Research review

The evolution of polymorphic sexual systems in daffodils (*Narcissus*)

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Summary

Narcissus, the daffodil genus, exhibits an unusual diversity of sexual systems, with populations that are monomorphic, dimorphic or trimorphic for style length. Associated with this variation are striking differences among species in floral morphology and pollination biology. This diversity provides an opportunity to investigate the evolution of mating polymorphisms, and to determine how floral morphology promotes transitions among sexual systems. Because of the absence of heteromorphic incompatibility in *Narcissus*, floral morphology plays a key role in governing patterns of outcrossed mating. Phylogenetic evidence indicates that stylar monomorphism is ancestral in the genus, with multiple origins of stylar polymorphism, including independent origins of stigma-height dimorphism, distyly and tristyly. Sexual polymorphisms have evolved only in lineages with narrow floral tubes that are pollinated by Lepidoptera and/or long-tongued bees. Populations of polymorphic *Narcissus* species are typically dominated by the long-styled morph and display imperfect reciprocity in the positions of sexual organs. These features are consequences of the unusual association between stylar polymorphism and a self-incompatibility system that permits intramorph mating.

Introduction

Populations of flowering plants typically contain a single sexual phenotype that is capable of mating with all other conspecific individuals. Although reproductive traits commonly vary in these sexually monomorphic populations, the variation is largely quantitative and probably insufficient to cause consistent non-random mating. In contrast, in species with sexual polymorphisms, populations are reproductively subdivided into separate sexes (e.g. dioecy), or into distinct mating groups that differ in morphology (e.g. enantiostyly),

physiology (e.g. homomorphic self-incompatibility), or both (e.g. heteromorphic incompatibility) (reviewed in Barrett, 2002). The morphological and/or physiological differences that distinguish the mating morphs in polymorphic hermaphrodite species are of particular significance, because they directly govern patterns of outcrossed mating, resulting in the negative frequency-dependent selection that maintains sexual diversity (McCauley & Brock, 1998; Thompson *et al.*, 2003). Therefore, the evolution of reproductive traits that promote intermorph (disassortative) mating is central to the origin and maintenance of sexual polymorphisms within plant populations.
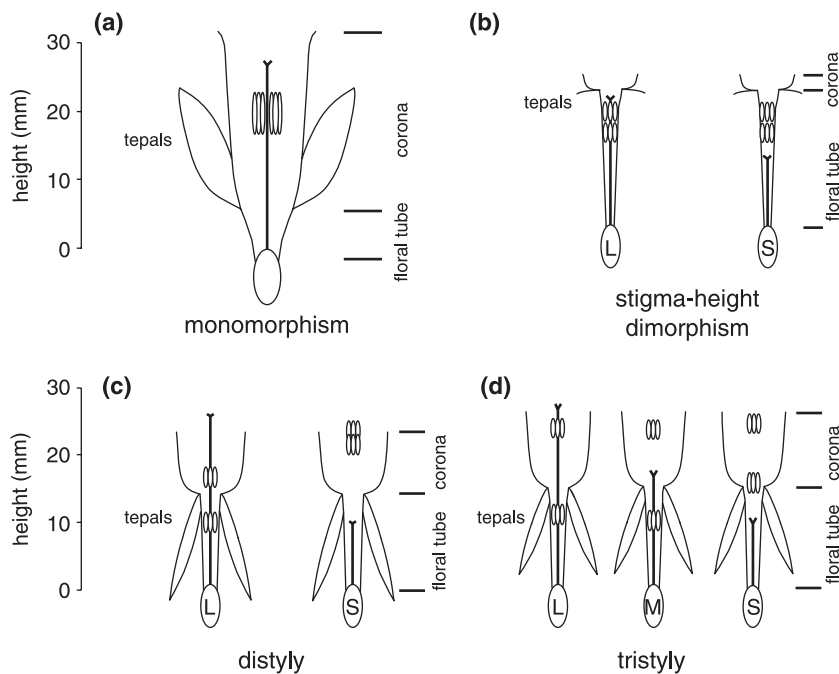
Fig. 1 Schematic examples of the four principal stylar conditions in *Narcissus*, illustrating variation in floral designs and the positions of sexual organs. L, M, and S refer to long-, mid-, and short-styled morphs. (a) Stylar monomorphism in the daffodil morphology, as seen in *N. longispathus*. Populations of this species display continuous variation in style length. (b) Stigma-height dimorphism in the paperwhite morphology, as illustrated by *Narcissus assoanus*. Populations of this species are bimodal for style length. (c) and (d) The triandrus morphology seen in (c) distylous *N. albimarginatus* and (d) tristylous *N. triandrus*. In *N. albimarginatus*, stigmas of the two morphs are positioned reciprocally with one or two anther levels in the opposite morph. Populations of *N. triandrus* are usually trimodal for style length and the position of the upper-level anthers of the L-morph are not in the 'mid' position typical of other tristylous species. Modified from Graham & Barrett (2004).

