion by S-morph	
						Pollen-limited	Resource-limited
Trt		q_{ll}	q_{sl}	q_{ls}	q_{ss}	$q_{ls} + q_{sl} > 2q_{ll}$	$q_{sl} > q_{ll}$
5L:1S	mean	8.70	10.17	41.72	39.41	4/4	3/4
	s.e.	0.94	1.21	7.00	6.86		
2L:1S	mean	16.28	23.00	32.46	28.27	4/4	4/4
	s.e.	1.76	2.77	2.34	1.94		

(b)						Conditions for invasion by L-morph	
						Pollen-limited	Resource-limited
Trt		q_{ll}	q_{sl}	q_{ls}	q_{ss}	$q_{ls} + q_{sl} > 2q_{ss}$	$q_{ls} > q_{ss}$
1L:5S	mean	47.44	39.68	8.17	4.71	4/4	4/4
	s.e.	5.16	4.59	0.33	0.96		
1L:2S	mean	31.59	43.30	13.56	11.56	4/4	2/4
	s.e.	4.66	2.52	1.36	2.33		

Table 6-5: Mean pollen transfer probabilities in (a) L-biased and (b) S-biased experimental populations of *Eichhornia paniculata* modified to exhibit a stigma-height dimorphism. *qij* was estimated from the genotypes of seeds and represents the pollen transfer probabilities from morph *i* to *j* and includes self pollen transfers. I compare the results with the Lloyd and Webb model (1992b) and indicate the fraction of times that their pollen transfer conditions are met. Data for each replicate is present in Appendix B.

(a)						Conditions for invasion by S-morph	
						Pollen-limited	Resource-limited
Trt		q_{ll}	q_{sl}	q_{ls}	q_{ss}	$q_{ls} + q_{sl} > 2q_{ll}$	$q_{sl} > q_{ll}$
5L:1S	mean	5.47	3.96	16.50	74.07	4/4	1/4
	s.e.	0.53	0.48	3.11	3.45		
2L:1S	mean	16.29	12.35	18.51	52.86	1/4	0/4
	s.e.	0.74	0.70	3.56	4.35		

(b)						Conditions for invasion by L-morph	
						Pollen-limited	Resource-limited
Trt		q_{ll}	q_{sl}	q_{ls}	q_{ss}	$q_{ls} + q_{sl} > 2q_{ss}$	$q_{ls} > q_{ss}$
1L:5S	mean	70.76	19.27	4.12	5.86	4/4	0/4
	s.e.	2.58	2.07	0.56	0.30		
1L:2S	mean	39.49	30.60	9.49	20.42	2/4	0/4
	s.e.	2.58	2.28	0.69	0.78		

eight, respectively, for the pollen and resource-limited cases (Table 6-5b). This finding indicates that the morph-specific differences in self-fertilization (overall L-$\hat{s}$=0.233, s.e.=0.030, and S-$\hat{s}$=0.469, s.e.=0.03) in populations of *E. paniculata* make it difficult for a stigma-height dimorphism to become established.

Although general trends are evident when simply counting the number of times that the pollen transfer inequalities of the Lloyd and Webb (1992b) model are satisfied, it is important to examine the data using a statistical approach. There are complications that make statistical analyses difficult and I outline the alternatives here. One potential analysis involves the use of ANOVA to examine mean pollen loads across replicates. However, since replicate populations show variation in the pollen transfer probabilities (Table 6-3, 6-4, and 6-5), this approach is problematic because it suggests that excess pollen donated to one flower can be transferred to another (see Stone & Thomson 1994). An alternative is a sign test. However because this test requires a minimum of six replicates and in six paired comparisons, all differences must be of the same sign for the test to be significant at the 5% level (Sokal & Rohlf 1995). Six replicate populations were used to examine pollen transfer in isoplethic populations but only four replicates of the various biased treatments (5L:1S, 2L:1S, 1L:2S, and 1L:5S) were conducted. It may be valid to combine the biased replicates in an analysis so that there are eight L-biased and eight S-biased replicates. These alternative analyses are currently being explored.

Establishment of an anther-height polymorphism

The pollen transfer data for the second stage of the Lloyd and Webb (1992b) model support the two pollen transfer inequalities ($q_{ss}>q_{ts}$ and $q_{tl} > q_{sl}$) required for the establishment of an anther-height polymorphism. In each of four replicate populations, both of these inequalities were satisfied (mean values are q_{ss}=7.20, s.e.=0.23 and q_{ts}=5.16, s.e.=0.46; and q_{tl}=10.32, s.e.=1.45 and q_{sl}=2.43, s.e.=1.23). The pollen transfer results for testing the inequality $q_{tl} > q_{sl}$ are complicated by the fact that long-level pollen of the S-morph sires a larger proportion of the seeds on L-styled plants than competing mid-level pollen (approx. 55%; G_{pooled}=4.63, df=1, P<0.05). The pollen transfer data indicate that pollen from the long-level anthers (i.e. the T-morph) sires approx. 80% of the seeds produced by intermorph mating to the L-morph and the S-morph sires 20%. This value is significantly higher than found in the pollen competition experiment ($G_{heterogeneity}$=13.8, df=1, P<0.001) suggesting that the observed effects are not only due to differences in siring ability but are also a function of more efficient pollen transfer from the T- to the L-morph because of the reciprocity of stigmas and anthers.

Discussion

One of the goals of this study was to examine the influence of sex-organ position on mating patterns and fertility in monomorphic and dimorphic populations. This was undertaken to understand the functional significance of stigma-height dimorphism. In monomorphic and dimorphic populations, I found consistent morph-specific differences in selfing rates and fertility (Fig. 6-3). Estimates of pollen transfer in dimorphic populations demonstrated that intermorph pollen transfer was more proficient than intramorph transfer. This suggests that a polymorphism could be maintained with these mating patterns. Another goal of the study was to estimate pollen transfer within and among the morphs to assess conditions required for Lloyd and Webb's (1992b) model for the evolution of distyly with stigma-height dimorphism as an intermediate stage. Conclusions regarding the ability of a mutant morph to invade a population differed depending on whether self-fertilization was included in the estimates of pollen transfer. I discuss the implications of these findings in relation to the various models of the evolution of distyly. However, I first discuss the validity of using modified tristylous flowers to examine the influence of sex-organ position on reproduction and to test hypotheses about the functional significance of stigma-height dimorphism.

