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EVOLUTIONARY MAINTENANCE OF STIGMA-HEIGHT DIMORPHISM IN *NARCISSUS PAPYRACEUS* (AMARYLLIDACEAE)[1]

JUAN ARROYO,[2] SPENCER C. H. BARRETT,[3,4] ROSARIO HIDALGO,[2] AND
WILLIAM W. COLE[3]

[2]Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Apartado 1095, E-41080 Sevilla, Spain; and
[3]Department of Botany, University of Toronto, 25 Willcocks Street, Toronto, Ontario, Canada, M5S 3B2

Stigma-height dimorphism is a sexual polymorphism in which plant populations are composed of two floral morphs that differ significantly in style length but not anther position. The morphs exhibit approach and reverse herkogamy, floral designs that in most species typically occur as monomorphic conditions. We investigated the floral biology of stigma-height dimorphism in the Mediterranean geophyte *Narcissus papyraceus* (Amaryllidaceae) in an effort to understand the evolutionary forces maintaining stylar polymorphism. Our survey of 66 populations in Spain, Portugal, and Morocco indicated that 56% were dimorphic with the long-styled morph at an average frequency of 0.79. The remaining 44% of populations sampled were monomorphic for the long-styled morph. In dimorphic populations there was a significant positive relation between population size and the frequency of the short-styled morph. Controlled pollinations demonstrated that *N. papyraceus* is self-sterile with no significant differences in female fertility between intra- and intermorph crosses. Prior self-pollination reduced seed set in flowers that were subsequently cross-pollinated. Estimates of mating patterns using allozyme markers in eight populations indicated that *N. papyraceus* is largely outcrossing (mean $t_m = 0.81$) with no significant differences between monomorphic and dimorphic populations or style morphs. Stigma-height dimorphism in *N. papyraceus* is maintained in populations by insect-mediated cross-pollination with biased morph ratios and stylar monomorphism likely resulting from the combined influence of the inheritance of the polymorphism, morph-specific differences in assortative mating and founder effects.

Key words: Amaryllidaceae; assortative and disassortative mating; founder events; heterostyly; *Narcissus papyraceus*; outcrossing rates; self-incompatibility; stigma-height dimorphism; style-morph ratios.

Interference between the reproductive functions of female and male sex organs in many animal-pollinated plants is reduced by herkogamy, the spatial separation of stigmas and anthers within flowers. Most commonly, stigmas are positioned above anthers, contacting pollinators first upon their entry into flowers. Less often the reverse sequence occurs, with anthers located above stigmas. These two conditions are referred to as approach and reverse herkogamy, respectively (reviewed in Webb and Lloyd, 1986). In the majority of angiosperm species, all populations are monomorphic for one or the other of these herkogamous conditions with individuals possessing flowers of similar floral design. More significant from an evolutionary perspective are species in which populations are polymorphic for approach and reverse herkogamy, because the maintenance of such sexual polymorphism implies adaptive significance and a balance of selective forces.

The most widely recognized herkogamy polymorphism is heterostyly, in which populations contain two (distyly) or three (tristyly) style morphs that differ from one another in stigma and anther positions and in a suite of ancillary morphological and physiological characters (Darwin, 1877; Barrett, 1992). Because of the reciprocal placement of stigmas and anthers in heterostylous flowers, this polymorphism has been described as reciprocal herkogamy (Richards, 1986; Webb and Lloyd, 1986). Experimental studies of heterostylous populations have provided convincing evidence that heterostyly is maintained by negative frequency-dependent selection and functions to promote pollinator-mediated cross-pollination and reduce pollen wastage (reviewed in Ganders, 1979; Lloyd and Webb, 1992a, b).

Less well known in comparison to heterostyly are species that are dimorphic for style length but in which anthers are positioned at *similar* heights in the two floral morphs. This polymorphism is referred to as stigma-height dimorphism and has been reported sporadically among a few angiosperm families (reviewed in Barrett, Jesson, and Baker, 2000). In some groups, stigma-height dimorphism appears to be an intermediate stage in the evolution of distyly, as predicted by theoretical models (Charlesworth and Charlesworth, 1979b; Lloyd and Webb, 1992b). However, the occurrence of the polymorphism in several non-heterostylous families (e.g., Ericaceae, Epacridaceae, Haemodoraceae, Liliaceae) indicates that stigma-height dimorphism is not always a transient polymorphism associated with the evolution of heterostyly (Barrett, Jesson, and Baker, 2000). The selective forces maintaining stigma-height dimorphism in flowering plants are still poorly understood.

Among taxonomic groups with stigma-height dimorphism the polymorphism is most commonly represented in the insect-pollinated Mediterranean geophyte *Narcissus* (Amaryllidaceae) occurring in at least a dozen species in three sections (Apodanthae, Jonquillae, and Tazettae) of the genus (Dulberger, 1964; Arroyo and Dafni, 1995; Barrett, Lloyd, and Ar-

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[4] Author for reprint requests (FAX 416-978-5878; e-mail: barrett@botany.utoronto.ca).

royo, 1996; Baker, Thompson, and Barrett, 2000a, b). In contrast, heterostyly occurs in only two species of *Narcissus*, distylous *N. albimarginatus* (Arroyo and Barrett, 2000) and tristylous *N. triandrus* (Barrett et al., 1997) although many spurious reports of the polymorphism in *Narcissus* have been made, because of a failure to recognize the distinguishing features of heterostyly and stigma-height dimorphism (reviewed in Barrett, Lloyd, and Arroyo, 1996). The contrasting frequencies and taxonomic distribution of the two polymorphisms in *Narcissus* suggest that stigma-height dimorphism is an evolutionarily stable floral strategy that only rarely becomes modified to heterostyly.

