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Stylar Polymorphisms and the Evolution of Heterostyly in *Narcissus* (Amaryllidaceae)

Spencer C.H. Barrett,* David G. Lloyd,[†]
and Juan Arroyo[‡]

Introduction

In outcrossing hermaphrodite plants, the separate functions of pollen dispersal and pollen receipt may interfere with one another so that fitness as a paternal or maternal parent is compromised (van der Pijl, 1978; Bawa and Opler, 1975; Lloyd and Yates, 1982; Lloyd and Webb, 1986; Webb and Lloyd, 1986; Bertin and Newman, 1993; Harder and Barrett, 1995). This is particularly likely in flowers in which the sex organs are close together and mature at the same time. Interference can potentially take several forms, including the obstruction by pistils of efficient pollen dispatch by pollinators, stamens restricting access by pollinators to stigmas, thus reducing pollen deposition, and the deleterious effects of self-pollination on maternal function due to stigmatic, stylar, or ovular clogging. Although there is some experimental evidence for self-pollen interference (Shore and Barrett, 1984; Barrett and Glover, 1985; Bertin and Sullivan, 1988; Palmer et al., 1989; Waser and Price, 1991; Scribailo and Barrett, 1994), the other two forms of pollen-pistil interference have seldom been investigated (see, however, Barrett and Glover, 1985; Kohn and Barrett, 1992a).

Selection to reduce interference by segregating sex organs in time or space has been proposed as a major influence on floral evolution, resulting in a variety of conditions including dichogamy, herkogamy, and various forms of gender expression such as monoecy (Lloyd and Webb, 1986; Webb and Lloyd, 1986; Bertin, 1993; Bertin and Newman, 1993; Harder and Barrett, 1995; also see

*Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2.

[†]Department of Plant & Microbial Sciences, University of Canterbury, Christchurch 1, New Zealand.

[‡]Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, 41080 Sevilla, Spain.

Chapter 6). Traditionally, these floral traits have been interpreted as antiselfing mechanisms, which reduce the harmful effects of inbreeding. However, their frequent occurrence in self-incompatible taxa casts doubt on this as a general explanation for their adaptive significance. Although selection to reduce pollen-pistil interference is likely to be of evolutionary significance in outcrossing plants, few studies in floral biology have examined this problem compared with the large literature on antiselfing mechanisms and inbreeding depression (reviewed in Richards, 1986; Charlesworth and Charlesworth, 1987; Jarne and Charlesworth, 1993; Thornhill, 1993).

In animal-pollinated plants, the spatial separation of female and male reproductive organs within flowers (*herkogamy*) may be constrained by the requirements of proficient cross-pollination. If stigmas and anthers are too widely separated, pollinators may contact only one set of sex organs or touch them with different body parts while visiting flowers. The conflict between the avoidance of self-interference and precision in pollination is reduced in heterostylous plants because of the occurrence within populations of two or three usually self-incompatible morphs that differ reciprocally in the placement of female and male reproductive organs within flowers. Plants of each morph are strongly herkogamous, thus avoiding self-interference; however, the placement of female and male sex organs in a reciprocal and complementary manner among the morphs promotes more precise cross-pollen transfer. Although, heterostyly has been studied intensively since Darwin's (1877) early work, there is still debate concerning its evolution and adaptive significance (reviewed in Barrett, 1992a). Models of the evolution of heterostyly differ in the pathways by which the polymorphism is thought to originate and in the emphasis placed on proficient cross-pollination, inbreeding avoidance and pollen-stigma interference as selective forces responsible for evolution of the principal features of the polymorphism (Charlesworth and Charlesworth, 1979b; Charlesworth, 1979; Ganders, 1979; Richards, 1986; Barrett, 1990; Lloyd and Webb, 1992a,b). Unfortunately, a major limitation in testing these models is the paucity of plant groups in which putative stages in the evolutionary assembly of the polymorphism can be identified unequivocally.

In this chapter, we investigate the patterns of sex-organ variation that occur in populations of the perennial geophyte *Narcissus* (Amaryllidaceae) and discuss the selective mechanisms responsible for the evolution and maintenance of this variation. Our interest in *Narcissus* was initially sparked by a historical debate concerning the nature of sexual polymorphisms in various species and whether "true" heterostyly occurs in the genus. Several early workers reported the occurrence of heterostyly in species of *Narcissus* (Henriques, 1887, 1888; Fernandes, 1935, 1964, 1965, also see Crié, 1884; Wolley-Dod, 1886; Des Abbayes, 1935). However, its occurrence in the genus was later disputed by Bateman (1952a) and most modern workers interested in the evolution of heterostyly. Skepticism over the presence of heterostyly in *Narcissus* was based on the concern that only a limited sampling of variation had been involved in early reports and continuous

variation in sex-organ position appeared to exist in natural populations (Bateman, 1952a; also see Baker, 1964). In addition, the finding that several *Narcissus* species possess an incompatibility system with many mating types, rather than a system with only two (distyly) or three (tristyly) (Bateman, 1954; Dulberger, 1964), probably biased modern workers against accepting the earlier reports. For many workers the association of diallelic (intramorph) incompatibility with the style-stamen polymorphism that characterizes heterostylous plants is a *sine qua non* for the occurrence of "true" heterostyly. Recently, however, Lloyd et al. (1990) reconsidered the evidence presented in earlier papers and also examined sex-organ variation in horticultural cultivars of *N. triandrus*. They concluded that both distyly and tristily occur in the genus, but suggested that field studies were necessary to confirm the true nature of the polymorphisms.

To clarify these issues, we have examined the patterns of sex-organ variation in natural populations of selected species of *Narcissus* from the Iberian peninsula. Of particular importance in these studies was to establish whether the relative positions of female and male sex organs vary continuously, or discrete morphs differing in sex-organ position occur. Our surveys reveal that three distinct types of sexual variation occur in *Narcissus*, with populations either monomorphic, dimorphic, or trimorphic for style length. These three fundamental conditions are illustrated in Figure 13.1. Here we document this variation in detail and address the following general questions: (1) What are the patterns of variation in stigma and anther position within and between species and do any species exhibit heterostyly? (2) What selective forces maintain style-length variation and what are the evolutionary relationships among different stylar conditions? (3) What floral traits are associated with the three types of sexual variation and which aspects of the pollination biology of species have influenced the evolution of stigma-height polymorphisms? Our results confirm that heterostyly occurs in *Narcissus*, but its expression differs in important ways from the widely held view of the polymorphism. These differences are of special significance because they not only provide clues as to how the polymorphism may have evolved in *Narcissus*, but they also give more general insights into the selective mechanisms responsible for the evolution of heterostyly in other groups.

Floral Biology of *Narcissus*

Before discussing the evolution of sexual polymorphisms in *Narcissus*, we briefly review the systematics and natural history of the genus, focusing in particular on floral biology. A remarkable feature of *Narcissus* is that although it is one of the most important perennials used in horticulture, with more than 20,000 registered names and over a century of breeding effort, the floral biology and pollination systems of most *Narcissus* species are unknown. Moreover, little is known of the phylogenetic relationships of taxa, and species circumscriptions

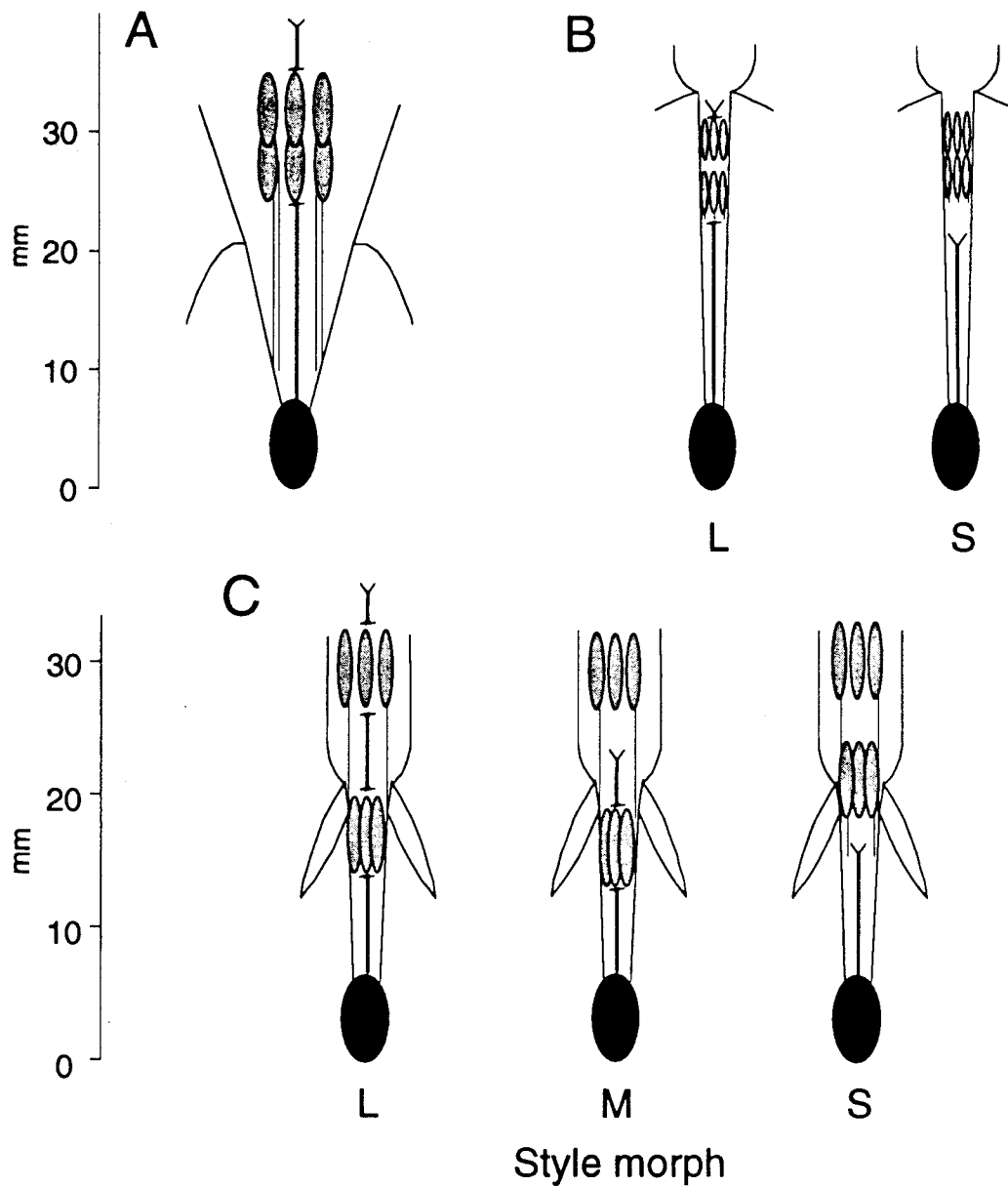


Figure 13.1. The three fundamental stilar conditions in *Narcissus*. Schematic illustrations of the flowers of *Narcissus* species monomorphic, dimorphic, and trimorphic for stigma height. All measurements are based on 50 flowers per morph sampled from a single population of each species (A) *N. bulbocodium*. Populations of this species are monomorphic for style length and possess approach herkogamous flowers with two anther levels. (B) *N. assoanus*. Two morphs that differ in stigma height are present in populations of this species. In the long-styled morph (L), stigmas are positioned above the two anther levels (approach herkogamy), whereas in the short-styled morph (S), stigmas are below the two anther levels (reverse herkogamy). (C) *N. triandrus*. Three morphs (L, M, and S) differing in style length occur in most populations of this species. Anther heights are similar in the long- and mid-styled morph, but differ in the short-styled morph. See text for further details.

vary widely, so there is little agreement on the number of species in the genus. Estimates have ranged from 16–150, with most authorities accepting between 35–70 species, divided among two subgenera and 10 sections (for further details, see Fernandes, 1951, 1967, 1975; Meyer, 1961; Webb, 1980; Valdés, 1987; Blanchard, 1990; Jefferson-Brown, 1991).