The use of modified tristylous Eichhornia paniculata *flowers*

The experiments described here involved floral manipulations of the long- and short-styled morphs of tristylous *E. paniculata* in an effort to simulate a population with stigma-height dimorphism. I used this approach because of the difficulties of employing natural populations of a species with stigma-height dimorphism discussed above. An important question to address before interpreting the data is whether the simulated phenotypes that I created through stamen removal capture a sufficient amount of biological realism so that inferences of pollen transfer patterns can be made to natural populations with stigma-height dimorphism. While I cannot answer this question definitively, several lines of evidence suggest that the experimental populations were similar to species possessing stigma-height dimorphism. First, the levels of intermorph pollen transfer that I measured would be sufficient to maintain the polymorphism

(Table 6-3; discussed in more detail below). Secondly, like *E. paniculata* in my experimental populations, most stigma-height dimorphic species are pollinated by bees (Ch. 2), and bees do not discriminate among the style morphs. This was also true in Kohn and Barrett's (1992) study of tristylous *E. paniculata* at the same location. Finally, since *E. paniculata* is heterostylous, it is possible that the species may have had a stigma-height dimorphism in its evolutionary history.

Inferences from this study to pollen transfer in natural populations are complicated by the morph-specific differences in mating patterns and pollen limitation in the experimental populations. Female selfing rates were considerably higher in the S-morph and probably because of this, the S-morph was less often pollen-limited than the L-morph.(Figs. 6-2, 6-3). There is little empirical data on natural populations of stigma-height dimorphic species to evaluate how common morph-specific differences occur in mating patterns and fertility. Data available for *Narcissus* spp. failed to detect morph-specific differences in female fertility (Ch. 4), female outcrossing rate (Ch. 4), or pollen-limitation (Ch. 5). However, differences in stigma position among the morphs are believed to strongly influence pollen transfer and mating within and among the morphs as argued in Chapter four. Furthermore, in distylous species there are many examples of morph-specific differences in components of reproduction (reviewed in Ganders 1979a, Barrett 1992a). Differences in components of reproduction do not invalidate the experiments described here as these are a common feature of polymorphic sexual systems, although these differences complicate data interpretation and evaluation of the Lloyd and Webb (1992a,b) model.

Effects of sex-organ position on reproduction

This is the first study to directly compare the reproductive consequences of approach and reverse herkogamy. The spatial separation of stigmas and anthers has been interpreted as a mechanism to reduce the interference of male and female function (e.g. Webb & Lloyd 1986) yet few studies have assessed the reproductive consequences of herkogamous floral designs. The most obvious consequence of self-interference is self-pollination. However, other effects also include the interference of pollen removal and receipt, stigma or stylar clogging with self pollen or pollen tubes, and ovule discounting (reviewed in Barrett *et al.* 2000b). Populations of *E. paniculata* modified to exhibit approach herkogamy experienced reduced fertility and higher outcrossing rates when compared with populations modified to exhibit reverse herkogamy (Fig.

6-3). This suggests that the long-styled morph of *E. paniculata* is more effective at preventing self-interference, especially self-fertilization, than the S-morph (and see Kohn & Barrett 1992). Webb and Lloyd (1986, p.172) speculated that approach herkogamy would be more effective in reducing self-interference than reverse herkogamy and the data from this study supports this hypothesis although the specific mechanisms involved are not known.

Numerous studies have suggested that the degree of stigma-anther separation has significant functional consequences on mating patterns (see Belaousoff & Shore 1995 and references contained within). However, it is unlikely that this explains the results found in modified *E. paniculata* since the degree of stigma-anther separation is similar in both the approach and reverse morph (from data on sex-organ position of intact flowers in Barrett 1985). Furthermore, since outcrossing rates in the monomorphic populations of modified *E. paniculata* are similar to those obtained in unmodified plants (Kohn & Barrett 1992), it is unlikely that manipulations influenced the manner in which pollinators positioned their body in comparison with tristylous flowers. In hypothesizing a mechanism for promoting legitimate cross-pollination in species with stigma-height dimorphism, Lloyd and Webb (1992b) suggested that the "free path" taken by the proboscis of the pollinators differs between the morphs because the position of the floral organs affects which portions of the proboscis comes into contact with the floral organs. It is possible that the manner in which *E. paniculata* flowers are probed differs between L- vs. S-styled flowers in a way that results in higher levels of self-pollination in flowers with short-level stigmas. Careful studies of pollen pickup by bees are required to test this hypothesis. This approach was used by videotaping bee visits by Stone & Thomson 1994.

Functional significance of stigma-height dimorphism

The hypothesis that stigma-height dimorphism promotes proficient cross-pollen transfer between the morphs was partially supported by the data from experimental populations of modified *E. paniculata*. In support of the hypothesis were the findings that estimated intermorph pollen transfers were typically greater than intramorph transfers (Table 6-3), outcrossing rates were greater for both morphs in dimorphic populations, and overall fertility and pollen loads on stigmas increased for the L-morph (Fig. 6-3) in dimorphic vs. monomorphic populations. If stigma-height dimorphism functions to promote a significant amount of pollen transfer between the morphs, an increase in pollen deposition rates and subsequent seed set in the S-morph should have also been observed. In fact, female fertility of the S-morph was reduced in dimorphic populations. Additionally, q_{ss} is consistently the least proficient outcross pollen transfer probability (Table 6-3) in dimorphic populations. However, the finding that more pollen is donated to the L-styled morph with the introduction of S-styled plants suggests that the presence of the S-styled mutant increases male fitness through more proficient pollen transfer from the S- to the L-morph.