Investigations of several *Narcissus* species have revealed two distinctive features associated with stylar polymorphism. First, self-sterility in the genus is associated with a late-acting ovarian self-incompatibility system that restricts self-fertilization but permits both intramorph and intermorph mating (Bateman, 1954; Dulberger, 1964; Barrett et al., 1997; Sage et al., 1999; Baker, Thompson, and Barrett, 2000b). Second, style-morph ratios in populations are commonly biased in favor of the approach herkogamous or long-styled morph (Arroyo and Dafni, 1995; Barrett, Lloyd, and Arroyo, 1996; Barrett et al., 1997; Baker, Thompson, and Barrett, 2000a). These features differ from the conventional patterns observed in heterostylous species in which intramorph mating is prevented because of diallelic self-incompatibility and style-morph ratios are often close to unity (reviewed in Barrett, 1992). Theoretical models on the evolutionary forces maintaining stigma-height dimorphism in *Narcissus* indicate that biased morph ratios likely arise because of morph-specific differences in assortative mating (Barrett, Lloyd, and Arroyo, 1996; Baker, Thompson, and Barrett, 2000b). Unlike most heterostylous species, such matings can occur in *Narcissus* species because of intramorph compatibility. Further studies of *Narcissus* species with stylar polymorphisms are required to determine whether these unusual features of the reproductive system occur more generally.

Here we examine the floral biology of stigma-height dimorphism in natural populations of *N. papyraceus* Ker-Gawler (section Tazettae). This widely grown ornamental bulb known as 'Paper White' is widely distributed in the Mediterranean region occurring from the southern Iberian Peninsula, Morocco, and Algeria to Southern France, Italy, Greece, and Yugoslavia (Blanchard, 1990). Our investigations focused on populations in southern Spain and Portugal and northern Morocco where the species is particularly common. Our study had four principle objectives: (1) to document patterns of style-length variation in natural populations and to determine whether biased morph ratios are a common feature of the species; (2) to investigate through controlled crosses whether *N. papyraceus* is self-sterile and whether, like other *Narcissus* species with stigma-height dimorphism, all cross-pollinations are fertile regardless of morph identity; (3) to examine whether the prior self-pollination of flowers had any negative influence on seed set following subsequent cross-pollination (such an effect has been demonstrated in tristylous *N. triandrus* and may be of evolutionary significance with respect to the maintenance of stylar polymorphisms in the genus [see Barrett, Lloyd, and Arroyo, 1996; Barrett et al., 1997; Sage et al., 1999]); (4) to quantify mating patterns in natural populations using genetic markers to determine whether outcrossing rates differ between dimorphic and monomorphic populations and between style morphs within population.

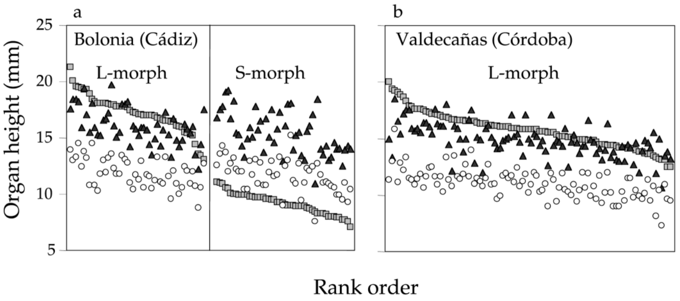

Fig. 1. Stylar dimorphism and monomorphism in *Narcissus papyraceus*. Stigma and anther heights of flowers sampled from 100 individuals from a representative (a) dimorphic (Bolonia, Cádiz Province) and (b) monomorphic (Valdecañas, Córdoba Province) population. Flowers are ranked by stigma height (squares) with upper- and lower-level anthers indicated by triangles and circles, respectively.

MATERIALS AND METHODS

Study species—*Narcissus papyraceus* is a long-lived, insect-pollinated geophyte that occurs commonly throughout the Mediterranean basin, particularly in the southern portions of the Iberian Peninsula and northern Morocco. Populations inhabit open grasslands and pastures or rocky hillsides, usually at altitudes below 2000 m, that are often very wet in winter but hot and dry in summer. In our study area, flowering varies considerably from late November to early March, usually peaking in December and January. Flowers have a mean of 48.4 ovules (SD 10.6, $N = 250$) and are white, sweetly scented, and displayed on umbels containing 6–15 flowers, each of which lasts for up to 3 wk depending on temperature and pollination. Flowers have a short corona and a long floral tube up to 20 mm long and are pollinated by a wide range of insect visitors including solitary bees (*Anthophora* sp.), honey bees (*Apis mellifera*), sphingid moths (*Macroglossum stellatarum*), butterflies (*Gonepteryx cleopatra*, *Pieris brassicae*), and flies (*Bombylius* sp., *Eristalis tenax*) (J. Arroyo, personal observations). Individual ramets usually produce a single inflorescence but clonal propagation through bulb splitting can result in multiple inflorescences being produced by a single genet. Human activities, particularly ploughing, have likely resulted in the multiplication of *N. papyraceus* clones in some highly disturbed habitats.

Population sampling—We sampled a total of 66 populations in southwest Iberian Peninsula and northwest Morocco during the late winter and early springs of 1991–1999 to determine the frequencies of the long- and short-styled morphs (hereafter L-morph and S-morph) in each population. Localities were obtained from surveys of herbarium specimens and from information in regional floras and checklists and can be obtained from the first author on request. We estimated morph ratios and the size of individual populations using previously described methods (see Barrett et al., 1997; Baker, Thompson, and Barrett, 2000a). We made a particular effort to avoid repeated sampling of genets by sampling only one inflorescence per clump of plants, but this may have been unavoidable in populations exposed to repeated human disturbance. We investigated the relationship between log population size (x) and the frequency of the S-morph (y) in dimorphic populations using linear and nonlinear regression models. For the nonlinear regression the predicted frequency of the S-morph $= 0.5(1 - e^{-K(x-B)})$, modified from von Bertalanffy (1938), and two parameters, B (x intercept) and K (the shape of the curve), were estimated using JMP's version 4.04 (SAS Institute, 2000).

In selected populations we preserved random samples of flowers in 70% ethanol to facilitate the measurement of style length and anther height of the style morphs following protocols outlined in detail in Baker, Thompson, and Barrett (2000a; see their Fig. 1).

