FLORAL DISPLAY IN *NARCISSUS*: VARIATION IN FLOWER SIZE AND NUMBER AT THE SPECIES, POPULATION, AND INDIVIDUAL LEVELS

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Floral display (the size, number, and arrangement of open flowers) influences pollinator visitation to animal-pollinated plants and should be an important determinant of reproductive success. We examined variation in the size and number of open flowers in wild daffodils (*Narcissus*). Our analysis of published data on 45 taxa showed that flower number varied negatively with flower diameter among *Narcissus* species, which supports the widespread assumption that there is a trade-off between these traits. In contrast, field measurements indicated a positive relation between flower number and diameter within two populations of *Narcissus dubius*, and no relation was evident after we controlled for variation in bulb size. The discrepancy between inter- and intraspecific patterns may have occurred because variable resource levels obscure trade-offs when variation in flower size is low (e.g., within species). Size-related increases in floral tube length were half as great as corresponding increases in flower diameter, a result that is consistent with stronger stabilizing selection on tube length. Staggered flowering within *N. dubius* inflorescences limited the mean number of open flowers to <66% of total flower number, and slow expansion by later opening flowers resulted in significant differences in flower size throughout flowering. Although pollinators preferred large flowers, experimental reductions in flower diameter did not affect seed production. Our results illustrate how the relative importance of the factors influencing floral display can vary among levels of biological organization. Interspecific variation in flower size and number appeared to be constrained by allocation trade-offs, but intraspecific variation in both traits was more greatly influenced by plant resource status. Within plants, the size and number of open flowers reflected the relative age of individual flowers and floral longevity.

Keywords: Amaryllidaceae, ecology, flowering phenology, plant size, reproductive biology, resource allocation, size-number trade-offs.

Introduction

Floral displays are composed of the size, number, and arrangement of open flowers on a plant. In animal-pollinated species, floral displays advertise rewards to potential pollinators, and therefore, floral displays have an important influence on mating patterns and reproductive success. Recognition of the fact that resources are finite has led theoreticians considering the evolution of floral display to assume that increases in flower size occur at the expense of flower number (Cohen and Dukas 1990; Morgan 1993; Harder and Barrett 1996). In their models, the benefits of increased flower number for pollinator attraction are countered by both decreased flower size and increased pollen transfer between flowers on the same plant. The latter has been empirically demonstrated to increase self-pollination via geitonogamy and to reduce pollen export through pollen discounting (de Jong et al. 1993; Harder and Barrett 1996; Snow et al. 1996).

Empirical support for flower size-number trade-offs lags behind theoretical models. Flower number varies negatively with flower size in *Raphanus sativus* (Stanton et al. 1991), *Begonia*

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involucrata (Schemske and Ågren 1995), and *Eichhornia paniculata* (A. C. Worley and S. C. H. Barrett, unpublished manuscript), but several other studies have not revealed trade-offs (Stanton and Preston 1988; Ågren and Schemske 1995; Schemske et al. 1996; Morgan 1998). Negative relations between flower size and number are sometimes evident between sexes of diclinous species (review by Delph 1996) or after artificial selection has created divergence in flower size and number (Meagher 1994; A. C. Worley and S. C. H. Barrett, unpublished manuscript). These examples indicate that, unless variation in flower size or number is high, trade-offs may often be obscured by variation in overall resource allocation to flowering (van Noordwijk and de Jong 1986; de Laguerie et al. 1991). Detecting trade-offs may, therefore, require that variation in resource levels be accounted for or that taxa differing widely in floral traits be compared. We know of no published studies that compare flower size and number among related species, nor do we know of any published studies that attempt to account for intraspecific variation in resource levels.

The evolution of flower size and number is usually approached in the context of size-number trade-offs because flowers should be analogous to other repeated parts or products (Lloyd 1987). In Smith and Fretwell's (1974) classic model, offspring number (n) depends on offspring size (s) according to the relation $n = E/s$, where E is the energy (re-

sources) available for reproduction. Size-number trade-offs are well documented as a general phenomenon, and they occur among eggs and live offspring per litter (review by Roff 1992), seeds per fruit (reviews by Roff 1992; Méndez 1997), and pollen grains per flower (Vonhof and Harder 1995).

The generality of size-number trade-offs has stimulated modifications of the original models, and two of these yield testable predictions regarding variation in offspring size within species. First, the above models assume that parental fitness is a linear function of offspring number, and they predict optimal offspring size to be independent of the resources available for reproduction (Lloyd 1987). However, optimal size can increase in response to enhanced resources when negative density-dependent interactions occur among "siblings" (Venable 1992). Geitonogamy and pollen discounting are two negative interactions that increase with flower number. They may, therefore, indirectly cause optimal flower size to vary positively with flower number in animal-pollinated plants. Second, when offspring number is small, dividing continuous floral resources among few offspring may increase variation in offspring size. The inverse relation between variance and offspring number should be strongest when fewer than six offspring are produced (Ebert 1994; Charnov et al. 1995). Accordingly, variation in flower size should decline as flower number increases, resulting in convergence toward the optimal flower size. Comparing mean flower size, and variation around each mean, among individuals differing in flower number should indicate whether convergence to an optimal size occurs and whether optimal size increases or remains constant with increased resource levels.

Both flower size and number are more complex traits than we have so far implied. First, although we have discussed flower size (which presumably reflects investment per flower) as a single trait, flowers are composites of multiple traits. Changes in the size of different floral organs may not always have equivalent effects on fitness. For example, flower parts that influence pollinator positioning and pollen removal/deposition (e.g., floral tube length) may experience strong stabilizing selection (Conner and Via 1993; Mazer and Hultgård 1993), regardless of selection on optimal investment per flower. Therefore, optimal tube length may remain constant even when geitonogamy and pollen discounting alter the optimal investment per flower.