Implications for models of the evolution of distyly

The models for the evolution of distyly proposed by Lloyd and Webb (1992a,b), Charlesworth and Charlesworth (1979), and Richards (1998) differ primarily in the assumed ancestor and in the order that reciprocal herkogamy and heteromorphic incompatibility evolve. Lloyd and Webb (1992a) assume that the ancestral condition was a population exhibiting approach herkogamy while Charlesworth and Charlesworth (1979) and Richards (1998) assume a non-herkogamous ancestor. The Charlesworths (1979) emphasize that selection on pollen receipt does not favour a stigma-height dimorphism in a self-compatible population, although they do state that a stigma-height dimorphism can evolve in a self-compatible population with reduced levels of selfing (Charlesworth & Charlesworth, 1979, p. 475). Because of this finding, they prefer the hypothesis that the ancestor is a homostylous plant which first evolves diallelic incompatibility whereas Richards (1998), who also assumes a homostylous ancestor, suggests that reciprocal herkogamy arises before the incompatibility system. Lloyd and Webb (1992a,b) conclude that a stigma-height dimorphism can become established in a fully outcrossing or partially selfing population.

Estimated outcross pollen transfers in experimental populations (Table 6-4) often met the inequalities required for the establishment of a stigma-height dimorphism (Lloyd & Webb 1992b), particularly when seed set was pollen-limited. Furthermore, negative frequency-dependence of fitness in both morphs was evident (Fig. 6-6), since when a morph was rare, individuals of that morph had higher fitness (total number of seeds sired and produced) than when the morph was common (and see Lloyd and Webb 1992b). This was true for the fitness of both morphs. Only outcross pollen transfers are relevant for examining the conditions for the second stage of the Lloyd and Webb model, the establishment of an anther-height polymorphism. Similar to the finding for the first stage of the model, the data support the necessary pollen transfer inequalities. This stage was described as "easy to achieve" by Lloyd & Webb (1992b, p. 194).

However, in deriving the pollen transfer inequalities for the establishment of a stigma-height dimorphism, Lloyd and Webb (1992b) assumed that selfing rates were equivalent among the morphs. In the experimental populations of modified *E. paniculata*, this assumption was satisfied in only nine of 22 comparisons. When self-pollen transfers were included in estimates of pollen transfer, the likelihood of the establishment of stigma-height dimorphism were met less often (Table 6-5). Selfing was greater in the S-morph than the L-morph in twelve of the experimental populations. If levels of intermorph mating were also sufficiently high this would increase the likelihood of invasion of an approach herkogamous population by the S-morph (Lloyd & Webb 1992b). These conditions are satisfied in Fig. 6-6, where the slope of the line representing negative frequency-dependent fitness of the S-morph is steeper than for the L-morph. The conditions for invasion under resource limitation were rarely satisfied while the pollen transfer inequalities required under pollen limitation of seed set were met more often. Lower and equivalent levels of selfing among the morphs would undoubtedly increase the number of times in which the conditions for the establishment of a stigma-height dimorphism were satisfied.

Do the estimates of pollen transfer in dimorphic populations support the Lloyd and Webb model? Lloyd and Webb (1992b, pp. 191-192) recognize that differences in selfing among the morphs would complicate the establishment of a stigma-height dimorphism. Although I observed this, I cannot conclude that my results necessarily falsify the Lloyd and Webb (1992b) model for the evolution of distyly. This is because the estimates of pollen transfer based only on outcross mating events typically meet the inequalities established by Lloyd and Webb (1992b). It is thus likely that natural populations with stigma-height dimorphism likely exhibit low rates of selfing or it would be difficult for the dimorphism to be maintained. Further studies are required to examine the floral biology and mating patterns in species exhibiting stigma-height dimorphism to assess how significant a role the relative amounts of outcrossing and selfing play in the establishment of the polymorphism and its subsequent role in the evolution of distyly.

CHAPTER 7

CONCLUDING DISCUSSION

In this thesis, I examined the ecology and evolution of stigma-height dimorphism. This plant sexual polymorphism has most commonly been described as an unusual form of distyly, or as an intermediate condition that is quickly passed through during the evolution of distyly. I compared and contrasted features of stigma-height dimorphism with the more common distylous syndrome (Chapter 2). I also documented the nature of stigma-height dimorphism in two species of *Narcissus* by examining floral morphology and population morph ratios (Chapter 3). In Chapter 4, I used empirical studies of mating patterns and female fertility in natural populations and computer simulations of mating to examine the likely evolutionary forces maintaining stigma-height dimorphism in *Narcissus* populations. Since pollen limitation is a component of a pollen transfer model for the evolution of distyly (Lloyd & Webb 1992b), I also examined a stigma-height dimorphic species for evidence of pollen limitation across spatial and temporal scales (Chapter 5). Finally, I used floral manipulations and experimental garden arrays of the annual herb *Eichhornia paniculata* to examine pollen transfer and morph-specific fitness in a population with stigma-height dimorphism. These data were used to assess the Lloyd and Webb (1992b) model for the evolution of distyly. In the following discussion, I begin by summarizing the major findings of my research and then present ideas for future research related to my studies.

