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Nonadditive effects of floral display and spur length on reproductive success in a deceptive orchid

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Abstract. Pollinators may mediate selection on traits affecting pollinator attraction and effectiveness, and while nonadditive effects of traits influencing the two components of pollination success are expected when seed production is pollen limited, they have been little studied. In a factorial design, we manipulated one putative attraction trait (number of flowers) and one putative efficiency trait (spur length) previously shown to be subject to pollinator-mediated selection in the deceptive orchid *Dactylorhiza lapponica*. Removal of half of the flowers reduced pollen removal, proportion of flowers receiving pollen, fruit set, and fruit mass compared to unmanipulated plants, while spur-tip removal increased fruit set and fruit mass but did not affect pollen removal or proportion of flowers receiving pollen. The effect of spur-tip removal on fruit mass was stronger among plants with intact number of flowers compared to plants with experimentally reduced number of flowers. The results demonstrate that number of flowers and spur length are direct targets of selection and may affect female fitness nonadditively. More generally, they show that the adaptive value of a given trait can depend on floral context and illustrate how experimental approaches can advance our understanding of the evolution of trait combinations.

Key words: adaptive significance; correlational selection; *Dactylorhiza lapponica*; floral evolution; mechanism of selection; phenotypic manipulation experiment; pollination by deceit; pollination efficiency; pollinator attraction; pollinator-mediated selection.

INTRODUCTION

Flowers and their ecological and evolutionary interactions provide a rich source for studies of evolution by natural selection (Darwin 1862, Fenster et al. 2004, Mitchell et al. 2009, Conner 2010, Sapir and Armbruster 2010). Interactions with pollinators may result in selection on a variety of floral traits (Sandring and Ågren 2009, Caruso et al. 2010, Parachnowitsch and Kessler 2010, Sletvold et al. 2010a), spatiotemporal variation in current selection on floral traits may to a large degree be mediated by pollinators (Sletvold and Ågren 2010), and variation in plant–pollinator interactions may drive plant population differentiation (Herrera et al. 2006, Anderson et al. 2010) and speciation (Schemske and Bradshaw 1999, Kay and Sargent 2009). However, the degree to which the adaptive value of a given trait is influenced by the expression of other floral traits remains poorly understood.

Floral traits may influence both attractiveness to pollinators (Ida and Kudo 2010) and morphological fit between flower and pollinator (Alexandersson and

Johnson 2002), and thereby the number of visits received and the efficiency of each visit in terms of pollen exchange with the pollinator. Plant reproductive success is expected to be a function of the product of these two components of pollination success (cf. Stebbins 1950, 1970), and this assumption underlies the frequent use of this product as an estimate of pollinator importance (cf. Fenster et al. 2004). If more than one visit per flower is required to complete female and male function, traits influencing attractiveness and traits affecting morphological fit are expected to have multiplicative effects on fitness, and should be subject to correlational selection (broadly defined as situations where the shape or magnitude of selection on a given trait depends on the expression of another trait). Multiplicative effects may be more likely to be observed for some fitness components than others. For example, while a single visit may be enough to initiate fruit development, several visits may be needed for all ovules to be fertilized. Multiplicative effects are thus more likely to be observed when effects on number of seeds per fruit and total seed production per plant are considered, than when the proportion of flowers producing a fruit is the response variable.

Despite the fact that most plants are likely to need multiple visits per flower for maximum pollen export and seed set, few studies have detected correlational selection on combinations of traits expected to influence

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attraction and efficiency (Benítez-Vieyra et al. 2006, Cuartas-Domínguez and Medel 2010, Vanhoenacker et al. 2010). This could reflect low statistical power in most phenotypic selection studies, since large sample sizes are needed to detect nonadditive effects of moderate strength (cf. Kingsolver et al. 2001). Moreover, those studies that do suggest correlational selection cannot rule out the possibility that relationships are due to effects of traits not included in the analysis (Mitchell-Olds and Shaw 1987), or to environmentally induced correlations between traits and fitness (Rausher 1992). To establish conclusively the targets of selection, an experimental approach is required (Wade and Kalisz 1990). Experimental manipulation of trait combinations also reduces covariances among traits, increasing the power to detect nonadditive effects.

Several studies have manipulated single floral traits expected to influence either pollinator attraction or effectiveness, and significant effects on fitness have been documented equally often for both types of traits (reviewed in Harder and Johnson 2009). However, the significance of floral trait combinations is still largely unknown, with few studies using experimental methods to investigate the adaptive value of suites of traits (reviewed in Campbell 2009). We are aware of only one study that has manipulated a putative attraction trait and efficiency trait simultaneously. Boberg and Ågren (2009) examined the independent and interactive effects of corolla size and spur length on pollen transfer and fruit and seed production in the orchid *Platanthera bifolia*. However, in that study, only spur length affected pollination success.

