

Pollinator-mediated selection on floral display, spur length and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*

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Summary

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- Nonrewarding animal-pollinated plants commonly experience severe pollen limitation, which should result in strong selection on traits affecting the success of pollination. However, the importance of pollinators as selective agents on floral traits in deceptive species has not been quantified experimentally.
- Here, we quantified pollinator-mediated selection ($\Delta\beta_{\text{poll}}$) on floral morphology and start of flowering in the deceptive orchid *Dactylorhiza lapponica* by subtracting estimates of selection gradients for plants receiving supplemental hand-pollination from estimates obtained for open-pollinated control plants.
- There was directional selection for taller plants with more flowers and longer spurs, but no statistically significant selection on corolla size or flowering start. Pollinator-mediated selection accounted for all observed selection on spur length ($\Delta\beta_{\text{poll}} = 0.32$), 76% of the selection on plant height ($\Delta\beta_{\text{poll}} = 0.19$) and 42% of the selection on number of flowers ($\Delta\beta_{\text{poll}} = 0.30$). Sixteen per cent of developing fruits were consumed by insect herbivores, but fruit herbivory had only minor effects on the strength of pollinator-mediated selection.
- Our results demonstrate that pollinators mediate selection on floral traits likely to affect both pollinator attraction and pollination efficiency, and are consistent with the hypothesis that deceptive species experience strong selection for increased display and mechanical fit between flower and pollinator.

Introduction

Plant–pollinator interactions are thought to have shaped patterns of floral diversity (Fenster *et al.*, 2004), and the prevalence of pollen limited female reproductive success (Knight *et al.*, 2005) suggests that pollinator-mediated selection on floral traits should be common (Ashman & Morgan, 2004). However, despite clear theoretical predictions, and the documentation of current selection on floral morphology and phenology in numerous plant populations (reviewed in Harder & Johnson, 2009), the importance of pollinators as selective agents has rarely been quantified experimentally, limiting our current understanding of their role in driving evolution of floral traits (Galen, 1996; Fishman & Willis, 2008; Sandring & Ågren, 2009).

Some of the most fascinating examples of floral adaptations to pollinators involve the orchid family (Darwin, 1862). A particularly striking feature of the orchid family is the fact that about one-third of the species are deceptive, that is, they produce no reward for their pollinators (Dafni, 1984). Although some deceptive orchids mimic female insects or co-occurring rewarding plants, the majority belong to the group of generalized food deceptive species (Nilsson, 1992), relying on pollination by naive insects searching for rewards. As pollinators often show strong abilities of associative learning (Biernaskie *et al.*, 2009), rewardless flowers may be avoided after a few visits (cf. Smithson & Macnair, 1997; Ferdy *et al.*, 1998), and food deceptive species are consequently expected to be strongly pollen limited. Indeed, very low natural levels of fruit set and pronounced pollen limitation have been documented

in several species (Neiland & Wilcock, 1998; Tremblay *et al.*, 2005), indicating conditions under which selection on floral traits contributing to pollinator attraction and pollination efficiency is expected (cf. Ashman & Morgan, 2004).

There is some evidence that measures of reproductive success are related to floral traits in deceptive species. Male and female pollination success was positively related to plant height in *Cypripedium acaule* (O'Connell & Johnston, 1998), the probability of fruit set was positively related to the number of flowers in *Anacamptis morio* (Johnson & Nilsson, 1999), spur length influenced the functional fit between pollinator and plant in the *Disa draconis* species complex (Johnson & Steiner, 1997), and frequency-dependent selection on petal colour has been documented in *Dactylorhiza sambucina* (Gigord *et al.*, 2001). Finally, a higher reproductive success for early-flowering individuals has been observed in some deceptive orchids (O'Connell & Johnston, 1998; Sun *et al.*, 2009). The common pattern of early spring-flowering in food deceptive species has been interpreted as an adaptation to secure pollination by inexperienced insects, avoiding competition from later-flowering rewarding species (Nilsson, 1980; Internicola *et al.*, 2008; but see Ruxton & Schaefer, 2009). Alternatively, reproductive success of deceptive species could be facilitated by flowering in synchrony with rewarding species, because the latter increase pollinator abundance in the area and may also increase visitation to nonrewarding co-occurring species (Alexandersson & Ågren, 1996; Johnson *et al.*, 2003).