General conclusions

Stigma-height dimorphism as a plant sexual polymorphism

A goal of this thesis was to compare and contrast features of stigma-height dimorphism with distyly. While the more common sexual polymorphisms distyly and tristyly have been studied intensively for well over a century, little was known about stigma-height dimorphism. The dimorphism was most commonly believed to be an ephemeral stage in the evolution of distyly, not an evolutionarily stable mating strategy. This thesis is the first comprehensive attempt to synthesize reports of unusual or anomalous cases of distyly, and suggest that the taxa with these traits share features which merit the recognition of stigma-height dimorphism as a distinct polymorphism. The defining feature of heterostyly is reciprocal herkogamy (Richards 1986; Lloyd *et al.* 1990; Lloyd & Webb 1992a,b; Barrett *et al.* 1997). This feature is absent from populations with stigma-height dimorphism (Figs. 2-4, 2-5). Features that usually occur in heterostylous species include a heteromorphic incompatibility system, and ancillary polymorphisms of the anthers and stigmas (reviewed in Ganders 1979a; Barrett 1992a; Richards 1997). Morph-specific differences in pollen size and stigmatic features are rarely associated with stigma-height dimorphism. Furthermore, in all taxa for which incompatibility reactions have been investigated, inter- and intramorph pollinations produce equivalent numbers of seed. Some species with stigma-height dimorphism exhibit self-compatibility (e.g. *Narcissus dubius*, *Primula boveana*) but most are self-sterile (Table 2-1).

Heterostylous species typically exhibit 1:1 morph ratios because of heteromorphic incompatibility and disassortative mating among the floral morphs (reviewed in Barrett 1992b). Isoplethic morph ratios also occur in some taxa with stigma-height dimorphism, indicating that high and equivalent levels of disassortative mating can occur in some populations even in the absence of heteromorphic incompatibility. However, morph ratios of stigma-height dimorphic species were commonly biased for the L-morph (Table 2-1). This suggests that assortative mating among plants of the L-morph occurs more often than other possible matings. This likely occurs because stigma-anther separation is commonly reduced in the L-morph compared to the S-morph. This difference favours more efficient pollen pick-up and deposition from anthers and stigmas, respectively, of the L-morph facilitating high levels of intramorph transfer.

Stigma-height dimorphism in Narcissus

I studied the reproductive ecology and evolution of stigma-height dimorphism in populations of *Narcissus* spp. (*N. assoanus* – section *Jonquillae* and *N. dubius* – section *Tazettae*) since stigma-height dimorphism is particularly well-established in the genus, occurring in at least a dozen species in three sections. Although the two species show similar patterns of sex-organ deployment, they differ in their incompatibility status, outcrossing rates, and population morph ratios. In both species, stigma-anther separation is greater in the S-morph than the L-morph (Fig. 3-3) and there is similar positioning among the long-level but not short-level organs (Fig. 3-1). However, population morph ratios vary dramatically among the species (Figs. 3-4, 3-5). Population morph-ratios for *N. assoanus* range from isoplethy to L-biased while *N. dubius* populations are strongly L-biased or monomorphic for this morph. Morph ratios in *N. assoanus* are associated with population size, but in *N. dubius* there was no relation with population size (Fig. 3-6).

I employed experimental methods and computer simulations to investigate the potential selective mechanisms responsible for variation in morph ratios of *N. assoanus* and *N. dubius*. I first established that *N. assoanus* was moderately self-sterile whereas *N. dubius* was self-compatible (Fig. 4-1). There were no morph-specific differences in female components of fertility in populations (fruit- and seed-set – Fig. 4-2; outcrossing rate – Fig. 4-3), indicating that differences between the morphs in male fertility were more likely to be responsible for the observed morph-ratio variation. Measuring pollen transfer in *Narcissus* populations was complicated by the absence of pollen size heteromorphism, so I employed a computer mating model to examine the effects of variation in the relative amount of assortative and disassortative mating on equilibrium morph ratios (Fig 4-4). The results indicate that high levels of disassortative mating result in isoplethic morph ratios. Biased populations result when assortative mating in one of the morphs is high. This appears to occur in the L-morph but not the S-morph since populations are commonly biased for the L-morph but never for the S-morph. Moderate self-sterility in *N. assoanus* favours the maintenance of stigma-height dimorphism since the vast majority of matings arise from outcrossing and in isoplethic populations, a sizeable fraction are likely to be disassortative. In contrast, disassortative mating appears to be more difficult to achieve in *N. dubius*, perhaps because of high levels of self-compatibility.

I also examined populations of *N. assoanus* for pollen limitation of fruit and seed set. Lloyd and Webb's (1992b) conditions for the establishment of a stigma-height dimorphism vary depending on whether fertility is pollen- or resource-limited. The study of *N. assoanus* is the first study examining pollen limitation of fertility in a species with stigma-height dimorphism. Pollen limitation is likely to vary across temporal and spatial scales and therefore affect the ecology and evolution of plant reproduction (e.g. Haig & Westoby 1988; Morgan & Schoen 1997). However, few studies have examined pollen limitation of fruit and seed set in more than one population or year (reviewed in Burd 1994). I examined the degree of pollen limitation in five populations over three years. There was significant variation in the degree of pollen limitation among populations (Table 5-3) for a given year and four of the five populations exhibited pollen limitation of fertility at some time (Figs. 5-2, 5-3). An unexpected finding of this study was the consistently low seed:ovule ratios in both open- and hand-pollinated capsules. Low fertility of open- and hand-pollinated flowers can result from resource-limitation or genetic load, in addition to pollen limitation (reviewed in Charlesworth 1989) but further work is required to assess the relative contributions of these mechanisms.

Pollen transfer and the role of stigma-height dimorphism in the evolution of distyly

I used experimental garden populations of modified *Eichhornia paniculata* to quantify pollen transfer and mating patterns in monomorphic populations and those with a stigma-height dimorphism. This study is one of the first to compare the reproductive consequences of approach vs. reverse herkogamy. The finding that outcrossing rates of the approach morph were consistently greater than the reverse morph (Fig. 6-3) support Webb and Lloyd's (1986, p. 172) speculation that approach herkogamy is more effective at promoting cross pollen dispersal than the reverse form. I then compared components of reproduction in monomorphic vs. dimorphic populations and found that stigma-height dimorphism increased outcrossing rates in both morphs. In addition, female fertility and pollen transfer to the L-morph was greater in dimorphic populations than in monomorphic populations (Fig. 6-3). Intermorph mating was typically greater than intramorph mating in dimorphic populations (Table 6-3). These findings partially support the hypothesis that stigma-height dimorphism functions to increase proficient pollen transfer among the morphs. Similar results were obtained by Stone and Thomson (1994) in artificial flowers, but the study of *E. paniculata* is the first using real flowers, albeit flowers that have been experimentally modified through emasculation.