Polymorphic sexual systems have evolved independently from sexual monomorphism in numerous plant lineages. The evolutionary pathways and selective mechanisms leading to the origin of dioecy are reasonably well understood (Geber *et al.*, 1999), but less is known about the evolution of populations polymorphic for style length or orientation (stylar polymorphism), including the heterostylous conditions of distyly and tristyly (Barrett, 1992). The paucity of groups displaying recognizable stages in the evolutionary assembly of heterostyly has limited inferences concerning ancestral states and the sequence in which morphological and physiological traits become associated.

Narcissus (daffodils–Amaryllidaceae), a monocot genus (~65 species) of perennial geophytes native to the Mediterranean, provides opportunities to investigate the evolution and maintenance of sexual polymorphisms. All *Narcissus* are insect pollinated, with outcrossing largely enforced by a late-acting (ovarian) self-incompatibility system (Bateman, 1954; Dulberger, 1964; Sage *et al.*, 1999), although a few species are self-compatible and produce mixtures of selfed and outcrossed seeds (*N. dubius*; Baker *et al.*, 2000b,c; *N. longispathus*; Barrett *et al.*, 2004a). Unlike most heterostylous species, in which plants are physiologically capable of mating only with individuals of an alternate morph (heteromorphic incompatibility), the incompatibility system in *Narcissus* permits intramorph outcrossing. Consequently, floral morphology plays a dominant role in governing mating patterns within populations. *Narcissus* displays a range of stylar conditions, with species that are monomorphic, dimorphic or trimorphic for style length (Fig. 1, Barrett *et al.*, 1996). Dimorphic species exhibit two distinct stylar polymorphisms: stigma-height

dimorphism and distyly. Both polymorphisms involve long- and short-styled morphs (hereafter L- and S-morphs), but only distyly has stigmas and anthers in reciprocal positions in the two morphs (reciprocal herkogamy). Stylar dimorphism is controlled by a single, diallelic locus, with the allele for short styles dominant to that for long styles (Dulberger, 1967). Trimorphic populations exhibit tristyly, with the additional morph possessing a mid-length style (hereafter M-morph). The sexual diversity in *Narcissus* is associated with differences among species in floral morphology and pollination biology, indicating that changes in pollen dispersal have likely triggered transitions among stylar conditions.

Here we summarize recent work on the evolution and maintenance of sexual systems in *Narcissus*. Through the use of phylogenetic and microevolutionary approaches we address three main topics: the correlated evolution of floral morphology and stylar polymorphisms; the role of pollen transfer in the evolution and maintenance of stylar polymorphisms; and the evolution of morph ratios in polymorphic populations. As this review demonstrates, polymorphic *Narcissus* species provide unique opportunities to study floral evolution, because of the functional links between morphology, mating and morph ratios.

Floral morphology and pollination

Floral diversity within *Narcissus* has evolved largely through changes in the relative sizes and positions of floral structures. The perianth of all *Narcissus* flowers (except *N. cavanillesii*) is comprised of three main components: the proximal tube, formed by fusion of basal segments of the tepals; the free

tepals, which are reflexed from the perianth; and the distal corona (Fig. 1). The insertion of the free tepals on the fused perianth demarcates the transition between the tube and corona. All *Narcissus* species produce nectar at the top of the inferior ovary. Two floral designs predominate among *Narcissus* species: the 'daffodil' form has a relatively short, broad tube and an elongate corona (Fig. 1a), whereas the 'paperwhite' morphology involves a relatively long, narrow tube and a short, flaring corona (Fig. 1b). Among the less common designs within the genus, the 'triandrus' morphology is of particular interest, as it is possessed by the only two heterostylous species in the genus, *N. albimarginatus* and *N. triandrus*. This morphology combines a long, narrow tube with a bell-shaped corona of almost equal length (Fig. 1c,d). In general, different floral designs in *Narcissus* are associated with specific suites of pollinators.

In the daffodil form, the tube grades into the corona, forming a broad, cylindrical or trumpet-shaped perianth that allows bees (*Bombus*, *Anthophora*, *Andrena*) to enter the flower completely as they search for nectar and/or pollen (Fig. 1a, Herrera, 1995). In these flowers, the stigma lies in the corona mouth, beyond the six anthers that are presented in a single whorl well within the corona (approach herkogamy – stigma positioned above anthers). While visiting such flowers, bees contact the anthers with their legs, thorax and abdomen. Because of approach herkogamy, cross-pollination occurs as bees enter flowers, contacting the stigma before the anthers. All species with this floral morphology are monomorphic for style length.