Several agents of selection in addition to pollinators may influence selection on floral morphology and flowering phenology. Selection on traits such as plant height and number of flowers, which are usually tightly linked with plant size, may reflect selection for increased resource acquisition rather than increased pollination success (Harder & Johnson, 2009). Moreover, if floral phenology and display traits also influence the intensity of herbivory, selection may be mediated by antagonists (Armbruster, 1997). Conflicting selection on floral morphology resulting from interactions with pollinators and herbivores or seed predators have been demonstrated in several systems (Gómez, 2003; Strauss & Irwin, 2004; Toräng *et al.*, 2008). Similarly, selection on flowering phenology can be influenced by seasonal changes in abiotic conditions (Franks *et al.*, 2007) and in the intensity of interactions with antagonists (Elzinga *et al.*, 2007). Quantifying the relative importance of different agents of selection therefore requires experimental manipulation (cf. Wade & Kalisz, 1990).

Pollinator-mediated phenotypic selection has been identified by comparing the strength of selection in open-pollinated control plants and in plants receiving supplemental hand-pollination (Galen, 1996; Fishman & Willis, 2008; Sandring & Ågren, 2009). We have recently proposed that

the strength of pollinator-mediated selection ($\Delta\beta_{\text{poll}}$) can be quantified by subtracting estimates of selection gradients for plants receiving supplemental hand-pollination from estimates obtained for open-pollinated control plants (N. Sletvold & J. Ågren, unpublished). Here, we use this approach to quantify the strength of pollinator-mediated selection on floral display, spur length and flowering phenology in the bumblebee-pollinated deceptive orchid *Dactylorhiza lapponica*. Fruit herbivory is common in *D. lapponica*, and capsules may be completely consumed during development by lepidopteran larvae. We quantify pollinator-mediated selection on morphological traits likely to affect pollinator attraction (plant height, number of flowers and corolla size) and pollination efficiency (spur length) and flowering start, and we ask whether fruit herbivory is related to floral morphology or flowering phenology, and thus influences patterns of selection on floral traits.

Materials and Methods

Study species and site

Dactylorhiza lapponica (Laest. ex Hartm.) Soó (Orchidaceae) is a long-lived, tuberous and nonclonal orchid, occurring in Fennoscandia, Scotland and alpine areas in central Europe (Øien & Moen, 2002; Delforge & Harrap, 2006). In Fennoscandia, *D. lapponica* is found in open lawn communities in calcareous fens and springs in the boreal zone. Aboveground parts emerge in late May to early June, and include a single inflorescence with *c.* 3–15 flowers that open acropetally. The cerise flowers have a short spur, but no nectar production. The two pollinaria are situated above the spur entrance, and are composed of numerous massulae (tightly packed pollen-masses; Nazarov & Gerlach, 1997). *Dactylorhiza lapponica* is self-compatible, but depends on pollinators for successful fruit set (N Sletvold, unpublished). Low levels of fruit set are typical (20–30%; Sletvold *et al.*, 2010). Fruits mature 3–5 wk after pollination, and the minute seeds are dispersed by wind in autumn.

The present study was conducted in a population of *c.* 500 flowering individuals located at *c.* 450 m asl within the nature reserve Tågdalen in central Norway (63°03'N, 9°05'E). Tågdalen has an oceanic climate, with mean temperature in July of 11.2°C, and fairly high annual precipitation (1507 mm). The study population is found in an open, wet fen, dominated by the bryophytes *Campylium stellatum* (Hedw.) Lange and Jensen and *Scorpidium cossonii* (Schimp.) Hedenäs, as well as the vascular plants *Eriophorum latifolium* Hoppe, *Molinia caerulea* (L.) Moench, *Succisa pratensis* Moench and *Trichophorum cespitosum* (L.) Hartm. The *D. lapponica* population flowers during 2–4 wk from mid June, and is pollinated by *Bombus pascuorum* and *B. lucorum*, both frequent visitors on the common, co-flowering rewarding species *Bartsia alpina* L.

and *Pedicularis palustris* L. (N. Sletvold, pers. obs.). Mean proboscis length of worker bees is 7.6 mm in *B. pascuorum* and 5.8 mm in *B. lucorum* (<http://www.bumblebee.org/bodyTongue.htm>). Herbivory by lepidopteran larvae (unknown spp.) that consume the entire fruit is relatively common.