Experimental pollinations—In November 1999, we carefully excavated plants with developing inflorescences in soil from a large population at Bolonia, Cádiz Province, Spain. The plants and attached soil were placed in plastic pots, watered thoroughly, and returned to an experimental garden at the University of Sevilla where they were used in controlled crosses. We collected the plants over a large area (5 ha) to maximize the number of genets in our sample. When flowering commenced in December we conducted three experimental hand-pollination treatments (self-pollination, intramorph pollination and intermorph pollination), using fine forceps, and recorded the position of each flower in the inflorescence. Each inflorescence received the three treatments, randomly assigned to different flowers, on two separate days. We used only the first 6–8 flowers opening on the inflorescence in hand-pollinations; the remaining flowers were left unpollinated. All flowers were emasculated just prior to flower opening before anthers had dehisced, and pollinations were conducted 1–2 d later. In cross-pollinations we employed a single pollen donor of the same or different style morph, depending on treatment. We carried out pollinations daily from 3 December–2 January on inflorescences from 16 long-styled and 16 shorted-styled plants that were bagged with fine mesh to prevent any potential pollen contamination. Just before seed maturity in February, we harvested fruits and counted the number of plump seeds and estimated total ovule number per flower. Results were analyzed using ANCOVA JMP version 4.04 (SAS Institute, 2000) on log-transformed data of seed set per pollination. Main effects were pollination treatment and style morph and their interaction, and covariates were ovule number per flower and floral position within an inflorescence.

To investigate the potential cost of prior self-pollination we compared the fruit and seed set of flowers that were cross-pollinated with those that were self-pollinated and then cross-pollinated 2–3 d later. We used a total of eight plants each of the L- and S-morph in these comparisons. All cross-pollinations were between morphs and used a single pollen donor. On a given day we randomly assigned treatments to flowers within an inflorescence and their position was recorded. Fruit and seed set were recorded as above and data were analyzed using ANCOVA.

Estimates of mating patterns—We estimated multilocus outcrossing rates using horizontal starch gel electrophoresis of allozymes from open-pollinated seed randomly sampled in eight populations of *N. papyraceus*, using electrophoretic procedures outlined in Wendel and Weeden (1989). Four populations were dimorphic (population 1, Bolonia, Cádiz Province, Spain; population 2, Facinas, Cádiz Province; population 5, Algeciras El Palancar, Cádiz Province; population 11, Oued Lediane, Tanger Province, Morocco), and four were monomorphic (population 10, Posadas, Cordoba Province, Spain; population 15, Carcabuey Valdecañas, Cordoba Province; population 18, Villanueva de los Castillejos, Huelva Province, Spain; population 19, Hinojos Coto del Rey, Huelva Province) for style morph. In dimorphic populations we sampled 8–10 maternal families of ten seeds from each style morph and in monomorphic populations we sampled 15 families of ten seeds. We used polymorphism at four loci (*Aco, Aat, Gdh, Idh*) in all populations, except populations 1 and 2, which were monomorphic at *Idh* and *Gdh,* respectively. In populations with polymorphic loci, 2 alleles were evident at *Aat* and *Gdh,* 2–4 at *Aco,* and 3–4 at *Idh.* We estimated multilocus population outcrossing rates (t_m) and their standard errors (based on 1000 bootstrap values) by employing Ritland's MLTR program (Ritland, 1990). In three dimorphic populations we compared outcrossing rates between the style morphs by pairwise comparisons of bootstrap estimates following the method of Eckert and Barrett (1994).

RESULTS

Patterns of style-length variation—Two fundamentally distinct patterns of style-morph variation were evident in populations of *N. papyraceus*. Populations were either monomorphic for plants with long styles or contained two morphs with long or short styles (Fig. 1). In the dimorphic population at Bolonia illustrated in Fig. 1a, mean stigma height (in millimeters) differs significantly between the style morphs (L-morph = 17.2 mm, S-morph = 9.1 mm, $F_{1,98} = 885.3$, $P < 0.0001$). In contrast, there were no significant differences in the mean heights of upper- and lower-level stamens between the style morphs (upper-level stamens: L-morph = 16.0 mm, S-morph = 15.4 mm, $F_{1,98} = 3.16$, $P = 0.08$; lower-level stamens: L-morph = 11.9 mm, S-morph = 11.6 mm, $F_{1,98} = 1.06$, $P = 0.31$).

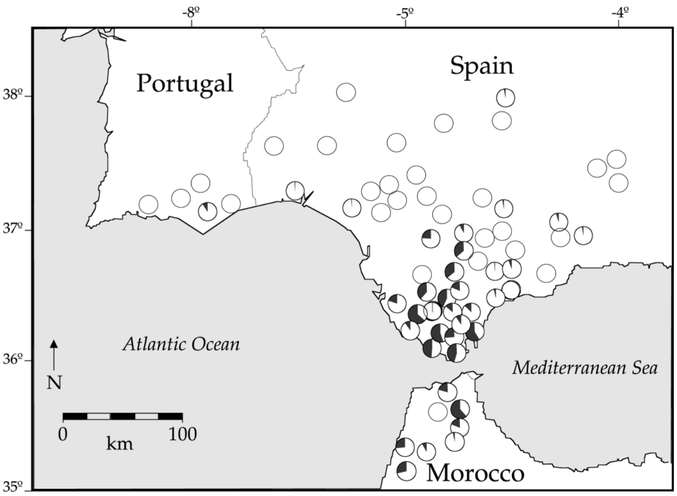

Fig. 2. The geographical distribution of dimorphic and monomorphic populations of *Narcissus papyraceus* in Spain, Portugal, and Morocco. Style-morph frequencies are indicated for the 37 dimorphic populations sampled, dark segments of each circle indicate the frequency of the S-morph. The remaining 29 populations contained only the L-morph and are indicated by open circles.

Of the 66 *N. papyraceus* populations we sampled, 44% were monomorphic and 56% were dimorphic for style length. Among dimorphic populations, the L-morph dominated with average frequencies of the L- and S-morphs of 0.79 and 0.21, respectively. Of the 37 dimorphic populations, 22 deviated significantly from a 1 : 1 morph ratio (*G* tests for each population using Bonferroni correction critical $P = 0.001$). Only a single population exhibited an S-biased morph ratio, the remainder being L-biased. No populations encountered in the survey were monomorphic for the S-morph.