I also quantified pollen transfer and morph-specific fitness in experimental populations with biased morph ratios. The conditions for both stages (1 – establishment of stigma-height dimorphism, and 2 – anther-height dimorphism) of the Lloyd and Webb (1992b) model are met when only outcrossed pollen transfers are considered (Table 6-4a). However, if selfing events are included in the estimates of pollen transfer, the establishment of stigma-height dimorphism is difficult to achieve (Table 6-4b). Average morph-specific fitness of an individual is negatively frequency-dependent (Fig. 6-6) and the strength of the relation is higher in the S-morph than the

L-morph when selfing is included. This suggests that it would be easier for an S-morph to invade a population of the L-morph than the reverse sequence. However, this reflects the higher selfing rates of the S-morph of *E. paniculata* and it is not known whether this pattern occurs in natural populations of species with stigma-height dimorphism. In fact, outcrossing rates in *N. dubius* and *N. assoanus* were not significantly different between the morphs (see above).

Collectively, the results of the empirical studies in populations of *Narcissus*, computer simulations, and the investigations of experimental garden populations of *E. paniculata* suggest that frequency-dependent male fertility plays a significant role in the establishment and maintenance of stigma-height dimorphism. Moreover, my results suggest that self-sterility may play an important role in the maintenance of stigma-height dimorphism since self-incompatible *N. assoanus* exhibited isoplethic morph ratios but self-compatible *N. dubius* did not. Additionally, the conditions of the Lloyd and Webb (1992b) model for the establishment of stigma-height dimorphism were more commonly met when only outcrossed pollen transfers were considered in experimental populations of *E. paniculata*. While Lloyd and Webb (1992b; p. 192) recognize that the stability of the stigma-height dimorphism increases with the outcrossing rate, they propose that self-incompatibility evolves after reciprocal herkogamy. The results of this thesis present an interesting contradiction to the Lloyd and Webb (1992b) model for the evolution of distyly. Clearly, the influence of selfing on the establishment and maintenance of stigma-height dimorphism warrants further attention (and see Charlesworth & Charlesworth 1979).

Future Directions

My thesis research concentrated on a genus (*Narcissus*) that contains both stigma-height dimorphism and distyly, but little is known about the reproductive consequences and maintenance of stigma-height dimorphism in non-heterostylous groups. Numerous questions remain, especially in non-heterostylous families where stigma-height dimorphism has evolved. Below I outline three main avenues of future research related to more general issues regarding pollination mechanisms and the maintenance of stigma-height dimorphism, the occurrence of the dimorphism in non-heterostylous families, and the evolution of stylar polymorphisms.

Mechanism of proficient disassortative pollen transfer

One of the most obvious questions arising from my research concerns the mechanism of proficient pollen transfer among the morphs in populations with stigma-height dimorphism. From a functional perspective, the presence of isoplethic morph ratios in populations with stigma-height dimorphism presents a puzzle. Stigma-height dimorphism is probably maintained in populations because it increases the proficiency of pollen transfer between the morphs and reduces self-pollination and self-interference (because of the spatial segregation of anthers and stigmas). However, studies of pollinators of heterostylous species have indicated that pollen is placed on the pollinators' body corresponding to the position of the anthers (e.g. Darwin 1877; Lloyd & Webb 1992b). If this is true in stigma-height dimorphic species, how are two distinct stigma heights maintained in populations? And how is disassortative pollen transfer achieved with anther monomorphism?

Lloyd and Webb (1992b) proposed a possible mechanism for the promotion of pollen transfer between the morphs of populations with stigma-height dimorphism. This involved the sequential contact of a pollinator's body with stigmas and anthers. The stigmas are first contacted in the L-morph whereas the anthers are first contacted in the S-morph. This sequence results in segregation of pollen on the pollinators' body. Lloyd and Webb (1992b) outline three ways in which segregation of pollen may occur in species with stigma-height dimorphism. These include differences in the "free path" of the probe (i.e. the curvature of entry/exit path of the proboscis), variations in the diameter of the probe along its length, and differences in the entry and exit paths of the probe. However, no empirical data are available to assess the likelihood of these explanations and they may not be mutually exclusive.

In Stone and Thomson's (1994) test of the Lloyd and Webb (1992b) model, they videotaped bees visiting glass-sided artificial flowers constructed to have approach or reverse herkogamy. Their results indicated that the long style affected bee posture so that pollen deposition onto the body usually occurred on a different region of the bee than for the S-morph. Similarly, contacts of the stigma differed among the morphs. The pattern of contacts provided a mechanism for efficient pollen transfer from the S-morph to the L-morph but short-level stigmas were contacted relatively infrequently and by an area of the pollinator that was contacted by anthers of both morphs. An alternate hypothesis that may explain the mechanism of proficient intermorph pollination in species with stigma-height dimorphism visited by Lepidoptera is coiling of the proboscis between visits to flowers (L.D. Harder, pers. comm.). While pollen pick-up by long-tongued pollinators occurs on a restricted location of the proboscis, pollen may be redistributed through proboscis coiling so that efficient deposition occurs on both stigma heights. Alternatively, pollen may be distributed over a considerable area of the pollinator during visits to the flower allowing maintenance of a wider range of variation in stigma height than typically occurs in most species (Barrett *et al.* 2000b). Careful observations using videotaped sequences of pollinator behaviour and pollen pick-up are required to understand the mechanism of pollen transfer in species with stigma-height dimorphism.

