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THE COST OF FLOWERS IN *NIGELLA DEGENII* INFERRED FROM FLOWER AND PERIANTH REMOVAL EXPERIMENTS

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I examined the costs of producing and maintaining floral structures in *Nigella degenii* (Ranunculaceae). To test for such costs, I subjected plants of two populations to flower or perianth removal and then evaluated allocation to subsequently produced flowers, fruits, and seeds. Based on data from a flower removal experiment, the amount of resources allocated to flowers during the staminate and pistillate stages was about half as great as the amount of resources devoted to fruit maturation. Plants on which the perianths were removed at the bud stage produced more flowers or had a higher percent fruit set and seed viability than did plants from which the perianths were removed immediately after the growth of the sepals and petals. Removal of fully developed perianths at the onset of flowering increased flower number, fruit set, or seed viability relative to plants on which the perianths remained until senescence. Some of these effects were population specific, but plants in low and high watering treatments responded similarly to perianth removal. Given these observations, I propose that the costs of producing and maintaining floral structures represent a significant drain on the water and/or energy budget of a *N. degenii* plant and that some trade-offs may be specific to the population in which they have been detected.

Keywords: Nigella degenii, floral evolution, pollination, reproduction, resource allocation.

Introduction

Large floral displays have been shown to enhance pollinator attraction, pollen receipt, and/or pollen donation (e.g., Bell 1985), but little is known about the extent to which such features have negative effects on other components of fitness. For instance, it is possible that the production or maintenance of large showy flowers requires an increased investment of essential resources (water and energy) compared to the investment required of smaller, less conspicuous flowers. According to this hypothesis, the optimum flower size of a population is expected to decline under resource-poor conditions (Galen et al. 1999) or as plants become less dependent on cross-pollination (Darwin 1877; Charlesworth and Charlesworth 1987; Lloyd 1987). To the extent that the display costs include resources invested in nectar production, maintenance respiration, and transpiration by floral structures ("maintenance costs"), one would also expect optimizing selection on flower longevity (Primack 1985; Ashman and Schoen 1994, 1996, 1997).

Despite evidence for greater investment of resources in large conspicuous flowers than in small flowers (e.g., Waller 1979; Lovett Doust and Cavers 1982; Ashman 1994; Schemske and Ågren 1995; Galen et al. 1999), only a few studies have documented genetically based trade-offs between floral advertising and other plant functions (Mossop et al. 1994; Robertson et al. 1994; Campbell 1997). Several authors have demonstrated that flowers and fruits compete for the same pool of resources by raising fruit production and observing a negative response in flower size (reviewed in Ashman and Schoen 1996). However, there have been relatively few experiments in which investigators have manipulated floral investments and then evaluated allocation to subsequently produced flowers, fruits, and seeds (Holtsford 1985; Pyke 1991; Ashman and Schoen 1997; Andersson 1999). Ashman and Schoen (1997) examined the effect of manipulating floral investment under two levels of resource availability, but so far no attempt has been made to include more than one population in flower manipulation experiments. Hence, it remains to be seen whether estimates of floral costs are specific to the population in which they have been measured or whether they also apply more generally.

In this study, I carried out a series of flower and perianth removal experiments in a greenhouse to determine whether plants of *Nigella degenii* respond to the loss of attractive structures by increasing the rate of flower, fruit, and seed production. By removing perianths or entire flowers at different stages, it was also possible to test for a cost of maintaining floral organs over time. All experiments involved plants from two geographically distant populations (different subspecies), and one experiment was carried out under two levels of water availability. Hence, my investigation provided an opportunity to evaluate the consistency of allocation patterns across populations and to examine whether the attracting structures incur a greater cost under stressful growth conditions, as suggested by Ashman and Schoen (1997) and Galen et al. (1999).