Floral Morphology

Members of the genus are perennial bulbs of rocky hills and montane areas of Europe and North Africa, particularly the Mediterranean region. The center of diversity of the genus is Spain and Portugal with a high concentration of species also occurring in Morocco. All species are winter-growing and summer-dormant with most blooming in early spring and a few (*N. cavanillesii*, *N. elegans*, *N. serotinus*, *N. broussonetii*, and *N. viridiflorus*) flowering in the autumn. Flowers vary in diameter from as little as 12 mm in forms of *N. bulbocodium* to over 12.5 cm in *N. nobilis* and, with the exception of *N. viridiflorus*, flowers usually possess white, yellow, or orange perianth parts. The most prominent features of *Narcissus* flowers are the corona, which forms a cylindrical cone extending beyond the tepals, and the fused perianth giving rise to a prominent floral tube. Corona morphology is quite variable, ranging from a tiny disk in *N. serotinus* to a long trumpetlike structure in *N. pseudonarcissus*. Floral tubes range from long and narrow in species of sections Jonquillae and Apodanthae to virtually absent in *N. cavanillesii*. Flower number per stem ranges from 1 in many species to 15–16 in *N. tazetta*, and flower orientation varies from ascending in *N. rupicola* to deflexed in *N. triandrus*.

Pollinator Visitation

Surprisingly little information has been collected on the pollination biology of *Narcissus* species, if we consider that many possess prominent showy flowers and some are highly scented. Our observations of *N. bulbocodium*, *N. papyraceus*, and *N. triandrus* from the Iberian peninsula indicate that flowers are visited primarily by bees and also butterflies and flies. The only night-flowering species in the genus, the strongly scented *N. viridiflorus*, is believed to be pollinated by small moths (Vogel and Müller-Doblies, 1975). One noteworthy aspect of our observations of insect visitors to *Narcissus* flowers is the very low frequency of visitation in populations, irrespective of the size of the floral display. For example, despite many hours observing populations of *N. assoanus*, *N. calcicola*, *N. gaditanus*, and *N. rupicola*, few pollinators were observed. Fernandes (1965) remarked on the curious lack of pollinators visiting *N. triandrus* and raised the possibility that the species may be pollinated by nocturnal insects. However, he doubted this was likely because of the cool night temperatures that occur during flowering in many of the montane sites that the species inhabits in central Portugal

and Spain. Experimental studies of *N. longispathus* by Herrera (1995) revealed that fruit set in this species was pollen-limited. Because many *Narcissus* species flower during early spring when pollinator activity is low, pollen limitation may often occur.

Narcissus flower longevities range from 5–20 days, depending on the species and environmental conditions during flowering. The adaptive significance of this variation has not been investigated, but is likely to be associated with the levels of pollinator activity experienced by individual species (see Chapter 5). There is some evidence to support this view for *N. tazetta*, one of the few *Narcissus* species in which the floral biology of natural populations has been studied. Populations in Israel differ in habitat, flowering time, floral morphology, style-morph ratios, and the types of pollinators that visit populations (Dulberger, 1967 and personal communication; Arroyo and Dafni, 1995). Populations occurring in lowland marshes have floral longevities of 14–20 days and are serviced by the sphingid *Macroglossum stellarum*, which visits flowers quite infrequently. In contrast, floral longevities of hill populations range from 5–7 days, and flowers are visited regularly by the syrphid *Erystalis tenax* and solitary bees, including *Anthophora* sp. and *Proxycopa olivieri*. Differences in the rates of fitness accrual as a result of contrasting levels of pollinator activity (see Chapter 5) may account for the differentiation of floral longevities in the two ecological races of *N. tazetta*. Herrera (1995) has recently conducted a detailed study of the floral biology of the early-flowering trumpet daffodil *N. longispathus* in southern Spain. Flowers of this species last for 17 days on average and are pollinated primarily by the andrenid bee *Andrena bicolor*. Herrera found that bees foraged only on sunny days when the temperature was above 12–17°C. Visitation to flowers was positively correlated to the average temperature inside coronas, which could be up to 8°C above ambient temperature.

Sexual Systems

Although detailed studies of dichogamy have not been conducted, our preliminary observations indicate that most *Narcissus* species are protandrous to varying degrees. Bateman (1954), however, reported strong protogyny in *N. bulbocodium* with anthers dehiscing “a day or more after the stigma becomes receptive.” In species with two distinct stamen levels (e.g., *N. bulbocodium* and *N. triandrus*), anthers of the upper stamens frequently dehisce before anthers of the lower stamens. This pattern has also been reported in tristylous *Pontederia cordata* (Harder and Barrett, 1993). In some species, dichogamy is associated with the prolonged growth of styles. In members of sections Tazettae, Jonquillae, and Apodanthae, style growth in long-styled plants often proceeds during anther dehiscence so that stigmas are at the same level as the anthers during early male function, but project above them during stigma receptivity. This pattern of development can be interpreted functionally as a mechanism to reduce pollen-

stigma interference. A similar phenomenon has been reported in distylous *Quinchamalium chilense* (Santalaceae) by Riveros et al. (1987).

Despite extensive breeding of *Narcissus* for ornamental purposes, there are few published studies on the compatibility or mating systems of wild or cultivated species. The most detailed study is that of Bateman (1954) published in the *The Daffodil and Tulip Year Book*. Based on controlled self- and cross-pollinations and observations of seed set, he reported the occurrence of self-sterility in *N. asturiensis*, *N. bulbocodium*, *N. calcicola*, *N. cyclamineus*, *N. poeticus*, *N. tazetta*, and *N. triandrus*. The strength of self-sterility varied in a few species (e.g., *N. bulbocodium* varieties and *N. triandrus*), with some plants setting few seeds upon selfing. This variation may explain the report of self-compatibility in *Narcissus triandrus* by Richards (1986, p. 241). Based on our own pollination studies, the following additional species can be added to the list of self-sterile taxa: *N. assoanus*, *N. elegans*, *N. jonquilla*, *N. papyraceus*, and *N. viridiflorus*. We have also confirmed the presence of self-sterility in *N. bulbocodium* and *N. triandrus*. In addition, we have shown that *N. serotinus* is thoroughly self-compatible, setting similar numbers of seeds following self- or cross-pollination. *Narcissus longispathus* is also highly self-compatible and flowers occasionally set fruit by autonomous self-pollination (Herrera, 1995).

Of relevance to the issue of heterostyly in *Narcissus* are the patterns of cross-compatibility found within and among members of different style-length morphs. In the four polymorphic species of *Narcissus* studied experimentally, individuals are self-sterile but cross-compatible with other members of the same style-length group. Hence, intramorph as well as intermorph crosses are fully interfertile, a pattern unexpected in heterostylous species that usually exhibit strong intramorph incompatibility. This situation was first found in trimorphic *N. triandrus* by Bateman (1952a) and has also been demonstrated in dimorphic *N. tazetta* by Dulberger (1964). We have confirmed the occurrence of intramorph compatibility in *N. triandrus*, as well as dimorphic *N. assoanus* and *N. papyraceus*. The absence of an association between floral heteromorphism and the incompatibility system responsible for self-sterility also occurs in distylous *Anchusa* of the Boraginaceae (Dulberger, 1970; Philipp and Schou, 1981; Schou and Philipp, 1983, 1984) and has implications for the evolution of heterostyly (see "Implications for Models of the Evolution of Heterostyly").

An interesting aspect of self-sterility in *Narcissus* concerns the underlying mechanism(s) responsible for reduced seed set upon self-pollination. Bateman (1954, p. 24) reported that "in *Narcissus* pollen tubes grow quite as fast on selfing as on crossing, for the full length of the style. The incompatibility must be late-acting, somewhere in the ovary, and perhaps even after fertilization." Dulberger (1964) found that self-sterility in *N. tazetta* was caused by a breakdown in ovule development after fertilization or at least after pollen tubes had grown through the micropyle and penetrated the embryo sac. Similar late-acting ovarian phenomena occur in *N. triandrus*, although in this species self-rejection appar-

ently precedes fertilization (Sage et al., unpublished). These findings raise the issue of whether self-sterility in *Narcissus* results from a true late-acting self-incompatibility system (Seavey and Bawa, 1986; Waser and Price, 1991; Gibbs and Bianchi, 1993) or self-rejection is a manifestation of early-acting embryo abortion owing to inbreeding depression (Sorensen, 1969; Wiens et al., 1989; Krebs and Hancock, 1991; Husband and Schemske, 1995). Of course, both mechanisms could also influence patterns of seed set following self- and cross-pollination. Careful histological studies will be required to assess these possibilities (see Sage et al., 1994).

Although the mechanism(s) of self-sterility in *Narcissus* is not known, the late-acting rejection may bear important reproductive costs. Because pollen-tube growth rates of self- and outcross pollen are similar, seed set can be reduced significantly if self-pollen is deposited on stigmas before outcross pollen. This effect has been demonstrated experimentally in *N. tazetta* (Dulberger, 1964) and *N. triandrus* (Barrett et al., unpublished). In the latter species, application of self-pollen to stigmas 24 h prior to cross-pollen reduces seed set by 75% compared with outcrossed controls. As discussed below, the wastage of ovules that may arise because of prior self-pollination in *Narcissus* could have important ecological and evolutionary consequences, particularly with regard to the evolution of sexual polymorphisms in the genus.

Patterns of Style-Length Variation in Natural Populations of *Narcissus*

During 1990 and 1991, we collected population samples in Spain and Portugal of 11 *Narcissus* species distributed among seven sections of the genus to investigate the patterns of sex-organ variation within and between populations. In addition, we surveyed herbarium specimens, performed a literature search, and grew additional *Narcissus* taxa under glasshouse conditions in an effort to determine whether species of *Narcissus* not encountered in our field survey possessed sexual polymorphisms involving stigma and anther heights. Field studies involved random collection of a large sample (when possible) of flowering stems from each population. Detailed localities of all populations reported in this study are available from the senior author on request. Population samples were used for two purposes. First, measurements of a range of floral traits including style length and anther height were made on a subsample of flowers. Measurements were either conducted in the field with digital calipers, or the laboratory on preserved material fixed in 70% alcohol using a binocular microscope and digitizing tablet. When sexual polymorphisms were evident, the complete sample of stems was used to estimate floral morph frequencies in each population.

Based on our studies, sexual polymorphisms involving stigma height occur in four of the 10 sections of *Narcissus*. In three sections (Tazettae, Jonquillae, and Apodanthae), both monomorphic and dimorphic species occur. In *N. trian-*

drus, the sole member of section *Ganymedes*, most populations are trimorphic, but dimorphic populations also occur in some parts of the range. In the remaining six sections of *Narcissus*, no evidence of sexual polymorphisms was found. Although our surveys are not exhaustive and we have not observed all species in the field, we are reasonably confident that sexual polymorphisms are unlikely to be discovered in the monospecific sections (*Tapeinanthus*, *Serotini*, and *Aurelia*) or small sections *Narcissus* and *Bulbocodium*. Further work on the larger section *Pseudonarcissus* is desirable, particularly as the rare *N. cyclamineus* was reported by Henriques (1887) as possessing homostylous and herkogamous morphs. However, for reasons discussed more fully below, we believe the floral morphology of species in section *Pseudonarcissus* is unlikely to be associated with the evolution of sexual polymorphisms. It would therefore not be surprising to us if our findings on the distribution of polymorphisms for stigma and anther height among the four sections of *Narcissus* remain unchanged despite further exploration.