Orchids are highly represented in studies of floral evolution, due to their diverse and specialized pollination mechanisms (cf. Tremblay et al. 2005). Trait manipulation experiments have demonstrated that shortening of nectar spurs reduces fitness (Nilsson 1988, Johnson and Steiner 1997, Boberg and Ågren 2009), plant height influences pollinator attraction (Peakall and Handel 1993), while modification of flower size may have minor effects on reproductive success (Boberg and Ågren 2009, Cuartas-Domínguez and Medel 2010). Despite this interest, we have little information on nonadditive effects on fitness of traits affecting visitation frequency and efficiency in orchids. Species with pollen aggregated into pollinia usually have considerably higher pollen transfer efficiency compared to species with granular pollen (Harder and Johnson 2008), suggesting that the former need only a few visits per flower to complete both sex functions. However, some studies have demonstrated that supplemental hand-pollination increases the number of seeds per fruit compared to open-pollinated controls (Sletvold and Ågren 2010, Sletvold et al. 2010a), indicating that individual flowers need multiple visits to receive enough pollen for maximum seed set. Moreover, Benítez-Vieyra et al. (2006) documented a multiplicative effect of number of flowers and nectary depth on pollen removal

in the orchid *Cyclopogon elatus*; the effect of nectar depth on pollen removal increased with the number of flowers. Taken together, these studies suggest that traits influencing pollinator attraction and pollination efficiency in orchids may have nonadditive effects on components of both female and male fitness.

The deceptive bumble bee-pollinated orchid *Dactylorhiza lapponica* (see Plate 1) experiences pollinator-mediated selection on plant height, number of flowers, and spur length (Sletvold et al. 2010a). Plant stature and flower production is likely to affect pollinator attraction, while spur length is likely to influence the functional fit between flower and pollinator, and thereby pollination efficiency. In a previous study, hand pollination increased seed number per fruit compared to natural pollination in *D. lapponica* (Sletvold et al. 2010a), indicating that individual flowers need more than one visit to attain maximum seed set. However, there was no indication of correlational selection on these traits in that study. This could be because (a) only some of the traits were direct targets of selection, (b) few visits are needed to saturate female fitness of individual flowers, or (c) the sample size was too low or trait covariance too strong for correlational selection to be detectable. In the present study, we manipulated number of flowers and spur length in a factorial design, to test the hypothesis that both traits are direct targets of selection, and to test for nonadditive effects on fitness of traits expected to influence pollinator attraction and pollination efficiency, respectively. We manipulated number of flowers by removing 50% of the flowers, expecting this to reduce pollinator visitation, and we manipulated spur length by cutting off the spur tip, expecting this to increase pollination efficiency because a pollinator is likely to force its head and proboscis deeper into flowers with open-ended spurs.

MATERIAL AND METHODS

Study species and site

Dactylorhiza lapponica (Laest. ex Hartm.) Soó (Orchidaceae) is a long-lived, non-clonal terrestrial orchid, growing in Fennoscandia, Scotland, and alpine areas in Central Europe. In Fennoscandia, *D. lapponica* is restricted to open lawn communities in calcareous fens and springs in the boreal vegetation zone. Plants emerge in late May to early June, and flowering individuals produce a single inflorescence with ~3–15 flowers that open from the base of the inflorescence and upward. The cerise flowers have a short spur, but no nectar production. Flowers do not closely resemble any sympatric rewarding species, and the pollination system is thus one of generalized food deception. The two pollinia are situated above the spur entrance, and each pollinium is composed of 88 ± 14 massulae (mean \pm SD, $n = 15$ individuals from the study population). In a survey of the study population, on average 1.26 pollinia were removed per flower ($n = 55$), 42% of the flowers had received pollen ($n = 55$), the total number of massulae