Second, both flower size and number may vary spatially and temporally within plants (Wyatt 1982; Ellstrand et al. 1984). Many plants display their flowers sequentially, introducing the distinction between daily (the number of open flowers) and total flower number per plant or inflorescence. Size-number trade-offs seem more likely to apply to total than to daily flower number because all flowers on the same inflorescence develop over a relatively short time and all should compete for resources. However, daily number is the functional display because only open flowers affect pollinator attraction, geitonogamy, and pollen discounting (Harder and Barrett 1995, 1996). In several species, flower size also varies with position or relative age of flowers, with later and more distal flowers often being smaller (Diggle 1992). Proximate factors that may reduce flower size include resource depletion by earlier, more proximal flowers (Stephenson 1981) and developmental changes in meristem size (Wolfe 1992). Temporal and spatial

variation in the number of open flowers, flower age, and flower size may also reflect differences in reproductive contribution. For example, early and late flowers sometimes differ in functional gender (Brunet 1996; Diggle 1997b). In addition, older flowers sometimes continue to attract pollinators after their own reproductive life span has passed (review by van Doorn 1997; Larson and Barrett 1999). Finally, flower size may influence pollinator attraction most when daily flower number is low.

In this study we examined floral display in the wild daffodil genus *Narcissus* (Amaryllidaceae), with particular emphasis on *Narcissus dubius*. This genus contains ca. 50 species and varieties (Blanchard 1990) of insect-pollinated bulbous geophytes that exhibit wide variation in flower size and number. Total flower production is easy to assess in relation to resource status because most bulbs produce a single inflorescence during the flowering season. We began by exploring the relation between flower size and number through an interspecific survey of 45 taxa. We first assessed whether accounting for plant size, as indicated by bulb size, helped to reveal a trade-off between flower size and number. Second, we considered whether relations between flower size and number remained consistent when taxonomic sections were included in the analysis. A situation in which flower size and number varied only among sections would imply relatively few evolutionary changes in these traits and would also imply that taxa within sections were not independent data points (Harvey and Pagel 1991; but see Westoby et al. 1995).

After describing large-scale patterns at the species level, we focused on intraspecific studies of *N. dubius*, keeping the following questions in mind: (1) What is the relation between flower size and number? Are trade-offs between flower size and number revealed by accounting for variation in resource status? (2) What are the patterns of variation in corolla size and floral tube length? Theoretical models predict that variation in both traits will decline as flower number increases, and selection for efficient pollination may further reduce variation in tube length. *Narcissus dubius* is particularly well suited to test predictions regarding variation in flower size when flower number is low because individuals most often produce between two and six flowers per inflorescence. (3) How do flowering phenology and floral longevity affect functional display size? Flowers of *N. dubius* open sequentially, thereby allowing us to document patterns of flowering phenology and their consequences for flower size and daily number (=functional display size). We also considered whether flowers opening at different times differed significantly in size and whether size differences affected maternal reproductive success.

Material and Methods

Interspecific Variation in Flower Size and Number in Narcissus

We compiled published data on flower diameter, flower number, and bulb size for 45 taxa (species and subspecies) distributed among six sections of the genus (Apodanthae, Bulbocodium, Ganymedes, Jonquillae, Pseudonarcissus, and Tazettae); data were taken from Tutin et al. (1980) and Blanchard

(1990). Measurements of bulb size were not provided by Blanchard (1990), but he did categorize bulbs as small, medium, or large. Our own measurements of fresh bulbs in several species (A. C. Worley, unpublished data) and those provided by Tutin et al. (1980) indicated that these categories corresponded to diameters of 10–25, 25–40, and >40 mm. Species were also classed by taxonomic section. Our original intention was to conduct two analyses, one of which would treat species as independent data points and the second of which would explicitly include information on phylogenetic relationships (e.g., independent contrasts, Felsenstein 1985; Purvis and Rambaut 1995). A recent phylogeny of the genus based on *ndh*F (Graham 1997) supported the monophyly of most sections but provided low resolution at the species level. Calculating independent contrasts with a poorly resolved phylogeny would result in the loss of most of the information in the data set, so we confined our consideration of phylogenetic relatedness to taxonomic sections.

We analyzed flower number in response to flower diameter and bulb size (fixed effect) using ANCOVA (PROC GLM; SAS Institute 1997). We also wanted to include taxonomic section in the analysis, but not all bulb size classes were represented in every section. Therefore, we conducted a second analysis of flower number with taxonomic section as a fixed effect and with flower diameter as a covariate. We did not include the three single-species sections (Aurelia, Serotini, and Tapeinanthus) in either analysis. In both analyses, the interaction between flower diameter and the fixed effect was initially included but was later removed because it was not significant at $P = 0.05$ in either analysis. Data were log transformed before analysis in order to stabilize variances. We report back-transformed means in the results, and we report their asymmetrical lower and upper standard errors as LSE and USE. The least-squares means from each analysis account for variation in the covariate (flower size) and are referred to as adjusted means.

Intraspecific Study of Narcissus dubius

Narcissus dubius and study sites. *Narcissus dubius* (section Tazettae) is a perennial geophyte that is native to southwest France and to southeast Spain. Plants overwinter as a subterranean bulb. Mature bulbs produce several leaves and a single inflorescence that bears two to six flowers with prominent coronas and long floral tubes. The flowers mature sequentially from the top to the bottom of the inflorescence. We refer to the top flower as the first flower, and we sequentially number the flowers in subsequent positions. Flower color ranges from greenish or cream colored to white, depending on age. *Narcissus dubius* plants are self-compatible, and recent studies indicate the presence of a stigma-height dimorphism (Baker et al. 2000a, 2000b). As in heterostyly, stigmas are positioned either above or below the anthers, but unlike the heterostylous condition, the two anther levels are similar in long (L)- and short (S)-styled morphs. Mean stigma-anther separation is 2.66 mm in the S morph but only 0.08 mm in the L morph (Baker et al. 2000a). Despite the increased proximity of stigma and anthers in the L morph, selfing rates are similar for both morphs (mean $s = 0.42$, $n = 3$ dimorphic populations and two monomorphic populations, Baker et al. 2000b). This result indicates that selfing may reflect geitono-

gamy rather than within-flower pollen transfer. Plants flower from mid-February to late March, with peak flowering occurring in mid-March. As in other *Narcissus* species, the inflorescence and flower buds differentiate in the fall preceding flowering. *Narcissus dubius* inflorescences are visited by sphingid moths (*Macroglossum stellatarum*), various hymenoptera (mostly *Anthophora* spp. and *Apis mellifera*), and flies.