Monomorphic Populations

Figure 13.2 illustrates continuous variation of style length in four species of *Narcissus*, each from a different section of the genus. In these species and all others we have observed that are monomorphic for style length, stigmas are positioned at or above the two anther levels. Flowers in which stigmas are regularly held above the anthers are described as exhibiting *approach herkogamy* (Webb and Lloyd, 1986). In some species of *Narcissus*, stigma-anther separation is quite substantial (e.g., *N. bulbocodium*, 5–10 mm), in others (e.g., section *Pseudonarcissus*), it is quite small (<5 mm), and in yet others (e.g., *N. serotinus*), there is no separation between the sex organs and stigmas are positioned at the same height as the two anther levels. In most monomorphic species that we have observed, the two anther levels are positioned close together within the flower (e.g., *N. viridiflorus*) or are not distinguished by height (e.g., section *Pseudonarcissus*). Figure 13.3 illustrates the range of variation in sex-organ position in a population of *N. bulbocodium*. There is continuous variation in both stigma height and the degree of herkogamy. *Narcissus bulbocodium* is atypical for most species without sex-organ polymorphisms in possessing two anther levels that are differentiated by height, particularly during early anthesis.

Dimorphic Populations

Figure 13.4 illustrates patterns of style-length variation in four species of *Narcissus* from three sections of the genus. The observed bimodality of style length demonstrates that each species possesses a population-level polymorphism involving two morphs that differ in style length. In the long-styled morph, anthers are positioned below the stigma, whereas in the short-styled morph, they are above

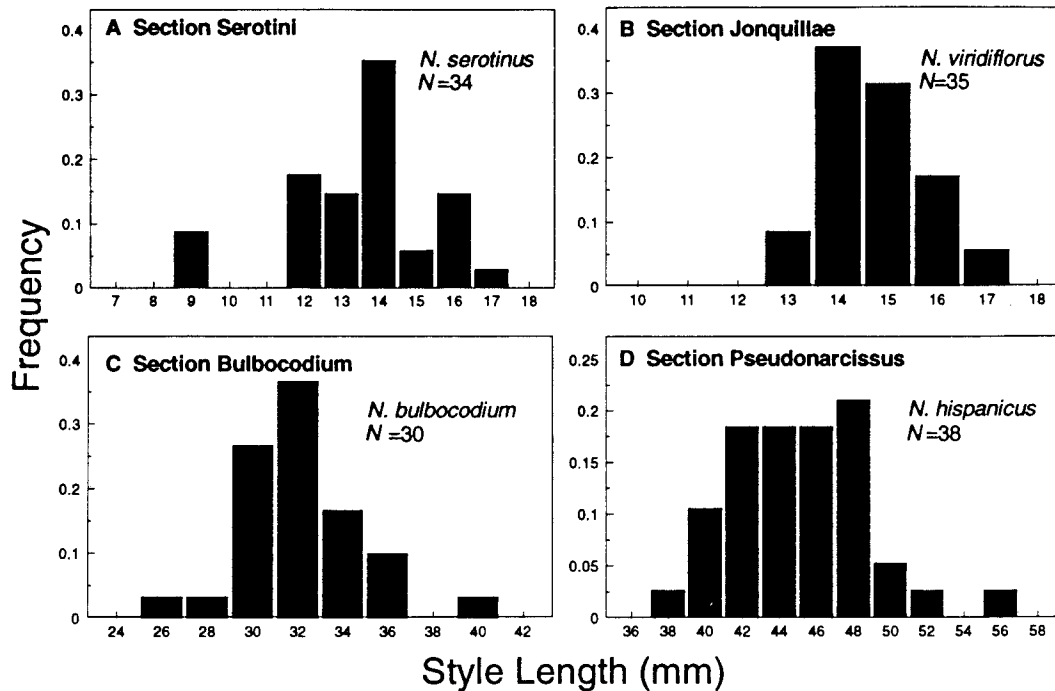


Figure 13.2. The distribution of style length in populations of four *Narcissus* species from different sections of the genus. Each species is monomorphic for style length. (A) *N. serotinus*. (B) *N. viridiflorus*. (C) *N. bulbocodium*. (D) *N. hispanicus*. *N* refers to the number of flowers sampled. In each population, a single flower was measured from plants sampled at random.

the stigma. The latter condition is referred to as *reverse herkogamy* (Webb and Lloyd, 1986). The occurrence of these two morphs within a population is characteristic of distylous species. Lloyd and Webb (1992a, p. 152) defined heterostyly in morphological terms 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.” This description would appear to fit the dimorphic *Narcissus* populations described above. However, in elaborating on this definition, Lloyd and Webb indicated that for a species to be considered truly heterostylous, both anther and stigma heights should differ in a reciprocal manner between the morphs and they introduced the term *reciprocal herkogamy* to describe this condition.

Although the floral variation in dimorphic *Narcissus* species resembles heterostyly, in our opinion it does not fully meet the criteria required to define that particular floral polymorphism. This is because anther heights are not sufficiently differentiated in the two floral morphs and there is little correspondence in height between the lower stamens of the long-styled morph and stigmas of the short-styled morph (see Fig. 13.1B). Measurements of sex-organ position in six dimorphic populations are presented in Table 13.1. Although mean stigma heights are markedly differentiated in each morph, the positions of upper and lower stamens

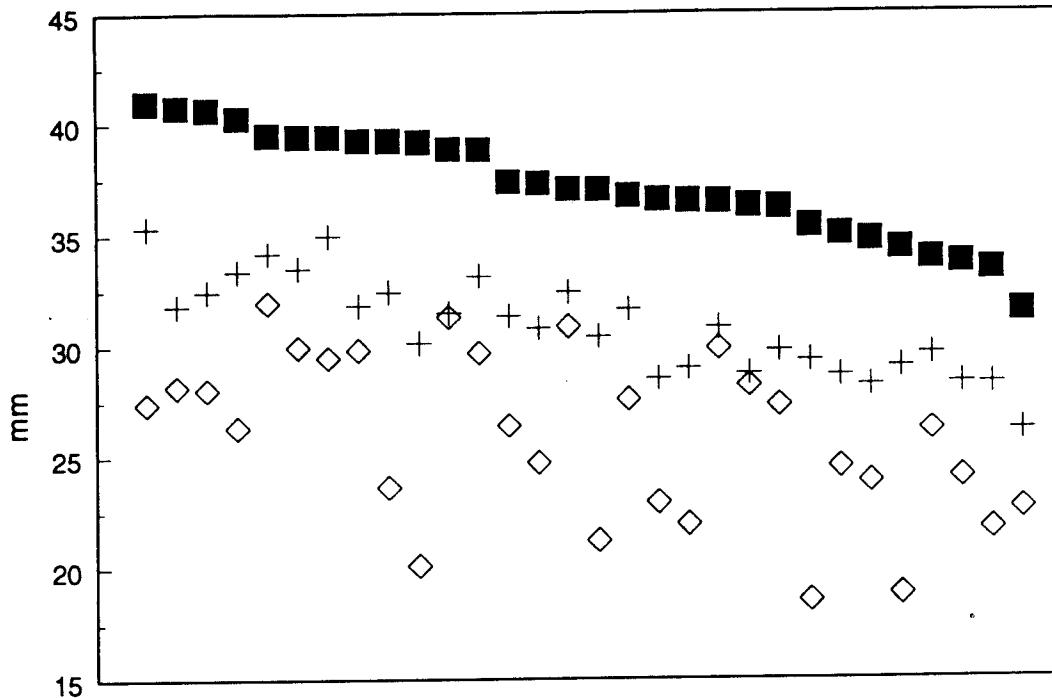


Figure 13.3. Variation in style length (■) and anther height (upper anthers +, lower anthers ◇) among individuals of *Narcissus bulbocodium* within a single population. Flowers are ranked by style length and each anther height measurement is the mean of the three anthers per level.

differ to only a limited degree. For example, in four of the six species examined, upper stamens in the two morphs are not significantly different in height. For lower stamens there are significant differences in all species, but the lower anthers of the short-styled morph are only a few millimetres higher in the floral tube than the corresponding anther level of the long-styled morph. Although these differences may be functionally important (see below), the clear lack of reciprocity in organ position probably disqualifies the dimorphic species of *Narcissus* from being considered truly distylous, particularly when a strictly morphological definition of the polymorphism is applied. Instead, we prefer to refer to the condition found in these species as involving a simple stigma-height dimorphism (also see Dulberger, 1964).

Considerable variation in the positions of stigmas and anthers occurs among individuals within dimorphic populations of *Narcissus* species. This is evident in Figure 13.5, which illustrates the range of variation in sex-organ position within a population of *N. assoanus*. Part of this variation is developmental in origin or associated with flower size differences among individuals; however, the basic dimorphism in stigma position is not obscured by these sources of variability. Among long-styled individuals, there is considerable variation in the degree of herkogamy. In most plants, the stigma is positioned above the two

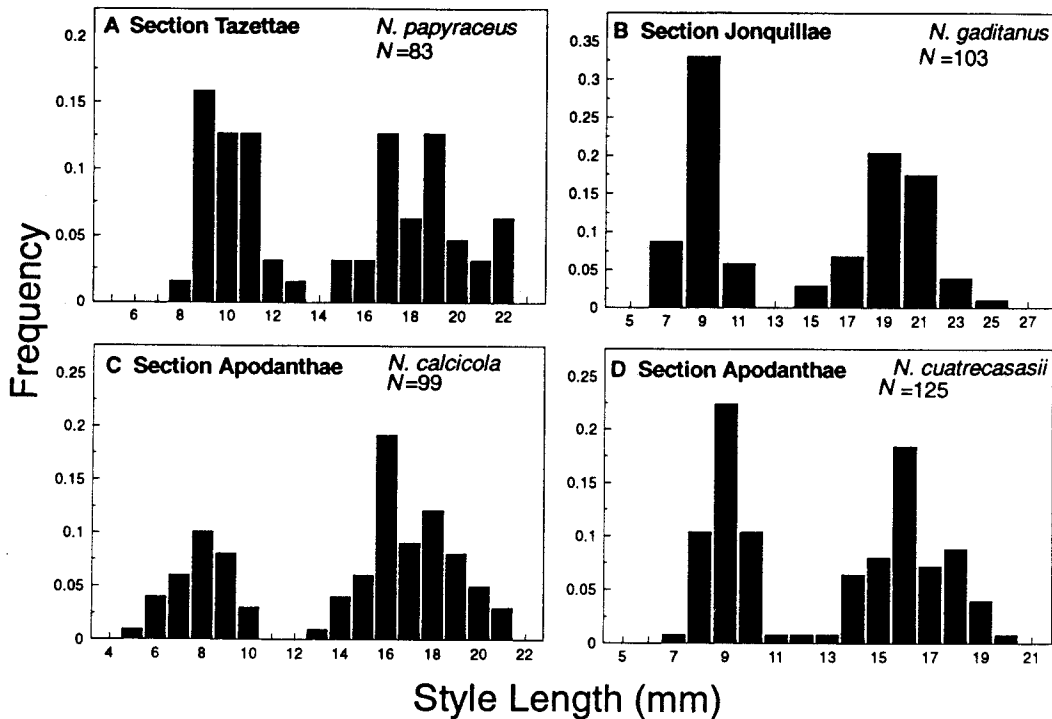


Figure 13.4. The distribution of style length in populations of four *Narcissus* species from three sections of the genus. Each population possesses a stigma-height dimorphism. (A) *N. papyraceus*. (B) *N. gaditanus*. (C) *N. calcicola*. (D) *N. cuatrecasasii*. *N* refers to the number of flowers sampled. In each population, a single flower was measured from plants sampled at random.

stamen levels; however, in some individuals the upper stamens are above the stigma and the lower stamens below it. This pattern was also evident in several other dimorphic species that were studied in detail (*N. calcicola*, *N. gaditanus*, *N. papyraceus*, and *N. rupicola*). Of particular functional significance was the finding that in all the dimorphic species studied, the average distance separating stigmas and anthers was considerably greater in the short-styled morph than the long-styled morph (Table 13.1). Averaged over the six species, the mean stigma-anther separation in the short-styled morph was approximately four times greater than in the long-styled morph. This consistent difference in the degree of herkogamy is likely to play an important role in influencing levels of self-pollination in the two sexual morphs. Elsewhere, there is good evidence that the distance separating stigmas and anthers has a strong effect on levels of self-pollination (e.g., Breese, 1959; Ennos, 1981; Thomson and Stratton, 1985; Barrett and Shore, 1987; Barrett and Husband, 1990; Murcia, 1990; Robertson and Lloyd, 1991).