Field experiment and measured traits

In mid June 2009, a total of 250 plants with flower buds were haphazardly chosen and individually tagged. We randomly assigned 85 plants to the supplemental hand-pollination treatment, and 165 to the open-pollinated control treatment. The study population was visited every day during the onset of flowering and approximately every third day throughout the rest of the flowering period. On each visit, all open flowers on plants in the experimental treatment were pollinated by hand with cross pollen, and all flowers received supplemental pollen at least once. Pollinations were performed by rubbing one or two pollinia across each stigma, saturating the surface with pollen. Pollinaria were collected haphazardly from within the group of plants receiving supplemental hand-pollination; the distance to pollen donors varied from a few dm to c. 50 m. During its flowering period, a hand-pollinated plant received pollen from multiple donors.

For each plant, we recorded start of flowering (reported as Julian day) and plant height to the nearest mm (distance from the ground to the topmost flower) on the day the first flower opened. On one of the two lowermost flowers on each individual we measured spur length (distance from corolla to spur tip) and maximum corolla width and height to the nearest 0.1 mm with digital callipers. Corolla size was quantified as the product of width and height. The number of flowers was noted at the end of the flowering period. We recorded the number of initiated fruits 7–10 d after the end of flowering, and the number of intact fruits (number of initiated fruits minus

number of fruits consumed by herbivores) at fruit maturation, and we harvested up to three intact nondehiscent capsules from each plant and determined mean fruit mass. 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 intact fruits and mean fruit mass. To estimate what female fitness would have been in the absence of insect herbivory, we multiplied number of initiated fruits by mean fruit mass. This estimate is based on the assumptions that consumed fruits were of average size, and that fruit herbivory did not affect the size of the remaining fruits. In the few cases where the herbivore left behind an intact capsule wall (containing a single exit hole), fruit volume did not differ between the consumed fruit and the one situated immediately below or above in the inflorescence (paired t -test, $P = 0.32$, $n = 10$). The second assumption seems reasonable as fruit herbivory occurred late during fruit development. We quantified pollen limitation (PL) before and after herbivory as $1 - (\text{mean female fitness of open-pollinated control plants} / \text{mean female fitness of hand-pollinated plants})$.

Flower production, corolla size and spur length did not differ between treatment groups, but plants receiving supplemental hand-pollination were, on average, somewhat taller and started to flower marginally later than open-pollinated control plants (Table 1).

Statistical analyses

The effect of pollination treatment on plant performance and herbivory was examined by one-way ANOVA. Fruit set (i.e. the proportion of flowers initiating fruits) and herbivory (the proportion of initiated fruits consumed by herbivores) were arcsine square-root transformed before analyses.

Selection was estimated following Lande & Arnold (1983), using multiple regression analyses with relative

Table 1 Trait mean \pm SD for open-pollinated control plants (C) and plants receiving supplemental hand-pollination (HP) in the *Dactylorhiza lapponica* population at Tågdalen, Norway

Trait	C ($n = 165$)	HP ($n = 85$)	<i>P</i>
Plant height (cm)	14.1 \pm 3.6	15.4 \pm 2.7	0.0039
Number of flowers	8.8 \pm 3.1	9.0 \pm 2.7	0.63
Corolla size (mm ²)	93.2 \pm 15.9	95.7 \pm 15.5	0.24
Spur length (mm)	7.6 \pm 0.9	7.7 \pm 0.8	0.44
Flowering start (Julian day)	172.8 \pm 1.6	173.4 \pm 1.6	0.0091
Fruit set (proportion of fruits initiating fruit development)	0.41 \pm 0.25	0.97 \pm 0.07	< 0.0001
Number of fruits consumed	0.70 \pm 1.09	0.72 \pm 1.38	0.91
Proportion of initiated fruits consumed	0.16 \pm 0.25	0.08 \pm 0.16	0.008
Number of intact fruits	3.2 \pm 2.6	8.0 \pm 2.8	< 0.0001
Fruit mass (mg)	0.0102 \pm 0.0060	0.0159 \pm 0.0045	< 0.0001
Fitness (number of intact fruits \times fruit mass)	0.045 \pm 0.061	0.133 \pm 0.073	< 0.0001