Material and Methods

Plant Material

The *Nigella arvensis* species complex (Ranunculaceae) consists of ca. 10 annual taxa located within the Mediterranean

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Table 1

The Mean Sepal and Petal Size at the First Removal Stage in the Perianth Removal Experiments and at the Onset of Flowering (Final Size)

	Area (mm^2)		Dry weight (mg)	
Category	Removal stage	Final size	Removal stage	Final size
Mikonos:				
Sepals	16.1 (0.27)	52.8 (1.94)	0.22	0.75
Petals	8.5 (0.43)	16.2 (0.25)	0.21	0.54
Siros:				
Sepals	13.2 (0.65)	53.4 (2.51)	0.19	1.05
Petals	8.0 (0.51)	16.1 (0.52)	0.22	0.61

Note. Values are means with SEs in parentheses based on separate measurements (area) or bulked samples (dry weight) of 10 sepals or petals. Areas measured with an image analysis system on a Macintosh computer.

region. Flowering is sequential, starting with the flower terminating the main shoot and ending with flowers on the lowermost branches; only one or two flowers per branch bloom at any given time. Each flower has a double perianth with five white petaloid sepals and eight conspicuously colored nectar-producing petals, a variable number of stamens, and a gynoecium with up to 10 carpels, partially united to form a capsule (Strid 1970). Plants of *Nigella* that have adapted for insect pollination (e.g., *Nigella degenii* Vierh.) have self-compatible, protandrous flowers (15–25 mm across) that fail to set fruit in the absence of pollinators (Strid 1969). Because of pollination-induced flower senescence, the "flower life span" of *N. degenii* ranges from 6 to 7 d for flowers that are pollinated at the onset of stigma receptivity to ca. 2 wk for unpollinated flowers. The ovaries continue to grow until the stigmas become receptive (S. Andersson, unpublished data). Fruit maturation in the terminal flowers begins while the basal flowers are still being formed.

This investigation is based on plant material from two populations of *N. degenii* in the Cyclades (Greece), one on the island of Mikonos (ca. 2.5 km north-northwest of the town) and the other on the island of Siros (south of Kini). These populations are separated by ca. 35 km and represent different subspecies that differ in flower and fruit morphology, but there are no obvious differences in reproductive ecology or in the type of habitat occupied by the two populations (open patches in shrubby heath; Strid 1970; S. Andersson, personal observation). The seeds used in the flower manipulation experiments are the product of several generations of random crosses in the greenhouse.

Flower Removal Experiment

In 1997, an attempt was made to estimate the maximum amount of resources that can be allocated to flowers during the pistillate and staminate stages. In April, ca. 200 plants from each population were planted in 125-cm^3 plastic pots containing a mix of peat soil and sand and were placed in random positions on two adjacent benches in an unheated greenhouse. When the first plants reached anthesis (June), ca. 50 plants per population were assigned to each of three treatments: (1) removal of flowers (flowers were clipped off with scissors) at the onset of flowering, (2) removal of flowers immediately after perianth abscission, and (3) hand pollination at the onset of female receptivity (with pollen from freshly dehisced anthers in flowers on one or two plants from the same population). Slow floral development made it necessary to repeat the experimental procedures every third to fourth day during a 2-mo period. After the flowering season, I counted the flowers initiated on each plant (including the removals).

Since flower removal in both removal groups occurred after the growth of the perianths, any increase in mean flower number after removal of flowers at the onset of flowering, relative to plants on which flowers were removed after perianth abscission, would indicate a cost of maintaining floral structures (including the perianths). Given the use of unpollinated plants in both treatment groups (few other "resource sinks") and the unusually long "life span" of flowers removed at the abscission stage (ca. 2 wk), this comparison provides a rough estimate of the maximum amount of resources that can be allocated to intact flowers during the staminate and pistillate stages. This floral maintenance cost was compared with the cost of fruit maturation, estimated as the difference in mean flower number between plants whose flowers were removed after the abscission of the perianths (no fruit production) and plants in the hand pollination group.

