

CORRELATED EVOLUTION OF FLORAL MORPHOLOGY AND MATING-TYPE FREQUENCIES IN A SEXUALLY POLYMORPHIC PLANT

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Abstract.—In sexually polymorphic species, reproductive morphology governs mating patterns and the character of negative frequency-dependent selection. If local environmental conditions cause sexual morphs to differ between populations, then frequency-dependent selection should create corresponding geographic variation in morph frequencies. We investigate this relation with a model of morph-ratio evolution and analysis of geographic variation in the heterostylous plant *Narcissus triandrus*. Unlike other tristylous species, *N. triandrus* possesses both imperfect reciprocity among morphs in sex-organ position and a self-incompatibility system that permits outcrossing within and between morphs. We sampled 137 populations throughout the Iberian Peninsula for floral-morph ratios, and measured floral morphology in 31 populations. Morph ratios exhibited three atypical features: (1) predominance of the long-styled (L) morph; (2) absence of the mid-styled (M) morph from 17.5% of populations; and (3) a negative relation between the frequencies of the L and M morphs among populations. Morph ratios varied geographically, with decreasing frequency of the M morph from the southeast to the northwest of the species' range. Much of this variation accompanied allometric change in the positions of sex organs, especially the mid-level organs, with the M morph declining in frequency and ultimately being lost in large-flowered populations. Using multivariate multiple regression, we demonstrate that variation in floral morphology among populations predicts this geographic variation in morph frequencies. Our theoretical analysis illustrates that patterns of pollen transfer governed by imperfect sex-organ reciprocity can select for unequal equilibrium morph ratios like those observed for *N. triandrus*. We interpret the L-biased morph ratios and the unusual morphology of *N. triandrus* as a consequence of its atypical intramorph compatibility system.

Key words.—Allometry, floral morphology, frequent-dependent selection, geographical variation, *Narcissus triandrus*, style-morph ratios, tristily.

Received July 11, 2003. Accepted January 5, 2004.

The maintenance of sexual polymorphism involves negative frequency-dependent selection, whereby a mating type's reproductive success depends on the frequency of the sexual morphs with which it can mate (e.g., Clarke et al. 1988; Eckert et al. 1996; McCauley and Brock 1998; Thompson et al. 2003). Frequency-dependent selection leads to an equal morph ratio at equilibrium (isoplethy: Finney 1952), when each morph can mate only with other sexual phenotypes, which are equally suitable mating partners (e.g., Fisher 1930). Alternatively, an equilibrium with unequal frequencies results when morphs differ in their ability to mate with each other. In either case, all morphs realize equal fitness at the equilibrium morph ratio. Thus, in polymorphic populations, the equilibrium morph ratio is causally linked to how the reproductive traits of each morph govern mating patterns.

Heterostylous plants illustrate the role of mating phenotype in controlling equilibrium morph ratios. Species with this polymorphism include two or three floral morphs that differ physiologically and morphologically. For example, typical tristylous species are characterized by three sexual morphs that differ reciprocally in stigma and anther height (hereafter long-, mid-, and short-styled or L, M, S morphs). Such species also possess heteromorphic incompatibility, which allows fertilization of ovules only by pollen from a different morph. This imposition of disassortative mating generally results in an isoplethic equilibrium (Fisher 1941, 1944; Charlesworth 1979; Heuch 1979; Barrett 1993). Because physiology strictly controls mating patterns, the morphological differences between morphs do not affect the frequency de-

pendence of mating outcomes, and so do not affect the equilibrium morph ratio. In contrast, some heterostylous species lack heteromorphic incompatibility (reviewed in Barrett and Cruzan 1994), so that differences in floral morphology between morphs influence mating patterns more strongly. The role of floral morphology in mating depends on the interaction of flowers with pollen vectors (e.g., Harder and Barrett 1993; Campbell et al. 1996; Morgan and Conner 2001) and is much less precise than a physiological incompatibility mechanism in enforcing intermorph mating. As a result, the equilibrium morph ratio in heterostylous species without heteromorphic incompatibility could deviate from isoplethy and differ extensively between populations, depending on the local mating environment.

If local conditions cause differences in the phenotypes of the floral morphs between populations and morphology is the main influence on mating patterns, then local frequency-dependent selection should create corresponding geographic variation in morph frequencies. These situations could be identified by an association between the specific morphology of each mating type within populations and the corresponding morph frequencies. Here we investigate this association in *Narcissus triandrus* L. (Amaryllidaceae), an unusual tristylous species with extensive geographic variation in morph ratios. Surveys in the Iberian Peninsula found most populations dominated by the L morph, with some populations lacking the M morph (Fernandes 1965; Barrett et al. 1997), a unique pattern for a tristylous species. *Narcissus triandrus* also exhibits atypical floral morphology and reproductive

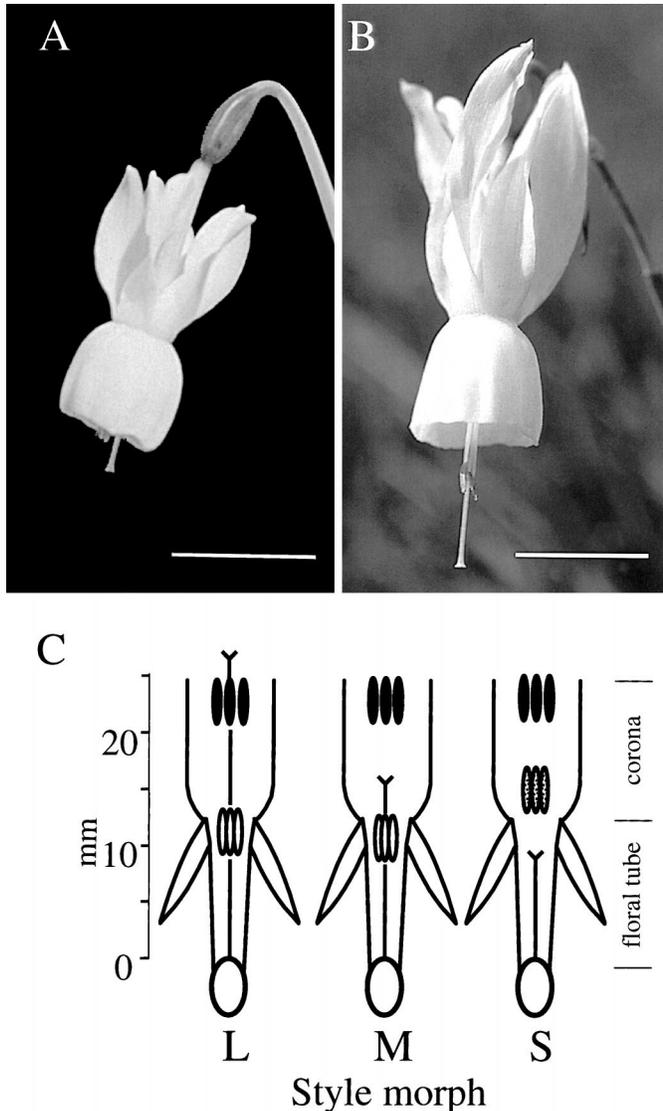


FIG. 1. Morphology of *Narcissus triandrus* flowers. Panels (A) and (B) illustrate flowers of the long-styled morph of *N. triandrus* var. *cernuus* and var. *triandrus*, respectively (scale bars = 10 mm). Note the striking difference between the two varieties in the exsertion of sex-organs beyond the corona mouth. As discussed in the text, this difference is the result of allometric relations between flower size and upper-level sex-organs. Panel (C) depicts the three floral morphs of *N. triandrus*, illustrating the imperfect reciprocity between anther and stigma positions that characterizes this species. Mean sex-organ heights are based on pooled data from all var. *cernuus* populations.

physiology. All three morphs of this species possess long-level anthers, so that the L and M morphs have similar anther positions (Fig. 1C). The anomalous position of the upper anthers of the L-morph effectively unbalances reciprocity, because different numbers of anther levels are aligned with stigmas of the three morphs (L morph, three anther levels; M morph, one anther level; S morph, two anther levels). In addition, this species' self-incompatibility system permits outcrossing both within and between morphs (Sage et al. 1999), which allows more diverse patterns of outcrossed mating than occurs in typical heterostylous species. The absence

of heteromorphic incompatibility in this species should allow floral morphology to govern the incidence of mating within and between morphs. Consequently, *N. triandrus* provides a rare opportunity to explore the functional association between floral morphology and population morph structure.