Theoretical and experimental approaches used in this thesis indicated that stigma-height dimorphism is likely maintained in populations because it promotes efficient pollen transfer between the morphs. Specifically, as Lloyd and Webb (1992b; and see Ch. 6) outlined q_{sl} and q_{ls} must be greater than q_{ll} and q_{ss}, respectively. However, this hypothesis remains untested in natural populations. Quantifying pollen transfer in natural populations was not possible in this thesis owing primarily to time constraints (and see below). However, I was able to set up experimental populations in the field that allowed for a preliminary examination of pollen transfer in *N. assoanus*. I set up replicates of three morph-ratio treatments: 1–monomorphic for the L-morph (i.e. q_{ll} transfers), 2–isoplethic (q_{ls} & q_{sl}), and 3–monomophic for the S-morph (q_{ss}). Given the floral biology of *Narcissus*, pollen transfer, and hence fertility should be reduced in treatment 3. Fertility was quantified using fruit and seed set. While there were no significant differences among the treatments in seed set, fruit set was lower in treatment 3 when compared to treatments 1 and 2 (χ^2=4.95, d.f.=1, P=0.084).This data suggest that q_{ss} is indeed the least proficient transfer in *N. assoanus*. However, these results are preliminary and a more detailed examination is required.

There are three ways in which assortative (q_{ll} & q_{ss}) and disassortative (q_{ls} & q_{sl}) pollen transfers can be quantified in natural populations. The approach that has proven successful in heterostylous populations has utilized the differences between the morphs in pollen size. Because there is little overlap in the size of pollen grains from different morphs, the source of pollen grains on stigmas can be determined with relative ease. Unfortunately, this option is not available in species with stigma-height dimorphism since they do not possess a pollen size heteromorphism. An alternate method involves the use of paternity analysis. In this method, the marker genotypes of the offspring, its maternal parent, and all potential fathers are used to estimate the true father (e.g. Smouse & Meagher 1994). However, to quantify morph-specific levels of assortative and disassortive pollen transfers, it is not required to identify individual male parents, so the detail provided by this method is not necessary. The most promising approach would involve using molecular markers to quantify mating events and estimate pollen transfer in a method similar to that employed in experimental populations of *E. paniculata*. Morph-specific markers would be required for this type of experiment but unfortunately I was unable to find morph-specific allozyme differences in *Narcissus*. However, with the ever increasing development of molecular markers (e.g. Snow & Lewis 1993; Jones *et al.* 1997) and their application, it is may be possible to identify morph-specific AFLP (amplified fragment length polymorphism) or microsatellite (SSRs – simple sequence repeats) markers. These markers are multi-allelic and show a great deal of polymorphism and hence may allow for the estimation of pollen transfer probabilities in natural populations.

Stigma-height dimorphism in non-heterostylous families

Stigma-height dimorphism is reported from three heterostylous families (Amaryllidaceae, Boraginaceae, Primulaceae) and may be a stage in the evolution of distyly in some cases. However the dimorphism is also reported from non-heterostylous families (Epacridaceae, Ericaceae, Haemodoracae, Liliaceae, and possibly the Scrophulariaceae) but virtually nothing is known about the reproductive biology of stigma-height dimorphism in these families. This group represents a heterogeneous assortment of animal-pollinated species, none of which express traits commonly associated with the heterostylous syndrome (see Ch. 2). How the polymorphism in maintained in these families is unclear but its occurrence demonstrates that stigma-height dimorphism does not always lead to the evolution of distyly as previously suggested (e.g. Lloyd & Webb 1992a). In most of these genera, very little is known about the pollination biology, incompatibility status or morph ratios of populations. Furthermore, there have been no detailed empirical studies examining the reproductive consequences of stigma-height dimorphism in natural populations. Such studies could provide valuable insight into the evolution and maintenance of stigma-height dimorphism in these groups and could determine whether similar selective forces are maintaining the polymorphism among diverse taxonomic groups.

Evolutionary relations between heterostyly and stigma-height dimorphism

I used experimental populations of *E. paniculata* to examine the plausibility of the Lloyd and Webb (1992b) model for the evolution of distyly. However, these experiments do not allow for speculation of the sequence of events in the evolution of distyly, or even the polarity of the relation between distyly and stigma-height dimorphism. Such question can only be addressed with the aid of phylogenetic reconstruction and character mapping methods (reviewed in Weller & Sakai 1999). Recently these approaches have been used to examine the evolution of numerous plant characters including breeding systems and reproductive characters in general (e.g. Sytsma *et al.* 1991; Graham & Barrett 1995; Kohn *et al.* 1996; Bruneau 1997; Schoen *et al.* 1997; Donoghue *et al.* 1998; Endress 1999; Weiblen *et al.* 2000). The development of well resolved phylogenies of groups of taxa where both stigma-height dimorphism and distyly (and tristyly) occur would be invaluable in addressing questions related to the sequence of events in the evolution of these polymorphisms, the number of transitions between the different stylar polymorphisms, and the evolution of correlated characters.

The occurrence of heterostyly and stigma-height dimorphism among closely related species (e.g. taxa of *Lithodora* and *Narcissus*) provides valuable systems for studying the evolution and type of incompatibility reaction present in these groups. Dulberger (1992) suggested that heteromorphisms of the pollen and stigmas in distylous species contribute to heteromorphic incompatibility reactions. In all heterostylous species in which hetermorphic incompatibility has been lost, pollen heteromorphisms have also diminished in expression (reviewed Barrett 1992a) suggesting that the heteromorphisms play a role in the incompatibility reactions. The incompatibility system found in species with stigma-height dimorphism is apparently not of the heteromorphic type, but rather is probably of the multi-allelic homomorphic type, and species generally lack pollen heteromorphism. It would be interesting to examine heterostylous members of *Narcissus* (and see below) and *Lithodora* for pollen heteromorphisms and incompatibility reactions. The existence of homomorphic and heteromorphic incompatibility in closely related taxa is relevant to hypotheses of the evolutionary relations between the two incompatibility types.