Perianth Removal Experiments

In 1998, I carried out a perianth removal experiment to estimate the cost of producing sepals and petals. About 35 plants per population (planted in the same type of pots and soil as were used in 1997) were assigned to each of two treatments: (1) removal of sepals and petals (sepals and petals were removed with a pair of tweezers) at the bud stage and (2) removal of fully expanded sepals and petals at the onset of flowering. The sepals and petals in the early removal group (treatment 1) were removed when they had reached 20%–30% (sepals) or 50% (petals) of their final size (table 1). The stamens and ovaries appeared to develop normally after the removal procedure (S. Andersson, personal observation), regardless of whether the perianths were removed at the bud stage or at the onset of flowering. All flowers were outcrossed by hand at the onset of female receptivity. This procedure enhanced the power

Table 2

Two-Way ANOVA on the Total Number of Flowers Initiated in the Flower Removal Experiment

Source	df	MS	*F*
Population	1	2.58	30.6^{***}
Treatment	2	10.72	127.3^{***}
Population × treatment	2	0.03	0.4 ns
Error	301	0.08	...

Note. Analysis based on ln-transformed data. $n = 50–52$ per population and treatment. ns = not significant ($P > 0.05$).

*** $P < 0.001$.

Table 3

The Mean Number of Flowers Produced at the End of the Flower Removal Experiment for Each Treatment Group

Treatment	Mikonos	Siros
Removal at onset of flowering	19.4	16.0
	(0.59)	(0.51)
Removal after perianth abscission	16.4	13.4
	(0.60)	(0.51)
Hand pollination (no removals)	10.1	8.9
	(0.60)	(0.52)

Note. Values are least-square means with SEs in parentheses. $n = 50–52$ per population and treatment.

to detect resource trade-offs between flower production and reproductive performance by increasing the number of resource sinks (increased fruit production) and the range of response variables (see below). After the flowering season (September), I measured the following response variables on each plant: the number of flowers initiated, the proportion of carpels setting seed (fruit set), the number of seeds in the terminal capsule, and "seed viability," estimated as the fraction of seeds in the terminal capsule that germinated within a 3-wk period (based on a sample of 20 seeds sown in moist sand in a petri dish at room temperature, with alternating 12-h periods of light and dark). The use of terminal capsules ensured that the position of the flower on the plant had little influence on the seed characters.

In 1999, I repeated the perianth removal experiment to estimate the costs of producing and maintaining sepals and petals under two levels of resource availability. Ca. 180 plants from each population were assigned to one of two watering treatments (high or low) and one of three flower manipulation treatments: (1) removal of sepals and petals at the bud stage, (2) removal of fully expanded sepals and petals at the onset of flowering, and (3) control (flowers left intact). All flowers were hand pollinated. Plants in the high water treatment were watered daily or as needed, whereas plants in the low water treatment were watered two or three times per week, depending on weather conditions. The low water treatment resulted in premature wilting of leaves and had a negative effect on flower production (see below), showing that this treatment provided less than adequate water. The plants were scored for the same variables as were used in 1998.

Given the size difference between perianths in the early and late removal groups (table 1), any increase in reproductive success after removal of sepals and petals at the bud stage, relative to plants on which sepals and petals were removed at the onset of flowering, would indicate a cost of fully developing sepals and/or petals. Similarly, given the difference in flower life span of the control plants (6–7 d) and plants from which perianths were removed at the onset of flowering (<1 d), any increase in reproductive success after late perianth removal (treatment 2), relative to the control group, would indicate a cost of maintaining sepals and/or petals over time.

Analyses and Assumptions

Differences between populations, environments, and flower manipulation treatments were tested by analyses of variance (ANOVA), with all factors considered as fixed and tested over the error term. Preliminary analyses revealed nonsignificant "greenhouse bench effects" ($P > 0.80$), so this factor was excluded from the final analyses. Analyses of data obtained in the 1997 and 1999 experiments also included planned comparisons between particular treatment groups to partition the floral costs into different components (see above). All analyses were performed using SuperANOVA (1989) on a Macintosh computer.