In this study, we specifically address two questions concerning the relation between morph ratios and morphology in *N. triandrus*. First, could the atypical morphology of the L morph lead to its general predominance in populations? We address this theoretically with a model of frequency-dependent selection that incorporates the influence of floral morphology on pollen transfer within and among morphs. Second, is variation in morph frequencies, including absence of the M morph, governed by parallel variation in floral morphology? We motivate this problem by quantifying geographical variation in morph ratios and floral morphology of *N. triandrus*. A consistent geographic pattern would support the influence of deterministic processes, such as frequency-dependent selection, in governing morph ratios. We then investigate the ability of floral morphology to predict variation in population morph ratios, using both multivariate multiple regression and our pollen-transfer model. These analyses identify ecological and evolutionary processes that could be responsible for the geographic patterns of morph-frequency variation in *N. triandrus*.

MODEL OF POLLEN TRANSFER AND MORPH RATIOS

Our model of frequency-dependent selection of morph ratios in heterostylous populations elaborates previous models by Charlesworth (1979) and Lloyd and Webb (1992). We assume that seed production is limited by resource availability, rather than by pollen receipt, so that fitness differences between individuals of different morphs arise from their relative performance as pollen donors. We also assume that individuals of all morphs export equivalent amounts of pollen. The proportion of exported pollen from morph i that reaches recipient morph j is represented by q_{ij} . Because of resource limitation, pollen from a given morph competes for access to ovules in proportion to its relative abundance on stigmas of the receiving morph. Given the pollen transfer coefficients, q_{ij} , and the current morph frequencies, f , the relative fitnesses for the three morphs are

$$w_L = \frac{1}{2} + \frac{q_{LL}f_L}{2(q_{LL}f_L + q_{ML}f_M + q_{SL}f_S)} + \frac{q_{LM}f_M}{2(q_{LM}f_L + q_{MM}f_M + q_{SM}f_S)} + \frac{q_{LS}f_S}{2(q_{LS}f_L + q_{MS}f_M + q_{SS}f_S)}, \quad (1a)$$

$$w_M = \frac{1}{2} + \frac{q_{ML}f_L}{2(q_{LL}f_L + q_{ML}f_M + q_{SL}f_S)} + \frac{q_{MM}f_M}{2(q_{LM}f_L + q_{MM}f_M + q_{SM}f_S)} + \frac{q_{MS}f_S}{2(q_{LS}f_L + q_{MS}f_M + q_{SS}f_S)}, \quad \text{and} \quad (1b)$$

$$\begin{aligned}
 w_S = & \frac{1}{2} + \frac{q_{SL}f_L}{2(q_{LL}f_L + q_{ML}f_M + q_{SL}f_S)} \\
 & + \frac{q_{SM}f_M}{2(q_{LM}f_L + q_{MM}f_M + q_{SM}f_S)} \\
 & + \frac{q_{SS}f_S}{2(q_{LS}f_L + q_{MS}f_M + q_{SS}f_S)}. \tag{1c}
 \end{aligned}$$

The initial 1/2 term represents a plant’s contributions of genes as a maternal parent. The remaining terms on the right side represent a morph’s paternal contributions realized through competition in the pistils of L, M, and S morphs, respectively. Because w_L , w_M , and w_S are relative fitnesses, the frequencies of the L, M, and S morphs in the next generation are $f'_L = w_L f_L$, $f'_M = w_M f_M$, and $f'_S = w_S f_S$, respectively. With frequency-dependent selection, the morph frequencies evolve until reaching a stable equilibrium at which $w_L = w_M = w_S = 1$. In its most general form, this model is analytically intractable, so that the equilibrium morph ratio must be identified by numerical simulation of the evolution of morph frequencies from a set of initial frequencies. Simulations of a genetically explicit model lead to the same equilibrium as this phenotypic model, when there is a single unique equilibrium, so we consider only the much simpler phenotypic model.

Our theoretical analysis is based on Darwin’s (1877) proposal that in heterostylous plants pollen disperses primarily between anthers and stigmas of equivalent height, because of segregated pollen placement on pollinators’ bodies. Empirical studies support this hypothesis (reviewed in Lloyd and Webb 1992). This role of morphology in pollination can be examined by using values of q_{ij} that reflect the organ positions of the donating and receiving morphs.

For example, consider the consequences of the anomalous position of the upper anthers of the L morph for the equilibrium morph ratio. We consider this situation by using different values of $q_{LL} = Q$ and $q_{LM} = 0.5 - Q$, with $0 \leq Q \leq 0.5$. Pollen-transfer coefficient between other organ positions are those expected with complete reciprocity (see details in Table 1A). In this special case (Table 1A), the equilibrium morph ratio is

$$f_L = \frac{1}{3 - 2Q}, \tag{2a}$$

$$f_M = \frac{3 - 4Q}{3(3 - 2Q)}, \text{ and} \tag{2b}$$

$$f_S = \frac{1}{3}, \tag{2c}$$

indicating that the position of the upper anthers of the L morph should affect equilibrium ratios of the L and M morphs strongly. With the complete reciprocity of typical tristylous species ($Q = 0$), each morph mates equally with the other two morphs. In this situation each morph realizes equal reproductive success when it is at equal frequency with the other morphs, so that the equilibrium morph ratio is $f_L = f_M = f_S = 1/3$ (Fig. 2). A higher position for the upper anthers of the L morph reduces pollen export from the L morph to the M morph and increases export between plants of the L

TABLE 1. Matrices of pollen-transfer proficiencies considered in the simulation model of equilibrium morph ratios in tristylous populations. Model A considers situations with different values of Q ranging from 0.0 (typical tristylous) to 0.5 (similar to *Narcissus triandrus*). Model B considers situations with different values of R ranging from 0.0 to 0.5, representing increased elevation of the lower anthers of the S morph. See Figure 2 for model results.

Donating morph	Receiving morph		
	L	M	S
Model A			
L	Q	$0.5 - Q$	0.5
M	0.5	0.0	0.5
S	0.5	0.5	0.0
Model B			
L	0.45	0.05	0.5
M	0.5	0.0	0.5
S	$0.5 + R$	$0.5 - R$	0.0

morph ($Q > 0$). This change results in equilibrium morph ratios with a predominance of the L morph and a relative deficiency of the M morph (Fig. 2). When the upper anthers of all three morphs occupy identical long-level positions and donate pollen only to the L morph ($Q = 0.5$), one equilibrium involves a 3:1:2 ratio of the L, M, and S morphs (Fig. 2). However, in this situation, the L and M morphs have identical anther positions and are interchangeable as pollen donors. As a consequence, different initial morph frequencies lead to the evolution of different equilibria, which satisfy the equalities $f_M = (1 - f_L)^2 / (2 - f_L)$ and $f_S = (1 - f_L) / (2 - f_L)$.

These results expose two conclusions. First, any change in pollen transfer between morphs resulting from morphological evolution bears predictable consequences for equilibrium morph frequencies. Second, an elevated position of the upper anthers of the L morph compared to the typical tristylous condition results in L-biased morph ratios, with a low frequency of the M morph. This change alone has no impact on the frequency of the S morph (eq. 2). Changes in morphology that affect other q_{ij} would have more diverse effects on equilibrium morph ratios.