In southwest Spain, where the majority of populations were sampled, the geographical distribution of monomorphic and dimorphic populations was nonrandom (Fig. 2). Dimorphic populations occurred more commonly on the Strait of Gibraltar peninsula, whereas stylar monomorphism predominated farther north. In the Strait of Gibraltar peninsula several very large dimorphic populations occurred that contained equal frequencies of the style morphs. On average, population sizes were significantly smaller in monomorphic than dimorphic populations (mean size = 690 and 41 512 in monomorphic and dimorphic populations, respectively; ANOVA on log-transformed population size $F_{1,65} = 3.87$, $P = 0.05$), although median sizes were identical (500) for the two types of population. There was a significant positive relation between log population size and the frequency of the S-morph in dimorphic populations (linear regression: predicted S-morph frequency = $-0.064 + 0.09$ log(population size), MSE = 0.0289, $F_{1,35} = 12.95$, $P = 0.001$). The nonlinear regression model gave a similar fit to the data (predicted S-morph frequency = $0.5[1 - e^{-K(\log(\text{pop size})-B)}]$, $K = 0.7$, SE = 0.13, $B = 1.57$, SE = 0.16, MSE = 0.0311; Fig. 3). This model asymptotically approaches isopethy (S-morph frequency = 0.5) with increasing population size (Fig. 3).

Experimental pollinations—Hand self- and cross-pollinations of the style morphs demonstrated that *N. papyraceus* is

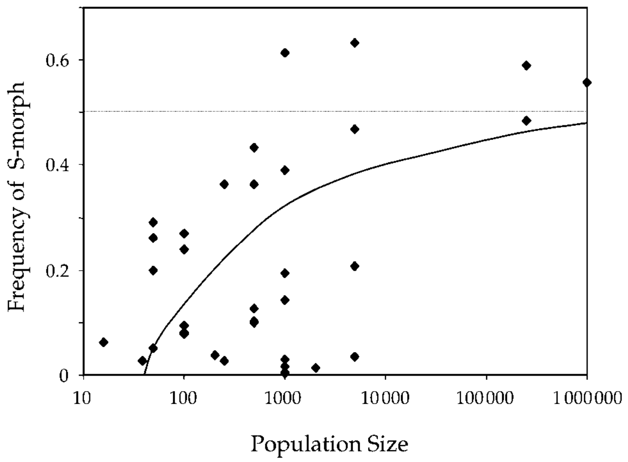

Fig. 3. Relation between the log of the number of individuals and the frequency of the S-morph in populations of *Narcissus papyraceus*. Mean values were used for populations sampled in more than 1 yr. The straight line indicates the equilibrium frequency (0.5) of the S-morph under symmetrical disassortative mating. The fitted line is the predicted frequency of the S-morph from a nonlinear regression model (see MATERIALS AND METHODS).

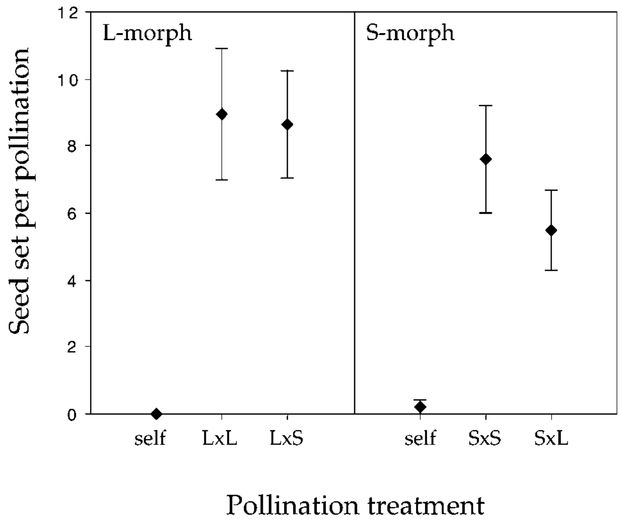

Fig. 4. The effect of controlled self- and cross-pollination on seed set in the style morphs of *Narcissus papyraceus*. Values indicated are the mean and standard error following self-, intramorph, and intermorph pollinations.

strongly self-sterile (Fig. 4). Only occasional fruits and seeds were obtained from self-pollinations. In contrast, both intramorph and intermorph crosses gave high fruit and seed set with no significant differences between the two classes of cross-pollination (contrast, intra- vs. intermorph outcross: $F_{1,236} = 0.103$, $P = 0.749$). ANCOVA indicated both covariates were significant (flower position: $F_{7,65} = 5.61$, $P < 0.0001$; ovule number: $F_{1,236} = 16.592$, $P < 0.0001$), although pollination treatment explained the largest amount of variation in seed set ($F_{2,236} = 43.156$, $P < 0.0001$). The influence of style-morph and the morph × pollination interaction were not significant (style morph: $F_{1,236} = 0.671$, $P = 0.414$; morph × pollination treatment: $F_{1,236} = 1.895$, $P = 0.153$). In general, the L- and S-morphs responded similarly to the pollination treatments, indicating that the strength of self-sterility is essentially the same in both morphs.

On average, prior self-pollination of cross-pollinated flowers reduced mean seed set per pollination by 60% in comparison with cross-pollinated controls (mean seed set per pollination; cross = 9.55, SE = 1.474; self and cross = 3.84, SE = 1.474). We were unable to detect a significant difference in the response of the two style morphs to prior self-pollination (Fig. 5). The ANCOVA for seed set per pollination with flower position ($F_{5,56} = 2.3$, $P = 0.057$) and ovule number ($F_{1,56} = 0.053$, $P = 0.819$) as covariates indicated a significant pollination treatment effect ($F_{1,56} = 7.165$, $P = 0.009$), whereas style-morph and the morph × pollination interaction were not significantly different (style morph $F_{1,56} = 0.698$, $P = 0.407$; morph × pollination treatment $F_{1,56} = 0.969$, $P = 0.\ 329$).

Mating patterns—Multilocus estimates of outcrossing rate in eight populations of *N. papyraceus* using allozyme markers demonstrated that the species is largely outcrossing (Fig. 6, mean $t_m = 0.81$). Among populations, selfing rates varied from 0.09 to 0.34 with no significant difference between monomorphic vs. dimorphic populations (mean $t_m = 0.826$, SE = 0.020 and 0.847, SE = 0.028, respectively; $F_{1,6} = 0.355$, $P = 0.573$; ANOVA on population estimates weighted by the inverse of the variance estimates; see Barrett, Harder, and Cole, 1994). We found no evidence for morph-specific differences in mating patterns within populations (population 1, $t_L = 0.81$, $t_S = 0.66$, $P = 0.44$; population 2, $t_L = 0.66$, $t_S = 0.84$, $P = 0.28$; population 11, $t_L = 0.91$, $t_S = 0.87$, $P = 0.29$). Although population 5 was dimorphic, the low frequency of the S-morph precluded estimating the outcrossing rate of this morph.