Central to this study are the assumptions that measures of flower, fruit, and seed production are positively correlated with lifetime fitness and that different reproductive functions compete for the same pool of limited resources, whether these

Table 4

Treatment Means and *F* Values from Two-Way ANOVA for Each of the Response Variables in the 1998 Perianth Removal Experiment

	Least-square means						
	Mikonos		Siros		Source of variation[a]		
Variable	Removal at bud stage	Removal at anthesis	Removal at bud stage	Removal at anthesis	Population	Treatment	Interaction
No. flowers	13.4	14.2	12.6^{A}	10.5^{B}	16.3^{***}	1.3 ns	6.5^{*}
	(0.6)	(0.6)	(0.6)	(0.6)			
Fruit set[b]	1.01^{A}	0.89^{B}	0.63	0.67	80.3^{***}	1.6 ns	6.5^{*}
	(0.03)	(0.03)	(0.03)	(0.03)			
Seeds per capsule	44.9	44.5	46.3	47.6	1.0 ns	0.0 ns	0.1 ns
	(2.3)	(2.3)	(2.3)	(2.3)			
Seed viability[b]	0.97^{A}	0.74^{B}	0.61	0.66	15.3^{***}	2.4 ns	6.0^{*}
	(0.06)	(0.06)	(0.06)	(0.06)			

Note. $n = 31–34$ per treatment and population. Values followed by different letters indicate significantly different treatment means within a population ($P < 0.05$ or 0.01, one-way ANOVA). SEs in parentheses. ns = not significant ($P > 0.05$).

[a] *F* values from ANOVA.

[b] Means and analyses based on arcsine square root–transformed data.

* $P < 0.05$.

*** $P < 0.001$.

resources are supplied by nearby leaves or by photosynthesis of the reproductive organs themselves. The first assumption seems reasonable in an annual plant like *N. degenii*, and the second assumption should be satisfied when the effects of removing floral parts (or entire flowers) are evaluated in the same "currency" (flowers, fruits, or seeds), as was the case in the present investigation. Finally, given the use of hand-pollinated plants in the two perianth removal experiments, my results are most relevant to situations in which fecundity is limited by resources rather than by pollination.

Results

Flower Removal Experiment

A two-factor ANOVA on flower production demonstrated that population and treatment each had a significant influence on the number of flowers initiated, whereas the population-by-treatment interaction was nonsignificant (table 2). Based on the treatment means (table 3), plants of the Mikonos population had higher flower production than did those from the Siros population. The average flower number was highest for plants in which flowers were removed at the onset of flowering, lowest for plants with hand-pollinated flowers, and intermediate for plants in which flowers were removed after perianth abscission. Differences between the means of the two removal groups and between the late removal and hand pollination groups were significant at $P < 0.001$, according to the planned comparison tests.

Perianth Removal Experiments

Removing the sepals and petals at the bud stage reduced the amount of biomass invested in these structures by more than 50% (table 1). Perianth removal had a population-specific effect on reproductive success in the 1998 experiment: the Siros plants responded to perianth removal by increasing flower production, whereas the Mikonos plants responded by increasing fruit set and seed viability (table 4). There was a significant main effect of population for all but one variable (seeds per capsule), with plants from the Mikonos population exceeding plants from the Siros population in flower number, fruit set, and seed viability (table 4).

The 1999 experiment verified the low reproductive performance of the Siros plants, although the between-population difference in flower number failed to reach significance (tables 5, 6). Low water availability had a negative effect on flower production (tables 5, 6), but there was no tendency for plants in the high and low water treatments to differ in their responses to perianth removal (no interaction between watering regime and perianth removal; $P > 0.45$). The main effect of perianth removal was significant for fruit set and seed viability (table 5), with manipulated plants exceeding the control plants in most cases (table 6). Perianth removal also increased flower number, but only for plants from the Siros population (tables 5, 6). According to the planned comparison tests, plants in which perianths were removed at the onset of flowering had a significantly higher flower production (Siros), fruit set (Mikonos), or seed viability (Siros) than did the control plants, whereas the differences between the two removal treatments failed to reach significance for any trait (table 6). Inspection of treatment means (tables 4, 6) indicated higher reproductive performance in 1998 than in 1999, especially for plants from the Siros population.