The prevailing view is that heteromorphic and homomorphic incompatibility are evolutionarily distinct with independent origins (e.g. Charlesworth 1982; Ganders 1979a; Barrett

1988; Lloyd & Webb 1992a) and therefore it is unlikely that the two systems would occur in closely related taxa. An alternate view suggests that heteromorphic incompatibility evolved from homomorphic incompatibility through the loss of alleles (e.g. Crowe 1964; Muenchow 1982; reviewed in Wyatt 1983) but this view is generally unsupported by data of the occurrence of both systems in closely related taxa (reviewed in Barrett & Cruzan 1994).

Tristylous *N. triandrus* does not exhibit pollen heteromorphisms or heteromorphic incompatibility (Bateman 1954; Barrett *et al.* 1997), and the incompatibility mechanism that occurs in the species does not readily fit into classical descriptions (Sage *et al.* 1999). However, nothing is known about ancillary polymorphisms and the incompatibility reactions of distylous *N. albimarginatus*, or of the mechanism of incompatibility in species with stigma-height dimorphism. Similarly, populations of stigma-height dimorphic *Lithodora fructicosum* exhibit self-sterility yet inter- and intramorph pollinations produce full seed set (J.D. Thompson unpubl. data), but little is known about the incompatibility status of distylous *Lithodora hispidula*. If self-incompatible stigma-height dimorphism was an intermediate stage in the evolution of distyly in these taxa, it would be at odds with the model for the evolution of distyly proposed by Lloyd and Webb (1992b), since they suggest that self-incompatibility evolves after reciprocal herkogamy. However, the Charlesworth and Charlesworth (1979) model involved the evolution of self-incompatibility before reciprocal herkogamy although their evolutionary sequence does not involve a stigma-height dimorphism. Further comparative and phylogenetic studies are obviously required to examine the sequence of events in the evolution of distyly. Moreover, these studies may prove useful in evaluating the evolutionary relationships between heteromorphic and homomorphic incompatibility.

APPENDIX A

Morph frequency and population size of *Narcissus assoanus* and *N. dubius* populations in southern France.

Table A-1: Morph frequency and population size of *Narcissus assoanus* populations in southern France. Significant *G*-tests indicate that the population morph ratio differs from 1L: 1S.

Population	L	S	Number Sampled	Pop Size	*G*-test
Col Hortus	0.72	0.28	128	1200	***
Labau	0.68	0.32	63	80	***
St-Guilhem-le-Desért	0.62	0.38	69	200	*
St-Bauzille-de-Montmel	0.87	0.13	193	500	***
Montagne d' Hortus	0.75	0.25	83	1200	***
Massargues	0.55	0.45	108	200	ns
Les Cognets	0.73	0.27	146	800	***
Pont sur Brestalou	0.56	0.44	259	500	ns
Bancel	0.71	0.29	175	800	***
Route de Villeseque	0.53	0.47	75	200	ns
Sauve	0.58	0.42	106	200	ns
Beaucous	0.55	0.45	147	500	ns
Les Camps	0.50	0.50	125	300	ns
Clapiers	0.91	0.09	33	50	***
St-Matieu-de-Tréviers	0.68	0.32	198	800	***
Gabriac Church	0.60	0.40	95	300	ns
Colline Geraniol	0.68	0.32	151	200	***
Fontanès	0.64	0.36	70	90	*
Aguzan	0.54	0.46	140	400	ns
Lauret	0.66	0.34	87	100	***

La Clause 1	0.76	0.24	93	300	***
La Clause 2	0.71	0.29	49	80	***
Coutach	0.72	0.28	72	300	***
Notre-Dame-de-Londres	0.64	0.36	70	200	*
Blandas	0.47	0.53	163	5000	ns
Rigalderie	0.49	0.51	186	10000	ns
Vissec	0.46	0.54	196	4000	ns
Rogues	0.48	0.52	140	15000	ns
St-Maurice-Navacelles1	0.52	0.48	161	4000	ns
St-Pierre-de-la-Fage	0.48	0.52	130	4000	ns
Le Caylar	0.53	0.47	80	3000	ns
Ceyrac	0.50	0.50	208	2000	ns
Pompignan	0.56	0.44	174	1000	ns
Montoulieu	0.46	0.54	80	800	ns
Laroque-Ganges	0.49	0.51	96	400	ns
Tourrès	0.47	0.53	95	600	ns
St-Clemént-de-Rivière	0.71	0.29	77	90	***
Restinclières	0.84	0.16	100	300	***
Le Tibilet	0.54	0.46	201	8000	ns
Vingrau	0.86	0.14	115	2000	***
Cases-de-Pene	0.93	0.07	56	400	***
Les Sidrières	0.86	0.14	57	200	***
St-Maurice-Navacelles2	0.49	0.51	313	2000	ns
Sorbs	0.54	0.46	224	1500	ns
Le Cros	0.48	0.52	336	1000	ns
Cabane	0.52	0.48	93	200	ns
TOTAL	0.59	0.41	6014		

$G_{pooled} = 204.56$ ***

$G_{heterogeneity} = 44\,603.18$ ***

ns = not significant, * $P < 0.05$, *** $P < 0.001$

Table A-2: Morph frequency and population size of *Narcissus dubius* populations in southern France. All dimorphic populations are biased for the L-morph.