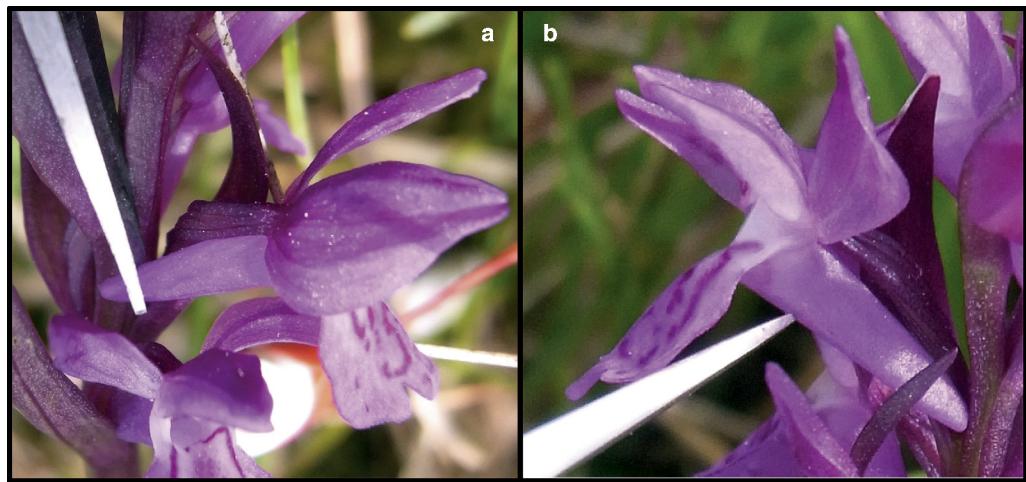


FIG. 1. *Dactylorhiza lapponica* flowers subject to (a) spur-tip removal and (b) control damage. Photos by J. Trunschke.

deposited per pollinated flower was 31 ± 29 (range 2–91, $n = 23$), and on average 12% of the removed pollen thus reached a conspecific stigma (pollen transfer efficiency). *Dactylorhiza lapponica* is self-compatible, but depends on pollinators for successful fruit set (N. Sletvold, *unpublished data*). Low levels of fruit set are common (20–30%; Sletvold et al. 2010b).

The present study was conducted in a population of approximately 500 flowering individuals located at Tågdalen, ~450 m above sea level in central Norway ($63^{\circ}03' N$, $9^{\circ}05' E$; see Sletvold et al. 2010a for a more detailed description of the study site). The population flowers during three to four weeks from mid to late June, and is pollinated by worker bees of the species *Bombus pascuorum* and *B. lucorum* (N. Sletvold, *personal observation*). Fruit production and fruit mass are strongly pollen limited in the study population (Sletvold et al. 2010a).

Field experiment

To examine the effects of number of flowers and spur length on pollination success and seed production, we manipulated both traits in a factorial design (natural vs. 50% reduction in number of flowers, natural vs. “infinite” spur length, respectively).

In late June 2010, a total of 250 plants with flower buds were haphazardly chosen and individually tagged. We randomly assigned 50 plants to each of the four treatment combinations in the factorial experiment, as well as to an additional control group that was left unmanipulated. For each plant, we recorded the number of flowers, start of flowering, and plant height to the nearest millimeter (distance from the ground to the topmost flower) on the day the first flower opened. In the number-of-flowers treatment, we removed 50% of the flowers by cutting off every other bud with scissors prior to flower opening. In the spur-length treatment, we cut off the spur tip with scissors as the flowers were

about to open, thereby producing open-ended spurs of “infinite” length (Fig. 1a). To account for possible effects of necrosis in the cut surfaces we used a foreceps and pinched a small hole at the lower side of the spur base in both treatment combinations with natural spur length (control damage, Fig. 1b). Plants were checked daily as flowering commenced, and floral manipulations were conducted as flowers opened. All flowers within a plant were manipulated. Pollinium removal and pollen receipt were recorded every 1–5 days throughout the flowering period, before flowers started wilting. For each flower, we counted the number of pollinia removed and noted whether pollen had been received or not. At fruit maturation, we recorded the number of mature fruits produced and harvested up to three mature fruits spread across the inflorescence to determine mean fruit mass for each plant. Fruit mass is positively correlated with number of seeds with embryos in *D. lapponica* ($r = 0.92$, $P < 0.001$, $n = 12$ fruits, each from a separate individual). For each plant, we estimated female fitness as the product of number of fruits and mean fruit mass, and male fitness as the total number of removed pollinia.

Flowering start (one-way ANOVA, $F_{3,188} = 0.35$, $P = 0.79$), plant height ($F_{3,188} = 0.42$, $P = 0.74$), and number of flowers prior to experimental manipulation ($F_{3,188} = 0.76$, $P = 0.52$) did not differ between the four treatment groups. Fruit herbivory by lepidopteran larvae has previously been recorded in the study population (Sletvold et al. 2010a), but was rare in the year of the present study (<1% fruits consumed), and did not differ between treatments.

Statistical analyses

We assessed the effect of control damage on plant reproductive success by one-way ANOVA, comparing the natural number of flowers \times natural spur length treatment with the unmanipulated controls. We used two-way ANOVA to examine the effects of number of

TABLE 1. ANOVA results of the effects of number of flowers (natural vs. 50% removal), spur length (natural vs. spur-tip removal), and their interaction.