We examined three populations (St. Bauzille, La Clause, and Hortus Mountain) that were located within 8 km of one another and ca. 20 km north of Montpellier, in the Languedoc region of southern France. Data were collected in 1996 and 1998 from St. Bauzille, in 1998 from La Clause, and in 1996 from Hortus Mountain. Data were collected in February and March of both years. Plants at St. Bauzille grow on the hillside above the village of St.-Bauzille-de-Montmel. La Clause is a roadside population (4 km north of St. Bauzille). Plants from Hortus Mountain grow on Montagne d'Hortus. All three sites are open, with well-drained, rocky, calcareous soil and southern exposure.

Variation in flower size, flower number, and floral tube length. We assessed the relation between flower number and flower diameter in *N. dubius* both before and after accounting for variation in plant size. We excavated 19 plants from Hortus Mountain and 28 plants from St. Bauzille in 1996; separated them into reproductive, aboveground vegetative, and belowground vegetative (bulbs) parts; and oven-dried them at 70°C for 1 wk, weighed them, and measured the diameter of their bulbs. Bulb diameter was the best vegetative predictor of both flower diameter and flower number per inflorescence. We used ANCOVA (PROC GLM; SAS Institute 1997) to assess responses of flower diameter and number to the effects of site (random effect), bulb diameter, and either flower number or diameter. We analyzed the first flower on each inflorescence in order to avoid confounding our analysis with position effects on flower diameter (see "Phenology of floral expansion and positional differences in flower size," the fourth subsection of "Intraspecific Study of *Narcissus dubius*").

We measured both flower diameter and tube length to assess whether variation in the size of floral organs declines with increased flower number per inflorescence and to test the expectation that tube length should vary less than flower diameter. Floral measurements were made on the first flower of plants with inflorescence sizes of two to six flowers. Approximately 30 plants in each flower-number category were measured at both the St. Bauzille and La Clause populations in 1998. We used ANOVA (PROC GLM; SAS Institute 1997) to assess the effects of site (random effect) and flower-number class (fixed effect) on the mean size of floral organs. We also used a paired sample *t*-test to compare coefficients of variation for flower diameter and tube length for each combination of site and flower number.

Functional display size and floral longevity. Flower counts were conducted at the St. Bauzille and La Clause populations to estimate the size of floral displays (flower number) through the 1998 flowering season. We marked 87 stems at St. Bauzille and 100 stems at La Clause before flowering and counted early-, mid-, and late-season display sizes as well as the total number of flowers per inflorescence. Observation dates were March 2, 10, and 21, 1998, and February 25, March 7, and

Table 1

Factors Affecting Floral Longevity and Flower Diameter in *Narcissus dubius*

Effect	Floral longevity	Flower diameter[a]	Flower diameter[b]
Between plant:			
Site[c]	$F_{1,83} = 0.01$	$F_{1,47} = 4.87^{**}$	$F_{1,22} = 3.24$
Flower number	$F_{3,83} = 0.58$	$F_{4,47} = 11.94^{**}$	...
Site × flower number	$F_{3,83} = 0.81$	$F_{4,47} = 2.73^{*}$	...
Within plant:			...
Position	$F_{2,82} = 0.26$	$F_{1,47} = 298.81^{***}$	$F_{2,21} = 42.77^{***}$
Position × site	$F_{2,82} = 2.97$	$F_{1,47} = 3.64$	$F_{2,21} = 0.88$
Position × flower number	$F_{6,164} = 1.36$	$F_{4,47} = 0.75$	...
Position × site × flower number	$F_{1,164} = 0.71$	$F_{4,47} = 2.02$	...
Age	...	$F_{1,47} = 23.33^{***}$	...
Age × site	...	$F_{1,47} = 0.66$	...
Age × flower number	...	$F_{4,47} = 1.28$	...
Age × site × flower number	...	$F_{4,47} = 0.24$	...
Position × age	...	$F_{1,47} = 46.37^{***}$	...
Position × age × site	...	$F_{1,47} = 0.07$	...
Position × age × flower number	...	$F_{4,47} = 0.84$	...
Position × age × site × flower number	...	$F_{4,47} = 1.07$	...

Note. Results are based on repeated-measures analyses because several flowers were measured on the same plant (flower position effect), and the same flowers were measured when inflorescences were 4, 7 (flower diameter[a] : age effect), and 10 d old (flower diameter[b]). Only multivariate tests for within-plant effects are reported because the data did not meet the assumptions of univariate analyses. See "Material and Methods" for further details. There were no qualitative differences between univariate and multivariate results.

[a] Analysis of perianth diameter in plants with flowers in positions 1 and 2 that were open both when inflorescences were 4 and 7 d old (see fig. 5).

[b] Analysis of perianth diameter on 10-d-old inflorescences with open flowers at all of positions 1, 2, and 3.

[c] Sites were St. Bauzille and La Clause in the analysis of floral longevity (1998 data) and St. Bauzille and Hortus Mountain in the analyses of flower diameter (1996 data).

[*] $P < 0.05$.

[**] $P < 0.01$.

[***] $P < 0.001$.

March 21, 1998, for St. Bauzille and La Clause populations, respectively.