In the paperwhite form, the tube is so narrow that it admits only a pollinator's proboscis and the short, flaring corona serves primarily as a funnel that guides the proboscis tip into perianth tube's mouth (Fig. 1b). In monomorphic species with this morphology, the stigma is positioned in the mouth of the perianth tube, slightly above two whorls of three anthers each (similar to the L-morph in Fig. 1b). About a dozen species with the paperwhite morphology exhibit style-length dimorphism: individuals of the L-morph have flowers much like those of monomorphic species; whereas in individuals of the S-morph the stigma lies hidden within the tube, well below the anthers (Fig. 1b). These species are not distylous, because both morphs have anthers in similar positions, and so lack reciprocal herkogamy. Both monomorphic and dimorphic species with the paperwhite morphology are pollinated primarily by Lepidoptera (Sphingidae, Pieridae, Nymphalidae: Vogel & Müller-Doblies, 1975; Arroyo & Dafni, 1995; Arroyo *et al.*, 2002; Thompson *et al.*, 2003), which transport pollen on their proboscides or faces. Long-tongued bees also visit these species; however, their tongues are too short to reach the nectar at the base of the tube, so they collect only pollen. These bees probably disperse pollen effectively between long-level organs, but in dimorphic species they are incapable of transferring pollen to stigmas of the short-styled morph. Flowers of species with this morphology are always presented horizontally and tend to be very fragrant (Dobson *et al.*, 1997).

The triandrus form combines features of both the daffodil and paperwhite designs (Fig. 1c,d). The large, bell-shaped corona allows long-tongued bees (*Bombus* queens, *Anthophora*) to crawl into the perianth, but the narrow tube impedes their progress and requires them to probe deeply to access nectar. Both species with this morphology have pendant flowers, and so are not visited by Lepidoptera. Sexual organs can be presented at three positions: within the floral tube (short level), near the junction of the tube and corona (mid level), and at the corona mouth (long level). In distylous *N. albimarginatus*, the L-morph has a long-level stigma and mid- and short-level anthers, whereas the S-morph has a short-level stigma and two sets of long-level anthers (Fig. 1c). In tristylous *N. triandrus*, the three morphs differ in stigma position and the positions of their lower anthers (L- and M-morph, short level; S-morph, mid level), but they all present their upper anthers at the long level (Fig. 1d). This incomplete reciprocity of sex-organ positions among the three morphs is unique for a tristylous species.

Evolutionary history of floral morphology and stylar polymorphisms

A recent phylogenetic reconstruction of *Narcissus* provides insight into the pathways that led to sexual diversification and the sequence of morphological changes associated with these events. Graham & Barrett (2004) examined DNA sequence variation in two rapidly evolving plastid regions in *Narcissus* species from all 10 sections of the genus recognized by Fernandes (1975) and Blanchard (1990). The plastid regions included a rapidly evolving portion at the 3′-end of *ndh*F, the gene for subunit F of the plastid NADH dehydrogenase, and *trn*L-F, which spans two plastid tRNA transferase (*trn*) genes, *trn*L(UAA) and *trn*F (GAA). Character reconstructions exposed several inferences about the evolutionary history of stylar variation, despite incomplete resolution of parts of the phylogenetic tree.

The inferred phylogeny (Fig. 2) reveals that floral morphology has been remarkably dynamic during the history of *Narcissus*. None of the daffodil, paperwhite or triandrus morphologies is restricted to single clades, indicating extensive and repeated diversification and convergent evolution. As these morphologies are associated with particular suites of pollinators, the recurring transitions from one floral design to another has probably accompanied pollinator transitions.

Polymorphic sexual systems in *Narcissus* have evolved independently from the ancestral monomorphic state on at least six occasions (Fig. 2). Because almost all monomorphic *Narcissus* species possess approach herkogamy, stigma-height dimorphism probably evolved from this floral condition through the spread of short-styled variants in ancestral long-styled populations. The single instances of distyly (*N. albimarginatus* section *Apodanthi*) and tristyly (*N. triandrus* section *Ganymedes*) are clearly not homologous and represent an evolutionary convergence unique to the Amaryllidaceae (Fig. 2; Pérez *et al.*,
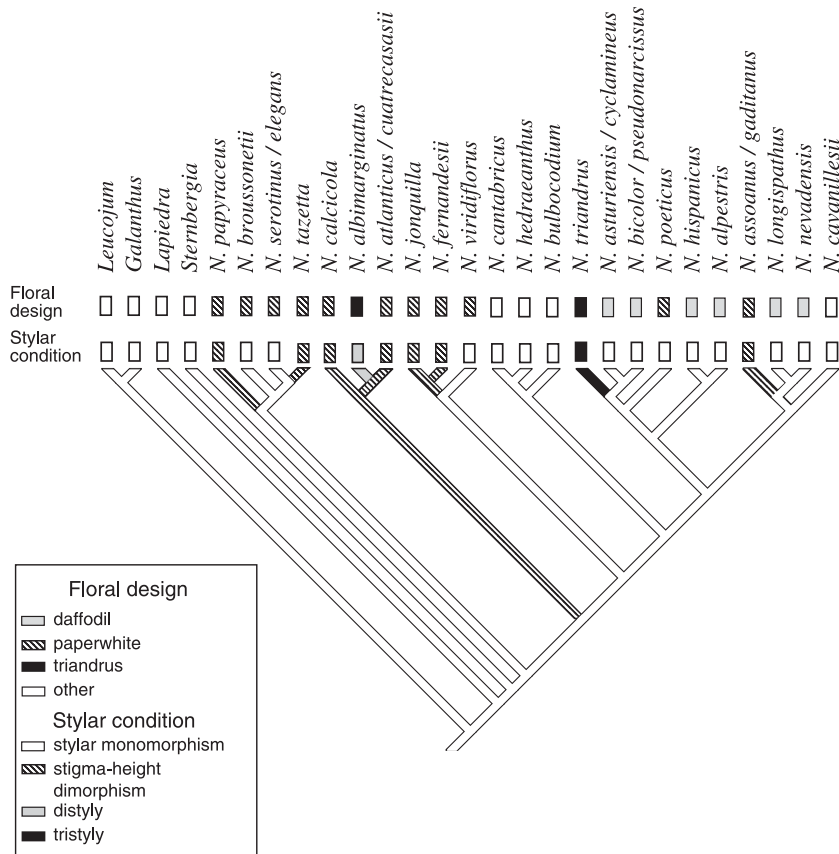
Fig. 2 Reconstruction of the evolutionary history of floral design and stylar polymorphisms in *Narcissus*, based on parsimony analysis of combined *ndh*F and *trn*L–*trn*F DNA sequences. Some taxa with identical stylar conditions are reduced to single termini; taxa of suspected hybrid origin are excluded. Six origins of stylar polymorphism (stigma-height dimorphism, distyly and tristyly) are inferred. For further details on tree construction and character optimization, see Graham & Barrett (2004).