Source of variation	df	MS	F	P
Proportion pollen removal				
Number of flowers	1	0.61	3.80	0.050
Spur length	1	0.041	0.26	0.61
Number of flowers × spur length	1	0.012	0.008	0.78
Error	188	0.16		
Total pollen removal				
Number of flowers	1	1311	65.8	<0.0001
Spur length	1	3.44	0.17	0.68
Number of flowers × spur length	1	5.85	0.29	0.59
Error	188	19.9		
Proportion of flowers receiving pollen				
Number of flowers	1	0.77	5.52	0.020
Spur length	1	0.084	0.60	0.44
Number of flowers × spur length	1	0.0002	0.0013	0.97
Error	188	0.14		
Fruit set				
Number of flowers	1	1.23	7.01	0.0088
Spur length	1	0.75	3.84	0.049
Number of flowers × spur length	1	0.016	0.05	0.70
Error	188	0.18		
Fruit mass				
Number of flowers	1	0.032	22.2	<0.0001
Spur length	1	0.048	29.3	<0.0001
Number of flowers × spur length	1	0.0083	5.74	0.018
Error	147	0.0014		
Female fitness				
Number of flowers	1	1.36	66.9	<0.0001
Spur length	1	0.33	16.2	<0.0001
Number of flowers × spur length	1	0.057	2.78	0.097
Error	188	0.020		

Notes: Response variables are proportion of pollinia removed, total pollen removal, proportion of flowers receiving pollen, proportion fruit set, mean fruit mass, and female fitness (number of fruits × mean fruit mass). Effects with $P < 0.05$ are shown in boldface type.

flowers (natural vs. 50% removal), spur length (natural vs. infinite) and their interaction on proportion of pollinia removed, proportion of flowers receiving pollen, proportion of flowers that set fruit, mean fruit mass, total pollen removal and female fitness. In these models, a significant interaction term between number of flowers and spur length demonstrates nonadditive effects of the two traits, and is consistent with the hypothesis that the two traits are subject to correlational selection. All proportional data were arcsine square-root transformed prior to analyses, and fruit mass and female fitness were square-root transformed to improve normality of residuals. A few flowers had been visited prior to manipulation (a total of 20 flowers in 12 individuals, no difference among treatments; one-way ANOVA, $F_{3,188} = 0.08$, $P = 0.87$), and were discarded from the analyses. Analyses were performed in SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Effects of control damage

The proportion of pollinia removed ($F_{1,94} = 1.25$, $P = 0.27$), the proportion of flowers receiving pollen ($F_{1,94} =$

0.16, $P = 0.69$), fruit set ($F_{1,94} = 0.01$, $P = 0.91$), fruit mass ($F_{1,82} = 0.63$, $P = 0.43$), total pollen removal ($F_{1,94} = 0.004$, $P = 0.95$), and female fitness ($F_{1,94} = 0.75$, $P = 0.39$) did not differ significantly between plants with control damage (hole pinched at the base of the spur) and unmanipulated control plants, indicating that the necrosis caused by damage to the spur did not affect plant reproductive success.

Number of flowers and spur length

The independent and combined effects of flower removal and spur length manipulation varied among components of reproductive success. In control plants, on average 58% of the pollinia were removed, and 36% of the flowers received pollen. The proportion and total number of pollinia removed, and the proportion of flowers receiving pollen were reduced following removal of 50% of the flowers, but were not affected by the spur length treatment (Table 1, Fig. 2a–c). The flower removal treatment was associated with a 58% decrease in total number of pollinia removed (Fig. 2b). Fruit set was on average 45% in control plants. It was significantly reduced by flower removal and significantly increased by spur-tip removal, and these effects were