MATERIALS AND METHODS

Study Species

Narcissus triandrus is a widespread and abundant geophyte in central and northern parts of the Iberian Peninsula. Populations begin flowering during late February, at the southern margin of the range in Andalucía, and continue into early May at higher elevations in central and northern Spain and Portugal. Plants produce a single flowering stem per season that bears between one and nine flowers, depending on variety (see below). Flowers of *N. triandrus* have a narrow floral tube, which expands into a broader corona with reflexed tepals (Fig. 1). Nectar-collecting long-tongued bees, primarily *Anthophora* and *Bombus* species, enter the corona and probe the tube to access the nectar at the top of the ovary. The long tubes of *N. triandrus* flowers exclude short-tongued visitors and the pendant flowers prevent visits by Lepidoptera. *Narcissus triandrus* flowers produce short-level sex organs within the floral tube, mid-level organs at or beyond the transition

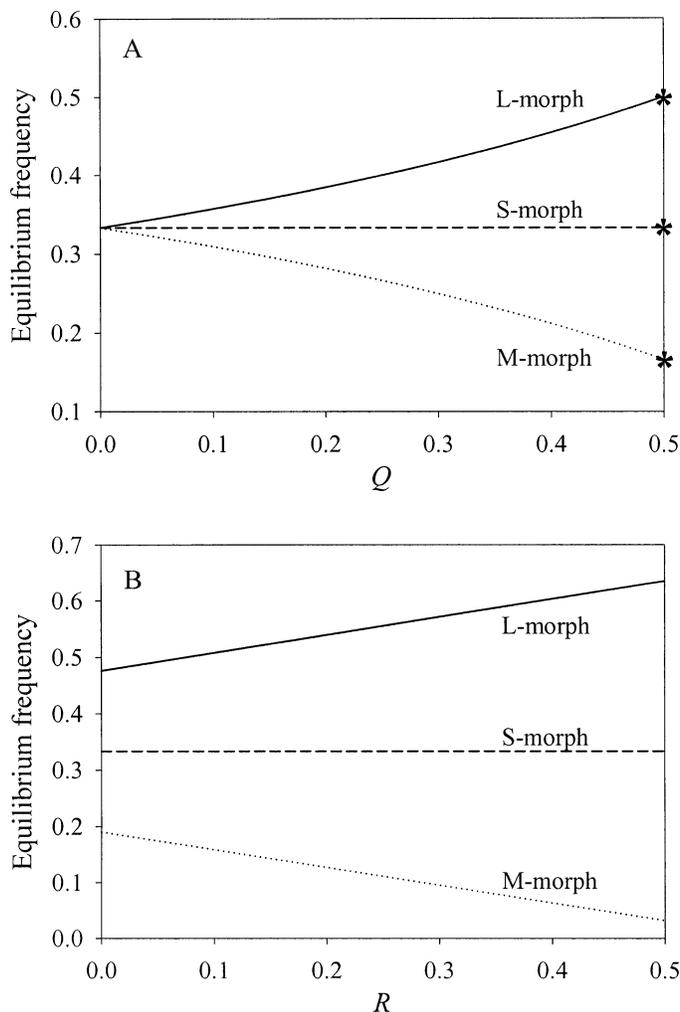


FIG. 2. Effects of different anther positions on the theoretical equilibrium morph frequencies in a tristylous population. (A) The consequence of elevating the upper anthers of long-styled plants, with Q representing pollen transfer from the L morph to other plants of the L and M morphs ($Q = 0$ represents typical tristily, $Q \approx 0.5$ represents *Narcissus triandrus*: see Table 1A and eq. 2). The asterisks at $Q = 0.5$ indicate that the illustrated equilibrium is one of many possibilities (see text for details). (B) The effects of elevating the lower anthers of the S morph for *N. triandrus* (see Discussion: *Correlated Evolution of Floral Morphology and Morph Ratios*). R represents the proportion of pollen dispersed from the S morph to the L morph that originated from the lower anthers, as depicted by the pollen-transfer proficiencies listed in Table 1B. Based on numerical simulation of equation (1).

from the tube to the corona, and long-level organs at or beyond the corona mouth (Fig. 1C). Flowers last for 7–14 days, depending on temperature and pollen receipt.

Various intraspecific taxa have been recognized in *N. triandrus* (reviewed by Blanchard 1990), but here we focus on the two common, wide-ranging varieties. *Narcissus triandrus* var. *cernuus* (Salisb.) Baker (Fig. 1A) occurs in central and southern parts of the species' range (Fig. 3A, closed symbols) and is characterized by small stature, pale lemon flowers, and usually one or two flowers per inflorescence (mean = 1.33, range = 1–5, $n = 38$ populations, 6806 plants). In contrast, *N. triandrus* L. var. *triandrus* (Fig. 1B) occurs in northern

Portugal and northwestern Spain (Fig. 3A, open symbols), is taller, with larger, white-to-cream flowers that are often produced in greater numbers per inflorescence (mean = 1.61, range = 1–9, $n = 81$ populations, 8775 plants). We also included two populations of the rare *N. triandrus* var. *concolor* (Haw.) Baker from central Portugal. This variety closely resembles var. *cernuus*, except for its deeper yellow flowers; we therefore aggregated morph-ratio data for var. *concolor* with var. *cernuus*.

Population Sampling

During the springs of 1990, 1991, 1995, 1997, and 1999, we sampled 137 populations of *N. triandrus* to determine the frequencies of floral morphs in each population and to investigate geographical patterns of floral-morph variation. Our total sample included 50 populations of var. *cernuus*, 85 populations of var. *triandrus*, and two populations of var. *concolor*. Most populations were separated by at least 5–10 km, and we attempted to sample evenly throughout the range of the species. In each population, we obtained a random sample of flowering stems and classified them according to floral morph. The mean number of plants sampled per population was 161 (range = 29–822). Because *N. triandrus* does not propagate clonally, the sampling of genets was unambiguous. Detailed localities and data on floral-morph ratios are available from S. C. H. Barrett on request.

To determine mean flower size and sex-organ positions of floral morphs within populations, we collected flowers of *N. triandrus* from a subsample of 31 populations that we also sampled for morph ratios ($n = 15$, var. *cernuus*; $n = 16$, var. *triandrus*). Flowers were preserved in fixative until we measured the height of the stigma and midpoint of the two anther levels above the ovary under a dissecting microscope (mean number of flowers measured per morph/population = 25, range = 9–68). For 17 populations, we also measured overall flower length (top of ovary to corona mouth) and floral-tube length as measures of flower size.

Statistical Analyses

We used likelihood-ratio (G) tests to explore variation in morph frequencies. Floral-morph ratios were compared to isoplethic equilibria with heterogeneity G -tests (Sokal and Rohlf 1995), with sequential Bonferroni adjustment to control the overall Type I error rate ($\alpha = 0.05$; Rice 1989). We used generalized linear models (McCullagh and Nelder 1989; Genmod procedure of SAS ver. 8.2, SAS Institute 2001) to assess the between-population variation in the relative frequencies of the M and S morphs. These analyses treated the frequencies of the M and S morphs as binomial dependent variables and used the logit transformation to linearize their relations with the frequency of the L morph. These analyses also considered variety (*cernuus* or *triandrus*) as a categorical independent variable.

To assess whether morph frequencies and floral traits exhibited consistent geographic variation, we used two spatially explicit techniques. We used Arc View 3.1 Geographic Information System (ESRI 1996) and Kriging interpolation (Kriging extension ver. 3.2, M. Boeringa, <http://www.nieuwland.nl>) to map the locations of all populations and to