Henriques (1887) reported long-, mid-, and short-styled morphs in *N. tazetta* of section Tazettae, and Fernandes (1964) “true trimorphic heterostyly” in several species in sections Jonquillae (*N. fernandesii*) and Apodantheae (*N. calcicola*,

Table 13.1. The positions of sexual organs in six species of *Narcissus*, each displaying genetic polymorphism for stigma height. Values given are the mean and standard error (in parentheses) of stigma height, upper and lower stamen heights, and stigma-anther separation in a single population of each species. Sample sizes for the long- and short-styled morphs (L, S morph, respectively) were *N. papyraceus*: 33, 30; *N. assoanus*: 46, 51; *N. gaditanus*: 52, 51; *N. calcicola*: 81, 80; *N. cuatrecasasii*: 68, 54; *N. rupicola*: 25, 75. Stigma and anther heights were measured from the base of the style and stigma-anther separation was based on the closest anther to the stigma. Significant differences were assessed using *t*-tests.

Species Style Morph	Stigma Height		Upper Anther Height		Lower Anther Height		Stigma-Anther Separation	
	L	S	L	S	L	S	L	S
<i>Narcissus papyraceus</i>	18.06	9.61	20.39	20.47	15.78	16.82	0.03	3.24
	(0.279)	(0.293)	(0.297)	(0.311)	(0.259)	(0.272)	(0.185)	(0.194)
<i>Narcissus assoanus</i>	24.53	13.15	22.63	23.15	18.06	19.71	0.84	4.91
	(0.322)	(0.305)	(0.345)	(0.327)	(0.307)	(0.292)	(0.237)	(0.225)
<i>Narcissus gaditanus</i>	18.62	8.10	15.95	16.24	10.89	12.69	1.64	3.16
	(0.219)	(0.221)	(0.209)	(0.211)	(0.192)	(0.194)	(0.165)	(0.167)
<i>Narcissus calcicola</i>	17.84	8.00	15.11	15.97	10.43	13.17	1.20	3.47
	(0.157)	(0.158)	(0.195)	(0.196)	(0.18)	(0.182)	(0.144)	(0.145)
<i>Narcissus cuatrecasasii</i>	15.67	8.47	16.39	17.26	11.90	15.08	0.25	2.64
	(0.155)	(0.174)	(0.131)	(0.147)	(0.144)	(0.162)	(0.088)	(0.098)
<i>Narcissus rupicola</i>	15.91	9.21	20.63	20.1	14.90	15.68	1.51	5.22
	(0.241)	(0.139)	(0.355)	(0.205)	(0.302)	(0.175)	(0.254)	(0.147)

ns = not significant.

***p* < 0.01.

****p* < 0.001.

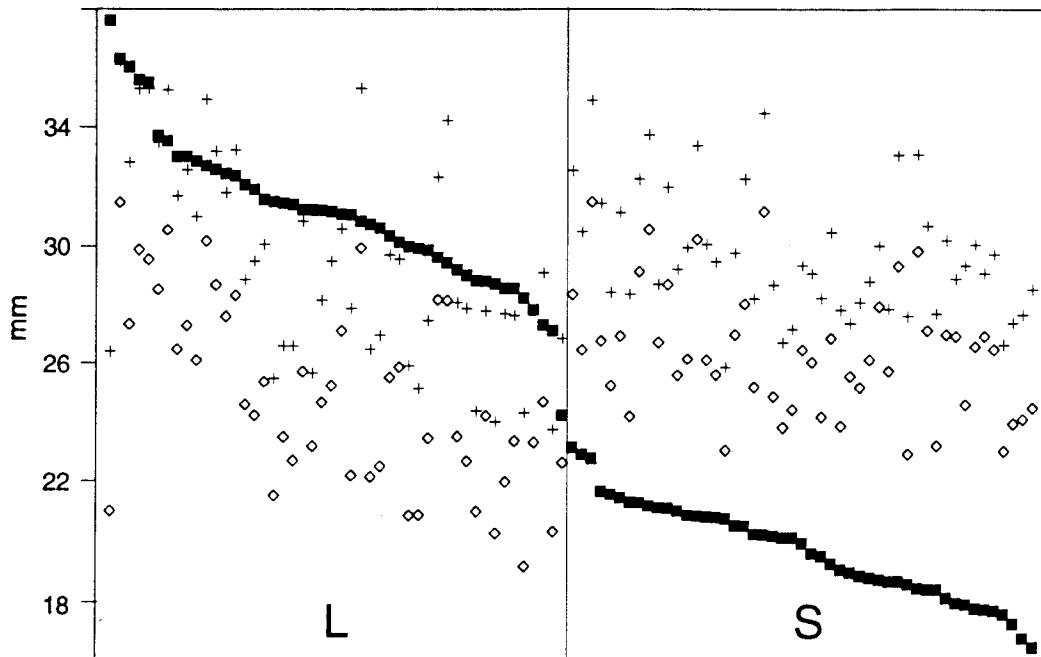


Figure 13.5. Variation in style length (■) and anther height (upper anthers +, lower anthers ◇) among individuals of *Narcissus assoanus* within a single population. Flowers are ranked by style length and each anther height measurement is the mean of the three anthers per level. L and S refer to the long- and short-styled morphs, respectively.

N. scaberulus, and *N. rupicola*). We suspect that this conclusion was based on observations of variation patterns similar to that illustrated in Figure 13.5. Individuals with stigmas positioned between the two stamen levels are likely to have been classified as mid-styled and the polymorphism interpreted as trimorphism. We believe this interpretation is erroneous and that only two sexual morphs are likely to occur in members of these three sections. The range of variation in stigma-anther position within the long-styled morph is continuous and the distance separating stamen levels does not warrant separation of a third class of plants classified as the mid-styled morph. Similarly, we see no merit in classifying individual plants with anthers and stigmas at similar heights as homostylous when they constitute part of the continuous variation displayed within one of the style-length morphs, as has been done for *N. tazetta* (see Yeo, 1975) and other polymorphic *Narcissus* species (Fernandes, 1965). Although this variation in sex-organ position may have important functional consequences for pollination, the use of these categories and the connotations from the heterostyly literature that they imply tend to obscure the fundamental dimorphism that occurs in populations of these species.

Floral Trimorphism

Narcissus triandrus appears to be the only species in the genus that displays a genuine genetic polymorphism for style length involving three discrete morphs

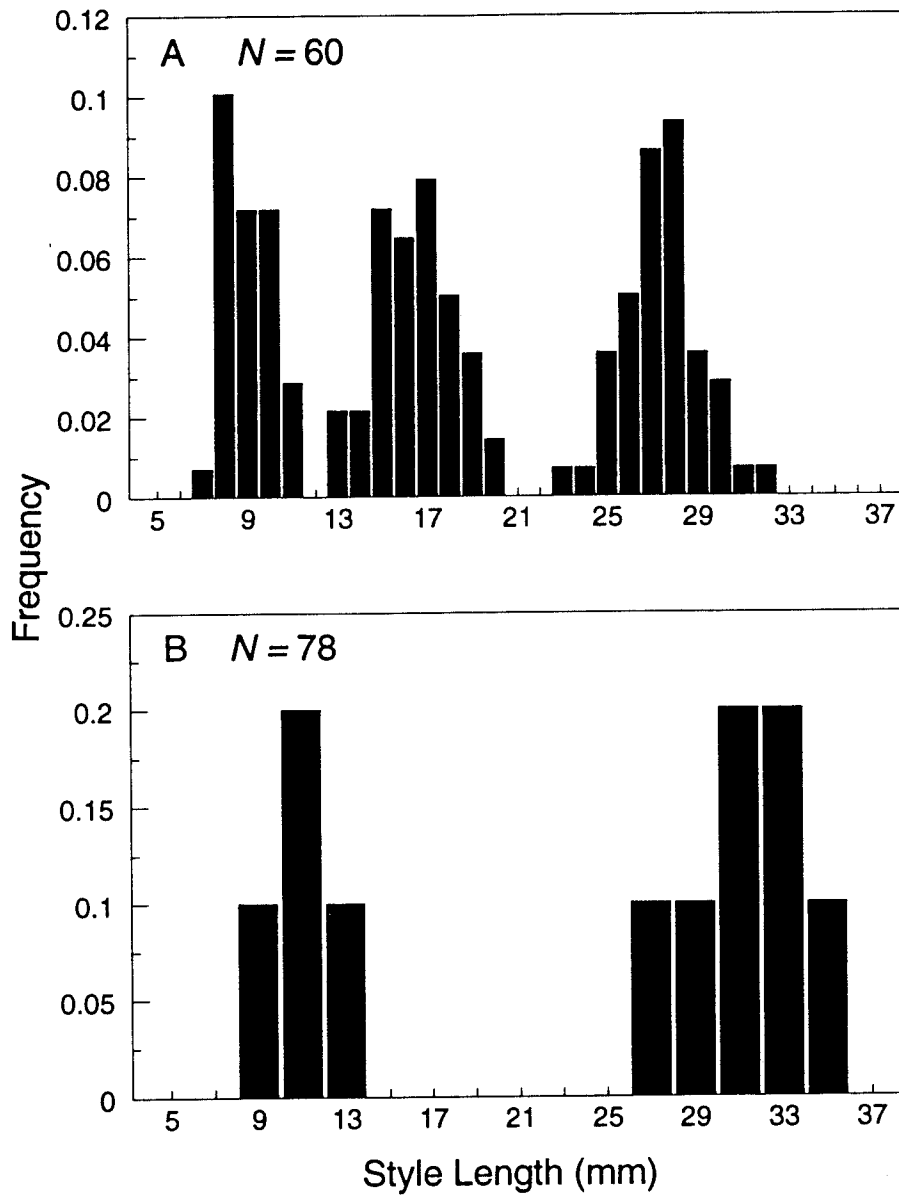


Figure 13.6. The distribution of style length in two populations of *Narcissus triandrus*. (A) A trimorphic population with long-, mid-, and short-styled plants (L, M, and S, respectively). (B) A dimorphic population with long- and short-styled plants (L and S, respectively). *N* refers to the number of flowers sampled. In each population, a single flower was measured from plants sampled at random.

(Fig. 13.6A). Most *N. triandrus* populations are comprised of long-, mid-, and short-styled individuals; however, dimorphic populations composed of long- and short-styled morphs (Fig. 13.6B) also occur in a restricted part of the geographical range of the species. In contrast to the dimorphic *Narcissus* species, in which stamen height differences between the morphs are slight, in *N. triandrus* three distinct stamen levels, which correspond to the positions of the three stigma

heights, are evident within trimorphic populations (Figs. 13.1C and 13.7A). This finding contradicts Bateman's (1952a) assertion that in *N. triandrus* "the anthers are at the same two levels in all individuals."

Because of the reciprocity between stigma and anther heights in populations of *N. triandrus*, we believe that the species warrants recognition as tristylous, as originally proposed by Henriques (1887) and Fernandes (1935). However, the anther positions in the three floral morphs of *N. triandrus* differ from those of any tristylous species that has been described in the literature. Although mid- and short-styled morphs exhibit reciprocal positioning of anther and stigma heights, in the long-styled morph the "mid-level" anthers are positioned above stigmas of the mid-styled morph and correspond in height to long-level stamens of the mid- and short-styled morphs. As a result, average stamen heights are identical in the long- and mid-styled morphs and only the short-styled morph possesses a distinct mid-level stamen position. A second atypical feature of tristily in *N. triandrus* is that, on average, stigmas in the long-styled morph are positioned above long-level anthers of the mid- and short-styled morphs. We believe these anomalies do not affect the conclusion that *N. triandrus* is tristylous; the sequence in which anthers and stigmas are presented within flowers is preserved, the sex organs occupy three distinct spatial location within each morph, and stigma-anther reciprocity is evident for two of the three organ levels.

