

BRIEF COMMUNICATION

DISCOVERY OF DISTYLY IN *NARCISSUS*
(AMARYLLIDACEAE)¹

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There has been disagreement concerning the nature of sexual polymorphisms in *Narcissus*, a genus of insect-pollinated geophytes native to the Mediterranean. The existence of trimorphic heterostyly in the genus has recently been confirmed, but the occurrence of distyly remains enigmatic. All sexually dimorphic species previously investigated possess two distinct style lengths but anthers of similar height. Stigma-height dimorphism does not qualify as true distyly because of the absence of a reciprocal correspondence in stigma and anther position in the floral morphs. Such reciprocal herkogamy is generally regarded as the defining feature of heterostyly. Here we report on distyly in *N. albimarginatus* (section Apodanthae), a rare species confined to a single mountain in northwestern Morocco. A population composed of equal numbers of long- and short-styled plants exhibited reciprocal herkogamy with lower anthers of the long-styled morph and upper anthers of the short-styled morph corresponding in height to stigmas of short- and long-styled plants, respectively. The presence of both stigma-height dimorphism and distyly in *Narcissus* is of general significance to theoretical models of the evolution of heterostyly.

Key words: Amaryllidaceae; distyly; Lloyd and Webb model; *Narcissus*; reciprocal herkogamy; stigma-height dimorphism.

Heterostyly has originated independently in at least 28 animal-pollinated flowering plant families but the evolutionary build-up of the polymorphism is still poorly understood (Ganders, 1979a; Barrett, 1992). Theoretical models differ in both the selective mechanisms invoked and in the sequence in which the morphological and physiological components of the syndrome are assembled (Charlesworth and Charlesworth, 1979; Lloyd and Webb, 1992a, b; Richards, 1998). Unfortunately, little comparative information is available to evaluate the different pathways by which heterostyly has evolved and there have been few experimental studies that have examined the contrasting predictions of theoretical models (Kohn and Barrett, 1992; Stone and Thomson, 1994).

Lloyd and Webb (1992a, b) argued that the first stage in the evolution of distyly from an approach herkogamous ancestor involves the establishment of a polymorphism for stigma height but not anther height. They suggested that the rarity of stigma-height dimorphism in heterostylous groups is because this stage was rapidly passed through during selection for complete reciprocal herkogamy. Selection for differentiation in anther height would likely increase the proficiency of pollen dispersal between morphs and be strongly selected in populations with only a dimorphism in stigma height. Charlesworth and Char-

lesworth (1979) also investigated the stability of stigma-height dimorphism and found that it was difficult to maintain in populations. Whether stigma-height dimorphism is an intermediate stage in the evolution of distyly has remained difficult to assess because of the absence of comparative data. One source of evidence in favor of the Lloyd and Webb (1992a, b) model would be the co-occurrence of stigma-height dimorphism and distyly among closely related species.

Narcissus (Amaryllidaceae) is composed of ~40 species of insect-pollinated geophytes native to the Mediterranean. Populations are either monomorphic, dimorphic, or trimorphic for style length (Barrett, Lloyd, and Arroyo, 1996) with at least a dozen species in three sections of the genus (Apodanthae, Jonquillae, and Tazettae) possessing length dimorphism of styles (Dulberger, 1964; Arroyo and Dafni, 1995; Baker, Thompson, and Barrett, 2000a, b). All species with stylar dimorphism possess two anther levels within a flower, but these are positioned similarly at the upper portion of the floral tube in both morphs [for example, see Fig. 1 in Dulberger (1964) and Baker, Thompson, and Barrett, (2000a)]. Because of the absence of reciprocal herkogamy, dimorphic species of *Narcissus* should not be considered distylous but instead are best described as possessing stigma-height dimorphism (reviewed in Barrett, Jesson, and Baker, 2000). In *N. triandrus* (section Ganymedes) three distinct style morphs commonly occur within populations and accompanying the differences in stigma height are three discrete anther levels. Because of the reciprocal nature of stigma and anther heights, Barrett et al. (1997) described this species as tristylous. In contrast to most heterostylous groups, heterostyly is rare in *Narcissus* whereas stigma-height dimorphism is common, implying that there are

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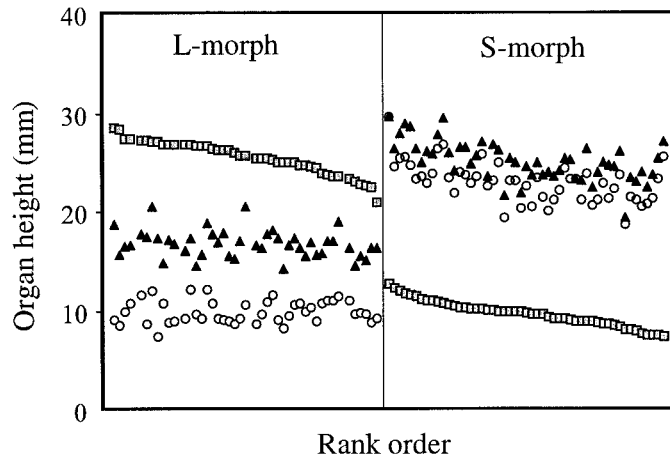