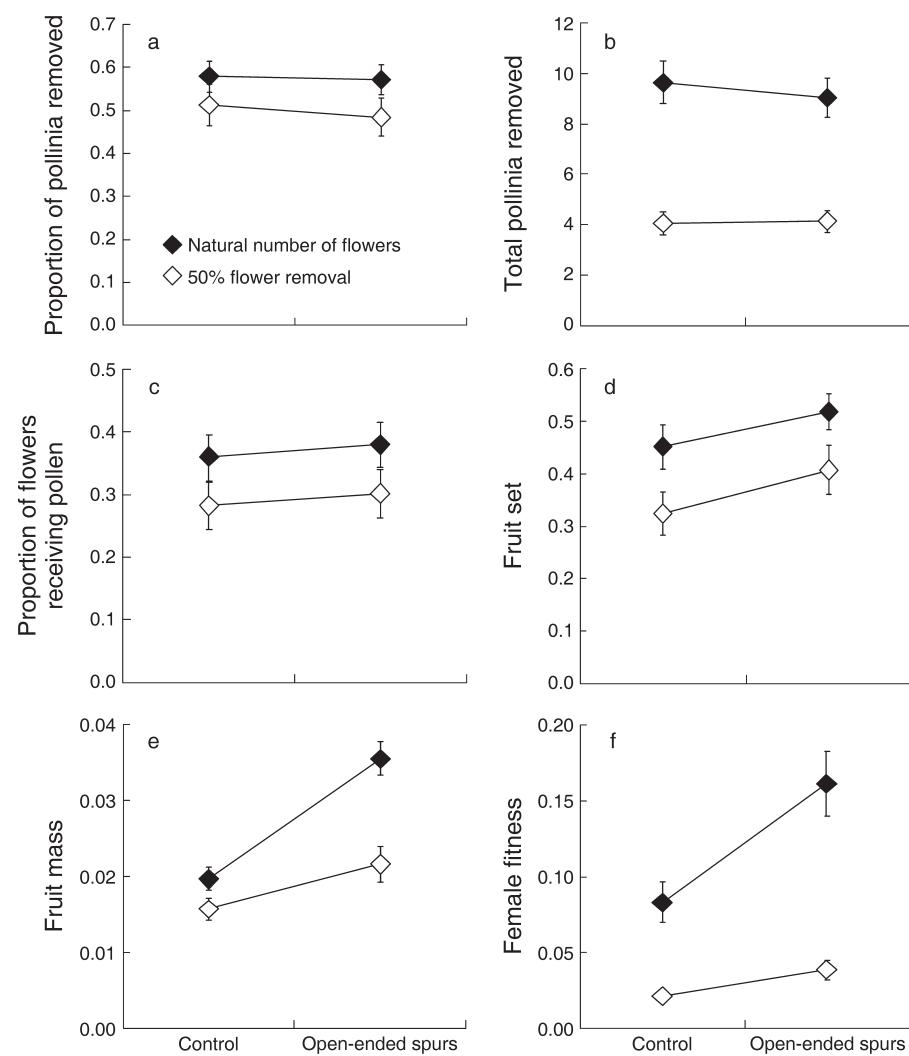


FIG. 2. The effects of number of flowers (natural vs. 50% removal) and spur length (natural vs. spur-tip removal) on (a) proportion of pollinia removed, (b) total number of pollinia removed, (c) proportion of flowers receiving pollen, (d) fruit set, (e) fruit mass, and (f) female fitness (number of fruits \times mean fruit mass) in the *Dactylorhiza lapponica* population at Tågdalen, Norway (means \pm SE).

additive (Table 1, Fig. 2d). Flower removal reduced fruit set by 29%, whereas the experimental increase in spur length increased fruit set by 16%. Flower removal also reduced mean fruit mass, while spur-tip removal increased fruit mass, and these effects were nonadditive (significant number of flowers \times spur length interaction in ANOVA; Table 1, Fig. 2e). The increase in fruit mass following spur manipulation was considerably stronger in plants with natural number of flowers (81%) than in plants with half of the flowers removed (38%; Fig. 2e). Removal of half of the flowers reduced female fitness, while spur-tip removal increased female fitness (Table 1, Fig. 2f). The increase in female fitness following spur manipulation tended to be larger in plants with natural number of flowers (94%) than in plants with half of the flowers removed (84%; Fig. 2f), but the interaction term was not statistically significant in the ANOVA (Table 1).

DISCUSSION

This study demonstrates that experimental manipulation of trait expression can reveal the mechanisms of pollinator-mediated selection, and help clarify how pollinator responses to variation in multiple traits translate into selection on floral trait associations. The studied population of *Dactylorhiza lapponica* has previously been shown to experience strong pollinator-mediated selection for more flowers and longer spurs, i.e., pollen limitation decreased both with increasing number of flowers and with increasing spur length (Sletvold et al. 2010a). The present results demonstrate that both traits are direct targets of selection, and that they partly influence different components of pollination success. The results also suggest nonadditive effects of flower production and spur length on female fitness,



PLATE 1. *Dactylorhiza lapponica* at the field site in Tågdalen, central Norway. Photo credit: N. Sletvold.

which is consistent with the prediction that traits influencing visitation rate and traits affecting pollination efficiency should be subject to correlational selection when seed production is limited by pollen.