P-value associated with the effect of pollination treatment in ANOVA.

female fitness (individual fitness divided by mean fitness) as the response variable and standardized trait values (with a mean of 0 and a variance of 1) as explanatory variables. Fitness was relativized and traits were standardized separately for each treatment. We initially included quadratic terms (γ_{ii}) to quantify nonlinear selection, but none of the quadratic gradients were statistically significant, and they improved model fit only marginally, as indicated by R^2 -values. We therefore report only linear gradients. Multicollinearity was assessed by inspection of variance inflation factors that in no case exceeded 2.5, indicating that the level of collinearity was not problematic (Quinn & Keough, 2002).

We used ANCOVA to determine whether pollination treatment influenced linear selection gradients. The model included relative fitness as the dependent variable and the five standardized traits (plant height, number of flowers, corolla size, spur length and start of flowering), pollination treatment (open-pollinated control vs hand-pollination) and the trait \times pollination treatment interaction as independent variables. To quantify the importance of pollinator-mediated selection, we subtracted for each trait the estimated selection gradient for plants receiving supplemental hand-pollination (β_{HP}) from the estimate obtained for open-pollinated controls (β_C), $\Delta\beta_{poll} = \beta_C - \beta_{HP}$. To determine whether fruit herbivory influenced patterns of selection, we first used linear regression to determine whether the proportion of fruits consumed by herbivores was related to floral display, phenology or fruit production. Second, we compared selection models using relative fitness after herbivory (number of intact fruits \times mean fruit mass) and relative fitness before herbivory (number of initiated fruits \times mean fruit mass) as the dependent variable. Finally, we conducted selective source analysis (Ridenhour, 2005) within each pollination treatment, where the effect of herbivory was included as the number of consumed fruits. Because the second and third approaches yielded identical conclusions, we report only the results from the former, mathematically simpler approach. Selection gradients were illustrated with added-variable plots, in which the residuals from a linear regression model of relative fitness on all traits

except the focal trait are plotted against the residuals from a regression model of the focal trait on the other traits (Cook, 1996). Analyses were performed in SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Floral traits and pollen limitation

Floral traits were moderately positively correlated, with the exception that plants with many flowers tended to start flowering early. Tall plants tended to have many flowers with large corollas and long spurs and started flowering late (Table 2).

On average, less than one fruit per plant was consumed by herbivores, and this did not differ between pollination treatments (Table 1). However, because fruit initiation was lower in the control treatment, this represented a higher proportion of damaged fruits among open-pollinated controls than among hand-pollinated plants (Table 1).

Hand-pollinated plants had about three times higher female fitness (number of intact fruits \times mean fruit mass) compared with open-pollinated controls (Table 1). Fruit set (the proportion of flowers initiating fruit development) and number of intact fruits were more than twice as high in hand-pollinated plants compared with open-pollinated controls, and hand-pollination increased fruit mass by > 50%. Fruit production, fruit mass and the combined fitness estimate were strongly pollen limited (Table 1). Pollen limitation (PL = 1 - (mean female fitness of open-pollinated control plants/mean female fitness of hand-pollinated plants)) was 0.64 before herbivory and 0.66 after herbivory.

Pollinator-mediated selection

There was directional selection for taller plants with more flowers and longer spurs in the study population (open-pollinated controls; Table 3, Fig. 1a,b,d). Corolla size and start of flowering were not subject to statistically significant selection (Table 3, Fig. 1c,e).

Table 2 Phenotypic correlations among traits in the *Dactylorhiza lapponica* population at Tågdalen, Norway, based on open-pollinated control plants (above diagonal, $n = 165$) and hand-pollinated plants (below diagonal, $n = 85$)

Trait	Plant height (cm)	Number of flowers	Corolla size (mm ²)	Start of flowering (JD)	Spur length
Plant height		0.42***	0.47***	0.45***	0.14***
Number of flowers	0.47***		0.36***	-0.24**	0.085*
Corolla size	0.55***	0.38***		0.063	0.37***
Start of flowering	0.26***	-0.19**	-0.056		0.22*
Spur length	0.32***	0.22**	0.36***	0.11	

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

JD, Julian day.