Fig. 1. Distily in *Narcissus albimarginatus*. Range of variation in stigma (□) and anther heights (upper-level stamens ▲, lower-level stamens ○) in a sample of 97 flowers from a population at Jebel Bouhachem mountain in northwestern Morocco. Note the reciprocal correspondence in height of stigmas of the long- and short-styled morphs with the upper- and lower-level stamens of the short- and long-styled morphs, respectively. Flowers are ranked by style length.

strong constraints on the evolution of reciprocal herkogamy in populations with stigma-height dimorphism. In addition, there is no evidence for the occurrence of diallelic incompatibility or ancillary polymorphisms of pollen and stigmas in any *Narcissus* species (Dulberger, 1964; Arroyo and Dafni, 1995; Barrett, Lloyd, and Arroyo, 1996; Sage et al., 1999; Baker, Thompson, and Barrett, 2000a, b).

Here we provide quantitative evidence for the existence of distily in a rare species of *Narcissus* from northwestern Morocco. *Narcissus albimarginatus* D. & U. Muller-Doblies was first described in 1989 from near the summit of the mountain Jebel (as in the original description) Bouhachem (Muller-Doblies and Muller-Doblies, 1989). The illustrations of *N. albimarginatus* accompanying the taxonomic description indicate two floral morphs differing in style length and anther position, and the authors describe the species as "heterostylic." However, population samples were not reported and many authors have described *Narcissus* species as heterostylous when in fact the species exhibit stigma-height dimorphism not distily (e.g., Henriques, 1887; Fernandes, 1964; Webb, 1980; Valdés, 1987; Richards, 1997; and see Barrett, Lloyd, and Arroyo, 1996, for a review). Because of this problem we decided to investigate sex-organ variation in *N. albimarginatus* further by attempting to locate the population described by D. and U. Muller-Doblies in Morocco and conducting detailed sampling to determine whether the species was indeed truly distylous.

METHODS

On 18 April 1999 the Djebel Bouhachem population was located in the Western Rif mountain range of northwestern Morocco. The population occurs along a sandstone ridge (not limestone as earlier reported) at 1500 m growing under *Cedrus atlantica* forest and was composed of ~100 flowering individuals in an approximate area of 200 × 100 m. At the time of the visit the population was past peak flowering, but a sufficient number of flowers was available for sampling. To determine

TABLE 1. Mean and (standard deviation) of stigma and anther heights (mm) in the two floral morphs of distylous *Narcissus albimarginatus*, a rare species from northwestern Morocco. See text for details.

Trait	Long-styled morph	Short-styled morph	<i>t</i> test
No. of flowers	45	52	
Style length	25.53 (1.87)	9.59 (1.50)	49.31 $P < 0.0001$
Upper-level stamen	16.78 (1.40)	25.11 (2.07)	38.06 $P < 0.0001$
Lower-level stamen	9.91 (1.13)	22.99 (2.05)	22.83 $P < 0.0001$

the nature of floral variation in the population a single flower was sampled from all individuals that were in anthesis and flowers were preserved in 70% ethanol. The sample involved 97 plants, of which 88 produced solitary-flowered inflorescences, eight had two flowers, and one had three flowers per inflorescence. *Narcissus albimarginatus* possesses pale-yellow pendulous flowers with a floral tube and a prominent corona with a white margin. No pollinators were observed visiting the population.

On return to the laboratory the style length (including stigma) and height of upper- and lower-level stamens (to anther base) of all flowers were measured using digital calipers to the nearest 0.1 mm. All measurements were made from the top of the ovary, and all stamens were measured and the average value taken for each stamen level. Data from *N. albimarginatus* were then compared with published measurements of sex-organ variation in seven *Narcissus* species with stigma-height dimorphism to assess variation in the degree of reciprocity in stigmas and anthers.

RESULTS AND DISCUSSION

The population of *N. albimarginatus* was composed of two discrete floral morphs differing in style length (Fig. 1). The population contained 45 long-styled plants and 52 short-styled plants. This ratio does not differ significantly from equality ($\chi^2 = 0.505$; $P = 0.477$). Of particular interest was the finding that the position of the two stamen levels within a flower differed significantly between the morphs, demonstrating that the population does not exhibit a simple stigma-height dimorphism (Table 1). In the long-styled morph the two stamen levels were included within the corona (upper-level stamens) and floral tube (lower-level stamens), whereas in the short-styled morph both stamen levels were exerted beyond the corona. Reciprocal herkogamy occurs in *N. albimarginatus* because lower-level stamens in the long-styled morph and upper-level stamens of the short-styled morph correspond in height to stigmas of the short- and long-styled morphs, respectively (Table 1). Because of this reciprocal correspondence in stigma and anther height *N. albimarginatus* qualifies as distylous.