We estimated floral longevity (in days) in relation to flower position (i.e., flower 1 is the first flower on an inflorescence to open) and total flower number. To do so, we recorded the longevity of all flowers on 37 plants at the St. Bauzille population and on 55 plants at the La Clause population, using plants that produced three or more flowers in 1998. Floral longevity was estimated as the number of days the corolla remained open and unwilted. Floral life span was analyzed with repeated measures ANOVA (SAS Institute 1997); site and total flower number were main effects, and position within each inflorescence was the repeated factor.

Phenology of floral expansion and positional differences in flower size. Casual observations indicated that flower diameter varied with position; the first flowers to open (position 1) were largest, and subsequent flowers that were lower down on the inflorescence were progressively smaller. Later flowers appeared to expand as they aged. We documented this pattern by following individual flowers on ca. 60 plants at St. Bauzille and Hortus Mountain in 1996. We measured the flower diameter of all open flowers when inflorescences were 1, 4, 7, 10, and 13 d old. By day 10, the first flower on roughly one-half of the inflorescences had wilted, and by day 13, only 15 plants still had open flowers at the first position.

Our main objective in analyzing these data was to determine whether and how rapidly second and third flowers attained the size of first flowers. We used repeated measures ANOVA (PROC GLM; SAS Institute 1997) to analyze flower diameter in response to site, flower number (both between-plant effects), position, and age (both repeated or within-plant effects). Most inflorescences had only one or two flowers for the first week of flowering, which resulted in missing values for the third position over much of flowering period. Therefore, our first analysis included 57 plants, for which we had measured the first two flowers on both days 4 and 7 (table 1, col. 3). This analysis allowed us to simultaneously assess the effects of position and age on flower diameter. Our second analysis included 24 plants, for which we had measured the first three flowers on day 10 (table 1, col. 4). This analysis tested whether position effects on flower diameter were evident throughout the life span of the first flower. The small sample size precluded inclusion of flower number in the second analysis. This omission did not affect our assessment of position effects because there was no evidence that position interacted with between-plant factors (see table 1).

We manipulated the corollas of first-position flowers to test two hypotheses regarding their contribution to floral display. First, the large size of first flowers may increase pollinator attraction early in flowering when first flowers are the entire floral display. Second, long-lived early flowers may enhance the attractiveness of later flowers by increasing display size.

On March 2 and 5, 1998, we marked 23 triplets of stems at La Clause. Plants within each triplet had the same total flower number and grew close together. At this time, the first flower on each inflorescence was newly opened with undehisced anthers. Plants within each triplet were randomly assigned to one of three treatments: control, small-sized (reduced flower diameter), and short-lived (reduced longevity of perianth) treatments. We clipped the corollas of first flowers at the beginning of anthesis in order to reduce flower diameter. In order to reduce the life span of attractive structures, we clipped the perianth (the corolla and corona but not the tube) from the first flower 4 or 5 d into anthesis. We collected mature infructescences in April and counted seeds and undeveloped ovules under a Zeiss dissecting microscope. We analyzed seed set of the first two flowers in response to treatment (fixed effect) and position (repeated factor) using repeated measures ANOVA (PROC GLM; SAS Institute 1997).

Results

Inter- and Intraspecific Relations between Flower Size and Number

Our survey of 45 taxa within the genus *Narcissus* revealed a negative relation between flower number and diameter among species ($F_{1,41} = 20.39$, $P < 0.001$; fig. 1). This relation did not differ among bulb sizes and was also present when bulb size was not taken into account. Bulb size also affected flower number significantly ($F_{2,41} = 10.82$, $P < 0.001$). For a given flower diameter, flower number in species with small bulbs (adjusted mean number = 1.5, LSE = 1.29, USE = 1.63) was lower than in species with medium (adjusted mean = 2.7, LSE = 2.28, USE = 3.22) or large bulbs (adjusted mean = 4.0, LSE = 3.24, USE = 4.84). In the analysis that included taxonomic section instead of bulb size, the negative effect of flower diameter on flower number was still present ($F_{1,38} = 7.11$, $P < 0.02$) and did not differ significantly among sections. Thus, the negative relation between flower diameter and number described above did not simply reflect sectional differences. For a given flower diameter, significant differences in flower number occurred among sections ($F_{5,38} = 5.71$, $P < 0.002$). Flower number was significantly greater in the section Tazettae (adjusted mean number = 3.9, LSE = 3.39, USE = 4.44) than in sections Apodanthae, Bulbocodium, Jonquillae, and Pseudonarcissus, which ranged in number from strictly solitary flowers in Bulbocodium to an average of 1.9 flowers in Jonquillae. Flower number in the section Ganymedes (adjusted mean = 3.1, LSE = 2.28, USE = 4.13) was significantly greater than that in Bulbocodium, but this number did not differ from that in any of the other sections.