DISCUSSION

This investigation of *N. papyraceus* 'Paper White' is the first detailed study of the reproductive biology of this widely grown ornamental. Our research revealed three important findings. First, many populations exhibit a stigma-height dimorphism, with a wide range of style-morph frequencies

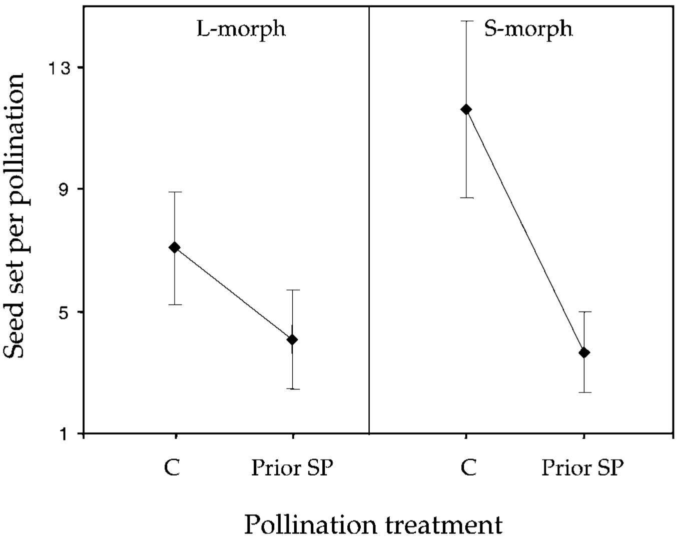

Fig. 5. The effect of prior self-pollination on outcrossed seed set in the style morphs of *Narcissus papyraceus*. Values indicated are the mean and standard error from cross-pollinated flowers (control, C) and for flowers self-pollinated and subsequently cross-pollinated (Prior SP) (see MATERIALS AND METHODS for details).

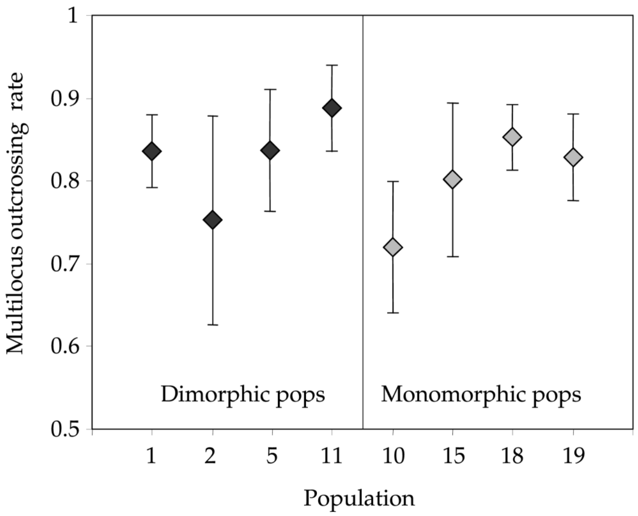

Fig. 6. Multilocus outcrossing rates estimated from allozyme markers in eight populations of *Narcissus papyraceus*. Values indicated are the mean and standard error based on 1000 bootstrap estimates.

from 1 : 1 ratios, through populations with L-biased ratios, to stylar monomorphism (Fig. 2). Second, *N. papyraceus* is strongly self-sterile with only limited seed set resulting from self-pollination in comparison with both within and between morph cross-pollination (Fig. 4). Estimates of mating patterns using genetic markers confirmed high levels of outcrossing in natural populations with no evidence of increased selfing in monomorphic populations (Fig. 6). Finally, prior self-pollination of cross-pollinated flowers resulted in a reduction in seed set in comparison with cross-pollinated controls (Fig. 5). This finding is likely to be associated with the occurrence of a late-acting ovarian self-incompatibility system in *N. papyraceus*. Below we compare our results with earlier work on other *Narcissus* spp. and discuss their significance for understanding variation in style-morph ratios and the evolutionary maintenance of stigma-height dimorphism.

Mating systems in Narcissus—In common with several other *Narcissus* species that have been examined experimentally (Bateman, 1954; Dulberger, 1964; Barrett et al., 1997; Sage et al., 1999; Baker, Thompson, and Barrett, 2000b), *N. papyraceus* is largely self-sterile. Moreover, our controlled crosses provided no evidence of heteromorphic incompatibility since cross-pollinations were equally fertile regardless of whether they were conducted within or between the style morphs. The absence of an association between self-sterility and style length, as commonly occurs in heterostylous plants, enables monomorphic populations of *Narcissus* to persist sexually through intramorph mating. This facility for assortative mating is undoubtedly the major cause of the wide range of style-morph ratios that are evident in natural populations of *Narcissus* species with stylar polymorphisms (Barrett, Lloyd, and Arroyo, 1996).

The low levels of seed set from self-pollination and the occurrence of related species with self-incompatibility suggest that the major factor governing self-sterility in *N. papyraceus* is the operation of a physiological self-incompatibility system. Although we cannot rule out early-acting inbreeding depression as a contributing factor to low fertility following self-pollination, it seems probable that the principle mechanism is self-incompatibility. Observations of pollen tube growth in *N. papyraceus* (R. Hidalgo and T. L. Sage, unpublished data) indicate similar patterns to those previously documented in the closely related *N. tazetta* (Dulberger, 1964) and also *N. triandrus* (Sage et al., 1999). In these species, pollen tube growth in the style is similar between self- and cross-pollination with self-rejection occurring in the ovary as a result of late-acting self-incompatibility. Experimental studies have shown that species with late-acting self-incompatibility systems are often susceptible to the inhibitory effects of prior self-pollination on outcrossed seed set (Waser and Price, 1991; Lloyd and Wells, 1992; Broyles and Wyatt, 1993; Seavey and Carter, 1993; Barrett et al., 1997). In *N. papyraceus*, we also demonstrated a reduction in outcrossed seed set following prior self-pollination, although the mechanism responsible, and whether this phenomenon influences female fertility under field conditions, is not known.