Lloyd and Webb (1992a) suggested that the reciprocal herkogamy of heterostylous populations does not necessarily require that the heights of anthers and stigmas coincide precisely in the floral morphs, and indeed in many species they do not. However, these considerations indicate that the decision to classify a species like *N. triandrus* as morphologically heterostylous but exclude others, such as the dimorphic *Narcissus* species, is to some extent arbitrary, because it rests on judgments on the degree of reciprocity displayed by particular species. Although numerical indices can be used to quantify sex-organ reciprocity (Lloyd et al., 1990; Richards and Koptur, 1993; Eckert and Barrett, 1994), survey data indicate that wide variation in reciprocity occurs among heterostylous plants and those with stigma-height polymorphisms (Richards and Koptur, 1993; Eckert and Barrett, 1994; Richards et al., unpublished). This problem is reminiscent of early attempts to classify the quantitative variation in gender that exists in many flowering plants into discrete categories (see Lloyd, 1980; Barrett, 1992b).

In all dimorphic populations of *N. triandrus* that we have observed, the mid-styled morph is absent (Fig. 13.7B). A similar pattern was also reported by Fernandes (1965). In some dimorphic populations of *N. triandrus*, mid-level stamens in the short-styled morph are positioned above the corresponding stamen level of this morph from trimorphic populations. This modification is similar to that described in populations of tristylous *Oxalis alpina* missing the mid-styled morph (Weller, 1992) and is probably a consequence of selection for efficient pollen transfer to stigmas of the long-styled morph. Differences between mid-level stamen position in the long-styled morph from trimorphic and dimorphic

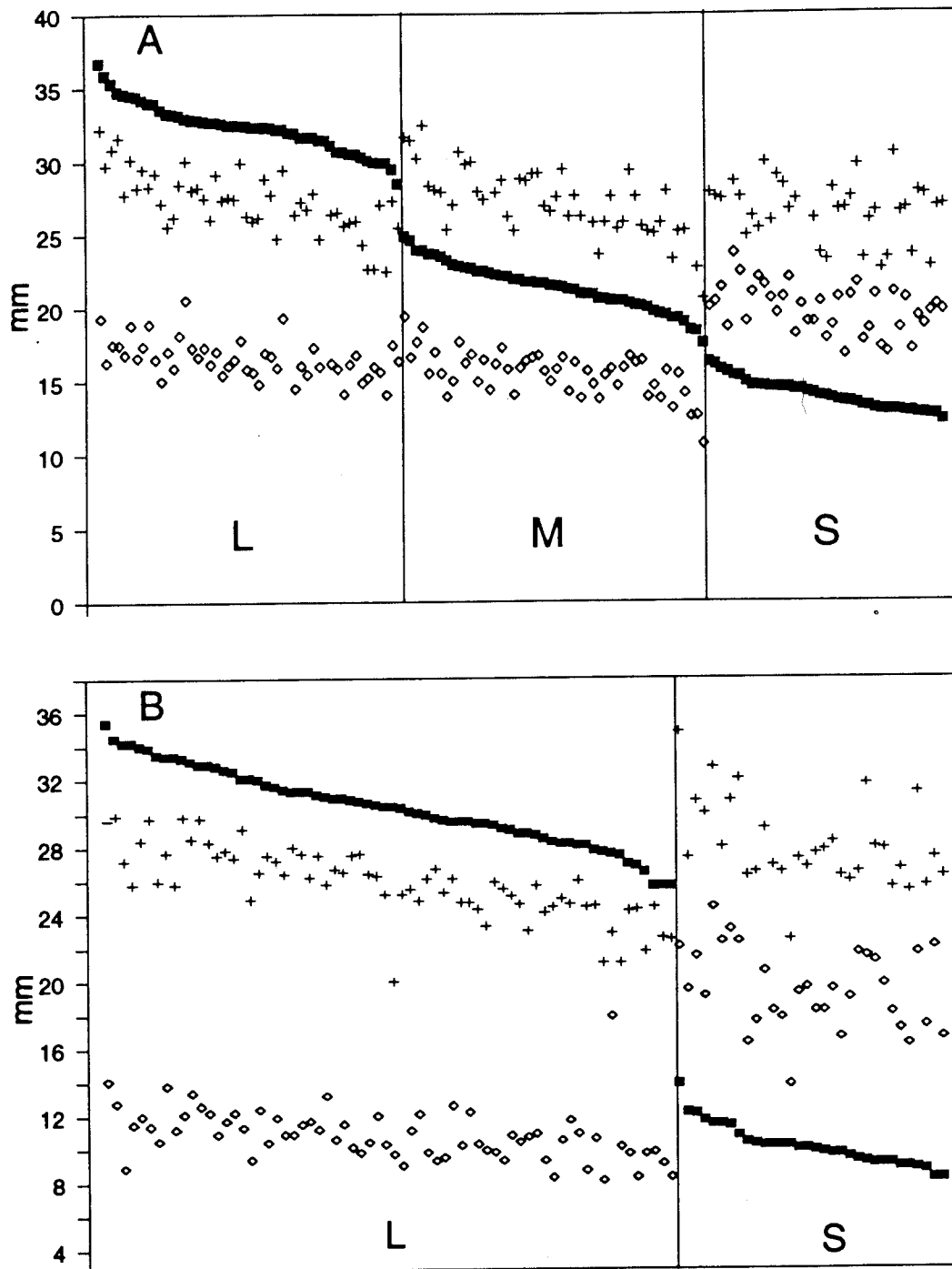


Figure 13.7. Variation in style length (■) and anther height (upper anthers +, lower anthers ◇) among individuals of *Narcissus triandrus* sampled from a trimorphic and dimorphic population. Flowers are ranked by style length and each anther height measurement is the mean of the three anthers per level. (A) In the trimorphic population, three distinct morphs are evident that differ in the sequence in which stigmas and anthers are presented within a flower. Note that unlike most tristylous species, anther heights are similar in the long-styled (L) and mid-styled (M) morphs. The short-styled (S) morph possesses distinct mid-level anthers corresponding in height to stigmas of the M morph. (B) In the dimorphic population, two distinct morphs (L and S) occur and the M morph is absent.

populations are less evident, presumably because this stamen level is already positioned above mid-level stigmas in trimorphic populations (see Fig. 13.1C).

Style-Morph Frequencies in Natural Populations

A considerable body of survey data exists on the frequencies of style morphs in populations of heterostylous plants (see Ganders, 1979; Barrett, 1993). In equilibrium populations with legitimate (intermorph) mating and no fitness differences among morphs, the frequencies of morphs should be equal for both the distylous and tristylous condition (Charlesworth and Charlesworth, 1979b; Heuch, 1979). This expectation is often observed, particularly in distylous populations. Because of the rarity of stigma-height polymorphisms in flowering plants, no comprehensive surveys have been conducted. To investigate morph ratios in *Narcissus*, we surveyed two widespread species (*N. papyraceus* and *N. triandrus*) extensively and several rarer species in less detail. Because style morphs are clearly distinguished morphologically, there is no ambiguity in classifying plants into different style-morph categories. Our results indicate an unusual pattern of style-morph structure that has not been reported elsewhere.

Dimorphic Populations

Surveys of nine populations of five dimorphic *Narcissus* species in sections Jonquillae and Apodanthae indicate a similar pattern (Table 13.2). In each population, the long-styled morph is most common with frequencies ranging from 0.61–

Table 13.2. Style-morph frequencies in nine dimorphic populations of five species of *Narcissus* from Spain and Portugal. L. and S refer to the long- and short-styled morphs, respectively.

Section	Species	Morph Frequency		L/S Ratio	Number of Plants Sampled
		L	S		
Jonquillae					
<i>N. assoanus</i>	Pop. 1	0.76	0.24	3.17	287
	Pop. 2	0.62	0.38	1.63	145
<i>N. gaditanus</i>	Pop. 1	0.76	0.24	3.17	255
	Pop. 2	0.94	0.06	15.7	55
<i>N. jonquilla</i>	Pop. 1	0.85	0.15	5.67	26
Apodanthae					
<i>N. calcicola</i>	Pop. 1	0.68	0.32	2.13	355
	Pop. 2	0.61	0.39	1.56	307
<i>N. cuatrecasasii</i>	Pop. 1	0.77	0.23	3.35	248
	Pop. 2	0.72	0.28	2.57	202

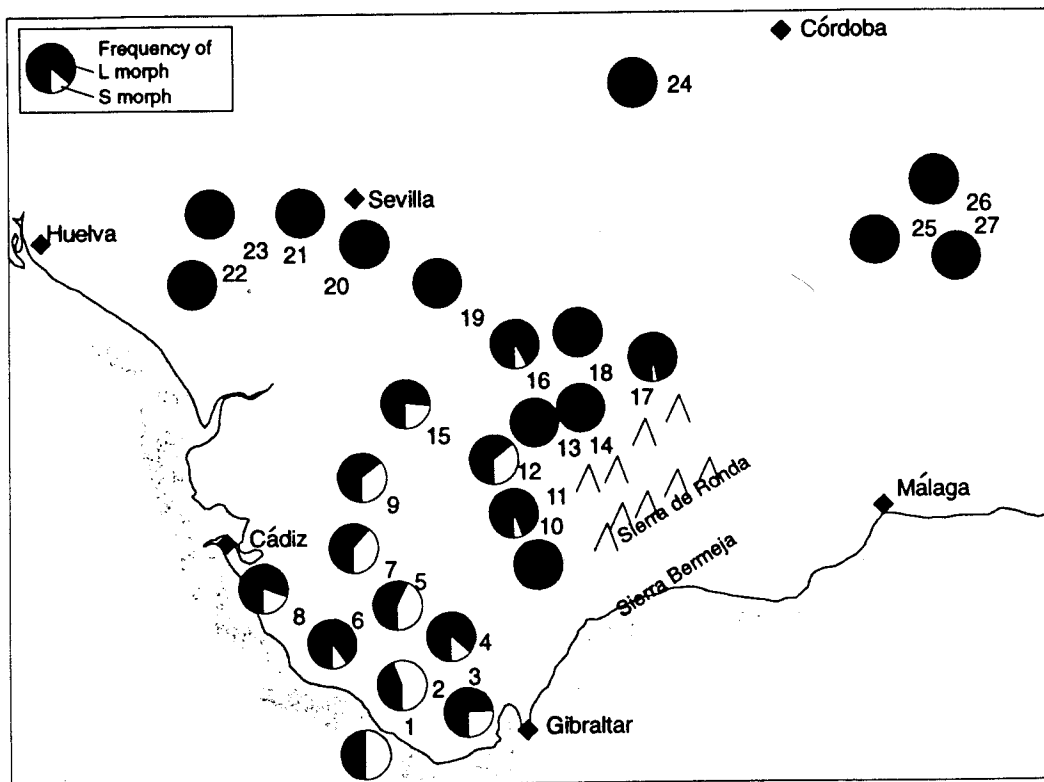


Figure 13.8. The frequencies of the long-styled (L) and short-styled (S) morphs of *Narcissus papyraceus* in a survey of 27 populations from southwest Spain. Populations occurring in the southern coastal portion of the region surveyed were dimorphic, whereas inland populations, particularly in mountainous areas, were more often monomorphic.

0.94. This pattern was also evident in the detailed survey of *N. papyraceus* populations from southern Spain (Fig. 13.8). Of 27 populations examined, 15 were dimorphic with the long-styled morph comprising 75% of all plants sampled ($N = 1305$ plants). All remaining populations contained long-styled plants only ($N = 913$ plants).