2003). *Narcissus albimarginatus* occurs within a clade with stigma-height dimorphism, so that distyly probably evolved from this ancestral state through the evolution of reciprocal herkogamy. Concentrated-changes tests indicate that long, narrow floral tubes (i.e. the paperwhite morphology) preceded the evolution of stigma-height dimorphism, and that this type of tube, in combination with the development of deep coronas, promoted, or at least was associated with, the independent origins of distyly and tristyly.

These historical reconstructions provide the first phylogenetic test of two competing hypotheses for the evolution of distyly from stylar monomorphism. Charlesworth & Charlesworth (1979) proposed that inbreeding depression selects for two physiologically distinct mating groups in a population of plants with equivalent stigma and anther positions, so that plants can outcross with members of the alternate group, but not with members of their own group. Subsequent selection to reduce pollen wastage leads to the evolution of reciprocal herkogamy. In contrast, Lloyd & Webb (1992a,b) emphasized the benefits of improved pollen transfer, proposing an evolutionary sequence that begins with approach herkogamy and involves an intermediate stage of stigma-height dimorphism, followed by complete reciprocal herkogamy. The absence of heteromorphic incompatibility in *Narcissus* and the findings that approach herkogamy is the ancestral stylar condition and distyly evolved from stigma-height dimorphism

support the evolutionary sequence proposed by Lloyd & Webb (1992a,b). Stigma-height dimorphism is rare in angiosperms and has usually been assumed to represent a transient stage that is replaced rapidly by distyly in response to selection for improved cross-pollination (Lloyd & Webb, 1992a,b). The occurrence of this polymorphism in ~12 species in three sections (*Apodanthi*, *Jonquillae* and *Tazettae*) of *Narcissus* raises the intriguing question of how two style morphs with uniform anther positions can be maintained. We return to this topic below in the Mating patterns and the evolution of morph ratios section.

Graham & Barrett's (2004) phylogenetic reconstruction of *Narcissus* provides less guidance in understanding the evolution of tristyly. Charlesworth (1979) predicted that distyly precedes the origin of tristyly. Unfortunately, the closest known relatives of the only tristylous *Narcissus*, *N. triandrus*, possess stylar monomorphism, so that the transition sequence from monomorphism to tristyly remains obscure. However, a direct transition from monomorphism to trimorphism seems extremely unlikely, so that tristyly probably evolved from a dimorphic intermediate stage that is now not represented in any extant *Narcissus* species. Some populations of *N. triandrus* are dimorphic, but they probably arose from loss of the M-morph, rather than being transitional states between monomorphism and tristyly (see below in the Mating patterns and the evolution of morph ratios section).