Transitions from stylar heteromorphism to monomorphism in heterostylous species are frequently associated with increased selfing rates. This can occur through breakdown of self-incompatibility associated with selection for reproductive assurance (Charlesworth and Charlesworth, 1979a; Ganders, 1979; Barrett, 1992). However, in *Narcissus* the facility for intramorph mating removes this selection pressure and, as long as pollinator service is reliable, there should be no reason why outcrossing rates might differ between dimorphic and monomorphic populations. Our results support this proposal. Outcrossing rates were generally high among all populations (Fig. 6), regardless of their morph structure, and there was no evidence for significantly increased selfing in monomorphic populations. These findings suggest that the self-incompatibility system in *Narcissus* buffers the maternal outcrossing rate against wide variation in style-morph ratios and population size without apparently impairing female fertility.

Hypotheses to explain variation in style-morph ratios—Our survey of style-morph ratios in 66 populations of *N. papyraceus* revealed several distinct patterns. First, the long-styled morph dominated in most dimorphic populations and was the only morph to occur in monomorphic populations. Second, style-morph ratios were associated with population size. Small populations were more likely to be monomorphic, and equal frequencies of the long- and short-styled morph characterized the very largest populations. Third, variation in style-morph ratios was nonrandomly distributed throughout the area sampled. Dimorphic populations were concentrated in the Strait of Gibraltar peninsula, and monomorphic populations occurred more commonly across the northern edge of the range sampled (Fig. 2). As discussed below, these patterns are strikingly similar to those found in several other *Narcissus* species with stylar polymorphisms, implicating similar ecological and evolutionary forces in structuring variation in sexual traits.

Five other surveys of style-morph ratios in *Narcissus* species with stylar polymorphism have been conducted (Table 1). In common with the results of this study all species are characterized by the frequent occurrence of populations with L-biased morph ratios. Indeed, more restricted surveys of an additional three species (*N. calcicola*, *N. gaditanus*, *N. jonquilla*) have also revealed L-biased morph ratios (Barrett, Lloyd, and Arroyo, 1996). This indicates that L-biased anisoplethy is a general feature of the population biology of *Narcissus* species with stylar polymorphisms. These species are distributed

TABLE 1. Patterns of style-morph variation in *Narcissus* species with stylar polymorphisms.

Species (Section)	Stylar polymorphism	Region sampled	*N* populations	Percentage monomorphic	Commonest morph in polymorphic populations	Mean frequency (%)
N. assoanus[a] (Jonquillae)	Stigma-height dimorphism	Southwest France	46	0.0	L (34/46)	62.0
N. cuatrecasasii[b] (Apodanthae)	Stigma-height dimorphism	Southern Spain	21	0.0	L (20/21)	73.0
N. dubius[a] (Tazettae)	Stigma-height dimorphism	Southwest France	21	28.6	L (15/15)	90.0
N. papyraceus[c] (Tazettae)	Stigma-height dimorphism	Spain, Portugal, Morocco	66	44.0	L (33/37)	79.0
N. tazetta[d] (Tazettae)	Stigma-height dimorphism	Israel	24	20.8	L (10/19)	58.2
N. triandrus[e] (Ganymedes)	tristyly	Spain, Portugal	137	0.0	L (113/137)	57.9

References: [a] Baker, Thompson, and Barrett, 2000a; [b] Arroyo, 2002; [c] This study; [d] Arroyo and Dafni, 1995; Dulberger 1964, 1967; [e] Barrett et al., 1997 and unpublished data.

among four sections of the genus (Apodanthae, Ganymedes, Jonquillae, Tazettae), and they differ in ecology, life history, and pollination biology. This raises the question of what ecological and genetic mechanisms might account for these similar patterns of style-morph variation.

Theoretical models of pollen transfer and mating indicate that the observed L-biased morph ratios in *Narcissus* populations could result from differences in the mating system of the style-morphs (Barrett, Lloyd, and Arroyo, 1996; Baker, Thomson, and Barrett, 2000b). Specifically, these models indicate that L-biased morph ratios will occur whenever levels of assortative (intramorph) mating are higher in the L-morph in comparison to the S-morph. As argued in Barrett, Lloyd, and Arroyo (1996) and Baker, Thomson, and Barrett (2000a, b), the contrasting spatial deployment of sex organs in the style morphs of *Narcissus* species seems likely to influence this potential difference in mating pattern. The significantly greater herkogamy of the S-morph in comparison with the L-morph (see Fig. 1 and Table 13.1 in Barrett, Lloyd, and Arroyo, 1996) should reduce the precision of intramorph pollen transfer in the S-morph, thus limiting assortative mating in this morph. In contrast, weaker herkogamy in the L-morph should promote intramorph transfers; indeed these transfers must predominate in monomorphic populations of *Narcissus* species because they are exclusively composed of the L-morph (Table 1). Unfortunately, in contrast to heterostylous species (see Lloyd and Webb, 1992b), measurements of morph-specific pollen transfer are difficult to make in *Narcissus* because of the absence of pollen-size heteromorphism (Barrett, Lloyd, and Arroyo, 1996; Baker, Thompson, and Barrett, 2000a; Sage et al., 1999). Therefore, estimates of assortative and disassortative mating using morph-specific genetic markers (e.g., Ganders, 1975; Barrett, Brown, and Shore, 1987; Kohn and Barrett, 1992) would be required to test the mating asymmetry hypothesis.

A second striking feature of the survey data on style-morph ratios concerns the morph representation in monomorphic populations. In each of the three species with monomorphic populations (*N. dubius*, *N. papyraceus*, *N. tazetta*; Table 1), all monomorphic populations sampled were composed of the L-morph. This raises the obvious question of what factors prevent the establishment and/or maintenance of populations composed exclusively of the S-morph. Two nonmutually exclusive hypotheses have been proposed to explain the absence of S-monomorphy in other *Narcissus* species (Baker, Thompson, and Barrett, 2000a). These hypotheses are clearly relevant to the data collected in this study and are briefly outlined below.