The study of variation in flower size and number within *Narcissus dubius* yielded results that contrasted with the interspecific survey. Both flower diameter and number varied positively with bulb size (flower diameter: $F_{1,46} = 14.18$, $P < 0.005$; flower number $F_{1,46} = 14.72$, $P < 0.001$; fig. 2a, 2b). These relations were similar for plants growing in the St. Bauzille and Hortus Mountain populations, although plants from the Hortus Mountain population generally had larger bulbs (see fig. 2) and slightly larger flowers for a given bulb size (site effect: $F_{1,46} = 3.17$, $P < 0.05$). The effects of bulb size resulted
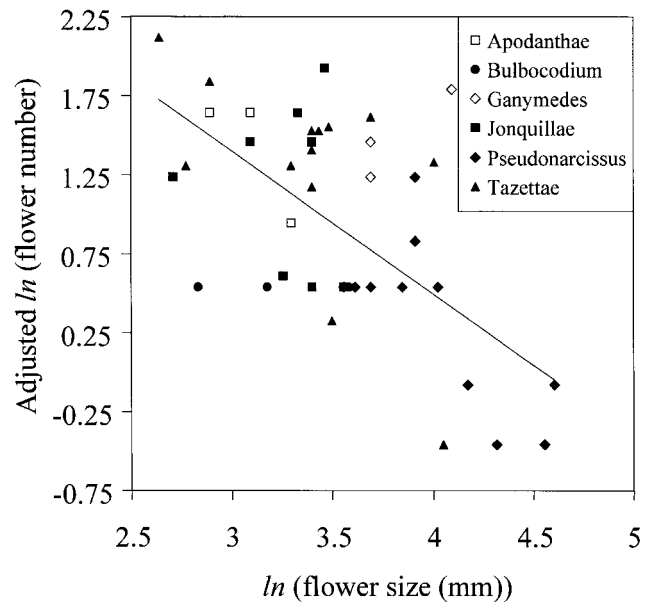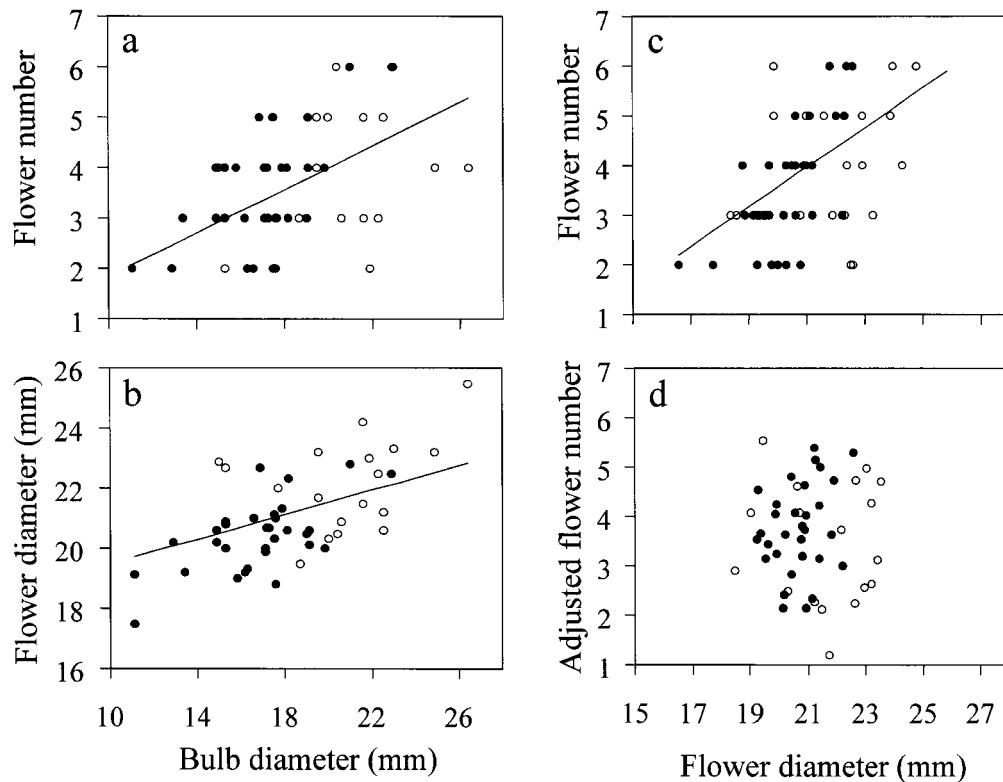

Fig. 1 Relation between flower number and flower diameter ($b \pm s_b = -0.90 \pm 0.199$) among 45 taxa of *Narcissus*. Flower number is adjusted for the effects of bulb size, and $R^2 = 0.45$ for the full model. Data are from Tutin et al. (1980) and Blanchard (1990). See "Material and Methods" for further details.

in a positive relation between flower diameter and number ($F_{1,47} = 6.39$, $P < 0.02$; fig. 2c), a relation that was no longer significant when variation in bulb diameter was included in the analysis (fig. 2d).

Variation in Flower Diameter and Tube Length within Narcissus dubius

Neither variation in flower diameter nor floral tube length decreased with increased flower number (fig. 3). Both mean flower diameter and mean tube length varied positively with flower number, but the relation was much stronger for flower diameter (flower diameter: $F_{4,294} = 19.5$, $P < 0.001$; tube length: $F_{4,294} = 4.32$, $P < 0.01$; fig. 3). Mean ($\pm$ SE) flower diameter increased from 20.0 ± 0.23 mm for two-flowered plants to 22.5 ± 0.29 mm for six-flowered plants, a 13% increase (fig. 3a). The corresponding change in tube length was only 6%, 14.5 ± 0.16 mm to 15.4 ± 0.20 mm (fig. 3b). Mean flower diameter did not differ between sites ($F_{1,294} = 0.14$, $P > 0.10$), but mean floral tube length was significantly greater at La Clause (mean $\pm$ SE; 15.1 ± 0.10 mm) than at St. Bauzille (14.7 ± 0.11 mm; $F_{1,294} = 8.31$, $P < 0.01$), even after accounting for differences in flower number. The effects of flower number on both traits did not differ between sites (site $\times$ flower number effect: $F_{4,294} \leq 1.10$, $P > 0.3$). Variation around each mean, as indicated by coefficients of variation (CVs), did not differ between tube length and flower diameter (mean difference in CV = 0.35 ± 0.250, $t_5 = 1.41$, $P > 0.19$). Thus, floral tube length varied less than flower diameter among inflorescences of different sizes but not within each flower number category.

Fig. 2 Relations between bulb diameter and flower number (*a*: $b \pm s_b = 0.22 \pm 0.056$) and flower diameter (*b*: $b \pm s_b = 0.20 \pm 0.064$) in *Narcissus dubius* plants at St. Bauzille (solid symbols) and Hortus Mountain (open symbols) in 1996. For analyses including site, $R^2 = 0.27$ and $R^2 = 0.43$, respectively. *c*, Relation between flower diameter and flower number ($b \pm s_b = 0.27 \pm 0.106$, $R^2 = 0.12$). *d*, No relation between flower diameter and number was evident after adjusting for variation in bulb diameter.