Fig. 3 The diversity and evolution of sex-organ arrangements in *Narcissus* species with paperwhite and triandrus floral designs. Hypothetical transitions from left to right involve repeated sequences of morph invasion and accommodation of floral morphology. Arrows beside anthers indicate changes in anther position as a result of morphological accommodation. Species illustrating different sex-organ arrangements include: (a) *Narcissus broussonetii* and *N. poeticus* (b) *N. calcicola* and *N. papyraceus* (c) *N. assoanus* and *N. cuatrecasasii* (d) *N. albimarginatus* (f) and (g) *N. triandrus*. Species a–c exhibit the paperwhite floral design, whereas species d–g have the triandrus floral design.
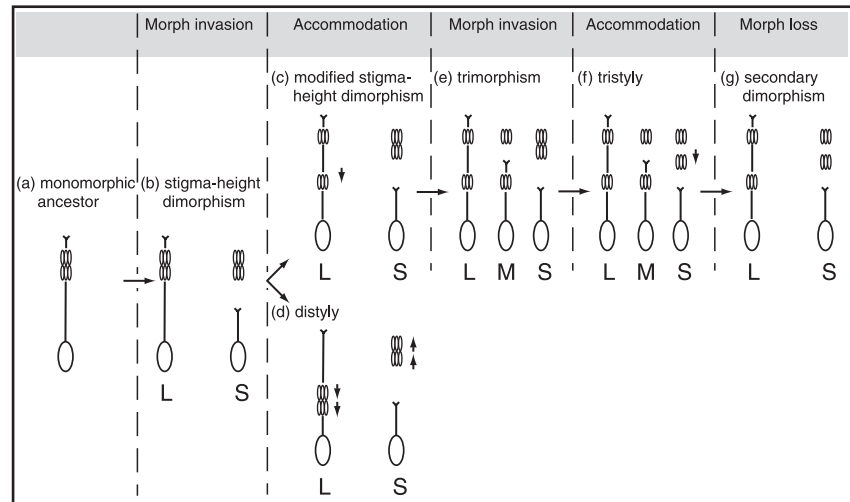
Floral design, pollen transfer and the evolution of polymorphisms

The diversity of sexual polymorphisms in *Narcissus* probably evolved as a result of recurring episodes of invasion of a novel variant into a population followed by adaptive accommodation in the floral morphology of the resident morph(s) to enhance mating with the variant. The accommodation process adjusts morph frequencies until all morphs realize equal mating success and may alter floral morphology to improve pollen transfer to the new morph. Figure 3 summarizes how repetition of this process could have resulted in the evolution of style-length dimorphism, distyly and tristyly in *Narcissus*.

Lloyd & Webb (1992a,b) proposed that stylar polymorphisms (including heterostyly) evolve most readily in species with approach herkogamy and well-developed floral tubes that conceal nectar sought by long-tongued pollinators. These features allow segregated pollen deposition on the proboscides and bodies of pollinators, facilitating cross-pollination when stigmas and anthers are widely separated and reciprocally positioned. This scenario seems applicable to *Narcissus*, as stylar polymorphism is restricted to clades with long tubes in which approach herkogamy is ancestral (Fig. 2). Indeed, it is hard to imagine how pollen deposition could be segregated in species with no significant floral tube. The main difficulty with Lloyd and Webb's hypothesis involves explaining the advantage of a short-styled variant in a population in which anther position of the long-styled morph has already been optimized for pollination (Fig. 3a,b).

Based on Graham & Barrett's (2004) phylogeny and functional considerations, stylar dimorphism probably evolved from ancestors with the paperwhite morphology. Existing species with this morphology and style-length dimorphism have floral tubes that are shorter than the tongues of their Lepidoptera pollinators, so that pollen is transported on the proboscis. Pollen becomes distributed along the length of the proboscis (L. D. Harder, unpublished data), either while distal

portions of the proboscis move past the anthers as the pollinator probes for nectar, or because of redistribution resulting from the pollinator coiling and uncoiling its tongue. In either case, pollen near the proboscis tip probably has little chance of contacting the stigma of flowers with approach herkogamy, and so is lost from the pollination process. In contrast, such pollen is well positioned for deposition on a stigma within the floral tube, which would favour the establishment of a short-styled variant (Fig. 3b). However, intramorph mating by S-morph plants is apparently less successful than either intramorph mating by the L-morph or intermorph mating (Thompson *et al.*, 2003), and few populations are dominated by the S-morph and monomorphic S-morph populations are very rare (the exceptions being populations of *N. rupicola*). Therefore, the mating success of the S-morph must decline as it becomes more common, leading to a polymorphic equilibrium.

In addition to functioning as a pollen recipient, a short-styled variant may experience less self-pollination than the long-styled morph. In species with stigma-height dimorphism, the stigma-anther separation of the S-morph is up to four times greater than that of the L-morph (Barrett *et al.*, 1996). Furthermore, in *Narcissus* species with stylar polymorphism, most of which possess late-acting self-incompatibility, self-pollination prior to cross-pollination reduces seed set in comparison to flowers that receive only cross pollen (e.g. *N. assoanus*, Cesaro *et al.*, 2004; *N. papyraceus*, Arroyo *et al.*, 2002; *N. triandrus*, Barrett *et al.*, 1997), probably by interfering with signalling between ovules and cross-pollen tubes (ovule discounting: Barrett *et al.*, 1996; Sage *et al.*, 1999). Therefore, both improved pollen transfer and reduced ovule discounting may have promoted the establishment and spread of the S-morph.