Monomorphic and dimorphic populations of *N. papyraceus* were nonrandomly distributed over the region surveyed. In the southern coastal area, populations were dimorphic with variable frequencies of the short-styled morph ranging from a slight excess in one population to deficiencies in most others. Populations further north, many of which occurred in more mountainous regions, were uniformly monomorphic. These patterns suggest that under certain ecological conditions the short-styled morph may be at a selective disadvantage. Elsewhere, Dulberger (1967) and Arroyo and Dafni (1995) found striking differences in style-morph ratios among dimorphic populations of the related *N. tazetta* from hill and marsh (lowland) habitats of Israel. In Dulberger's survey, morph ratios exhibited a slight excess of the short-styled morph in lowland populations growing on alluvial

soils (mean frequencies: L morph = 0.45, S morph = 0.55; $N = 6$ populations, and $N = 1814$ plants). In contrast, hill populations occurring on rocky slopes displayed a predominance of the L morph (mean frequencies: L morph = 0.86, S morph = 0.14; $N = 6$ populations, and $N = 711$ plants). Arroyo and Dafni (1995) have suggested that pollinator-mediated selection may account for the observed patterns. It would seem worthwhile to investigate this possibility in *N. tazetta* and in *N. papyraceus* populations from southern Spain.

Trimorphic Populations

Narcissus triandrus is widely distributed throughout the Iberian peninsula. We surveyed 80 populations from four major geographical regions including southern, central, and Atlantic Spain and central and northern Portugal. Our survey of style-morph ratios revealed three distinct patterns: (1) The long-styled morph predominated in both trimorphic and dimorphic populations (mean frequencies in trimorphic populations: L morph = 0.57, M morph = 0.22, S morph = 0.21; $N = 68$ populations, and $N = 14,515$ plants; dimorphic populations: L morph = 0.71, S morph = 0.29; $N = 12$ populations, and $N = 1535$ plants). Fernandes (1935, 1965) found a similar pattern involving the predominance of the long-styled morph in a small sample of populations from central Portugal. (2) In trimorphic populations, a decrease in the average frequency of the mid-styled morph among the different regions was associated with an increase in the long-styled morph. In contrast, the average frequency of the short-styled morph was remarkably similar throughout the range (Table 13.3). (3) All dimorphic populations were concentrated in central and northern Portugal and Atlantic Spain. In these regions, the average frequency of the mid-styled morph in trimorphic populations was low in comparison with its frequency in central and southern Spain. Hence, as in dimorphic species of *Narcissus*, the long-styled morph in *N. triandrus* has a fitness advantage over the short-styled morph. In addition, in the northern part of the range of *N. triandrus*, the ecological conditions are apparently often unsuitable for the maintenance of floral trimorphism. When this

Table 13.3. Style-morph frequencies (standard deviation in parentheses) in 68 trimorphic populations of *Narcissus triandrus* sampled from different regions of Spain and Portugal. L, M, and S refer to the long-, mid-, and short-styled morphs, respectively.

Region	Morph Frequency			Number of Populations Sampled	Number of Plants Sampled
	L	M	S		
Southern Spain	0.45 (0.10)	0.35 (0.09)	0.20 (0.04)	10	2962
Central Spain	0.46 (0.13)	0.32 (0.11)	0.22 (0.05)	18	5536
Central Portugal	0.57 (0.11)	0.21 (0.10)	0.22 (0.06)	17	3512
Northern Spain and Portugal	0.70 (0.08)	0.09 (0.07)	0.22 (0.06)	23	2505

occurs, it is always the mid-styled morph that is lower in frequency or missing from populations.

Field studies of the floral biology of the morphs are required to determine the selective mechanisms responsible for the particular morph ratios found in *N. triandrus* and other species of *Narcissus*. Floral morphology and incompatibility in *Narcissus* species are not associated, hence, it is not unexpected that equilibrium morph ratios are quite different from those found in heterostylous species with diallelic incompatibility. Because all cross-pollinations in *Narcissus* are compatible, morph ratios are likely to be governed by the genetic system controlling floral polymorphism and the relative fitness of the morphs as male and female parents. Interestingly, in dimorphic *Anchusa officinalis* in which style length and incompatibility are apparently also uncoupled, population surveys indicate that the frequency of the long-styled morph also greatly exceeds that of the short-styled morph (Philipp and Schou, 1981).

Bateman (1968) proposed pollinator-mediated selection as a mechanism to account for variation in the frequencies of style morphs among populations of *N. triandrus*. He suggested that the style morphs may be adapted to different pollinators that vary in their abundance among populations. He also suggested that monomorphic species such as *N. bulbocodium* and *N. pseudonarcissus* were adapted to more "regular" pollinators and styles in these species had therefore been selected for an optimum length. Although it seems improbable that the style morphs represent "different ecotypes adapted to different pollination conditions" as Bateman (1968, p. 646) suggested, we believe that information on the interactions between pollinators and the floral morphs is likely to be the key to understanding morph ratios in *Narcissus* populations. In this regard, it is worth noting that our preliminary observations of pollinators visiting *N. triandrus* in different regions of the Iberian peninsula revealed a striking pattern associated with the distribution of trimorphic vs. dimorphic populations. Throughout much of the central and southern range of the species occupied exclusively by trimorphic populations, the most common floral visitor was the bee *Anthophora pilipes* Fabr. (= *A. acervorum* L.). In contrast, in the cooler Atlantic zone where dimorphic populations are primarily located, this bee was not observed and, instead, bumble bees (*Bombus* spp.) were the main floral visitors. It is possible that species of *Bombus* are less effective at mediating segregated pollen transfer among the three morphs and this may account for the apparent selective disadvantage to the mid-styled morph at the northern edge of the range.

Evolutionary Considerations

The inter- and intraspecific patterns of style-morph variation in *Narcissus* described above raise many questions concerning the evolutionary origins and maintenance of the polymorphisms. For example, what are the evolutionary

relationships between stylar monomorphism, dimorphism, and trimorphism and do these represent stages in the assembly of tristily in the genus? What selective forces are responsible for the evolution of stigma-height polymorphisms and why does the long-styled morph predominate in most populations? What evolutionary constraints might account for the rarity of heterostyly in *Narcissus* compared with the more common occurrence of stigma-height dimorphism in the genus? To clarify some of these issues, we next develop several hypotheses and a quantitative selection model of the evolution of sexual polymorphisms in *Narcissus* to guide future studies aimed at answering these questions.

Evolutionary Relationships Among Stylar Conditions

The occurrence of stylar monomorphism, dimorphism, and trimorphism among taxa of *Narcissus* is markedly uneven. Stylar monomorphism represents the most widespread condition, occurring in most species and sections, dimorphism is restricted to four sections, and trimorphism is apparently limited to the single species (*N. triandrus*) of section Ganymedes. Although phylogenetic analysis is required to determine the evolutionary relationships of taxa and polarity of stylar conditions, we assume as a working hypothesis that monomorphism represents the ancestral stylar condition in the genus and stylar polymorphisms have evolved from this state. Whether stylar trimorphism is derived from stylar dimorphism or directly from a monomorphic condition is not known. However, we consider the former to be more likely. We take this position because we find it difficult to imagine that a complex polymorphism like tristily would not evolve via an intermediate condition involving two morphs. This was also the view taken by Charlesworth (1979) in her theoretical model of the evolution of tristily.

The occurrence of stigma-height polymorphisms in a genus that contains heterostyly is relevant to recent models of the evolution of heterostyly developed by Lloyd and Webb (1992 *a, b*). In their models, they considered establishment of a stigma-height polymorphism to be the first stage in the evolution of reciprocal herkogamy. Stylar dimorphisms in *Narcissus* therefore take on added significance because they may represent an intermediate stage in the evolution of tristily in section Ganymedes. It is not clear how similar the stylar dimorphisms that occur in extant members of the genus are to putative transitional stages that gave rise to tristily in *N. triandrus*. Furthermore, the postulated sequence from monomorphism via dimorphism to trimorphism does not preclude the possibility that secondary reversions from dimorphism to monomorphism or from trimorphism to dimorphism have occurred through the loss of style morphs from populations. Indeed, it seems likely that reversions of this type might account for some of the monomorphic and dimorphic populations that occur in *N. papyraceus* and *N. triandrus*, respectively.

If we assume that stylar monomorphism is the ancestral state in *Narcissus*, it

is of interest to consider what other floral characters occurred in the ancestors of species that now possess stylar polymorphisms. We consider three conditions particularly likely: wide perianths, approach herkogamy, and self-sterility.

Most *Narcissus* species with stylar monomorphism are characterized by either bowl-shaped or broadly tubular flowers. Pollinators visiting these species frequently enter the flower and contact anthers in an imprecise manner, rather than probing from the mouth of the tube. It seems likely that flowers of this type would not effectively promote reciprocal pollen transfer based on segregated contact of stigmas and anthers (see Lloyd and Webb, 1992b, Fig. 13.1) Accordingly, stylar polymorphisms in *Narcissus* are probably not associated with these types of floral shapes. In contrast, all species with stylar polymorphisms possess well-developed floral tubes that force pollinators into stereotypical positions while probing for nectar. This raises the possibility that a change from a relatively unspecialized pollination syndrome to one with greater precision, involving constricted floral tubes, long-tongued pollinators, and segregated contact of reproductive organs, may have provided the stimulus for the evolution of sexual polymorphisms in *Narcissus*. In this context, it is worth noting that among heterostylous families the types of flowers that have become heterostylous are predominantly those that possess prominent floral tubes. This is presumably because tube formation is advantageous in positioning insect mouth parts for segregated contact and efficient cross-pollination (Ganders, 1979; Lloyd and Webb, 1992a).

Both approach herkogamy and self-sterility are common in *Narcissus* species and occur in most of the sections for which information is available. It would seem unlikely given the rarity of their alternate states, namely, self-compatibility and reverse herkogamy, that the evolution of stylar polymorphisms involved species with these latter features. These arguments lead to the hypothesis that the reproductive systems of the ancestors of *Narcissus* taxa that currently possess stylar polymorphisms were largely outcrossing through self-sterility and possessed a floral morphology involving approach herkogamy and two stamen levels. This morphological phenotype was used as the ancestral state in Charlesworth's (1979) model of the evolution of tristylly. Lloyd and Webb (1992a) also concluded, based on comparative evidence, that the ancestors of heterostylous species were most likely to possess approach herkogamy. Following this view, the first step in the evolution of sexual polymorphisms was the invasion of an approach herkogamous population by a reverse herkogamous variant. Since the population may have been largely outcrossing, it seems likely that the selective forces responsible for the evolution of stylar polymorphisms in *Narcissus* did not involve inbreeding avoidance, but rather concerned aspects of pollen dispersal and possibly also pollen-stigma interference. To explore whether these features of floral biology may have been important, we next develop quantitative phenotypic selection models of this invasion process, in an effort to account for the evolution of stable stigma-height polymorphisms in *Narcissus*.

Selection of a Stigma-Height Dimorphism

As part of their theoretical work on the evolution of distyly, Lloyd and Webb (1992b) developed a phenotypic selection model for the evolution of a stable stigma-height polymorphism. Here we modify this model to take into account particular features of the floral biology of *Narcissus*. Specifically, we investigate to what extent the morph-specific differences in floral morphology, interacting with the particular type of late-acting ovarian self-sterility that occurs in *Narcissus*, may assist the invasion of herkogamous variants into populations monomorphic for style length. Recall that prior self-pollination of outcrossed *Narcissus* flowers can result in a significant reduction of seed set. This arises because the growth rates of self- and outcross pollen tubes are similar, and early arrival of self-pollen renders ovules that would otherwise be fertilized by outcross pollen tubes nonfunctional in some way. Hence, self-pollination can have significant reproductive consequences, depending on the arrival schedule of self- and outcross pollen. We refer to this loss of female fertility as *ovule discounting*. This differs from *seed discounting* (Lloyd, 1992), in which an increase in the amount of self-fertilized seed is accompanied by a concomitant decrease in some or all of the cross-fertilized seed. Because stigma-anther separation is much greater in the reverse herkogamous morph compared with the approach herkogamous morph (Table 13.1), we explore whether differences in ovule discounting between the morphs resulting from differential self-pollination favor the initial invasion of a reverse herkogamous morph. In addition, we examine whether features of the floral biology of *Narcissus* could result in the observed predominance of the long-styled morph (hereafter approach herkogamous morph) relative to the short-styled morph (hereafter reverse herkogamous morph).