Visual signals affecting long-distance pollinator attraction should be important for reproductive success in deceptive plants, but we are not aware of any previous study that has experimentally investigated the adaptive value of number of flowers in a deceptive orchid. Flower removal may influence female fitness through several mechanisms. It should decrease attractiveness to pollinators, but may also reduce attractiveness to herbivores and induce reallocation of resources to remaining flowers. In the present study, fruit herbivory was rare and removal of half of the flowers reduced female fitness of *D. lapponica* by on average 75%, i.e., by markedly more than expected from the reduction in number of flowers alone. The results thus suggest that the effect on pollination success dominated over any reallocation of resources. Both proportion of flowers forming a mature fruit and fruit size were reduced in the flower-removal treatment, which is consistent with reduced pollination success in plants with a small floral display. In contrast, the reduction in pollen removal was only marginally

stronger than expected by the direct effect of flower removal (58% vs. 50%). Since pollen packaging in orchids is associated with high pollen transfer efficiency compared to species with granular pollen (Harder and Johnson 2008), a low per-flower visitation rate is expected to suffice for successful completion of both sex functions. However, orchids produce many ovules per flower, and in *D. lapponica*, visitors often deposit only a few massulae per visit (J. Trunschke, personal communication; N. Sletvold, personal observation). The present results suggest that investment in display is more critical for pollen receipt than for pollen removal in the study population.

Traits such as corolla tube length (Alexandersson and Johnson 2002, Bloch and Erhardt 2008), and spur length (Nilsson 1988, Johnson and Steiner 1997) are known to influence the functional fit between pollinator and plant. In orchids like *D. lapponica*, where pollinia are placed on the head of the pollinator, spurs need to be at least as long as the pollinator proboscis to ensure effective contact with the stigma and anthers (Darwin 1862), and both female and male sex functions should be favored by long spurs. However, in *D. lapponica* only the estimate of female fitness was positively affected by the experimental increase in spur length. In contrast, experimental reduction in spur length has been documented to decrease pollen removal in several studies (Nilsson 1988, Boberg and Ågren 2009, Ellis and Johnson 2010). This suggests that most pollinator visits to intact flowers result in successful pollen removal, making it more difficult to detect an effect of improved fit than of reduced fit. However, spur length may still affect the precise placement of pollinia on the pollinator, thereby influencing pollen deposition and siring success. Spur manipulation increased all female fitness components except proportion of flowers receiving pollen. This suggests that the probability of at least some pollen receipt is unaffected by spur length, but that the quantity of pollen deposited can be too low to initiate fruit production. The proportion of flowers for which pollen receipt was recorded was lower than the proportion of flowers developing into fruits (Figs. 2c and d). Because pollen receipt is required for fruit development, this indicates low accuracy of the nondestructive recording of pollen receipt. However, the probability of detecting deposited massulae is not likely to have differed among experimental treatments. Manipulation of spur length should not affect the number of pollinator visits received, and the increase in fruit set following spur-tip removal suggests that closer contact between flower and pollinator is associated with more pollen being deposited per visit. A substantially higher efficiency of pollination in plants with open-ended spurs is also indicated by the 81% and 38% increase in fruit mass observed in the control and flower removal treatments, respectively (Fig. 2e). These relationships suggest that spur length limits the efficiency of pollen deposition, but not pollen removal in the study population.

The results of the present study suggest that floral traits in *D. lapponica* may evolve primarily through selection via female function, in contrast to the “male-function hypothesis” for floral evolution (reviewed in Burd and Callahan 2000). This is not surprising considering the strong pollen limitation of female fertility previously documented in the study population (Sletvold et al. 2010a) and commonly observed in deceptive species (Tremblay et al. 2005). However, only a low proportion of removed pollen reaches conspecific stigmas (on average 12% in the study population), and pollen removal is sometimes a poor proxy for male fitness also in orchids (Johnson et al. 2005, Ellis and Johnson 2010). The combination of floral manipulations and pollen tracking (cf. Ellis and Johnson 2010) or paternity analyses (Morgan and Conner 2001) can reveal selection through male function, and would allow a more stringent comparison of the strength of selection through male and female function in this and other deceptive and rewarding species.

The adaptive significance of floral traits is likely to depend on the expression of other floral traits (Meléndez-Ackerman and Campbell 1998, Vanhoenacker et al. 2010), and the fitness effects of traits influencing pollinator attraction and effectiveness should be inherently nonadditive when multiple visits are necessary to maximize reproductive success. In *D. lapponica*, combined trait manipulation resulted in a highly significant interaction effect on fruit mass, whereas the effect on fruit set was strictly additive. This suggests that relatively few visits are necessary to initiate fruit production, but that each flower needs multiple visits to maximize seed production. The results are consistent with seed production per fruit being proportional to the product of visitation frequency and pollen deposition per visit, and pollen transfer in the population being below saturation. Fruit set of control plants was 45%, which is comparable to the previous year (41%) when supplemental hand-pollination increased fruit set to 97%, indicating strong pollen limitation (Sletvold et al. 2010a). However, although the increase in female fitness following spur tip removal tended to be stronger in many-flowered plants (Fig. 2f), the interaction term was not statistically significant ($P = 0.097$). This is in accordance with the results of the previous study, which did not detect correlational selection on number of flowers and spur length in the studied *D. lapponica* population (Sletvold et al. 2010a), and suggests that only very strong nonadditive effects are likely to be detected by studies with sample sizes typical for estimates of phenotypic selection in the field (cf. Kingsolver et al. 2001).