Establishment of the short-styled morph could precipitate two changes within a population. First, the short-styled morph will become more common at the expense of the long-styled morph, if it has a mating advantage. Second, the increased frequency of an alternative mating partner may select for repositioning of the anthers of the long-styled morph to enhance

intermorph pollination. Because species with the paperwhite morphology have two anther levels, this morphological accommodation could follow one of two pathways. In one case, only the lower anthers of the L-morph move down the floral tube (modified stigma-height dimorphism, Fig. 3c), resulting in a situation seen in several extant species (e.g. *N. assoanus*, Baker *et al.*, 2000b). In the other case, anthers at both levels move down the floral tube, resulting in distyly (Fig. 3d). As the first case is seen only in Lepidoptera-pollinated species, whereas the sole distylous species is likely bee-pollinated, the evolution of distyly probably involved a pollinator transition early during the accommodation phase (Arroyo & Barrett, 2000).

The most probable pathway to stylar trimorphism involves establishment of a mid-styled variant of the L-morph in a population with modified style-length dimorphism (Fig. 3e). Unlike any known tristylous species, in *N. triandrus* the L- and M-morphs differ primarily in style length (Fig. 1d). Because *N. triandrus* is bee-pollinated, the evolution of trimorphism probably involved a transition from Lepidoptera pollination. After establishment of the M-morph, the form of tristyly seen in *N. triandrus* can evolve simply by lowering of the lower anthers of the S-morph to the mid-position, increasing its ability to pollinate the M-morph (Fig. 3f). Although some populations of *N. triandrus* lack the M-morph, they are unlikely to represent the ancestral dimorphic state. Instead, dimorphism probably resulted from loss of the M-morph (Fig. 3g), because the S-morph in dimorphic populations has anthers in the same position as in trimorphic populations.

Mating patterns and the evolution of morph ratios

Mating success in outcrossing species depends on the frequencies of suitable mates and competitors for those mates. In polymorphic populations, negative frequency-dependent selection leads to an equilibrium morph ratio, at which all morphs realize equal mating success. When mating is strictly disassortative and all morphs compete equally for mating opportunities, the equilibrium involves equal morph frequencies. Typical heterostylous species exhibit this pattern, because heteromorphic incompatibility permits ovule fertilization only by pollen from a different morph. As physiology is the sole determinant of frequency-dependent selection in these species, morphological differences between the morphs have little influence on mating patterns. In contrast, *Narcissus* species lack heteromorphic incompatibility, which allows intramorph outcrossing. This uncoupling of incompatibility and style-morph identity allows floral morphology to take on the principal role in governing mating patterns by mediating the interaction of plants and pollinators. Pollen-transfer efficiency between morphs may differ because of the interaction between floral form and pollinator contact with reproductive organs. As a result, the equilibrium morph ratio need not involve equal frequencies of all morphs. Additionally,

equilibrium morph ratios could vary considerably among populations, because of the influence of local environmental conditions on both floral phenotypes and insect visitors, with corresponding effects on mating patterns. Indeed, extensive surveys of morph ratios in five *Narcissus* species with stigma-height dimorphism have revealed striking variation among populations (Arroyo & Dafni, 1995; Barrett *et al.*, 1996; Baker *et al.*, 2000b; Arroyo *et al.*, 2002; Arroyo, 2002).

Surveys of species with stigma-height dimorphism in *Narcissus* reveal three general patterns: (i) in most populations the L-morph is more numerous than the S-morph; (ii) monomorphic populations almost always involve the L-morph; and (iii) equal morph frequencies are infrequent and are associated with large populations, which may often be pollen limited (e.g. Baker *et al.*, 2000a). Because no other genus with stylar polymorphism exhibits these consistent patterns, effort has been directed to understand the selective mechanisms that maintain these unusual morph ratios in *Narcissus*.

Models of the relation of equilibrium morph ratios to patterns of pollen transfer and mating indicate that greater intramorph mating in the L-morph than in other morphs results in L-biased morph ratios (Barrett *et al.*, 1996, 2004b; Baker *et al.*, 2000c). This pattern of outcrossed mating seems likely in *Narcissus* for two reasons. First, in all polymorphic *Narcissus* species the upper anthers and stigma of the L-morph occupy more similar positions than the anthers and stigmas of other morphs (see Fig. 1b), thereby facilitating effective intramorph pollen transfer. In contrast, the greater separation of anthers and stigmas in other morphs increases the chance that they contact different parts of a pollinator, limiting intramorph pollen transfer and enhancing intermorph transfer. Second, monomorphic populations of dimorphic species composed of the L-morph are highly outcrossing, so that individuals of this morph seem quite capable of mating with each other (e.g. *N. papyraceus*, Arroyo *et al.*, 2002). The near absence of populations monomorphic for the S-morph in *Narcissus* suggests that this morphology is poorly adapted for effective intramorph mating.