The model describes a dimorphic population containing a fraction a of approach herkogamous plants and a complementary fraction, $r = 1 - a$, of reverse herkogamous plants. Both morphs are assumed to be identical, except in style length, with each plant producing f flowers with o ovules each. We additionally assume that pollinators visit morphs randomly and pollen is transported only to the next plant visited by a pollinator. The average number of pollen grains transported between two plants (per flower) is q_{ij} , where i indicates the style morph of the donor plant and j identifies the morph of the recipient plant (e.g., q_{aa} . . . , whereas q_{ra} . . .) Given these morph frequencies and pollen-transfer proficiencies, the average approach herkogamous plant imports $f(aq_{aa} + rq_{ra})$ pollen grains and exports $f(aq_{aa} + rq_{ar})$ grains. Correspondingly, the average reverse herkogamous plant imports $f(aq_{ar} + rq_{rr})$ pollen grains and exports $f(aq_{ra} + rq_{rr})$ grains. For both morphs, we assume that the intensity of pollen dispersal is not sufficient to fertilize all available ovules.

Postpollination processes now act to determine seed production. Due to various causes (e.g., inviability and germination failure) only a fraction k of outcross pollen grains produce pollen tubes that successfully enter the ovary. We assume

that a pollen tube growing toward a particular ovule cannot alter its course if another pollen tube has already entered the micropyle. Consequently, not all the pollen received by a plant fertilizes ovules, even though seed production is pollen-limited. Of particular interest is the reduced ovule availability that results when a self-pollen tube disables an ovule. Because of ovule discounting, the average proportion of ovules available after self-pollination is v_a for approach plants and v_r for reverse plants.

The average fitness of plants of a given morph depends on the sum of female and male fertilities. For the approach herkogamous morph, female fertility is the number of ovules available per plant (fov_a) times the average pollen receipt ($aq_{aa} + rq_{ra}$) times the probability that a pollen grain received fertilizes an ovule (k), or $fov_a k(aq_{aa} + rq_{ra})$. The corresponding male fertility is the sum of siring success on other approach herkogamous flowers ($afov_a kq_{aa}$) and reverse herkogamous flowers ($rfov_r kq_{ar}$), or $fok(av_a q_{aa} + rv_r q_{ar})$. Hence, the total fitness of approach herkogamous plants is therefore:

$$w_a = fok[v_a(aq_{aa} + rq_{ra}) + av_a q_{aa} + rv_r q_{ar}]$$

Similarly, the total fitness for reverse herkogamous plants is:

$$w_r = fok[v_r(aq_{ar} + rq_{rr}) + av_a q_{ra} + rv_r q_{rr}]$$

The fitness advantage of reverse herkogamous plants is thus

$$w_r - w_a = fok\{a[q_{ar}v_r + v_a(q_{ra} - 2q_{aa})] - r[q_{ra}v_a + v_r(q_{ar} - 2q_{rr})]\}$$

Now consider whether the reverse herkogamous morph could invade a population of approach herkogamous plants (i.e., when $a \cong 1$ and $r \cong 0$). Invasion occurs when $w_r - w_a > 0$, or $q_{ar}v_r + v_a(q_{ra} - 2q_{aa}) > 0$, or alternatively,

Condition 1

$$\frac{q_{ar}\left(\frac{v_r}{v_a}\right) + q_{ra}}{2} > q_{aa}$$

If relative ovule availability is equal for the two morphs (i.e., $v_a = v_r$), this condition specifies that the average proficiency of pollen transfer between morphs must exceed the transfer proficiency among approach herkogamous plants (Eq. 5a of Lloyd and Webb, 1992b). Between-morph differences in ovule discounting modify this condition, so that invasion by the reverse herkogamous morph is

more likely if approach herkogamous flowers lose relatively more ovules as a result of self-pollination (i.e., $v_a < v_r$). The approach herkogamous morph can invade a population of reverse herkogamous plants (i.e., when $a \cong 0$ and $r \cong 1$) if $w_r - w_a < 0$, or $-q_{ra}v_a - v_r(q_{ar} - 2q_{rr}) < 0$, or alternatively,

Condition 2

$$\frac{q_{ar} + q_{ra} \left(\frac{v_a}{v_r} \right)}{2} > q_{rr}$$

As with condition 1, the weighted average proficiency of pollen transfer between morphs must exceed transfer among reverse herkogamous plants; however, in this case greater ovule discounting by the approach herkogamous morph renders invasion less likely. Once one morph invades a population of the other morph, a stable stigma-height dimorphism persists only if both conditions 1 and 2 are satisfied.

Two questions now arise:

1. Are between-morph differences in ovule discounting sufficient to allow persistence of a dimorphic population? If stigma position affects only ovule discounting and not pollen dispersal, then $q_{ar} = q_{ra} = q_{aa} = q_{rr}$. Now if, for example, approach herkogamous flowers suffer more ovule discounting than reverse herkogamous flowers (i.e., $v_a < v_r$) so that $v_r/v_a > 1$, condition 1 holds, but condition 2 does not. As a result, the reverse herkogamous morph could not only invade a population monomorphic for the approach herkogamous condition, but it should spread to fixation. Hence, based on this model, a balanced dimorphism *cannot* be maintained solely by between-morph differences in ovule discounting, whereas differences in pollen-transfer probabilities are both necessary and sufficient.

2. If a balanced dimorphism develops, what is the equilibrium morph ratio (a^*/r^*)? At equilibrium $w_r - w_a = 0$ and the expected morph ratio is therefore

$$\frac{a^*}{r^*} = \frac{q_{ra}v_a + v_r(q_{ar} - 2q_{rr})}{q_{ar}v_r + v_a(q_{ra} - 2q_{aa})}$$

For *Narcissus*, we expect the proficiency of pollen transfer onto reverse herkogamous flowers to be independent of the donor morph (i.e., $q_{ar} = q_{rr} = q_{.r}$). Given this simplification, the equilibrium frequency of approach herkogamous plants is

$$a^* = \frac{q_{ra}v_a - q_{.r}v_r}{2v_a(q_{ra} - q_{aa})}$$

According to this expression, approach herkogamous plants will tend to predominate at equilibrium if (1) pollen transfer from reverse to approach herkogamous

plants is more proficient than transfer among approach herkogamous plants (i.e., $q_{ra} > q_{aa}$ or $q_{ra}/q_{aa} > 1$). (2) Pollen transfer onto reverse herkogamous plants is less proficient than among approach herkogamous plants (i.e., $q_{aa} > q_r$ or $q_r/q_{aa} < 1$). (3) The ratio of ovule availability on reverse herkogamous plants relative to approach herkogamous plants is less than the ratio of pollen transfer among approach herkogamous plants to transfer onto reverse herkogamous plants (i.e., $v_r/v_a < q_{aa}/q_r$).

The first of these conditions could occur in *Narcissus* because of two floral mechanisms. In the reverse herkogamous morph, both absence of stylar interference with pollen dispatch by pollinators or the greater average height of anthers in this morph (Table 13.1) may result in more proficient cross-pollination to the approach herkogamous morph. The second condition involves pollen dispersal onto reverse herkogamous plants. Because of the lack of reciprocity between stigmas of reverse herkogamous plants and anthers in either morph, it seems likely that pollen transfer onto reverse herkogamous plants may be less proficient than that onto approach herkogamous plants. This suggestion was first proposed for *Narcissus* by Yeo (1975). In contrast to the first two conditions, the third requirement may not apply for *Narcissus* because the pattern of ovule discounting, if indeed it occurs, will likely be greater on approach herkogamous flowers and hence v_r/v_a will be >1 , whereas if condition 1 is true, q_{aa}/q_r will be <1 .

The effects of different combinations of pollen transfer probabilities and ovule discounting on equilibrium morph ratios are illustrated in Figure 13.9. Over a broad range of conditions, the approach herkogamous morph predominates, particularly when pollen transfer between approach herkogamous plants is more proficient than that onto reverse herkogamous plants. A simple numerical example serves to illustrate these effects. If pollen transfer from reverse to approach herkogamous flowers is 50% more proficient than among approach flowers (i.e., $q_{ra} = 1.5q_{aa}$) and transfer onto reverse herkogamous flowers is 40% less proficient (i.e., $q_r = 0.6q_{aa}$), then the ratio of approach to reverse herkogamous morphs would be 0.90:0.10, in the absence of ovule discounting (i.e., $v_a = v_r = 1$). However, if the approach herkogamous morph has 20% fewer ovules available for outcrossing owing to ovule discounting (i.e., $v_a = 0.8v_r$), then the same transfer probabilities result in an equilibrium morph ratio of 0.75:0.25. Thus, although the maintenance of a stable stigma-height polymorphism requires frequency-dependent selection resulting from the promotion of intermorph pollen transfer, the potential occurrence of less ovule discounting in the reverse herkogamous morph of *Narcissus* removes the necessity for both morphs to have a promotion advantage. In addition, it reduces the minimum size of such an advantage, especially during the early stages of the establishment of the polymorphism.

Constraints on the Evolution of Heterostyly

The first step in Lloyd and Webb's (1992b) model for the evolution of distyly is the establishment of a stigma-height polymorphism. This type of polymorphism

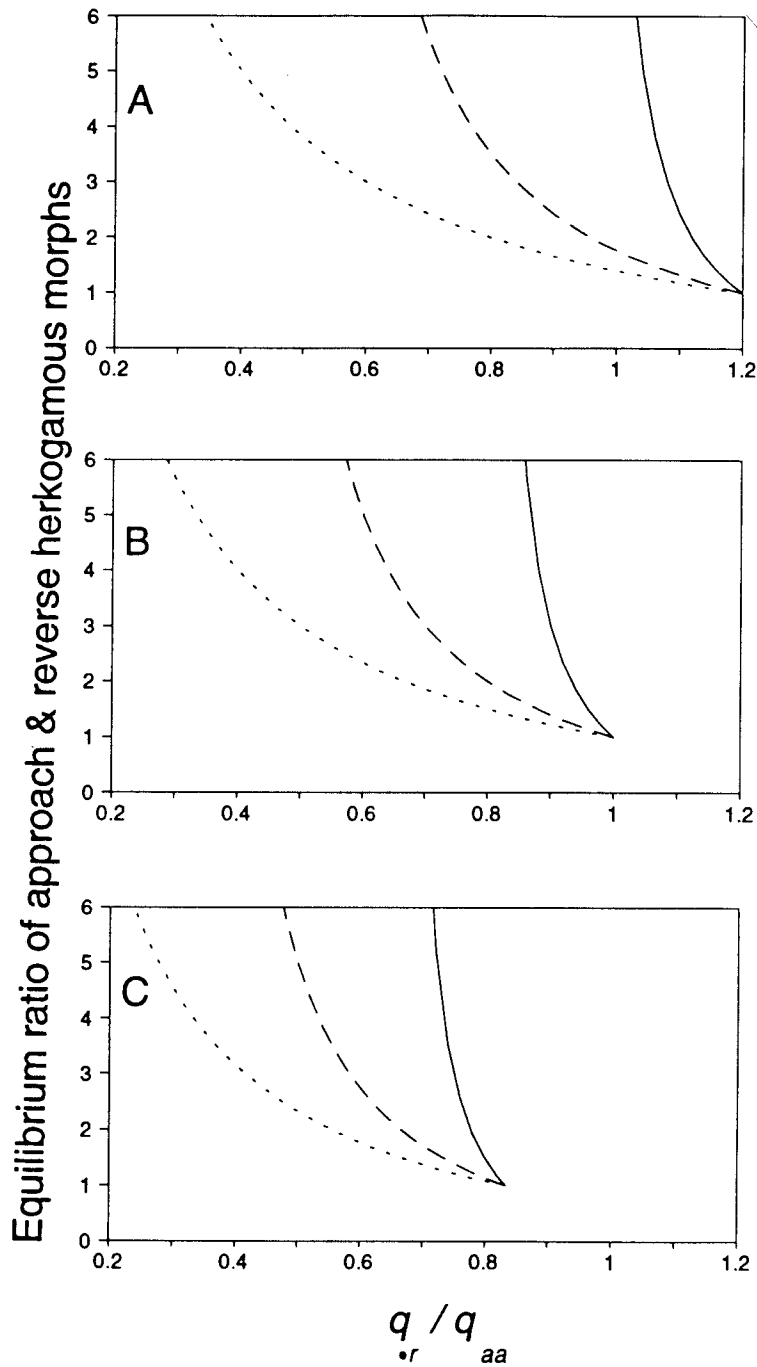


Figure 13.9. Examples of combinations of pollen-transfer probabilities and ovule discounting that result in an excess of approach herkogamous plants (L morph) compared with reverse herkogamous plants (S morph) at equilibrium in populations with a stigma-height dimorphism. The three panels illustrate the effects of different ratios of ovule availability on reverse vs. approach herkogamous plants: (A) $v_r/v_a=0.833$; (B) $v_r/v_a=1$; (C) $v_r/v_a=1.2$. Within each panel, the three curves illustrate equilibria for different values of q_{rd}/q_{aa} (solid curve, $q_{rd}/q_{aa}=1.2$; dashed curve, $q_{rd}/q_{aa}=1.6$; dotted curve, $q_{rd}/q_{aa}=2$).