Functional Display Size and Floral Longevity

Mean total flower number ($\pm$SE) was 3.7 ± 0.13 at La Clause and 3.1 ± 0.12 at St. Bauzille, with 83% and 65% of plants, respectively, producing three or more flowers. No plants produced more than six flowers (fig. 4). Daily flower number (functional display size) of flowering plants was always lower than total flower number, with the largest display sizes occurring midway through the flowering season (mean $\pm$ SE; 2.7 ± 0.11 and 2.1 ± 0.11 at La Clause and St. Bauzille, respectively). At peak flowering, 56% and 35% of flowering plants produced three or more flowers at the respective sites. Daily display sizes of four or five flowers were relatively uncommon (fig. 4).

Mean flower longevity (11 ± 0.1 d) was similar at both sites and across all flower positions (table 1). Because flowers open sequentially, with each flower opening 3–6 d after the previous one (see fig. 5), an average inflorescence of three flowers was in bloom for close to 3 wk.

Phenology of Floral Expansion and Positional Differences in Flower Size

Flower diameter depended on the combination of flower age and position (table 1, position × age interaction; fig. 5). The first flowers to open were relatively large on opening (day 1: mean $\pm$ SE; 20.8 ± 0.27 mm) and remained constant in size

throughout anthesis (day 10: 20.7 ± 0.29 mm). Later flowers opened at progressively smaller sizes (18.7 ± 0.22 and 17.8 ± 0.29 mm for flowers at positions 2 and 3 on the first day that they were measured; fig. 5). Although later flowers increased in size more than did earlier flowers, they were still significantly smaller when early flowers were 10 d old and at the end of their life spans. Thus, position greatly influenced flower diameter throughout flowering; earlier flowers were the largest and later flowers the smallest on the inflorescence (table 1; fig. 5).

As in earlier analyses, differences in flower diameter among plants depended on total flower number and site (table 1, site × flower number interaction). Flower diameters were generally smaller at St. Bauzille, and mean flower diameter varied positively with flower number. At Hortus Mountain, no clear relation between flower diameter and number was evident. This result probably reflected the small number of plants in each flower-number category ($n \leq 4$ for three of the five classes). Much larger samples that were specifically collected to assess flower number and site effects showed a more consistent relation between flower diameter and number (fig. 3).

Manipulating the size and longevity of first flowers did not significantly affect seed set, although the following trends were observed: mean seed number in first-position fruit was higher in control flowers (mean $\pm$ SE; 22.1 ± 2.32, $n = 14$) than in small-sized (19.6 ± 3.00, $n = 12$) or short-lived flowers
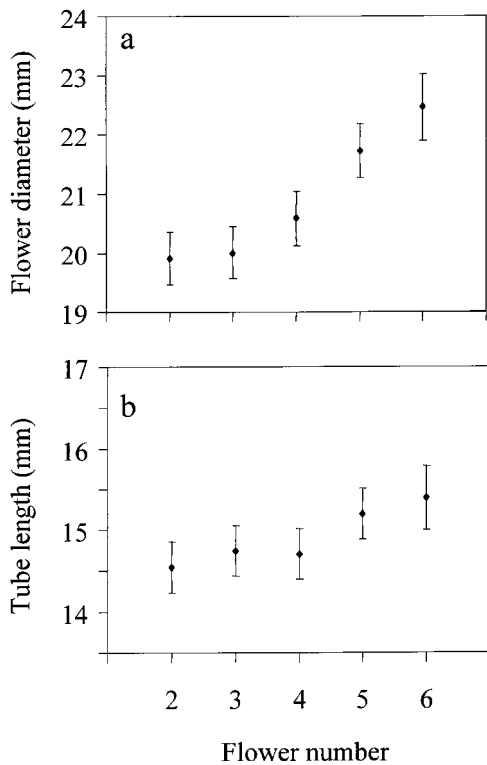
Fig. 3 Relations between floral organ size and flower number in *Narcissus dubius* plants at St. Bauzille and La Clause in 1998. *a*, Mean (±95% CI) flower diameter; and *b*, tube length for plants with different inflorescence sizes. Although the scales for each plot differ in absolute value, they are the same relative to the mean of each trait, so that equivalent relative change would appear to be similar in the two plots. For plants with two to five flowers, *n* = 61–64, and, for plants with six flowers, *n* = 44.

(16.2 ± 2.73, *n* = 10); mean seed numbers in second-position fruit were always lower (position effect: $F_{1,33}$ = 3.86, $P < 0.06$) and followed a similar trend among treatments, but the smallest and largest mean differed by less than two seeds (overall mean = 15.6 ± 1.51, *n* = 36). The overall treatment effect in the repeated-measures analysis was not significant (treatment effect: $F_{1,33}$ = 0.72, $P > 0.4$). Seed numbers in both first and second fruits were positively related to ovule number, which was also lower in second-position flowers. However, controlling for ovule number in separate analyses of seed number in first and second flowers did not reveal significant treatment effects (results not shown).

Discussion

This study considered variation in floral display at several levels of biological organization. Relations between flower size and number among *Narcissus* species supported the occurrence of trade-offs between these floral traits. In contrast, variation in both traits within *Narcissus dubius* depended on plant size, but no trade-off was apparent. Below, we first discuss how negative relations between flower size and number could develop among species in the absence of trade-offs. Second, we

consider how preformation may obscure trade-offs between flower size and number within *N. dubius*. We also discuss the possibility of size-related changes in optimal flower size and evidence for stabilizing selection on floral tube length in *N. dubius*. Finally, we address the effects of staggered flowering phenology on variation in flower size and number within individuals.