Discussion

Although genetic studies have documented negative correlations between floral advertising and other fitness traits (Mossop et al. 1994; Robertson et al. 1994; Schemske and Ågren 1995; Campbell 1997), the experimental evidence for such trade-offs is still limited. In this investigation of *Nigella degenii*, I manipulated floral investment of plants by removing flowers or floral parts at particular stages and then evaluated allocation to subsequently produced flowers, fruits, and seeds. The design of this study not only enhanced the power to detect causal relationships between variables (Mitchell-Olds and Shaw 1987), but it also enabled me to assess the response to flower manipulation for plants exposed to different levels of stress and for plants derived from two genetically distinct populations.

Plants of *N. degenii* in which the sepals and petals were removed at the bud stage generally had higher reproductive success than did plants in which the sepals and petals were removed after the growth of these organs, especially in the 1998 experiment and for plants representing the Mikonos population. Since all of these plants were hand pollinated, I propose that plants from which sepals and petals were removed at the bud stage increased their reproductive performance by using resources that would otherwise have been invested in the attracting structures. Hence, it seems that developing sepals and petals represent an important sink for assimilates, at least under greenhouse conditions.

Table 5

F **Values from Three-Way ANOVA for Each of the Response Variables in the 1999 Perianth Removal Experiment**

Variable	Population (P)	Watering (W)	Removal (R)	$P \times W$	$P \times R$	$W \times R$	$P \times W \times R$
No. flowers	2.0 ns	40.5***	2.6 ns	1.0 ns	4.8**	0.1 ns	2.0 ns
Fruit set[a]	248.6***	0.2 ns	6.5**	0.7 ns	2.5 ns	0.4 ns	0.5 ns
Seeds per capsule	56.5***	0.0 ns	0.4 ns	0.1 ns	0.2 ns	0.8 ns	0.0 ns
Seed viability[a]	70.1***	0.1 ns	3.5*	5.3*	0.7 ns	0.7 ns	1.0 ns

Note. ns = not significant ($P > 0.05$).

[a] Analyses based on arcsine square root–transformed data.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 6

Least-Square Means from Three-Way ANOVA for Each of the Response Variables in the 1999 Perianth Removal Experiment

	High water			Low water			Planned contrasts[a]	
Population and variable	Removal at bud stage (1)	Removal at anthesis (2)	Control (3)	Removal at bud stage (1)	Removal at anthesis (2)	Control (3)	(1) vs. (2)	(2) vs. (3)
Mikonos:								
No. flowers	3.5	4.1	3.9	3.2	3.0	3.1	1.0 ns	0.1 ns
	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)		
Fruit set[b]	0.99	0.84	0.73	1.01	0.97	0.74	1.9 ns	6.3*
	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)		
Seeds per capsule	32.3	32.5	32.4	34.8	34.9	30.2	0.0 ns	0.5 ns
	(3.3)	(3.3)	(3.3)	(3.3)	(3.3)	(3.3)		
Seed viability[b]	0.52	0.64	0.52	0.73	0.65	0.63	0.1 ns	1.1 ns
	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)		
Siros:								
No. flowers	4.3	3.8	3.2	3.0	2.9	2.3	1.8 ns	5.6*
	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)		
Fruit set[b]	0.25	0.34	0.26	0.31	0.31	0.19	0.4 ns	2.2 ns
	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)		
Seeds per capsule	17.2	19.2	20.0	18.2	20.9	16.0	0.5 ns	0.4 ns
	(3.3)	(3.3)	(3.3)	(3.3)	(3.3)	(3.3)		
Seed viability[b]	0.30	0.38	0.27	0.25	0.36	0.10	2.0 ns	7.3**
	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)		

Note. SEs in parentheses. ns = not significant ($P > 0.05$). n = 29–30 per treatment and population.