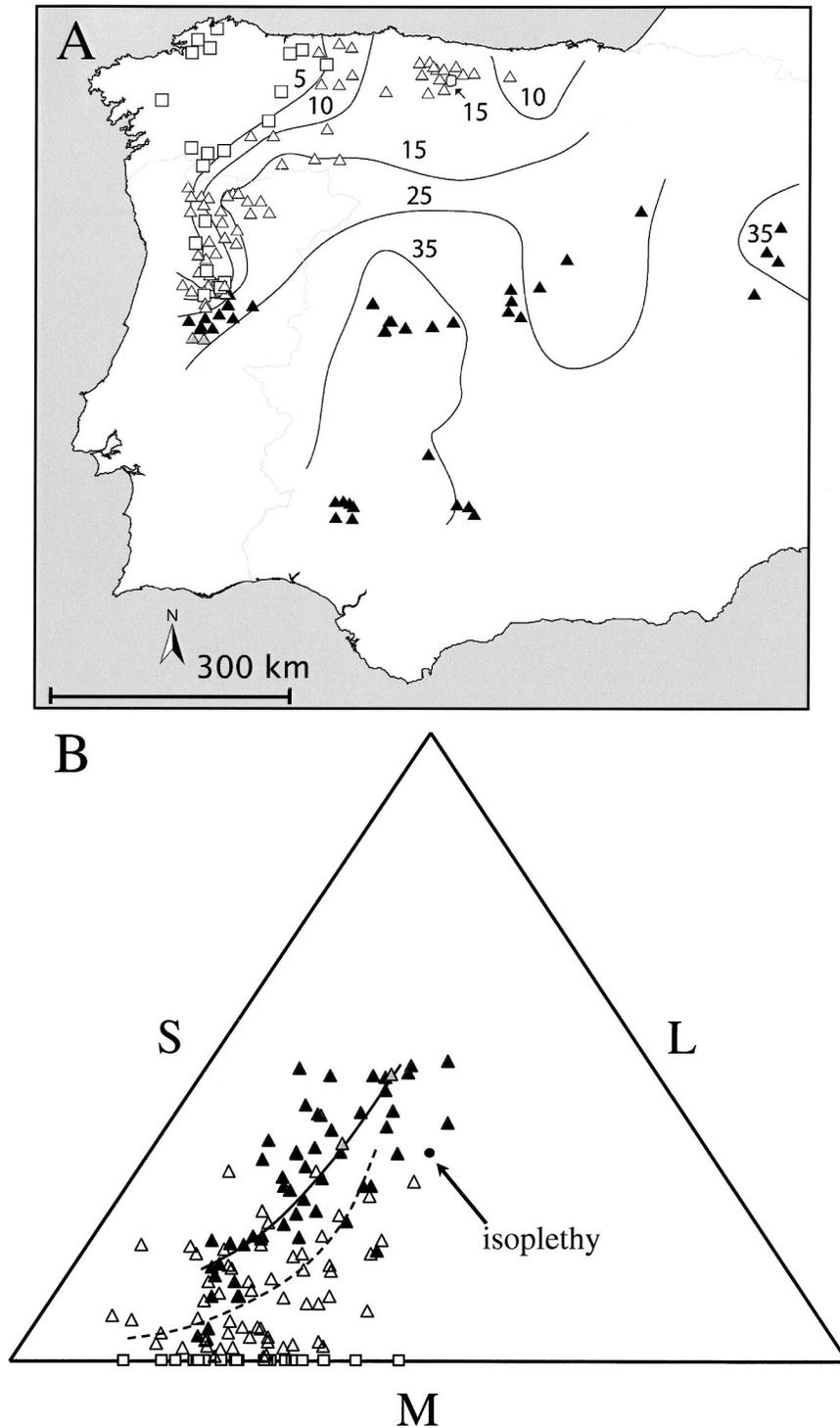


FIG. 3. Variation in the frequencies of floral morphs among 137 populations of *Narcissus triandrus*. (A) The nonrandom distribution of the M morph throughout the range of *N. triandrus* in the Iberian Peninsula; (B) the relative frequencies of all morphs. In both panels, filled, open, and gray symbols represent *var. cernuus*, *triandrus*, and *concolor*, respectively, with triangles identifying trimorphic populations and squares distinguishing dimorphic populations. Lines in panel (A) are isoclines of the percentage of individuals of the M morph estimated with the Kriging interpolation algorithm (see Materials and Methods). In panel (B) the distance from a point to an axis is proportional to the frequency of a morph in a population (e.g., populations lacking the M morph lie along the M axis). The lines in panel (B) depict regression relations of the frequency of the M morph (f_M) to that of the L morph (f_L ; $f_S = 1 - f_L - f_M$) for trimorphic populations of *var. cernuus* (solid line) and *var. triandrus* (dashed line).

calculate isoclines of the frequency of the M morph (see Royal et al. 1981; Oliver 1990). We calculated a semivariogram using Boeringa's Kriging Interpolator extension to fit the ordinary Kriging model (linear with sill, lag = 2°) to our data ($n = 137$ populations). For interpolation, we used a fixed search diameter of 1.2° and a sample count of six populations. The semivariogram and measures of goodness-of-fit indicated that a linear model with a sill provided the best fit to geographic variation in M-morph frequency. To evaluate systematic geographic variation in floral traits, we used multiple regressions (Reg procedure of SAS ver. 8.2, SAS Institute 2001), which considered variation in ln-transformed traits with respect to rectangular east (E) and north (N) coordinates (as opposed to latitude and longitude). The initial model for each analysis assessed the effects of E , N , E^2 , N^2 , and EN , and we then used backward elimination ($\alpha = 0.05$) to select the final regression model (Neter et al. 1996).

Our analyses of morphometric relations among floral traits involved two approaches, both of which considered ln-transformed data. We used linear, Model II regression to assess whether floral traits varied isometrically (regression coefficient, $b = 1$) or allometrically ($b \neq 1$) with either total perianth length or the length of the floral tube. These analyses considered population means and were conducted with the Mixed procedure of SAS (ver. 8.2, SAS Institute 2001), with the independent size trait treated as a random variable. We tested the estimated regression coefficient (\hat{b}) against the value expected for isometry ($b = 1$) with $F = (\hat{b} - 1)^2/s_b^2$, where s_b is the estimated standard error of the regression coefficient. Second, we characterized the Pearson's product-moment correlations between pairs of variables (based on population means), with sequential Bonferroni adjustment of the Type I error rate ($\alpha = 0.05$ over all correlations; Rice 1989).

If differences in the positions of sexual organs among floral morphs govern frequency-dependent selection on morph ratios in *Narcissus* populations, then morph ratios should vary predictably with floral morphology. To investigate this hypothesis, we performed multivariate multiple regression analysis (Aitchison 1986; GLM procedure, SAS ver. 8.2, SAS Institute 2001) on data from 31 populations. The data used in the analysis involved the mean heights of stigmas and upper- and lower-level anthers based on ln-transformed measurements for each population and floral morph. We did not include measurements of the M morph in the analysis, because dimorphic populations lacked this morph. We simultaneously analyzed two dependent variables, the log-odds of the L morph relative to the S morph, $\mathcal{L}_L = \ln(f_L/f_S)$, and the log-odds of the M morph relative to the S morph, $\mathcal{L}_M = \ln(f_M/f_S)$, where f_L , f_M , and f_S are the relative frequencies of the L, M, and S morphs, respectively. For dimorphic populations \mathcal{L}_M is undefined, so before calculating \mathcal{L}_L and \mathcal{L}_M we assigned $f_M = 4/9N$ and decremented f_L and f_S by $2/9N$, where N is the number of plants sampled to estimate morph frequencies (see Aitchison 1986). Because of this adjustment, our method cannot predict purely dimorphic populations. The regression used backward elimination (Neter et al. 1996) to find the set of independent variables that explained significant proportions of the joint variation in both dependent variables, based on Wilks' lambda. Given predicted values for the dependent

variables from the regression equations, $\hat{\mathcal{L}}_L$ and $\hat{\mathcal{L}}_M$, the predicted morph frequencies for a population are given by

$$\hat{f}_L = \frac{e^{\hat{\mathcal{L}}_L}}{1 + e^{\hat{\mathcal{L}}_L} + e^{\hat{\mathcal{L}}_M}}, \quad (3a)$$

$$\hat{f}_M = \frac{e^{\hat{\mathcal{L}}_M}}{1 + e^{\hat{\mathcal{L}}_L} + e^{\hat{\mathcal{L}}_M}}, \quad \text{and} \quad (3b)$$

$$\hat{f}_S = \frac{1}{1 + e^{\hat{\mathcal{L}}_L} + e^{\hat{\mathcal{L}}_M}}. \quad (3c)$$

RESULTS

Variation in Floral-Morph Frequencies

The morph frequencies in the 137 *N. triandrus* populations differed considerably from the isoplethic state found in typical tristylous species (Fig. 3B). Of these populations, 82.5% were trimorphic and the remainder was dimorphic. All dimorphic populations belonged to var. *triandrus* and lacked the M morph (Fig. 3B). Overall, the L morph predominated in all dimorphic populations and in 91% of the trimorphic populations sampled. The M morph was most frequent in 9% of trimorphic populations. The S morph was never the most frequent morph in a population. Within the 113 trimorphic populations, the mean frequencies of the floral morphs were L morph = 0.58, M morph = 0.19, and S morph = 0.23, whereas in the 24 dimorphic populations L morph = 0.71 and S morph = 0.29. These morph ratios differed significantly from isoplethy (trimorphic populations, $G_{\text{pooled}} = 3366.8$, 2 df, $P < 0.001$, $G_{\text{het}} = 2739.1$, 224 df, $P < 0.001$; dimorphic populations, $G_{\text{pooled}} = 498.2$, 1 df, $P < 0.001$, $G_{\text{het}} = 52.74$, 23 df, $P < 0.001$). Overall, 107 of the 113 trimorphic populations deviated significantly from a 1:1:1 ratio, and 16 of 24 dimorphic populations deviated significantly from a 1:1 ratio. Deviations from isoplethy were equally common in trimorphic populations of var. *cernuus* (47 of 52 populations) and var. *triandrus* (57 of 61 populations).