The degree of stigma-anther reciprocity in *N. albimarginatus* is much greater than in *Narcissus* species with stigma-height dimorphism (Fig. 2). This is largely because lower-level stamens in the long-styled morph correspond more closely in height to stigmas of the short-styled morph, whereas in species with stigma-height dimorphism these stamens are usually positioned higher in the floral tube. Interestingly, the mean difference in stigma height between the floral morphs in *N. albimarginatus* is the largest of the eight dimorphic species of *Narcissus* illustrated in Fig. 2. Disruptive selection on stigma height during the evolution of reciprocal herkogamy may have

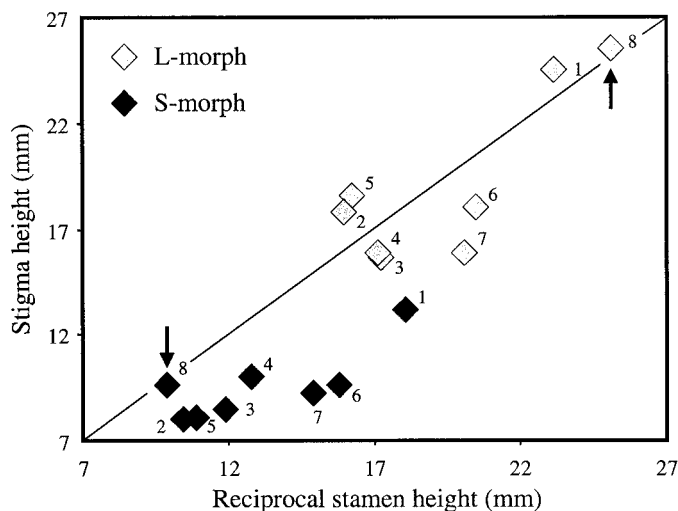


Fig. 2. The degree of reciprocity of stigma and anther heights in the long- and short-styled morphs of eight *Narcissus* species with style-length dimorphism. For each morph, the stamen level that most closely corresponds in height to the stigma of the alternate morph is plotted. 1. *N. assoanus*, 2. *N. calcicola*, 3. *N. cuatrecasasi*, 4. *N. dubius*, 5. *N. gaditanus*, 6. *N. papyraceus*, 7. *N. rupicola*, 8. *N. albimarginatus*. Data from a single population of each species were used in the comparison. The diagonal line represents complete reciprocity between stigma and anther heights. Note that both floral morphs of distylous *N. albimarginatus* (identified by arrows) fall on the line, whereas the remaining species, all of which possess stigma-height dimorphism, are either above or below the line. Source: 1–3, 5–7—Barrett, Lloyd, and Arroyo (1996); 4—Baker, Thompson, and Barrett (2000a); 8—this study.

been more intense in comparison with species possessing only stigma-height dimorphism.

Most distylous species possess a single stamen level and therefore the assessment of the degree of reciprocal herkogamy in the floral morphs is straightforward. However, dimorphic *Narcissus* species exhibit two stamen levels within a flower and therefore it is of interest to compare the extent to which the two stamen levels correspond in height to stigmas of the opposite morph. In *N. albimarginatus* one of the two stamen levels within a flower corresponds closely in height, whereas the other is either above (long-styled morph) or slightly below (short-styled morph) stigmas of the alternate morph. This pattern is also evident in *Erythroxyllum coca* (Ganders, 1979b), an atypical distylous species with two stamen levels. It is important to appreciate that there are often considerable disparities in the precise positions of anthers and stigmas in heterostylous plants and the degree of reciprocal herkogamy is rarely exact (Lloyd and Webb, 1992a). Nevertheless it seems likely that in *N. albimarginatus*, sex-organ positioning functions to promote significant intermorph pollen transfer despite a lack of complete reciprocal herkogamy of all stamen levels. The 1:1 morph ratio provides strong evidence that significant disassortative (intermorph) mating occurs in the population as commonly occurs in distylous species.