Pollen-transfer models also demonstrate that stigma-height dimorphism can be maintained in a population through negative frequency-dependent selection if intermorph mating exceeds intramorph mating (Lloyd & Webb, 1992b; Barrett *et al.*, 1996). Equivalent intermorph mating in the L- and S-morphs leads to an equilibrium morph ratio of 1 : 1, as in distylous species (Baker *et al.*, 2000c). Two observations indicate that stigma-height dimorphism in *Narcissus* can promote effective cross-pollen transfer between the style morphs. First, although biased morph ratios characterize polymorphic *Narcissus* species, large populations commonly exhibit 1 : 1 morph ratios (*N. assoanus*, Baker *et al.*, 2000b; *N. papyraceus*, Arroyo *et al.*, 2002), indicating that symmetrical intermorph mating can be achieved without sex-organ reciprocity. Second, experimental studies in which style-morph composition was manipulated in naturally occurring patches of *N. assoanus*

demonstrated frequency-dependent reproductive success and higher seed set in patches with 1 : 1 morph ratios than in monomorphic patches (Thompson *et al.*, 2003). This is expected if stigma-height dimorphism functions to improve cross-pollen transfer.

Both ecological and genetic factors have been proposed to account for the variation in morph frequencies among populations of dimorphic *Narcissus* species. These factors include differences in the pollinator fauna among populations, particularly the presence of short- vs long-tongued pollinators (Arroyo & Dafni, 1995), and the role of the inheritance of style length on morph representation in monomorphic populations following founder effects (Baker *et al.*, 2000b; Arroyo *et al.*, 2002). Interestingly, in several species (*N. assoanus* in France, Baker *et al.*, 2000c; *N. papyraceus* in Spain, Arroyo *et al.*, 2002; *N. tazetta* in Israel, Arroyo & Dafni, 1995) morph frequencies vary geographically in association with contrasting environmental conditions and population sizes. This geographical variation could be exploited to investigate the role of ecological factors in affecting patterns of outcrossed mating and the evolution of morph ratios.

Narcissus triandrus exhibits the most complex patterns of morph-frequency variation in the genus. This wide-ranging tristylous species from the Iberian Peninsula varies extensively in floral morphology, particularly in flower size and the relative positions of sexual organs (Barrett *et al.*, 2004b). In contrast to tristylous species in other families, *N. triandrus* possesses imperfect reciprocity in the positions of sex organs (Fig. 1d) and, like dimorphic *Narcissus*, a late-acting self-incompatibility system that permits outcrossing within and between morphs. These unusual features led Bateman (1952) to doubt early reports of tristyly in *N. triandrus* (Henriques, 1887; Fernandes, 1935), although this has now been confirmed (Lloyd *et al.*, 1990; Barrett *et al.*, 1997; Sage *et al.*, 1999).

The correlated evolution of floral morphology and morph ratios in *N. triandrus* has been recently investigated through an extensive analysis of a large sample of populations (*n* = 137) throughout the species' range (Barrett *et al.*, 2004b). This study revealed three features of morph ratios that differ from other tristylous species (Fig. 4): (i) L-biased morph ratios, as seen in dimorphic *Narcissus*; (ii) the frequencies of the L- and M-morphs vary negatively among populations; and (iii) dimorphic populations that lack the M-morph. Morph ratios vary geographically, with a decreasing frequency of the M-morph from the southeast to the northwest of the range. This gradient accompanies a steep increase in the L-morph, with less change in the frequency of the S-morph. As a result, the average frequency of the S-morph varies little between trimorphic and dimorphic populations. This variation in morph ratios parallels geographic variation in flower size and the positions of sex organs, so that the M-morph is rare or absent in large-flowered populations in the northwest portion of the species' range. This geographical association enabled statistical prediction of population morph ratios based on the mean
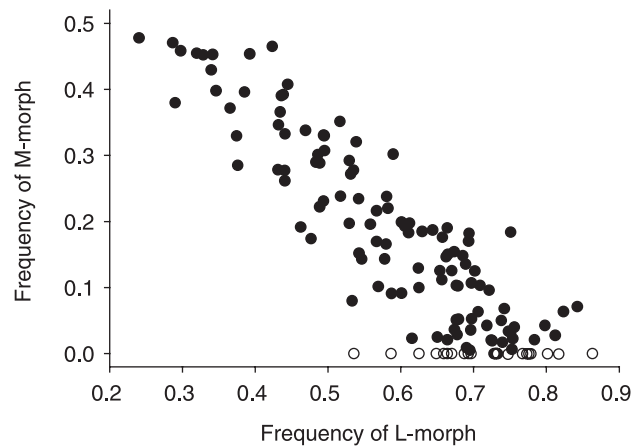

Fig. 4 Variation in the frequencies of the M-morph in relation to the frequency of the L-morph in 137 *Narcissus triandrus* populations. Open symbols indicate dimorphic populations, whereas closed symbols indicate trimorphic populations. From data in Barrett *et al*. (2004b).

positions of sex organs within flowers (Barrett *et al.*, 2004b), providing indirect evidence that floral morphology governs mating patterns and morph ratios.