The genetic hypothesis assumes that the control of style-length in *Narcissus* spp. is governed by a single Mendelian locus with the short-styled allele dominant to the long-styled allele. This pattern of inheritance has been demonstrated in *N. tazetta* (Dulberger, 1964, 1967), a species that in the past has been considered conspecific with *N. papyraceus* (see Blanchard, 1990). This is also the common mode of inheritance for distyly (Lewis and Jones, 1992) and for stigma-height dimorphism in *Anchusa* (Schou and Philipp, 1984). With this mode of inheritance and L-biased morph ratios the vast majority of short-styled plants should be heterozygous (*Ss*) at the style-length locus. Interestingly, Dulberger (1964) found no homozygous plants of the S-morph in her genetic studies of *N. tazetta* despite the absence of intrinsic barrier to intramorph mating (and see Schou and Philipp, 1984). Populations founded by *Ss* plants should always segregate long-styled plants (*ss*), thus reestablishing stylar dimorphism. In contrast, in the absence of gene flow populations founded by long-styled plants will always remain monomorphic for this form because of homozygosity at the style-length locus. Once populations monomophic for the L-morph establish in a region, founder events will further magnify L-monomorphism over larger areas, as appears to have happened around the perimeter of the range of *N. papyraceus*. Therefore, according to this hypothesis, the dominance relationships of alleles at the style-length locus plays a major role in governing which morph can establish monomorphic populations.

The ecological hypothesis proposes that plants of the S-morph are poor at founding populations because their stigmas are concealed deep within the floral tube and therefore require long-tongued bees and lepidoptera for cross-pollination. These types of pollinators may be less inclined to visit small populations. In contrast, the sexual organs of plants of the L-morph are easily accessible to a wide range of pollinators, including pollen-collecting bees and flies, and cross-pollination is less likely to limit population establishment. According to this hypothesis, fertility selection against the S-morph accounts for L-monomorphy. This hypothesis could be tested experimentally by establishing small monomorphic populations of the two morphs. The hypothesis predicts limited reproductive success of the S-morph compared with the L-morph under these conditions. Field observations of pollinators visiting *N. papyraceus* are consistent with aspects of this hypothesis (J. Arroyo, personal observations). A wide range of generalist pollinators including flies, bees, and lepidoptera have been observed visiting flowers in *N. papyraceus*; however, long-tongued bees and lepidoptera pollinators have only been commonly observed in large dimorphic populations in the Strait of Gibraltar peninsula. Similar patterns of insect visitation related to morph structure also occur in the closely related *N. tazetta* in Israel (Arroyo and Dafni, 1995).

The final pattern that emerges from our comparison of style-morph variation among *Narcissus* species is evidence for geographical structuring of the style morphs (Table 1). In four of the six species examined, the distribution of style-morph variation is nonrandomly distributed throughout the regions sampled. In *N. papyraceus*, monomorphic populations predominate along the northern perimeter of the region sampled and populations with similar frequencies of long- and short-styled morphs are concentrated in the Strait of Gibraltar peninsula (Fig. 2). In Israel, *N. tazetta* populations are dimorphic in lowland sites in marshlands, but have L-biased morph ratios or are monomorphic in hilly regions (Dulberger, 1967; Arroyo and Dafni, 1995). In *N. assoanus*, L-biased morph ratios predominate in small populations in lowland sites around Montpellier; in contrast, populations at upland sites are very large and have equal frequencies of the style morphs (Baker, Thompson, and Barrett, 2000a). Finally, in *N. triandrus*, trimorphic populations occur throughout the geographical distribution of the species in the Iberian peninsula, but dimorphic populations are restricted to the northwest corner of this range in central and northern Portugal and northern Spain (Barrett et al., 1997, and unpublished data). Various forms of pollinator-mediated selection, arising from spatial variation in the types of pollinators visiting populations, have been proposed as a potential explanation for these patterns (e.g., Arroyo and Dafni, 1995; Barrett, Lloyd, and Arroyo, 1996). However, the detailed studies required to substantiate this hypothesis have yet to be undertaken.

Maintenance of stigma-height dimorphism—From a functional perspective, stigma-height dimorphism represents a puzzling sexual polymorphism. The absence of clear sex-organ reciprocity, the defining feature of heterostyly, raises the obvious question of how stigma-height dimorphism functions and is maintained in populations. Theoretical models indicate that for stigma-height dimorphism to be stable the frequency of disassortative mating must exceed the frequency of assortative mating in a population (Lloyd and Webb, 1992b; Barrett, Lloyd, and Arroyo, 1996; Baker, Thompson, and Barrett, 2000b). Indeed, this general rule applies to the maintenance of all stylar polymorphisms in plants with equal morph ratios resulting from symmetrical disassortative mating (Barrett, Jesson, and Baker, 2000). What is unclear is how a simple difference in stigma height, unaccompanied by a corresponding anther-height dimorphism or diallelic self-incompatibility, would be sufficient to promote more between-morph than within-morph mating. To answer this question will require detailed investigations of the mechanics of pollen dispersal. These studies should include data on pollinator types and their positioning within flowers with respect to the sexual organs and information on the precise locations of pollen deposition on pollinators.

Despite our ignorance of the precise mechanism(s) by which stigma-height dimorphism promotes disassortative mating, the occurrence of 1 : 1 style-morph ratios in populations of *N. papyraceus* indicates that under some circumstances this mating pattern can be achieved. Very large populations all possessed 1 : 1 morph ratios, a condition also evident in *N. assoanus* (Baker, Thompson, and Barrett 2000a). The discovery of isoplethic morph ratios in *Narcissus* spp. is significant for two reasons. First, the promotion of disassortative mating by stigma-height dimorphism is a critical requirement for Lloyd and Webb's (1992b) model for the evolution of distyly. Our results therefore lend some empirical support to their ideas, despite the fact that the transition from stigma-height dimorphism to heterostyly rarely occurs in *Narcissus*. Second, 1 : 1 morph ratios indicate that symmetrical disassortative mating, in which levels of between-morph mating are equivalent, can be promoted by stigma-height dimorphism alone. In heterostylous plants, between-morph mating is jointly promoted by both heteromorphic incompatibility and reciprocal herkogamy. In contrast, our results for *N. papyraceus* are remarkable because they demonstrate that under the appropriate ecological and demographic conditions a simple morphological difference in style length, probably governed by a single allele, can have a significant influence on the mating biology of populations.

LITERATURE CITED

ARROYO, J. 2002. *Narcissus*, la evolución de los polimorfismos florales y la conservación más allá de las 'listas rojas'. *Revista Chilena de Historia Natural,* in press.

ARROYO, J., AND S. C. H. BARRETT. 2000. Discovery of distyly in *Narcissus* (Amaryllidaceae). *American Journal of Botany* 87: 748–751.

ARROYO, J., AND A. DAFNI. 1995. Variation in habitat, season, flower traits, and pollinators in dimorphic *Narcissus tazetta* L. (Amaryllidaceae) in Israel. *New Phytologist* 129: 135–145.