Morph frequencies varied considerably among populations (Fig. 3B: L morph, range = 23.9–86.3%; M morph, 0–47.9%; S morph, 6.5–46.5%). Among trimorphic populations, the frequencies of both the M and S morphs declined with increasing frequency of the L morph (Fig. 3B), with steeper declines for the M morph in var. *triandrus* than in var. *cernuus* (variety \times L-frequency interaction: M morph, $G_1 = 18.2$, $P < 0.001$; S morph, $G_1 = 5.8$, $P < 0.025$). This association was stronger for the M morph ($G_1 = 593.0$, $P < 0.001$) than for the S morph ($G_1 = 72.0$, $P < 0.001$; Fig. 3B). As a consequence, the average frequency of the S morph varied little between trimorphic and dimorphic populations (0.227 and 0.290, respectively), whereas trimorphic populations had a much lower frequency of the L morph (0.579) than did dimorphic populations (0.710).

The two varieties of *N. triandrus* exhibited contrasting geographical patterns in morph-frequency variation (Fig. 3A). In var. *cernuus*, the frequency of the M morph is highest around Sevilla and in the Sierra de Gredos, west of Madrid, but is lower near Madrid and in central Portugal. In contrast, in var. *triandrus* a general decrease in the frequency of the M morph was evident in central and northern Portugal and

northwest Spain (Fig. 3A). These areas were characterized by dimorphic and trimorphic populations with low frequencies of the M morph within a few kilometers of each other (Fig. 3A). Therefore, the transition between dimorphic and trimorphic areas was contiguous, rather than disjunct.

Morphological Variation

In addition to morph ratios, floral morphology varied extensively throughout the range of *N. triandrus* (Fig. 4). The most prominent feature of this variation involved a general increase in flower size from southeastern to northwestern portions of the range, which was accompanied by correlated changes in the positions of sex organs. Multiple regression detected significant influences of both east and north coordinates on flower length (Fig. 4A, $F_{3,13} = 15.11$, $P < 0.001$, $R^2 = 0.777$), the position of mid- and upper-level organs (e.g., position of the mid-level anthers of the S morph, Fig. 4B, $F_{2,25} = 56.23$, $P < 0.001$, $R^2 = 0.818$), and the position of short-level organs (e.g., stigma position of the S morph, Fig. 4C, $F_{2,25} = 6.60$, $P < 0.01$, $R^2 = 0.346$). The general conclusion of continuous geographic variation was not affected by whether the general linear model also included variety.

The floral tube and the sex organs within it varied less among populations with overall flower length than did more distal organs (Fig. 5). Mean floral-tube length had a regression coefficient less than one, indicating that it changed proportionally less than overall mean flower size (Table 2). In addition, the mean positions of the stigma of the S morph and the anthers produced within the floral tube (short-level anthers of the L and M morphs) did not vary significantly with mean flower size (Table 2). In contrast to its lack of relation to overall flower size, the mean position of the stigma of the S morph varied in direct proportion with mean floral-tube length (Table 2). Because of these relations, short-level anthers and stigmas remained at corresponding heights in all populations (Fig. 5, open symbols).

The corona and all mid- and long-level sex organs increased proportionally more among populations than overall flower size (Table 2: regression coefficients > 1). This positive allometry among populations had several consequences. Increased exertion of long-level anthers and stigmas accompanied increased flower size among populations, so that in the largest-flowered populations the stigma projected about 1 cm from the corona mouth (Fig. 5, gray symbols). The extent to which long-level stigmas projected beyond long-level anthers remained relatively constant, regardless of flower size (Table 2, note the similar regression coefficients for stigma position of the L morph and the positions of the long-level anthers of all morphs). Interestingly, the long-level anthers of the L morph occupied a significantly lower position than the L stigma, (mean difference in positions = 4.4 mm among populations) compared to the long-level anthers of the M and S morphs (3.6 and 3.2 mm, respectively), which did not differ from each other (repeated-measures ANOVA, $F_{2,41} = 7.06$, $P < 0.005$). Mid-level organs exhibited the greatest variation among populations in their average positions within flowers (Fig. 5, black symbols). In var. *cernuus*, these organs occupied the transition between the floral tube

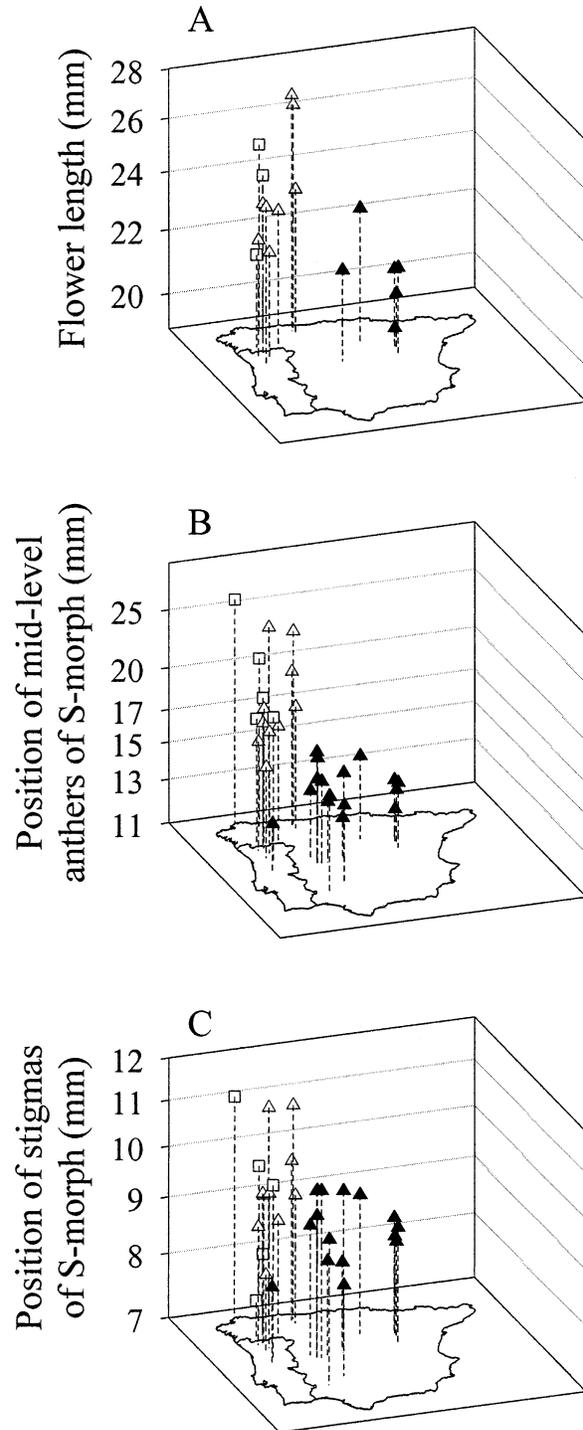


FIG. 4. Geographic variation in (A) mean flower length, (B) the position of mid-level anthers in short-styled flowers, and (C) stigmas in short-styled flowers of *Narcissus triandrus* among 31 population from Spain and Portugal. Closed symbols depict var. *cernuus*, and open triangles and squares indicate trimorphic and dimorphic populations of var. *triandrus*, respectively.