Population	L	S	Number Sampled	Pop Size
St-Bauzille-de-Montmel	0.88	0.12	106	500
Montagne d'Hortus	0.77	0.23	62	300
La Clause 1	0.91	0.09	151	800
La Clause 2	0.85	0.15	121	1000
St-Guilhem-le-Desért	0.90	0.10	115	1000
St-Jean-de-Bueges	0.86	0.14	117	500
Pont du Gard	1.00	0	198	1500
La Baume	1.00	0	176	1000
Puech Bouquet	0.91	0.09	137	300
Grote de l'Hortus	0.97	0.03	132	200
Les Cognets	0.95	0.05	41	80
Bancel	1.00	0	26	30
Ile Ste Lucie	0.91	0.09	82	200
La Clape	0.98	0.02	131	800
Le Castellas	1.00	0	93	300
Fourès	1.00	0	156	500
Vingrau	1.00	0	26	30
Cases-de-Pene	0.84	0.16	45	500
Les Sidrières	0.87	0.13	174	4000
Cap Leucate	0.94	0.06	129	2000
Pont du Diable	0.95	0.05	44	70
TOTAL	0.90	0.10	1607	
	$G_{\text{pooled}} = 1208.23$ ***			
	$G_{\text{heterogeneity}} = 52.45$ ***			

*** $P < 0.001$

APPENDIX B

Estimates of pollen transfer from garden populations of *Eichhornia paniculata* (Pontederiaceae) modified to exhibit stigma-height dimorphism

Table B-1: Pollen transfer probabilities based on outcrossing events only in (a) L-biased (this page) and (b) S-biased (following page) populations of *Eichhornia paniculata* modified to exhibit a stigma-height dimorphism. *qij* represents outcross pollen transfer probabilities of pollen dispersal from morph *i* to morph *j* and was inferred from the genotypes of seeds. The results are compared with the conditions for the invasion of a mutant morph outlined in Lloyd and Webb (1992b).

(a)						Conditions for invasion by S-morph	
						Pollen-limited	Resource-limited
Trt	Rep	q_{ll}	q_{sl}	q_{ls}	q_{ss}	$q_{ls} + q_{sl} > 2q_{ll}$	$q_{sl} > q_{ll}$
5L:1S	1	11.33	8.61	57.18	22.87	yes	no
	2	6.94	13.49	34.93	44.64	yes	yes
	3	8.65	10.45	25.85	55.05	yes	yes
	4	7.89	8.14	48.92	35.06	yes	yes
mean		8.70	10.17	41.72	39.41		
s.e.		0.94	1.21	7.00	6.86		
2L:1S	1	16.57	19.27	35.13	29.03	yes	yes
	2	17.37	30.15	28.96	23.51	yes	yes
	3	19.77	24.54	28.07	27.62	yes	yes
	4	11.40	18.02	37.68	32.90	yes	yes
mean		16.28	23.00	32.46	28.27		
s.e.		1.76	2.77	2.34	1.94		

Table B-1b: Pollen transfer probabilities based on outcrossing events only in S-biased populations of *Eichhornia paniculata* modified to exhibit a stigma-height dimorphism.

(b)						Conditions for invasion by L-morph	
						Pollen-limited	Resource-limited
Trt	Rep	q_{ll}	q_{sl}	q_{ls}	q_{ss}	$q_{ls} + q_{sl} > 2q_{ss}$	$q_{ls} > q_{ss}$
1L:5S	1	59.98	27.76	8.81	3.45	yes	yes
	2	47.91	40.41	7.64	4.03	yes	yes
	3	47.16	40.42	8.65	3.77	yes	yes
	4	34.71	50.14	7.58	7.57	yes	yes
	mean	47.44	39.68	8.17	4.71		
	s.e.	5.16	4.59	0.33	0.96		
1L:2S	1	29.06	46.79	11.7	12.45	yes	no
	2	24.57	42.32	17.49	15.62	yes	yes
	3	45.28	36.58	13.26	4.87	yes	yes
	4	27.43	47.5	11.77	13.30	yes	no
	mean	31.59	43.30	13.56	11.56		
	s.e.	4.66	2.52	1.36	2.33		

Table B-2: Pollen transfer probabilities in (a) L-biased (this page) and (b) S-biased (following page) experimental populations of *Eichhornia paniculata* modified to exhibit a stigma-height dimorphism. *qij* was estimated from the genotypes of seeds and represents the pollen transfer probabilities from morph *i* to *j* and includes self pollen transfers. I compare the results with the Lloyd and Webb model (1992b)

(a)						Conditions for invasion by S-morph	
						Pollen-limited	Resource-limited
Trt	Rep	q_{ll}	q_{sl}	q_{ls}	q_{ss}	$q_{ls} + q_{sl} > 2q_{ll}$	$q_{sl} > q_{ll}$
5L:1S	1	6.60	3.48	23.09	66.84	yes	no
	2	4.75	5.37	13.91	75.97	yes	yes
	3	4.40	3.69	9.13	82.77	yes	no
	4	6.14	3.30	19.87	70.69	yes	no
mean	5.47	3.96	16.50	74.07			
s.e.	0.53	0.48	3.11	3.45			
2L:1S	1	17.54	10.45	19.04	52.98	no	no
	2	14.60	13.23	12.71	59.46	no	no
	3	15.49	12.16	13.91	58.45	no	no
	4	17.53	13.57	28.37	40.53	yes	no
mean	16.29	12.35	18.51	52.86			
s.e.	0.74	0.70	3.56	4.35			

Table B-2b: Pollen transfer probabilities based on outcrossing and selfing events in S-biased populations of *Eichhornia paniculata* modified to exhibit a stigma-height dimorphism.

(b)						Conditions for invasion by L-morph	
						Pollen-limited	Resource-limited
Trt	Rep	q_{ll}	q_{sl}	q_{ls}	q_{ss}	$q_{ls} + q_{sl} > 2q_{ss}$	$q_{ls} > q_{ss}$
1L:5S	1	74.40	15.56	4.94	5.10	yes	no
	2	64.33	24.49	4.63	6.54	yes	no
	3	68.90	20.67	4.42	6.01	yes	no
	4	75.4	16.34	2.47	5.79	yes	no
	mean	70.76	19.27	4.12	5.86		
	s.e.	2.58	2.07	0.56	0.30		
1L:2S	1	32.78	36.32	9.08	21.82	yes	no
	2	42.72	27.31	11.28	18.69	yes	no
	3	44.27	26.56	9.63	19.53	no	no
	4	38.18	32.19	7.98	21.65	no	no
	mean	39.49	30.60	9.49	20.42		
	s.e.	2.58	2.28	0.69	0.78		

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