Relations between Flower Size and Number among Narcissus *Species*

The strong inverse relation between flower diameter and number among *Narcissus* species supports the widespread assumption of size-number trade-offs. Our analyses did not fully account for the possible lack of independence resulting from phylogenetic relatedness among taxa. Thus, our analyses provide a preliminary assessment of interspecific relations between flower size and number, although the analysis, including sections, indicated that the relation we measured could not be entirely explained by taxonomy. Still, the tendency for species to produce many small, or few large, flowers could reflect historical resemblance among closely related species rather than a functional relation or trade-off between the traits (Harvey and Pagel 1991; Reeve and Sherman 1993). However, continuous traits such as flower size and number seem likely to
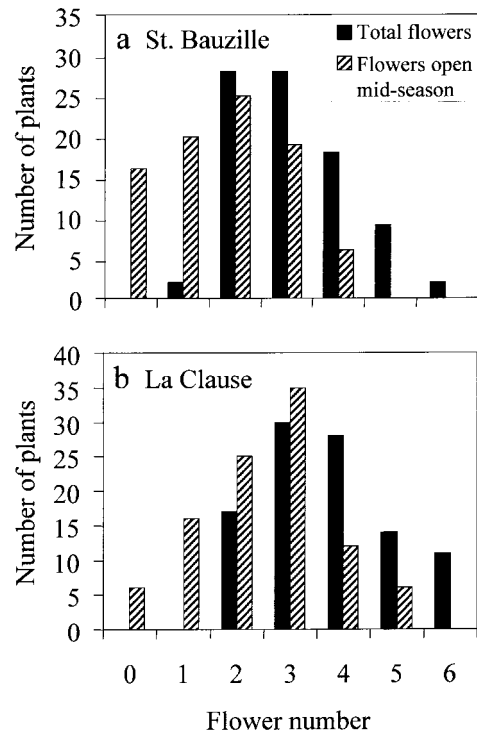
Fig. 4 Total flower number per inflorescence and the number of open flowers (daily flower number) midway through the flowering season on *Narcissus dubius* plants at St. Bauzille (*a*) and La Clause (*b*). Daily flower counts shown here were conducted on March 10 and March 7, 1998, at the respective sites. The distributions for daily flower number are shifted down by approximately one flower, compared with those for total flower number, thus indicating that plants do not display all flowers, even at peak flowering.
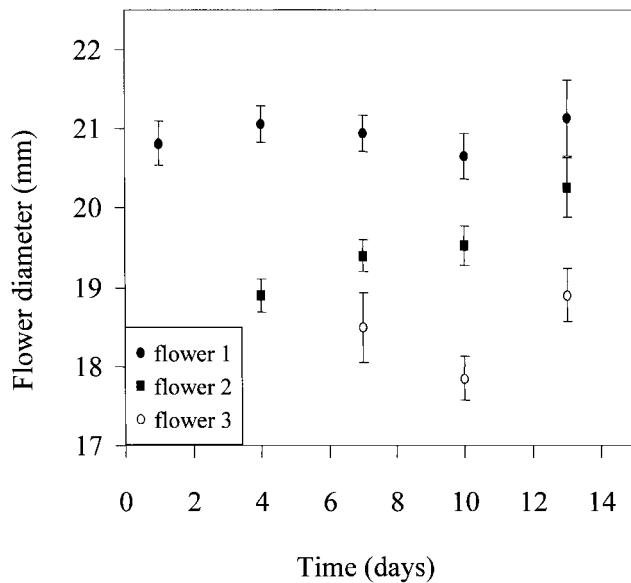
Fig. 5 Variation in flower diameter within *Narcissus dubius* inflorescences from St. Bauzille and Hortus Mountain during February and March 1996. *a*, Mean (± SE) size of flowers at different positions over the life of the inflorescence. Sample sizes for flower 1 were *n* = 53, 75, 58, 35, and 15 for days 1, 4, 7, 10, and 13, respectively. For flower 2, *n* = 75, 60, 37, and 16 for days 4, 7, 10, and 13, respectively. For flower 3, *n* = 15, 26, and 14 for days 7, 10, and 13, respectively. Age and position effects were assessed by repeated-measures analyses (see table 1).

be evolutionarily labile traits, rather than developmentally or physiologically constrained traits (discussed in Westoby et al. 1995; Barrett et al. 1996*a*). Both traits respond rapidly to artificial selection (Meagher 1994; A. C. Worley and S. C. H. Barrett, unpublished manuscript) and have been the target of selective breeding in *Narcissus* cultivars (Jefferson-Brown 1991). Also, in other groups, only one or two genes appear to govern the transition from single to multiple flowers by determining whether meristems form terminal flowers or remain indeterminate inflorescence meristems. Examples include *Antirrhinum* (Bradley et al. 1996), *Arabidopsis* (Bradley et al. 1997), and *Petunia* (Souer et al. 1998). Thus, the inverse relation between flower size and number among species likely reflects correlated evolution of these two traits. More comparative analyses, preferably ones involving resolved phylogenies, are needed to assess the extent of inverse relations between flower size and number among diverse taxa.

The strong negative relation between flower size and number that is evident among taxonomically diverse *Narcissus* species contrasts with the results obtained for *N. dubius*. This contrast is consistent with the suggestion that considerable genetic divergence in flower size may be necessary before trade-offs between flower size and number become apparent (see "Introduction"). However, it also raises the possibility that these floral traits do not directly compete for resources. If flower size and number vary independently within species, the negative interspecific correlation may reflect contrasting selection pressures among species (Stanton and Young 1994; Armbrus-

ter and Schwaegerle 1996). Although plants with more flowers can receive more visits from pollinators (reviewed by Harder and Barrett 1996), additional flowers also increase geitonogamy and pollen discounting (de Jong et al. 1993; Harder and Barrett 1995; Snow et al. 1996). Species with floral adaptations that reduce these mating costs (e.g., dichogamy, stylar polymorphisms, and separation of the sexes; Harder and Barrett 1996) may enhance their floral displays by producing multiple flowers, whereas those without these mechanisms may be more likely to produce a few large flowers. Interestingly, *Narcissus* species with stylar polymorphisms generally produce multiple flowers, whereas species with solitary flowers are usually monomorphic for style length (Barrett et al. 1996*b*).