and the corona. In contrast, in var. *triandrus*, the positive allometry resulted in mid-level organs being positioned well within the corona and, in trimorphic populations with the largest flowers, the stigma of the M morph occupied the

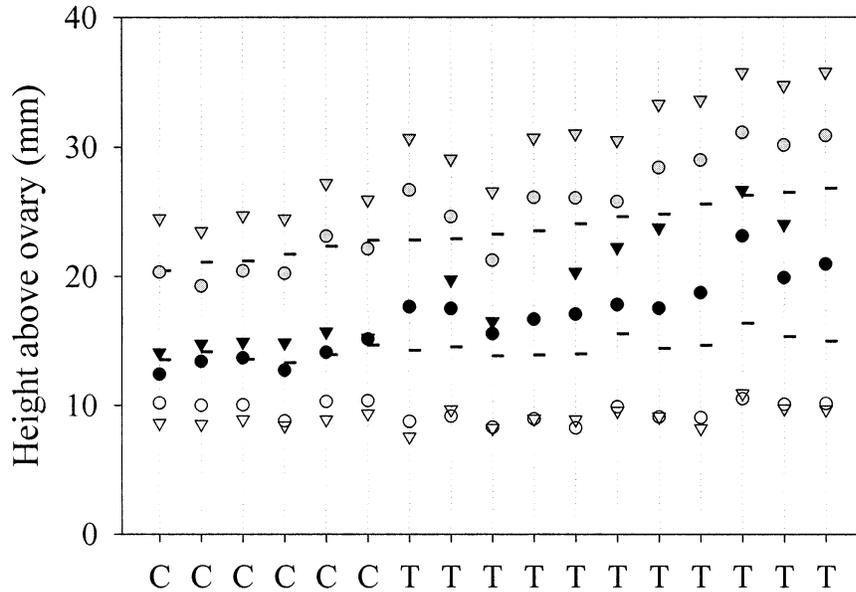


FIG. 5. Variation in mean flower size and the positions of sexual organs from 17 populations over the range of *Narcissus triandrus*. Populations have been ordered with respect to increasing flower length (tube length + corona length). The lower and upper dashes indicate the tops of the floral tube and corona, respectively. Triangular and circular symbols depict stigmas and anthers, respectively, for long-level organs (gray symbols, anthers represented by upper anthers of the L morph), mid-level organs (black symbols), and short-level organs (open symbols, anthers represented by lower anthers of the L morph). Four dimorphic populations lacked the M morph. C and T along the x-axis indicate whether a population belonged to var. *cernuus* (C) or var. *triandrus* (T).

corona mouth. This strong shift in position is reflected in the very large allometric coefficient for the stigma of the M morph (Table 2). Because of this change, the stigma of the M morph became increasingly separated from the mean position of mid-level anthers of the S morph.

Based on all 31 populations for which we measured the positions of sexual organs, we detected three distinct patterns of correlation. First, the mean positions of long- and mid-level organs (stigmas of the L and M morphs, upper anthers in all three morphs, and lowers anthers in the S morph) correlated almost perfectly with each other ($r \geq 0.93, P < 0.001$

in all 15 cases). This association represents the overwhelming influence of differences in flower size among populations, as all of the organs also varied strongly with flower length ($r \geq 0.86, P < 0.05$ in all six cases). The second pattern involves a strong correlation between mean positions of short-level anthers in the L and M morphs ($r = 0.822, P < 0.001$), but no correlations of these anthers with other organs ($r \leq 0.617, P > 0.1$ in all 15 cases). The final pattern involved the mean stigma position in the S morph, which exhibited moderate, but mostly nonsignificant, correlations with flower size and the positions of all organs in all morphs ($0.49 < r < 0.77$

TABLE 2. Floral allometry among 13 trimorphic and four dimorphic populations of *Narcissus triandrus*. Each regression coefficient ($b \pm SE$) depicts the relation between the listed trait and size variable, based on means of ln-transformed data. Tests of $b = 0$ identify whether the two traits varied significantly, whereas tests of $b = 1$ assess whether significant covariance involved isometry ($b = 1$), negative allometry ($b < 1$), or positive allometry ($b > 1$). All regressions considered the size variable as a random variable. Partial regression coefficients did not differ significantly between var. *cernuus* and var. *triandrus* ($P > 0.1$ in all cases).

Morph and trait	Size variable	$b \pm SE$	$H_0: b = 0$	$H_0: b = 1$
Corona length ¹	flower length	1.79 ± 0.170	$F_{1,15} = 110.76^{***}$	$F_{1,15} = 21.70^{**}$
Tube length ²	flower length	0.50 ± 0.106	$F_{1,15} = 22.20^{**}$	$F_{1,15} = 22.04^{**}$
L stigma	flower length	1.62 ± 0.146	$F_{1,14} = 122.81^{***}$	$F_{1,14} = 17.87^{**}$
L lower anthers	flower length	-0.01 ± 0.251	$F_{1,15} = 0.00$	
L upper anthers	flower length	1.80 ± 0.186	$F_{1,15} = 93.83^{***}$	$F_{1,15} = 18.56^{**}$
M stigma	flower length	2.53 ± 0.281	$F_{1,11} = 81.43^{***}$	$F_{1,11} = 29.83^{**}$
M lower anthers	flower length	0.02 ± 0.330	$F_{1,11} = 0.00$	
M upper anthers	flower length	1.69 ± 0.194	$F_{1,11} = 75.62^{***}$	$F_{1,11} = 12.52^{**}$
S stigma	flower length	0.51 ± 0.226	$F_{1,15} = 5.19$	
S lower anthers	flower length	1.98 ± 0.199	$F_{1,15} = 99.51^{***}$	$F_{1,15} = 24.44^{***}$
S upper anthers	flower length	1.78 ± 0.175	$F_{1,15} = 103.87^{***}$	$F_{1,15} = 20.08^{**}$
L lower anthers	tube length	0.64 ± 0.347	$F_{1,15} = 3.37$	
M lower anthers	tube length	0.86 ± 0.377	$F_{1,11} = 5.22$	
S stigma	tube length	1.10 ± 0.289	$F_{1,15} = 14.40^{***}$	$F_{1,15} = 0.11$

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, after sequential Bonferroni adjustment.

¹ Partial regression coefficient did not differ significantly between morphs ($F_{2,23} = 1.68, P > 0.2$).

² Partial regression coefficient did not differ significantly between morphs ($F_{2,23} = 0.72, P > 0.4$).

in all nine cases). Overall, the associations between sex-organ positions indicate strong correlations between stigmas and anthers at the same level and between competing anthers at the same level on different morphs.

Association of Morph Ratios with Floral Morphology

Morph ratios varied strongly with mean floral morphology among the 31 populations for which we measured sex-organ position (Fig. 6). The ability of the overall regression model to predict morph frequencies varied among the morphs, being strongest for the L morph (Fig. 6A) and weakest for the S morph (Fig. 6C). After backward elimination, only two morphological variables contributed significantly to the joint statistical prediction of morph ratios: the mean positions of the mid-level anthers (Wilks' lambda, $F_{1,27} = 23.89$, $P < 0.001$) and stigma of the S morph (Wilks' lambda, $F_{1,27} = 7.12$, $P < 0.005$). These relations did not differ between the var. *cernuus* and var. *triandrus* (Wilks' lambda, $F_{1,26} = 0.66$, $P > 0.5$). The apparent dependence of morph frequencies on only two features of the S morph must be interpreted carefully. In particular, recall that the mean position of the mid-level anthers of the S morph correlates strongly with the positions of the stigmas of the L and M morphs and the positions of the long-level anthers of all morphs. Given this multicollinearity, the mean position of the mid-level anthers of the S morph may act as a surrogate for variation in all of these organs in the regression analysis.