Table 3 Phenotypic linear selection gradients (\pm SE) for open-pollinated control plants (β_C) and for plants receiving supplemental hand-pollination (β_{HP}) in a population of *Dactylorhiza lapponica*

Trait	β_C ($n = 165$)	β_{HP} ($n = 85$)	$\Delta\beta_{poll}$	P Trait \times Poll
Plant height	$0.25 \pm 0.066^{**}$	0.064 ± 0.051	0.19	0.042
Number of flowers	$0.71 \pm 0.092^{***}$	$0.41 \pm 0.044^{***}$	0.30	< 0.0001
Corolla size	-0.081 ± 0.086	0.060 ± 0.042	-0.14	0.45
Spur length	$0.30 \pm 0.068^{***}$	-0.024 ± 0.037	0.32	< 0.0001
Start of flowering	-0.071 ± 0.080	0.007 ± 0.045	-0.078	0.58

P -values associated with the effect of trait \times pollination treatment interaction in ANCOVA are indicated ($P < 0.05$ in bold).

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

$\Delta\beta_{poll}$ is the strength of pollinator-mediated selection ($\Delta\beta_{poll} = \beta_C - \beta_{HP}$).

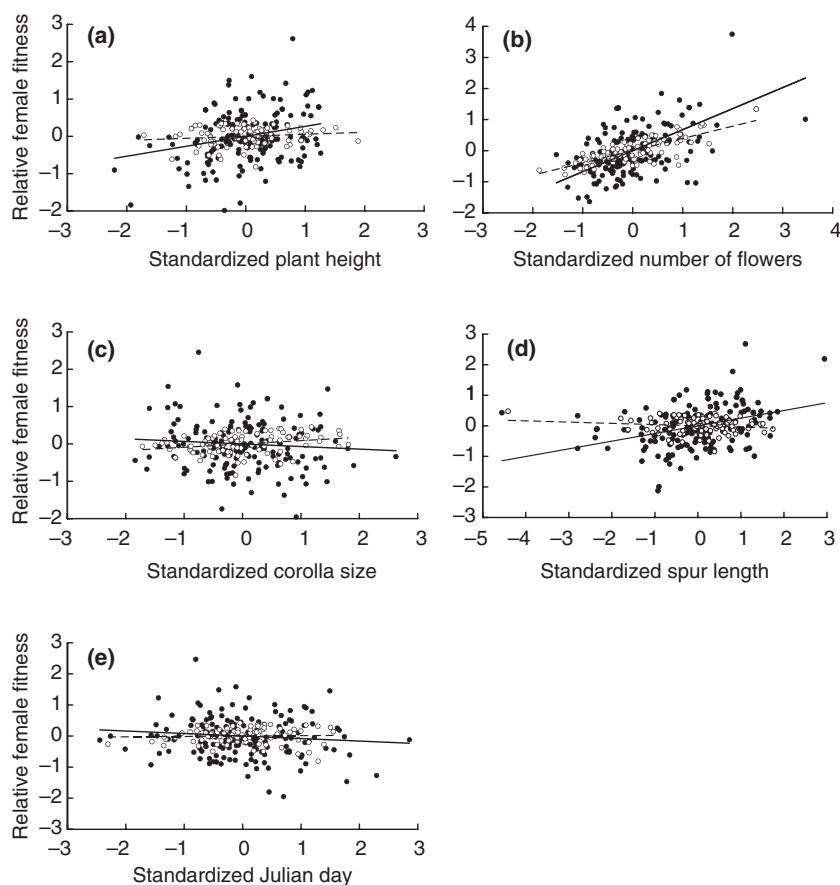


Fig. 1 Standardized linear phenotypic selection gradients for (a) plant height, (b) number of flowers, (c) corolla size, (d) spur length and (e) start of flowering in open-pollinated control plants (closed circles, solid line) and in plants receiving supplemental hand pollination (open circles, dashed line) in the *Dactylorhiza lapponica* population at Tågdalen, Norway. Selection gradients are illustrated with added-variable plots, in which the residuals from a linear regression model of relative fitness on all traits except the focal trait are plotted against the residuals from a regression model of the focal trait on the other four traits.