BAKER, A. M., J. D. THOMPSON, AND S. C. H. BARRETT. 2000a. Evolution and maintenance of stigma-height dimorphism in *Narcissus*. I. Floral variation and style-morph ratios. *Heredity* 84: 502–513.

BAKER, A. M., J. D. THOMPSON, AND S. C. H. BARRETT. 2000b. Evolution and maintenance of stigma-height dimorphism in *Narcissus*. II. Fitness comparisons between style morphs. *Heredity* 84: 514–524.

BARRETT, S. C. H. [ED.]. 1992. Evolution and function of heterostyly. Spring-Verlag, Berlin, Germany.

BARRETT, S. C. H., A. H. D. BROWN, AND J. S. SHORE. 1987. Disassortative mating in tristylous *Eichhornia paniculata* (Pontederiaceae). *Heredity* 58: 49–55.

BARRETT, S. C. H., W. W. COLE, J. ARROYO, M. B. CRUZAN, AND D. G. LLOYD. 1997. Sexual polymorphisms in *Narcissus triandrus*: is this species tristylous? *Heredity* 78: 135–145.

BARRETT, S. C. H., L. D. HARDER, AND W. W. COLE. 1994. Effects of flower number and position on self-fertilization in experimental populations of *Eichhornia paniculata* (Pontederiaceae). *Functional Ecology,* 8: 526–535.

BARRETT, S. C. H., L. K. JESSON, AND A. M. BAKER. 2000. The evolution and function of stylar polymorphisms in flowering plants. *Annals of Botany* 85: 253–265.

BARRETT, S. C. H., D. G. LLOYD, AND J. ARROYO. 1996. Stylar polymorphisms and the evolution of heterostyly. *In* D. G. Lloyd and S. C. H. Barrett [eds.], Floral biology: studies on floral evolution in animal-pollinated plants, 339–376. Chapman and Hall, New York, New York, USA.

BATEMAN, A. J. 1954. The genetics of *Narcissus*. I. Sterility. *Daffodil and Tulip Year Book* 1954: 23–29.

BLANCHARD, J. W. 1990. *Narcissus*: a guide to wild daffodils. Alpine Garden Society, Surrey, UK.

BROYLES, S. B., AND R. WYATT. 1993. The consequences of self-pollination in *Asclepias exaltata*, a self-incompatible milkweed. *American Journal of Botany* 80: 41–44.

CHARLESWORTH, B., AND D. CHARLESWORTH. 1979a. The maintenance and breakdown of distyly. *American Naturalist* 114: 499–513.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1979b. A model for the evolution of distyly. *American Naturalist* 114: 467–498.

DARWIN, C. 1877. The different forms of flowers on plants of the same species. John Murray, London, UK.

DULBERGER, R. 1964. Flower dimorphism and self-incompatibility in *Narcissus tazetta* L. *Evolution* 18: 361–363.

DULBERGER, R. 1967. Pollination systems in plants in Israel. Ph.D. dissertation, Hebrew University, Jerusalem, Israel.

ECKERT, C. G., AND S. C. H. BARRETT. 1994. Post-pollination mechanisms and the maintenance of outcrossing in self-compatible, tristylous *Decodon verticillatus* (Lythraceae). *Heredity* 72: 396–411.

GANDERS, F. R. 1975. Mating patterns in self-compatible distylous populations of *Amsinckia* (Boraginaceae). *Canadian Journal of Botany* 53: 773–779.

GANDERS, F. R. 1979. The biology of heterostyly. *New Zealand Journal of Botany* 17: 607–635.

KOHN, J. R., AND S. C. H. BARRETT. 1992. Experimental studies on the functional significance of heterostyly. *Evolution* 46: 43–55.

LEWIS, D., AND D. A. JONES. 1992. The genetics of heterostyly. *In* S. C. H. Barrett [ed.], Evolution and function of heterostyly, 129–150. Springer-Verlag, Berlin, Germany.

LLOYD, D. G., AND C. J. WEBB. 1992a. The evolution of heterostyly. *In* S. C. H. Barrett [ed.], Evolution and function of heterostyly, 151–178. Springer-Verlag, Berlin, Germany.

LLOYD, D. G., AND C. J. WEBB. 1992b. The selection of heterostyly. *In* S. C. H. Barrett [ed.], Evolution and function of heterostyly, 179–208. Springer-Verlag, Berlin, Germany.

LLOYD, D. G., AND M. S. WELLS. 1992. Reproductive biology of a primitive angiosperm, *Pseudowintera colorata* (Winteraceae) and the evolution of pollination systems in the Anthophyta. *Plant Systematics and Evolution* 181: 77–95.

RICHARDS, A. J. 1986. Plant breeding systems. Allen and Unwin, London, UK.

RITLAND, K. 1990. A series of FORTRAN computer programs for estimating mating systems. *Journal of Heredity* 81: 235–237.

SAGE, T. L., F. STRUMAS, W. W. COLE, AND S. C. H. BARRETT. 1999. Differential ovule development following self- and cross-fertilization in *Narcissus triandrus*. *American Journal of Botany* 86: 855–870.

SAS INSTITUTE. 2000. Statistics and graphics guide, version 4.04. SAS Institute, Cary, North Carolina, USA.

SCHOU, O., AND M. PHILIPP. 1984. An unusual heteromorphic incompatibility system. III. On the genetic control of distyly and self-incompatibility in *Anchusa officinalis* L. *Theoretical and Applied Genetics* 68: 139–144.

SEAVEY, S. F., AND S. K. CARTER. 1993. Self-sterility in *Epilobium obcordatum* (Onagraceae). *American Journal of Botany* 81: 331–338.

VON BERTALANFFY, L. 1938. A quantitative theory of organic growth. Inquiries on growth laws. II. *Human Biology* 10: 181–213.

WASER, N. M., AND M. V. PRICE. 1991. Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaceae). *American Journal of Botany* 78: 1036–1043.

WEBB, C. J., AND D. G. LLOYD. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. II. Herkogamy. *New Zealand Journal of Botany* 24: 163–178.

WENDEL, J. F., AND N. F. WEEDEN. 1989. Visualization and interpretation of plant isozymes. *In* D. E. Soltis and P. S. Soltis [eds.], Isozymes in plant biology, 5–45. Dioscorides Press, Portland, Oregon, USA.