Two ecological mechanisms are probably responsible for the geographical associations between floral morphology and morph ratios in *N. triandrus*. First, the increase in flower size from southeast to northwest correlates with an increase in plant size (Worley *et al.*, 2000; Barrett *et al.*, 2004b). This variation probably reflects a rainfall gradient from hot Mediterranean climates in the southeast to cooler, wetter Atlantic climates in the northwest. Second, changes in flower size are accompanied by differences in the pollinator fauna: *Anthophora* spp. are the dominant flower visitors in southern populations, but are replaced by larger-bodied *Bombus* spp. in the north. These differences in pollinator size (and perhaps behaviour) should affect selection on the positions of sex organs to maintain effective cross-pollen transfer. Evidence of divergent selection on floral morphology over the range of *N. triandrus* can be seen in the contrasting allometry of organ position within and between populations. For example, the stigma position of the M-morph varies with flower size more strongly among populations than would be expected from an extrapolation of the allometric relations within populations (Fig. 5). Thus the geographic variation in floral morphology does not result simply from phenotypic responses to local growing conditions.

In the absence of heteromorphic incompatibility, the atypical arrangement of sex organs in *N. triandrus* flowers should strongly influence pollen dispersal and mating. Barrett *et al.* (2004b) explored this proposition with a model of frequency-dependent selection on morph ratios, which assumed that pollinators disperse more pollen between anthers and stigmas at similar heights than between sex organs at dissimilar positions. This model demonstrated that the elevated anthers of
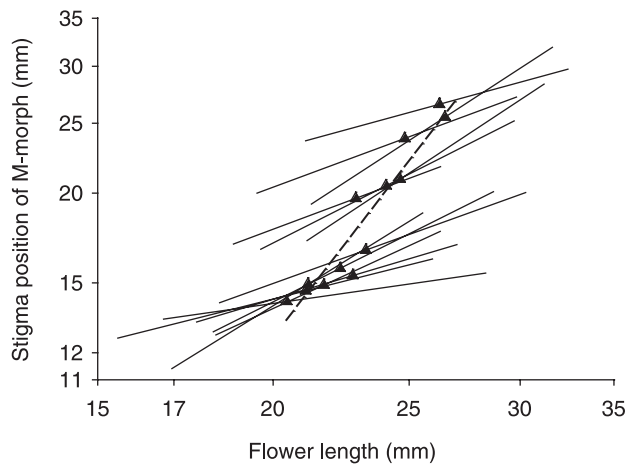
Fig. 5 Contrasting allometric relations within (solid lines) and among 13 populations (dashed line) of *Narcissus triandrus* in the position of the stigma of the M-morph and flower length. Note the ln-scaled axes. Details of data collection and measurements in Barrett *et al.* (2004b).

the L-morph (Fig. 1d) favours L-biased equilibrium morph ratios, and that the observed increase in height of the lower anthers of the S-morph toward the northwest of the geographical range of *N. triandrus* reduces the equilibrium frequency of the M-morph. Given these findings, why has the reciprocal morphology typical of most heterostylous species not evolved in *N. triandrus*? The answer is probably associated with the contrasting incompatibility systems associated with heterostyly. Because of intramorph compatibility in *N. triandrus*, selection for complete reciprocity should be relatively weak, as all cross-pollinations are fertile (Barrett *et al.*, 1997). In contrast, in tristylous species with heteromorphic incompatibility any pollen dispersed to incompatible stigmas is wasted, which should select for more precise sex-organ reciprocity. This explanation may also account for the imperfect sex-organ reciprocity and L-biased morph ratios that occur in dimorphic *Anchusa officinalis* (Boraginaceae), the only other known heteromorphic species with a late-acting self-incompatibility system and intramorph mating (Philipp & Schou, 1981; Schou & Philipp, 1984).

Concluding remarks

Sexual polymorphism in *Narcissus* has been known for over a century, but the merits of the genus for studies of floral evolution have been recognized only recently. The occurrence of diverse stylar conditions has provided insight into the pathways by which distyly has evolved in the genus. Some lessons from *Narcissus* are clearly relevant to the evolution of heterostyly in other groups, particularly the critical role that long-tongued pollinators play in shaping floral evolution and in facilitating intermorph pollen transfer. However, because of the unusual form of incompatibility in *Narcissus*, the genus provides little guidance on how the morphological and

physiological components of the heterostylous syndrome become tightly associated in other heterostylous families. Notwithstanding this issue, most *Narcissus* species are self-incompatible, so that inbreeding avoidance has probably played a minor role in the evolution of stylar polymorphisms, as Lloyd & Webb (1992a,b) also argued for heterostylous plants in general. Rather, fitness gain through male function resulting from more effective cross-pollen transfer seems the most probable adaptive outcome of the evolution of stylar polymorphism. Morph ratios in at least four species of *Narcissus* exhibit striking patterns of geographical variation unlike those reported in any other family with stylar polymorphisms. Morph ratios provide a visible signature of mating in preceding generations, so that this variation offers outstanding opportunities to examine the influence of ecology on morphological variation and its consequences for patterns of outcrossed mating in plant populations.

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