Morphology had contrasting effects on the frequencies of different morphs. The log-odds for the L morph relative to the S morph varied positively with anther position (partial regression coefficient, $b \pm SE = 1.534 \pm 0.455$, $t_{28} = 3.37$, $P < 0.005$), but not with stigma position ($t_{28} = 1.61$, $P > 0.1$, $R^2 = 0.300$). In contrast, the log-odds for the M morph relative to the S morph varied negatively with anther position ($b \pm SE = -9.244 \pm 1.667$, $t_{28} = 5.55$, $P < 0.001$), and positively with stigma position ($b \pm SE = 10.778 \pm 3.388$, $t_{28} = 3.18$, $P < 0.005$, $R^2 = 0.527$).

The regression surface for the frequency of the M morph identifies that trimorphic and dimorphic populations differ in the position of mid-level anthers relative to that of the stigma of the S morph (Fig. 7). Trimorphic populations have low mid-level anthers, whereas in populations lacking the M morph, mid-level anthers are much higher than the position of the short-level stigmas. These contrasting anther positions should alter the opportunity for pollen transfer to stigmas of the L morph.

DISCUSSION

Narcissus triandrus exhibits unusual floral morphology (Fig. 1C) and morph frequencies (Fig. 3B) for a tristylous species. These unique features vary extensively and in a correlated manner over the species' range (Figs. 3A, 4, 7). Flower size increases from southeast to northwest within the Iberian Peninsula (Fig. 4A), whereas the frequency of the M morph declines along this gradient, with a concomitant increase in the L morph (Fig. 3B). In addition to general variation in flower size, we found evidence for modification of sex-organ positions, particularly those occupying the mid-level within flowers (stigma of the M morph and lower an-

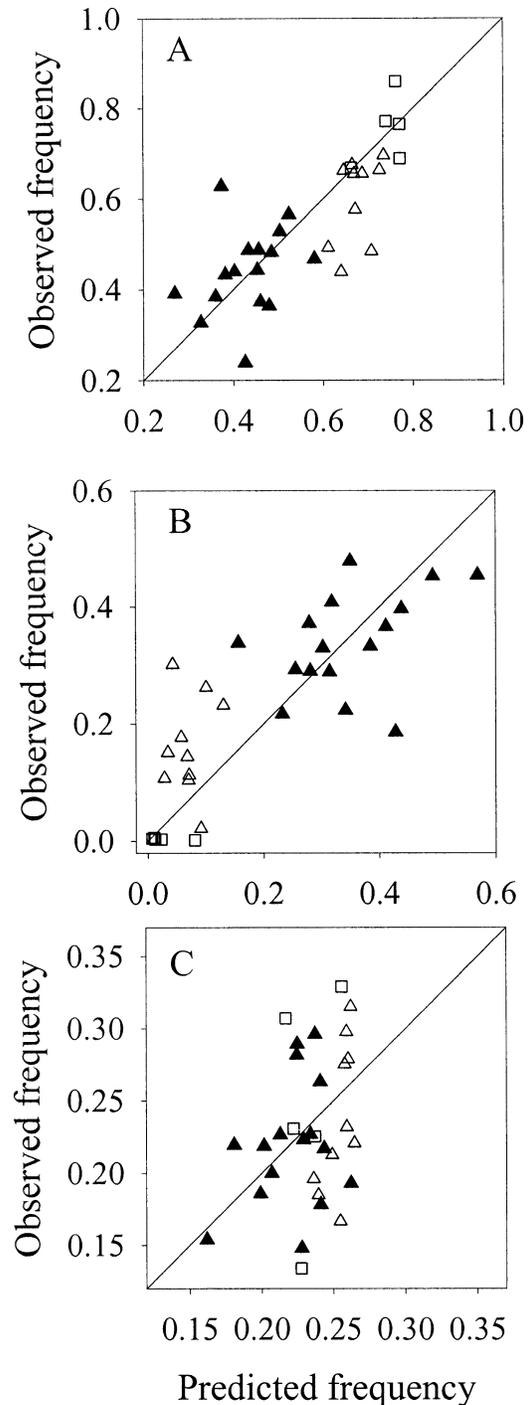


FIG. 6. Associations between observed and predicted frequencies for the (A) L morph, (B) M morph, and (C) S morph in 31 populations of *Narcissus triandrus*. Predicted frequencies were derived from a multivariate, multiple regression that considered the mean positions of the stigma and lower anthers of the S morph as independent variables. The diagonal lines indicate equality of observed and predicted frequencies. Closed symbols depict var. *cernuus*, and open triangles and squares indicate trimorphic and dimorphic populations of var. *triandrus*, respectively.

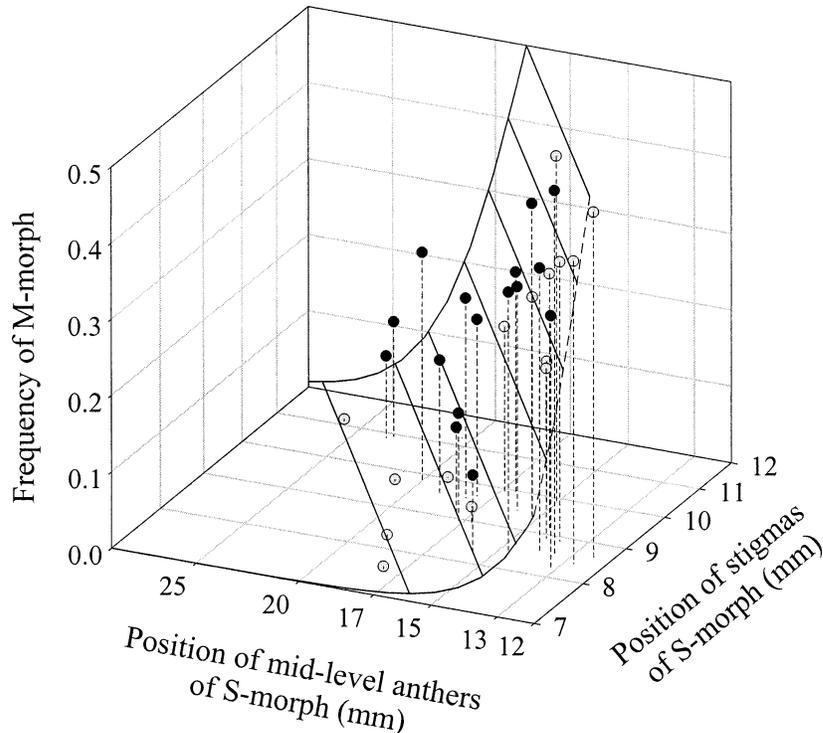


FIG. 7. Relation of the frequency of the M morph to the mean positions of the stigma and mid-level anthers in the S morph for 31 populations of *Narcissus triandrus*. Gray and black symbols indicate observed frequencies of the M morph that are respectively smaller or larger than the frequencies predicted by multivariate multiple regression (eq. 3), represented by the prediction surface.

thers of the S morph: Fig. 5, Table 2). These geographic associations allowed us to predict floral-morph frequencies statistically, based on the mean floral morphology of populations for the first time in a heterostylous species (Figs. 6, 7). Such a clear geographic pattern is unlikely to result from stochastic processes, but instead suggests changes in the operation of frequency-dependent selection on morph ratios in association with changes in flower size. Below we discuss the functional consequences of this correlated variation and the ecological and evolutionary mechanisms that may be responsible for both the anomalous floral morphology and patterns of variation in morph frequencies of *N. triandrus*.

Unusual Floral Morphology and Morph Ratios in Narcissus triandrus

Two features distinguish *N. triandrus* from other known tristylous species: imperfect reciprocity of the positions of sex organs and an incompatibility system that permits intramorph matings. Deviations from perfect reciprocity occur in other tristylous species, but they are usually associated with the breakdown of tristily and increased selfing rates (reviewed in Weller 1992). In contrast, in *N. triandrus* imperfect reciprocity occurs throughout the range and is associated with a mating system based on predominant outcrossing (Barrett et al. 1997). Therefore, imperfect reciprocity is clearly an evolutionarily stable feature of *N. triandrus*. This unusual arrangement of sex organs in the morphs will largely control the character of pollen dispersal and mating, given the absence of heteromorphic incompatibility.