Interactions with pollinators contributed to selection on all three traits that experienced selection, that is, linear selection gradients for plant height, number of flowers and spur length all differed significantly between the two pollination treatments (Table 3, Fig. 1a,b,d). The strength of pollinator-mediated selection on the three traits was of similar

magnitude ($\Delta\beta_{poll} = 0.19$ – 0.32 ; Table 3), but represented variable proportions of the selection documented among open-pollinated plants. All selection on spur length could be attributed to interactions with pollinators; among hand-pollinated plants, the estimated selection gradient for spur length was low and not significantly different from zero

(Table 3). Pollinators also mediated most of the selection for taller plants (76%), and the selection gradient in the hand-pollination treatment was weak and not statistically significant. A smaller proportion of the selection on number of flowers was caused by plant–pollinator interactions (42%), and there was also directional selection for more flowers in the hand-pollinated treatment (Table 3).

Herbivory had only minor effects on patterns of selection. The proportion of fruits consumed by herbivores was not significantly related to any of the traits included in the selection analysis (linear regressions, $P > 0.10$), to the number of initiated fruits or to mean fruit mass ($P > 0.30$). Selection gradients estimated with fitness calculated from number of intact fruits were in all cases similar to those estimated with fitness calculated from number of initiated fruits, and herbivory had little effect on the estimated strength of pollinator-mediated selection (maximum change in $\Delta\beta_{\text{poll}}$ was 0.03; data not shown).

Discussion

Deceptive animal-pollinated species commonly experience severe pollen limitation, which should result in pollinator-mediated selection for increased attractiveness and pollination efficiency. Consistent with this prediction, we demonstrate that interactions with pollinators are responsible for much of the selection observed on plant stature, flower production and spur length in a natural population of the deceptive orchid *D. lapponica*. This is the first study to quantify experimentally the strength of pollinator-mediated selection in a deceptive species.

There was directional selection for more flowers both among open-pollinated controls and among plants receiving supplemental hand-pollination. As flower production sets an upper limit to seed production, selection for more flowers is expected when seed production is used as a proxy for female fitness. However, the number of open flowers should also influence attractiveness to pollinators (Mitchell *et al.*, 2004; Grindeland *et al.*, 2005), and in the present study, pollinators accounted for 42% of the strong directional selection for more flowers ($\Delta\beta_{\text{poll}} = 0.30$). Substantial pollinator-mediated selection on number of flowers has also been documented in the insect-pollinated rewarding orchid *Gymnadenia conopsea* ($\Delta\beta_{\text{poll}}$, range 0.05–0.21, N Sletvold & J Ågren, unpublished) and in the insect-pollinated, self-incompatible herb *Arabidopsis lyrata* ($\Delta\beta_{\text{poll}}$, range 0.21–0.81; Sandring & Ågren, 2009), suggesting that flower production frequently affects fitness via positive effects on pollination success.

There was also directional selection for taller plants in the study population, and 76% of the observed selection on plant height could be attributed to interactions with pollinators ($\Delta\beta_{\text{poll}} = 0.19$). The importance of plant stature for pollination success should depend on vegetation height

(Toräng *et al.*, 2006). The study site is dominated by herbs that are taller than *D. lapponica*, and plant height is likely to affect the probability of pollinator visitation. Phenotypic selection for taller plants has previously been documented in the food deceptive orchid *Cypripedium acaule* (O'Connell & Johnston, 1998), where selection varied among microhabitats that differed in canopy cover and shrub density. Taken together, the present results demonstrate that visual cues such as flower production and plant height strongly influence pollination success in *D. lapponica*. Our results are consistent with the view that visual signals affecting long-distance attraction should be of major importance for pollination success in food deceptive plants.