is reported from only a handful of angiosperm species (e.g., *Chlorogalum angustifolium*: Jernstedt, 1982; *Epacris impressa*: O'Brien and Calder, 1989), and there is no evidence that this condition occurs commonly in heterostylous families. The rarity of stigma-height polymorphisms in heterostylous families led Lloyd and Webb (1992a) to suggest that this stage may be passed through quite rapidly during the evolution of heterostyly. In contrast, in *Narcissus* stigma-height polymorphisms are well established, occurring in at least a dozen or so species in four sections of the genus. On the other hand, heterostyly is very rare in *Narcissus*. If we assume that the postulated stages in the Lloyd and Webb model are correct, then the patterns in *Narcissus* indicate that there must be severe constraints preventing the transition from a stigma-height polymorphism to reciprocal herkogamy in the genus. It is not clear what constraints are involved, but they are likely to be associated with the morphology and pollination biology of *Narcissus* flowers.

The evolution of reciprocal herkogamy in groups with a stigma-height polymorphism requires selective modification of anther heights. Such modifications can be brought about by changes in flower size, alterations of filament attachment sites on the perianth, or elongation of the filaments themselves. Examples of such changes to anther position in heterostylous species are discussed in Richards and Barrett (1992) and Richards and Koptur (1993). Selection reducing anther height in the approach herkogamous morph of *Narcissus* may be developmentally constrained because of either packing restrictions associated with the very narrow diameter of the perianth tube in dimorphic species, or difficulties in the alteration of filament attachment sites. Shortening of filaments is unlikely because in dimorphic species these are already of limited length. Selection on the position of upper-level anthers may also be restricted because of the small coronas typical of species from dimorphic sections. However, because long-level anthers and stigmas of the long-styled morph are at similar heights, this alteration is likely to be of less selective importance. It is perhaps no coincidence that the floral morphology of tristylous *N. triandrus* is quite distinct from species in sections of the genus with stigma-height dimorphisms. Flowers of this species exhibit large tubular coronas and wider perianth tubes that permit a longer vertical area for the positioning of long-, mid-, and short-level stamens. The stigma-height polymorphisms in *Narcissus* may be evolutionarily stable because developmental aspects of floral architecture constrain selection of the distinct anther-height polymorphism that typifies most heterostylous plants.

In dimorphic *Narcissus* populations, there may be a limited zone within flowers for deployment of the two anther positions for optimal pollen dispatch by pollinators. A lowering of anther height in the approach herkogamous morph increasing reciprocity with short-level stigmas might impair effective pollen dispersal from this morph. Data collected by Harder and Barrett (1993) on pollen removal by long-tongued bees from the three anther levels in tristylous *Pontederia cordata* are relevant to these ideas. They found that removal of pollen from short-level

anthers was highly unpredictable in comparison with removal from mid- and long-level anthers and depended on the extent to which the deeply recessed anthers contacted insect mouth parts. Anthers positioned within the opening of the perianth tube were considered to be in the most beneficial location for pollen dispersal, in part because anthers in this position contact pollinators more consistently than more inserted or exerted anthers. According to this hypothesis, anther position should be selected to maximize pollen dispersal and the position(s) occupied then determines the location(s) of the stigma to reduce self-pollination and self-interference. The occurrence of stigma-height dimorphism is an interesting floral condition in this regard. The presence of two stigma heights but only one anther height implies that the benefits associated with the avoidance of self-pollination in the reverse herkogamous morph owing to greater herkogamy may outweigh any losses in fitness incurred from a lack of precision in pollen dispersal to short-level stigmas.

Implications for Models of the Evolution of Heterostyly

Despite the isolated occurrence of heterostyly in *Narcissus*, its presence in the genus has an important bearing on models for the evolution of heterostyly. Until recently, most workers considered that reciprocal herkogamy in heterostylous plants evolved *after* the origin of diallelic incompatibility (Bateman, 1952*b*; Baker, 1966; Yeo, 1975; Ganders, 1979; Lewis and Jones, 1992). This was based on the idea that the style-stamen polymorphism primarily reduces the large wastage of incompatible pollen that would occur in a monomorphic population with only two or three incompatibility groups. Hence, diallelic incompatibility is generally considered a prerequisite for the evolution of reciprocal herkogamy, and indeed quantitative models of the evolution of distyly by Charlesworth and Charlesworth (1979*b*) require its establishment for the evolution of stamen and style polymorphisms. In these models, the primary selective force responsible for the evolution of diallelic incompatibility in heterostylous populations is inbreeding depression.

Particular anomalies for this interpretation of the evolution of heterostyly are taxa such as *Narcissus* and *Anchusa* in which heterostyly is associated with a self-sterility system in which all cross-pollinations are fertile. In these taxa, the Darwinian concepts of legitimate and illegitimate pollination do not apply, and selection of reciprocal herkogamy requires alternative explanations. These difficulties are to some extent reconciled by the Lloyd and Webb models (1992*a, b*), which put greater emphasis on the pollination biology of the morphs than the genetic consequences of particular mating patterns. They propose that reciprocal herkogamy is selected because it promotes efficient cross-pollination among plants rather than mating types. The selective forces in these models are independent of the type of compatibility system present in the ancestral population because inbreeding avoidance is considered unimportant for the evolution of

reciprocal herkogamy. Hence, reciprocal herkogamy could evolve prior to the establishment of the incompatibility system in groups with diallelic incompatibility. This perspective differs from earlier interpretations that largely viewed heterostyly as an antiselfing mechanism, rather than a mechanical device that increases male fitness through active promotion of proficient cross-pollination (but see Kohn and Barrett, 1992b; Stone and Thomson, 1994). Although it seems likely that cross-promotion played the principal role in the evolution and maintenance of reciprocal herkogamy in *N. triandrus*, the reproductive costs associated with ovule discounting owing to self-pollination may have also been important, particularly during establishment of stigma-height polymorphisms in the genus.

Future Studies

Our preliminary survey of the floral biology of *Narcissus* species has exposed considerable diversity in floral traits associated with their pollination biology and sexual systems. Our studies confirm earlier reports of the occurrence of tristily in *Narcissus* and clarify the nature of other stylar polymorphisms in the genus. Three fundamentally distinct patterns of stylar variation involving monomorphism, dimorphism, and trimorphism occur. This variation offers outstanding opportunities to investigate the adaptive significance of reproductive characters, particularly in species such as *N. papyraceus* and *N. triandrus*, where intraspecific variation is geographically structured and possibly correlated with ecological factors that could influence the pollination biology of populations. Field studies are required to determine whether pollinator-mediated selection on pollen dispersal can account for the transitions from one stylar condition to another in *Narcissus*.

The models we developed to account for the evolution of stylar polymorphisms in *Narcissus* incorporated various features of floral biology that require experimental verification. Our arguments concerning the likelihood that particular pollen transfers are more proficient than others (e.g., $q_{ra} > q_{aa} > q_{ar} = q_{rr}$) were based in part on evidence from other heterostylous plants. In taxa with conspicuous pollen-size heteromorphism, studies of pollen loads of naturally pollinated stigmas have demonstrated that the transport of pollen between anthers and stigmas of similar heights is considerably more proficient than between those at different heights (Ganders, 1974; Barrett and Glover, 1985; Piper and Charlesworth, 1986; Lloyd and Webb, 1992b). In light of these findings, we hypothesized that the transfer of pollen onto approach herkogamous flowers may be more proficient than that onto reverse herkogamous flowers, because anthers correspond more closely in height to the stigmas of the approach herkogamous morph. Unfortunately, polymorphic *Narcissus* species lack pollen-size heteromorphism (Barrett, unpublished), precluding opportunities to use these techniques to investigate the dynamics of pollen transfer. Alternative experimental

approaches will therefore be required to verify the specific pollen-transfer relationships proposed for *Narcissus*.

The proposition that the pollen transfer $q_{ra} > q_{aa}$ was based, in part, on the assumption that stylar interference reduces the male fertility of approach herkogamous plants because of the close proximity of sex organs in the constricted floral tubes of dimorphic species (see Fig. 13.1B). According to this hypothesis, the placement of styles well below the anthers in reverse herkogamous flowers relieves this constraint and pollen dispatch by pollinators is therefore more proficient. There are virtually no data on this form of pollen-stigma interference to evaluate this possibility. In the only experimental study that has investigated whether stylar interference reduces male fertility, Kohn and Barrett (1992a) found no significant differences in the siring ability of long-styled plants of tristylous *Eichhornia paniculata*, with intact vs. excised styles. However, because of the close proximity of anthers and styles in dimorphic *Narcissus* species, we believe that stylar interference is likely to be considerably more important in comparison to *E. paniculata*, which possesses strongly herkogamous flowers and a flaring perianth tube. Experimental studies involving floral manipulations and estimates of male reproductive success are needed to determine the evolutionary significance of stylar interference.

The proximate mechanisms and ecological consequences of late-acting self-sterility in *Narcissus* species merit detailed consideration. Late-acting self-sterility systems appear to be much more widespread than previously appreciated (reviewed in Seavey and Bawa, 1986; Sage et al., 1994), but as yet there has been little discussion of their evolution and functional significance. To explore the implications of the ovule wastage that is frequently associated with late-acting systems (e.g., Cope, 1962; Dulberger, 1964; Crowe, 1971; Yeo, 1975; Waser and Price, 1991; Broyles and Wyatt, 1993), we introduced the concept of ovule discounting to describe the situation where ovules are excluded from cross-fertilization because they are rendered nonfunctional by self-pollen tubes as a consequence of prior self-pollination. Like pollen discounting (see Holsinger et al., 1984; Ritland, 1991; Kohn and Barrett, 1994; also see Chapter 6), ovule discounting has the potential to exert significant reproductive costs, particularly for species in which flowers receive abundant self-pollen.

In *Narcissus* the approximately 4-fold difference between the approach and reverse herkogamous morphs in the distance separating stigmas and anthers is likely to strongly affect levels of self-pollination through either autonomous or pollinator-mediated influences (see Dulberger, 1964; Yeo, 1975). Indeed, field observations of dimorphic species frequently revealed considerable self-pollen on stigmas of long-styled plants because of the close proximity of reproductive organs in this morph. Such effects are likely to be particularly pronounced in polymorphic species with horizontal or pendulous flowers, such as *N. tazetta* and *N. triandrus*, respectively. Although protandry could potentially reduce self-pollen interference, populations frequently experience low pollinator visitation

rates, resulting in delays in the removal of self-pollen. Under these circumstances, ovule discounting could be ecologically important. As demonstrated in our models, differences in ovule discounting between the morphs in conjunction with asymmetrical pollen transfer could help explain the predominance of the long-styled morph that characterizes many sexually polymorphic populations of *Narcissus* species. Investigations of the schedule and amount of self- and outcross pollen deposition on stigmas of the morphs and measures of ovule discounting are required to assess whether self-pollen interference plays a significant role in the floral biology of *Narcissus*.

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