[a] Values are F values (df = 1).

[b] Means and analyses based on arcsine square root–transformed data.

* $P < 0.05$.

** $P < 0.01$.

By removing perianths or entire flowers at later stages, it was also possible to document a cost of maintaining functional flowers. First, hand-pollinated plants in which the sepals and petals were removed at the onset of flowering usually had a higher reproductive performance than did hand-pollinated plants in which sepals and petals remained intact until senescence. Indeed, most of the significant treatment effects in the 1999 experiment could be attributed to these differences. Second, plants in which the flowers were removed at the onset of flowering produced more flowers than did plants in which the flowers were removed after the abscission of the perianths. Given the unusually long flower life span of the unpollinated plants in the latter group (ca. 2 wk), this difference is likely to set an upper limit for the amount of resources allocated to flowers during the staminate and pistillate stages (when evaluated as a trade-off with subsequent flower production). Judging from the decline in flower production of plants in which flowers were removed after perianth abscission, relative to plants in which flowers were removed at the onset of flowering, and from the corresponding effect of hand pollination (table 3), the maximum amount of resources allocated to functional flowers was about half as great as the amount of resources devoted to fruit maturation.

I found no support for the hypothesis that attracting structures put a greater drain on a plant's resources under stressful conditions (Ashman and Schoen 1997; Galen et al. 1999). First, even though water deficiency had a negative influence on flower production, there was no evidence that plants in low and high water treatments responded differently to perianth removal. Second, there was no tendency for the "production costs" in the perianth removal experiments to be most severe in the 1999 season, when reproductive performance was unusually low (tables 4, 6). In this context, it is important to emphasize that treatment effects were detectable in some, but not all, variables and that some responses differed in magnitude between the Siros and Mikonos populations. For instance, perianth removal generally had a stronger positive effect on flower production for the Siros plants than for the Mikonos plants. Based on comparative evidence (Strid 1970; Andersson 1997; this study), there are no obvious between-population differences in habitat specificity, plant morphology, or breeding system that could explain these differences, except that Siros plants usually produce fewer (but larger) flowers than do plants from the Mikonos population.

Relatively few experiments have been carried out to examine whether resource allocation to floral display can be costly in terms of flower and seed production. In a study of *Calochortus leichtlinii*, the number of seeds produced by a plant's second flower was negatively correlated with the age at which the first flower was removed (Holtsford 1985). Other examples include Ashman and Schoen (1997), who documented an energy cost of maintaining flowers in *Clarkia tembloriensis* by manipulating the timing of pollination; Pyke (1991), who detected a cost of floral nectar in *Blandfordia grandiflora* by manipulating nectar secretion; and Andersson (1999), who performed a ray removal experiment to assess the overall cost of floral display in the composite *Achillea ptarmica*. The results of the present study not only support the hypothesis that attracting structures can be costly to produce and maintain but they also indicate that some trade-offs may be specific to the population in which they have been detected.

By minimizing expenditure on floral structures, more resources can be diverted to fruit and seed production, allowing more economic seed production under stressful growth conditions (Galen et al. 1999) or at sites in which attractive flowers are unnecessary because conditions are unfavorable for cross-pollination (Charlesworth and Charlesworth 1987; Lloyd 1987). Hence, given that small inconspicuous flowers require a smaller investment of resources than do large showy flowers, and given that heritable variation for flower size exists or has existed in the past (Andersson 1997), I propose resource trade-offs as the basis for some of the reduction in flower size that characterizes selfing taxa in the *Nigella arvensis* complex (Strid 1969; for other hypotheses, see Andersson 1997).

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