Pollinators were responsible for all selection for increased spur length in the study population ($\Delta\beta_{\text{poll}} = 0.32$). This could be the result of an enhanced mechanical fit between the pollinator and the flower in long-spurred plants, increasing pollination efficiency. In orchids, precise pollen transfer usually requires a close morphological match between flower and pollinator. As far as we know, no previous study has documented phenotypic selection on spur length in natural populations of food deceptive species, but experimental shortening of spurs significantly reduced pollen deposition and fruit set in the deceptive, fly-pollinated *Disa draconis* (Johnson & Steiner, 1997). A correlation between tongue length of the local pollinator and spur length has also been documented in *Disa ferruginea* (Johnson, 1994) and *Disa draconis* (Johnson & Steiner, 1997), in line with expectations of pollinator-driven diversification. In both these species, the pollinaria are placed along the length of the fly's proboscis, and pollination will occur even if flower spurs are shorter than the proboscis length of the pollinators. In bee-pollinated species such as *D. lapponica*, pollinaria are usually attached to the head of the bee, and pollen deposition (and removal) should be unlikely if spurs are shorter than the bee's proboscis. Minimum spur length in the present study population (3.8 mm) was shorter than the proboscis length of the shorter-tongued pollinator *B. lucorum* (5.8 mm), suggesting that failure to contact the stigma may have caused low pollination success in short-spurred plants. Pollinator observations and spur length manipulations should be conducted to reveal the mechanisms underlying the relationship between spur length and female fitness in *D. lapponica*.

In contrast to earlier studies of deceptive species (O'Connell & Johnston, 1998; Sun *et al.*, 2009), we found no statistically significant selection on start of flowering in the study population. The low and nonsignificant estimate of pollinator-mediated selection on start of flowering in *D. lapponica* ($\Delta\beta_{\text{poll}} = -0.09$) indicates limited seasonal variation in pollination success. This may be related to slow learning when bees forage on deceptive plants intermingled with rewarding species. Johnson *et al.*

(2003) found that queens of *Bombus lapidarius* continued to probe flowers of the deceptive orchid *Anacamptis morio* after several weeks of experience, despite evidence of earlier visits to the species.

Conflicting selection on floral characters resulting from interactions with pollinators and herbivores has been demonstrated in several systems (Gómez, 2003; Strauss & Irwin, 2004; Toräng *et al.*, 2008), indicating that shifts in the direction of selection on floral traits may be common in plants that interact with several animals. In *D. lapponica*, there was no indication that herbivores were attracted by the same cues as pollinators. Tall, many-flowered plants did not experience a higher proportion of damage, and there was no indication that herbivores preferred plants with high pollination success. As a result, herbivory had little effect on patterns of selection and the strength of pollinator-mediated selection. Damage intensity was rather low in the present study. However, because damage was unrelated to display size, it is unlikely that herbivory would lead to conflicting selection on floral traits even at higher damage intensities.

The present approach to quantify pollinator-mediated selection shares a general problem with supplemental hand-pollination experiments (Aizen & Harder, 2007) in that it cannot by itself distinguish between effects of trait variation on the quantity and on the quality of pollen received. However, the effect of our hand-pollination treatment on the quality component may have been limited. Because *D. lapponica* is deceptive, the rate of self-pollination is likely to be low (cf. Johnson *et al.*, 2004), and pollen-transport between nearest neighbours is also likely to be lower than in species with rewarding flowers. Our hand-pollination treatment is thus not likely to have dramatically changed the frequency of self-pollination or matings between close relatives. Moreover, the difference in quality of pollen deposited is likely to be low because hand-pollinated plants received a mixture of pollen transferred by bees and pollen added by hand (cf. Ashman *et al.*, 2004). To conclude, the documented pollinator-mediated selection on floral traits may to a large extent reflect variation in the quantity of pollen deposited.

While the evolutionary origin and maintenance of pollination by deceit has been a topic of major research interest (reviewed in Cozzolino & Widmer, 2005; Schiestl, 2005), few have studied phenotypic selection on floral traits in deceptive species. Our results have shown experimentally that pollinators contribute strongly to selection on flower production and that they are the main selective agents on plant height and spur length in *D. lapponica*. Selection on traits affecting display and pollination efficiency is consistent with expectations in species that experience few pollinator visits. Although a deceptive pollination system should preclude coevolution of pollinators and plants, the present results suggest a strong role of pollinators as drivers of floral evolution in deceptive species.

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