In accord with Lloyd and Webb's (1992a, b) model of the evolution of distyly we propose that distyly in *N. albimarginatus* is derived from an ancestral condition involving stigma-height dimorphism. First, on functional grounds it would be difficult to imagine the selective

mechanisms that might favor the reverse evolutionary sequence since this would likely reduce the proficiency of cross-pollen dispersal between the floral morphs. Second, the widespread occurrence of stigma-height dimorphism in *Narcissus*, including species with varying degrees of reciprocal herkogamy (Fig. 2), implies that variation exists for the selection of distyly under the appropriate pollination environment. Finally, the isolated occurrence of distyly in a species apparently restricted to a single mountain in northwestern Morocco seems an unlikely place to locate the ancestral polymorphic condition in *Narcissus*, unless of course this taxon represents a relictual species and all other distylous taxa have gone extinct. Clearly, a phylogeny of the genus would be required to fully resolve these issues. In the meantime it seems to us that the weight of evidence favors the hypothesis that distyly in *Narcissus* is derived from stigma-height dimorphism through selection for more proficient cross-pollen transfer.

If this evolutionary scenario is correct, an important question that arises is why the transition to heterostyly is rare given the widespread occurrence of stigma-height dimorphism in *Narcissus*. The only other well-documented case of heterostyly in the genus involves *N. triandrus*, a tristylous species with occasional dimorphic populations (Barrett et al., 1997). *Narcissus albimarginatus* and *N. triandrus*, despite occurring in different sections of the genus, share a number of apparently convergent floral characters suggesting a similar pollination syndrome. Both have pale-yellow pendulous flowers of similar size with a prominent corona. In addition, the fragrance of both species is similar, at least as perceived by us, and both flower during the same period. The major pollinators of *N. triandrus* are large bees (*Anthophora* and *Bombus*), and it seems likely given their similarities in floral morphology that *N. albimarginatus* may also be adapted to bee pollination. The floral characteristics of both species are markedly different from *Narcissus* species with stigma-height dimorphism. These are mostly adapted to hawk moths and butterflies and differ mainly in having smaller coronas with reduced depth and longer floral tubes (Arroyo and Dafni, 1995; Barrett, Lloyd, and Arroyo, 1996; Baker, Thompson, and Barrett, 2000a, b). A shift of pollination system in *Narcissus* from lepidopteran to bee pollination could have provided the evolutionary stimulus for the evolution of distyly from stigma-height dimorphism. Elsewhere, Stone (1996) demonstrated that bees were more effective than lepidoptera at promoting intermorph pollen transfer in *Psychotria suerrensis*, a distylous species with narrow-tubular corollas.

The evolution of a deep corona in association with the shift to bee pollination may have been critical for the evolution of heterostyly in *Narcissus*. The corona provides a longer vertical area for separation of the two stamen levels within a flower. This would enable more segregated pollen deposition on a pollinator's body than is possible in species with stigma-height dimorphism where anthers are closer together at the mouth of the narrow floral tube and packing constraints may restrict opportunities for divergence in anther position (Barrett, Lloyd, and Arroyo, 1996). Deep coronas also occur elsewhere in *Narcissus* in the predominantly bee-pollinated sections

Bulbocodium and Pseudonarcissus, but species in these sections are uniformly monomorphic for style length. Why then has heterostyly not developed in these groups? We suggest it is the combination of a deep corona and a long floral tube (absent from sections Bulbocodium and Pseudonarcissus) that is necessary for the evolution of heterostyly in *Narcissus*. These traits function to provide more precise pollinator positioning and likely enable anther height divergence and the segregated pollen transfer required for the origin and maintenance of the polymorphism.

Because of its polyphyletic origins there is considerable variation in the heterostylous syndrome among angiosperm families and this has led to differing opinions on how best to define the polymorphism (reviewed in Barrett, 1992). We believe that a morphological definition that concerns the relative positioning of sexual organs is the most useful perspective. In our opinion a population that contains two or three floral morphs possessing a reciprocal arrangement of stigma and anther heights qualifies a species as heterostylous regardless of whether it possesses diallelic incompatibility or ancillary polymorphisms of pollen and stigmas. This broader view emphasizes the basic function of the polymorphism as a structural adaptation that increases the proficiency of pollinator-mediated pollen transfer between plants.

Lloyd and Webb (1992a, b) also emphasized functional criteria in their treatment of the evolution of heterostyly and proposed that distyly evolved from stigma-height dimorphism because of the influence of floral morphology on pollen-transfer patterns. The occurrence of both stigma-height dimorphism and distyly in *Narcissus* provides evidence in support of their model. This association is of particular significance because of the absence of diallelic incompatibility in *Narcissus* (Dulberger, 1964; Sage et al., 1999; Baker, Thompson, and Barrett, 2000b). While diallelic incompatibility is generally a prerequisite for the evolution of reciprocal herkogamy in some models for the evolution of distyly (Charlesworth and Charlesworth, 1979), those of Lloyd and Webb (1992a, b) are independent of the type of compatibility system present in ancestral populations. Reciprocal herkogamy probably evolved in *N. albimarginatus* to increase the effectiveness of pollen dispersal among plants because of altered pollination conditions, perhaps associated with the isolated geographical distribution of this species.

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