Our pollen-transfer model illustrates how the positions of

sex organs affect morph ratios in tristylous populations without heteromorphic incompatibility. Given pollen-transfer probabilities that reflect complete reciprocity, negative frequency-dependent selection results in an isoplethic equilibrium (Fig. 2, $Q = 0$). In contrast, elevation of the upper anthers of the L morph from the mid-level position leads to an equilibrium in which the L morph predominates (Fig. 2, $Q > 0$), if all morphs have equal total pollen-transfer proficiency. In all cases, the opportunity for siring seeds increases with the frequency of receptive stigmas in the population, whereas the competition for siring opportunities increases with the proficiency of pollen transfer between anthers and stigmas at the same and different levels. Given a polymorphic equilibrium maintained by frequency-dependent selection, individuals of all morphs realize equal mating success. Therefore, at the equilibrium morph ratio the intensity of competition must be balanced by the availability of mating opportunities. In *N. triandrus*, all three morphs have long-level anthers, so that competition should be most intense for access to ovules in pistils of the L morph. This competition is balanced by mating opportunities if the L morph is relatively common. In contrast, only the S morph has mid-level anthers, so that it experiences relatively little competition for access to ovules of the M morph. To balance this competitive advantage of the S morph, the M morph will be the least common in the equilibrium state. The specific equilibrium frequency will depend on how the particular floral morphology in a population affects pollen export between anthers and stigmas at the same and different positions.

Why has the reciprocal morphology typical of most tris-

tylous species not evolved in *N. triandrus*? With heteromorphic incompatibility, a nonreciprocal morphology is clearly costly, because pollen transferred to incompatible stigmas cannot fertilize ovules. This pollen wastage should select for more proficient pollen transfer between sex organs at the same level, resulting in more precise reciprocity of organ positions. In contrast, in *N. triandrus* all cross-pollinations are compatible (Barrett et al. 1997), so that incomplete reciprocity probably does not result in pollen wastage. For any combination of morphologies that allows coexistence of morphs, frequency-dependent selection produces an equilibrium morph ratio at which each morph realizes equal reproductive success. Therefore, because of intramorph compatibility, *N. triandrus* likely experiences weak selection for complete reciprocity.

Geographical Variation in Floral Morphology and Function

The continuous geographic variation in floral morphology (Fig. 4) and morph ratios (Fig. 3) among *N. triandrus* populations probably reflect contrasting ecological and evolutionary influences throughout the species' range. The dominant feature of the variation in floral traits is the increase in the average size of flowers along a southeast–northwest gradient (Figs. 4, 5). This geographic pattern is associated with a parallel increase in plant size (K. Hodgins and S. C. H. Barrett, unpubl. data). The size of *N. triandrus* plants varies along a climatic gradient from small, narrow-leaved individuals in southeastern Mediterranean environments with hot, dry summers to larger plants with broader leaves in northwestern, more temperate, Atlantic environments. Therefore, the geographic variation in flower size probably represents responses to local growing conditions, with plants in productive environments investing more resources in reproduction.

The variation in flower size in *N. triandrus* is accompanied by differing allometric relations among sex-organ positions, suggesting contrasting patterns of selection (Table 2, Fig. 5). The mean positions of the stigma of the S morph and the short-level anthers of the L and M morphs are strikingly insensitive to variation in flower size between populations. The positions of long-level organs vary more between populations, as the projection of stigmas of the L morph from the flower is exaggerated in large-flowered populations (Figs. 1A,B; Fig. 5, gray triangles). However, the positions of the upper anthers of all morphs and stigmas of the L morph maintain equivalent separation in all populations (Fig. 5, gray circles), as a result of similar allometric coefficients (Table 2). The relative constancy of position of short-level organs and the parallel variation in positions of long-level anthers and the stigma of the L morph should maintain within-level pollen transfer for these positions, despite considerable geographic variation in flower size.

In contrast to the apparent targeting between long-level organs and between short-level organs, changes in flower size cause extensive unequal shifts in the relative positions of mid-level organs (Fig. 5, black symbols), which probably alter patterns of pollen dispersal. In small-flowered populations, particularly in var. *cernuus*, the mid-level anthers of the S morph and the M stigma occupy similar positions at

the mouth of the floral tube, where they interact with bees' faces. As flower size increases between populations, both organs move into the corona; however, the position of the M stigma (Fig. 5, black triangles) changes more than that of the mid-level anthers of the S morph (Fig. 5, black circles). Consequently, in large-flowered populations the M stigma occupies the corona mouth, whereas mid-level anthers of the S morph lie in the middle of the corona. In these positions, mid-level organs likely interact with bee's thoraces, or even abdomens. Thus, over the range of *N. triandrus* the mid-level organs probably change from functioning similarly to short-level organs to functioning like long-level organs.

Correlated Evolution of Floral Morphology and Morph Ratios

We have proposed a causal link between floral morphology, patterns of pollen transfer and morph ratios in *N. triandrus*. Two lines of evidence support this hypothesis. First, our phenotypic model demonstrates the dependence of the equilibrium morph ratio on the proficiencies of pollen transfer within and among morphs. In particular, we have shown that a matrix of pollen-transfer proficiencies that reflects the unique position of the upper anthers of the L morph in *N. triandrus* (Table 1A) results in L-biased equilibrium morph ratios similar to those observed throughout this species' range (Fig. 2A). Second, the parallel geographic patterns in floral morphology (Fig. 4) and morph ratios (Fig. 3) and our ability to predict morph ratios statistically among *N. triandrus* populations (Figs. 6, 7) provide empirical evidence that geographic variation in sex-organ positions has modified frequency-dependent selection on morph ratios within populations.

Most of the observed variation in morph ratios results from a negative relation between the frequencies of the L and M morphs (Fig. 3B), and morphology predicts this relation much better than variation in the frequency of the S morph (cf. Figs. 6A and 6B with 6C). Notably, the M morph is rare or missing from populations of var. *triandrus* in which the mid-level organs are well exerted from the mouth of the floral tube. The elevated position of the lower anthers of the S morph in these populations (Fig. 7) should allow them to disperse relatively more pollen to L stigmas (increased q_{SL}) and relatively less to M stigmas (decreased q_{SM}), than do the equivalent anthers in var. *cernuus* populations. If negative frequency-dependent selection balances this change in competition for L and M stigmas with shifts in mating opportunities, then the frequency of the L morph must increase and that of the M morph must decrease. This scenario is depicted theoretically by the matrix of pollen-transfer proficiencies in Table 1B in which R represents the proportion of S-morph pollen exported to the L morph that originates from the lower anthers. Simulations based on equation (1) illustrate that as the S morph increases its siring success on the L morph the frequency of the M morph declines and the L morph increases (Fig. 2B). Interestingly, these changes do not alter the frequency of the S morph. These simulations do not replicate all aspects of the variation in the frequency of the M morph (including the absence of this morph at western and northern margins of the range), because they ignore other

aspects of observed morphological variation, such as the elevated position of the M stigma in large-flowered populations. Nevertheless, this example captures several features of the association between floral morphology and morph ratios, notably the negative correlation between the frequencies of the L and M morphs and the relatively limited variation in the frequency of the S morph (Fig. 3B).

Our ability to predict variation in morph ratios from floral morphology implies extensive geographical variation in patterns of outcrossed mating within populations. The observed variation in floral morphology probably results from regional differences in climatic conditions and the composition of the pollinator fauna. Our theoretical analysis illustrates that small changes in sex-organ position in response to such external ecological influences can profoundly influence mating patterns. *Narcissus triandrus* is valuable for exposing this dependence, because the unique variation in morph ratios provides a visible signature of the aggregate mating patterns within populations during preceding generations. Similar geographic variation in mating patterns within populations seems likely for most wide-ranging species, but is less evident because of the sexual uniformity of most flowering plants.

ACKNOWLEDGMENTS

We thank J. Arroyo and the late A. Fernandes for assistance in locating populations of *N. triandrus*, R. Kruus for help with mapping and Kriging interpolation, S. Richards for conceptual discussion, M. Geber for comments on the manuscript, and research grants from the Natural Sciences and Engineering Research Council of Canada to SCHB and LDH that funded this work.

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