Flower Number and the Size of Floral Organs in *Narcissus dubius*

In contrast to the negative relation among species, flower diameter and number within *N. dubius* populations were positively related. Mutual dependence on resource status likely caused this positive relation (van Noordwijk and de Jong 1986), as is indicated by positive relations between both traits and bulb diameter and by the removal of the positive relation between floral traits when we controlled for bulb diameter. However, adjusting floral measurements for variation in bulb size did not reveal a trade-off between the two traits. The positive influence of bulb size on flower size and number occurs within several other *Narcissus* species (A. C. Worley, unpublished data), and this positive influence indicates either that flower size and number vary independently or that bulb diameter may not have been an adequate index of resource status. The former possibility is discussed above. The latter would be surprising, given that bulb size measurements for *N. dubius* were more precise than they were in the comparative data set. However, this difference could reflect the fact that *Narcissus* flowers are preformed in the bulb during the autumn that precedes flowering (Blanchard 1990), so that floral differentiation occurs before resource status in the year of flowering is fully determined. Flower size may, in part, be governed by temperature, light, and water availability in the year of flowering, whereas flower number in species with preformation often reflects conditions and resource status in the seasons preceding flowering (Diggle 1997*a*; Geber et al. 1997; Worley and Harder 1999). This difference in short-term flexibility between flower size and number may obscure trade-offs between the two traits. In order to test whether current conditions affect flower size more than they affect flower number, an assessment of resource status and floral traits (over several years) would be required. Designing experiments to reveal trade-offs between flower size and number in a species with preformation would be more difficult.

The prediction that variation in flower size should be highest when continuous resources are divided among few products was not supported for either flower diameter or tube length. The absence of clear convergence toward an optimal size makes it difficult to rule out the possibility that increased flower size in plants with more flowers reflects allometric effects of plant size rather than changes in optimal flower size. The potential for geitonogamous pollination indicates that the efficiency of pollen export in *N. dubius* may indeed be reduced

by additional flowers. However, reductions in daily display size as a result of sequential maturation of flowers may reduce the mating costs associated with multiple flowers. Further studies investigating the phenology of anther dehiscence and stigma receptivity are needed to fully assess the potential for geitonogamy in *N. dubius*. In addition, studies comparing pollen transfer and selfing rates among plants with different flower numbers, as well as the relative fitness of selfed versus outcrossed progeny, are required in order to assess the fitness consequences of variation in flower size.

Although flower diameter increased with enhanced resources, floral tube length was more stable. Selection for efficient pollination should cause tube length to be less variable than the flower size as well as less likely to change with resource status. Data on *Raphanus sativus* (Conner and Via 1993) and on four European *Primula* spp. (Mazer and Hultgård 1993) support the first expectation. Our data confirmed the prediction that tube length in *N. dubius* changes less with resource status (indicated by flower number) than does perianth diameter. This result was consistent with the prediction that there is stronger stabilizing selection on tube length in *N. dubius*, although variation within flower-number classes did not differ from that associated with flower diameter.

Phenology of Floral Expansion and Positional Differences in Flower Size

The strongly staggered flowering phenology in *N. dubius* resulted in considerably fewer open flowers than the total number per inflorescence throughout flowering. Prolonged floral expansion may in part reflect cool temperatures in early spring. Sequential opening probably also distributes resource expenditure on flowering and fruiting over a longer period and may increase the chance that the plant will receive visits from pollinators in unpredictable spring weather. Because daily flower number is low, each *N. dubius* flower makes a relatively large contribution to floral display, especially the first flower, which can be solitary for up to 6 d.

Position-dependent variation in flower size in *N. dubius* could reflect differences in sink strength between flowers of different ages, especially given that first flowers produce larger fruit with larger and more plentiful seeds (A. M. Baker, unpublished data; but see Brunet 1996). The large corolla size of early flowers may have the added benefit of enhancing the visibility of these flowers when they are the only flowers that are open. The solitary position of early flowers led us to hypothesize that the larger size of these flowers might influence their attractiveness to pollinators. Plants with larger flowers

attract more pollinators in several species, including *Fragaria virginiana* (Bell 1985), *R. sativus* (Stanton and Preston 1988), *Phacelia linearis* (Eckhart 1991), and *Wurmbea dioica* (Vaughton and Ramsey 1998). Anecdotal observations of pollinator preferences (*Anthophora* sp., *Macroglossum stellatarum*) for control flowers from our experiment supported this expectation (A. M. Baker and J. D. Thompson, personal observation).

Despite apparent pollinator preferences for larger flowers, seed set did not differ between control and small-sized flowers, between control and short-lived flowers, or between second flowers associated with control and manipulated flowers. It is conceivable that direct effects of clipping on seed production may have confounded our results. However, clipping seems most likely to reduce investment in the manipulated flower, and our clipped flowers did not set significantly fewer seeds than the unmanipulated controls. These results indicate that the size and longevity of early flowers do not influence fitness as a maternal parent, although the possibility of yearly variation in pollen limitation of seed set remains. Indeed, fruit and seed set in the same populations varied significantly among years (Baker et al. 2000*b*). If our results represent an average season, any fitness advantage provided by larger flowers must be through male rather than female function. Positive relations between flower size and reproductive fitness, as indicated by pollen removal/deposition and seed set, occur in some species (e.g., *Polemonium viscosum* [Galen and Stanton 1989], *Raphanus raphanistrum* [Conner et al. 1996*a*], and *W. dioica* [Vaughton and Ramsey 1998]). However, empirical relations between floral morphology and fitness measures are not always evident and can vary spatially and temporally (Schemske and Horvitz 1989; Eckhart 1991; Conner et al. 1996*a*, 1996*b*), making it difficult to assess net selection on reproductive traits within a single season.

Acknowledgments

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