Floral traits can often be expected to evolve in association, with selection acting on suites of traits resulting in character correlations (cf. Fægri and van der Pijl 1966, Armbruster et al. 2004). So far, this idea has been addressed mainly with observational approaches (but see Fenster et al. 2006, Valdivia and Niemeyer 2006). The experimental approach used here was

successful in distinguishing different mechanisms of pollinator-mediated selection, and allowed us to evaluate trait–fitness correlations both for individual traits and for trait combinations. To advance our understanding of floral integration and pollinator-mediated evolution, there is clearly a need for further detailed experimental studies of the importance of floral context for selection on floral characters (Campbell 2009).

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LITERATURE CITED

- Alexandersson, R., and S. D. Johnson. 2002. Pollinator-mediated selection on flower-tube length in a hawkmoth-pollinated *Gladiolus* (Iridaceae). *Proceedings of the Royal Society B* 269:631–636.
- Anderson, B., R. Alexandersson, and S. D. Johnson. 2010. Evolution and coexistence of pollination ecotypes in an African *Gladiolus* (Iridaceae). *Evolution* 64:960–972.
- Armbruster, W. S., C. Pélabon, T. F. Hansen, and C. P. H. Mulder. 2004. Floral integration, modularity, and accuracy: distinguishing complex adaptations from genetic constraints. Pages 23–79 in M. Pigliucci and K. Preston, editors. *Phenotypic integration: studying the ecology and evolution of complex phenotypes*. Oxford University Press, New York, New York, USA.
- Benítez-Vieyra, S., A. M. Medina, E. Glinos, and A. A. Cocucci. 2006. Pollinator-mediated selection on floral traits and size of floral display in *Cyclopogon elatus*, a sweat bee-pollinated orchid. *Functional Ecology* 20:948–957.
- Bloch, D., and A. Erhardt. 2008. Selection toward shorter flowers by butterflies whose proboscis are shorter than floral tubes. *Ecology* 89:2453–2460.
- Boberg, E., and J. Ågren. 2009. Despite their apparent integration, spur length but not perianth size affects reproductive success in the moth-pollinated orchid *Platanthera bifolia*. *Functional Ecology* 23:1022–1028.
- Burd, M., and H. S. Callahan. 2000. What does the male function hypothesis claim? *Journal of Evolutionary Biology* 13:735–742.
- Campbell, D. R. 2009. Using phenotypic manipulations to study multivariate selection of floral trait associations. *Annals of Botany* 103:1557–1566.
- Caruso, C. M., S. L. Scott, J. C. Wray, and C. A. Walsh. 2010. Pollinators, herbivores, and the maintenance of flower color variation: a case study with *Lobelia siphilitica*. *International Journal of Plant Sciences* 171:1020–1028.
- Conner, J. K. 2010. Natural selection in plants 151 years after the Origin: introduction. *International Journal of Plant Sciences* 171:927–929.
- Cuartas-Domínguez, M., and R. Medel. 2010. Pollinator-mediated selection and experimental manipulation of the flower phenotype in *Chloraea bletioides*. *Functional Ecology* 24:1219–1227.
- Darwin, C. R. 1862. On the various contrivances by which British and foreign orchids are fertilised by insects. Murray, London, UK.
- Ellis, A. G., and S. D. Johnson. 2010. Gender differences in the effects of floral spur length manipulation on fitness in a hermaphrodite orchid. *International Journal of Plant Sciences* 171:1010–1019.
- Fægri, K., and L. van der Pijl. 1966. The principles of pollination ecology. Pergamon, Oxford, UK.

- Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics* 35:375–403.
- Fenster, C. B., G. Cheely, M. R. Dudash, and R. T. Reynolds. 2006. Nectar reward and advertisement in hummingbird-pollinated *Silene virginica* (Caryophyllaceae). *American Journal of Botany* 93:1800–1807.
- Harder, L. D., and S. D. Johnson. 2008. Function and evolution of aggregated pollen in angiosperms. *International Journal of Plant Sciences* 169:59–78.
- Harder, L. D., and S. D. Johnson. 2009. Darwin's beautiful contrivances: evolutionary and functional evidence for floral adaptation. *New Phytologist* 183:530–545.
- Herrera, C. M., M. C. Castellanos, and M. Medrano. 2006. Geographical context of floral evolution: towards an improved research programme in floral diversification. Pages 278–294 in L. D. Harder and S. C. H. Barrett, editors. *Ecology and evolution of flowers*. Oxford University Press, New York, New York, USA.
- Ida, T. I., and G. Kudo. 2010. Modification of bumblebee behavior by floral color change and implications for pollen transfer in *Weigela middendorffiana*. *Evolutionary Ecology* 24:671–684.
- Johnson, S. D., P. R. Neal, and L. D. Harder. 2005. Pollen fates and the limits on male reproductive success in an orchid population. *Biological Journal of the Linnean Society* 86:175–190.
- Johnson, S. D., and K. E. Steiner. 1997. Long-tongued fly pollination and evolution of floral spur length in the *Disa draconis* complex (Orchidaceae). *Evolution* 51:45–53.
- Kay, K. M., and R. D. Sargent. 2009. The role of animal pollination in plant speciation: integrating ecology, geography, and genetics. *Annual Review of Ecology, Evolution and Systematics* 40:637–656.
- Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. *American Naturalist* 157:245–261.
- Meléndez-Ackerman, E. J., and D. R. Campbell. 1998. Adaptive significance of flower color and inter-trait correlations in an *Ipomopsis* hybrid zone. *Evolution* 52:1293–1303.
- Mitchell, R. J., R. E. Irwin, R. J. Flanagan, and J. D. Karron. 2009. Ecology and evolution of plant-pollinator interactions. *Annals of Botany* 103:1355–1363.
- Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural-selection: statistical inference and biological interpretation. *Evolution* 41:1149–1161.
- Morgan, M. T., and J. K. Conner. 2001. Using genetic markers to directly estimate male selection gradients. *Evolution* 55:272–281.
- Nilsson, L. A. 1988. The evolution of flowers with deep corolla tubes. *Nature* 334:147–149.
- Parachnowitsch, A. L., and A. Kessler. 2010. Pollinators exert natural selection on flower size and floral display in *Penstemon digitalis*. *New Phytologist* 188:393–402.
- Peakall, R., and S. N. Handel. 1993. Pollinators discriminate among floral heights of a sexually deceptive orchid: implications for selection. *Evolution* 47:1681–1687.
- Rausher, M. D. 1992. The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* 46:616–626.
- Sandring, S., and J. Ågren. 2009. Pollinator-mediated selection on floral display and flowering time in the perennial herb *Arabidopsis lyrata*. *Evolution* 63:1292–1300.
- Sapir, Y., and W. S. Armbruster. 2010. Pollinator-mediated selection and floral evolution: from pollination ecology to macroevolution. *New Phytologist* 188:303–306.
- Schemske, D. W., and H. D. Bradshaw. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences USA* 96:11910–11915.
- Sletvold, N., and J. Ågren. 2010. Pollinator-mediated selection on floral display and spur length in the orchid *Gymnadenia conopsea*. *International Journal of Plant Sciences* 171:999–1009.
- Sletvold, N., J. M. Grindeland, and J. Ågren. 2010a. Pollinator-mediated selection on floral traits and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*. *New Phytologist* 188:385–392.
- Sletvold, N., D.-I. Øien, and A. Moen. 2010b. Long-term influence of mowing on population dynamics in the rare orchid *Dactylorhiza lapponica*: the importance of recruitment and seed production. *Biological Conservation* 143:747–755.
- Stebbins, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York, New York, USA.
- Stebbins, G. L. 1970. Adaptive radiation of reproductive characteristics in angiosperms. I: Pollination mechanisms. *Annual Review of Ecology and Systematics* 1:307–326.
- Tremblay, R. L., J. D. Ackerman, J. K. Zimmerman, and R. N. Calvo. 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biological Journal of the Linnean Society* 84:1–54.
- Valdivia, C. E., and H. M. Niemeyer. 2006. Do pollinators simultaneously select for inflorescence size and amount of floral scents? An experimental assessment on *Escallonia myrtoidea*. *Austral Ecology* 31:897–903.
- Vanhoeacker, D., P. Toräng, J. Ågren, and J. Ehrlén. 2010. Morph-specific selection on floral traits in a polymorphic plant. *Journal of Evolutionary Biology* 23:1251–1260.
- Wade, M. J., and S. Kalisz. 1990. The causes of natural selection. *Evolution* 44:1947–1955.