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## **Resource- and pollinator-mediated selection on floral traits**

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RT: Resource- and pollinator-mediated selection

### **Summary**

1. Female reproductive success is predicted to be simultaneously limited by the availability of pollen and resources. Selection on floral traits results from both factors, but their relative importance and interaction is poorly understood.

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2. We increased nutrient and pollen availability of the orchid *Dactylorhiza lapponica* in a factorial experiment to quantify resource- and pollinator-mediated selection on floral traits. Hand-pollination increased female fitness (number of fruits  $\times$  mean fruit mass) by 74% in both nutrient treatments, whereas nutrient addition did not significantly affect female fitness.
3. There was selection for more flowers and longer spurs, and selection on spur length was significantly pollinator-mediated and of similar strength across nutrient treatments ( $\Delta\beta_{\text{poll}} = 0.54$  and  $\Delta\beta_{\text{poll\_NPK}} = 0.59$ ). There was no statistically significant resource-mediated selection. Nutrient addition increased flower size the following year, but did not affect flower or fruit production, or selection on any trait.
4. The results demonstrate that *D. lapponica* does not increase flower production in response to nutrient addition, that the increase in female fitness in response to hand-pollination is not resource limited, and suggest that natural resource variation does not influence selection on floral traits. The study illustrates that crossed manipulations of pollen and resources can clarify their relative importance for selection on floral traits.

**Key-words:** biotic interactions, floral display, hand-pollination, natural selection, nutrient-addition, pollen limitation, resource limitation

## Introduction

Female reproductive success is predicted to be simultaneously limited by pollen and resources over a plant's lifetime (Haig & Westoby 1988; Ashman *et al.* 2004). Thus, a plant with optimal allocation to female function is not expected to respond strongly to increased pollen availability, because resources should rapidly become limiting (Haig & Westoby 1988).

Contrary to this prediction, a multitude of studies have found that seed production in a single year can be substantially increased by supplemental hand-pollination, indicating that the resources needed to capitalize on increased pollen loads are available (Ashman *et al.* 2004; Knight *et al.* 2005). This prevalence of pollen limitation can be reconciled with the Haig & Westoby model if stochastic variation in pollination success and resource supply is considered (Burd 2008; Rosenheim, Williams & Schreiber 2014), and sets the stage for pollinator-mediated selection on floral traits that enhance pollen receipt (Sletvold & Ågren 2014, and references therein).

A positive response to pollen supplementation does not preclude the existence of resource limitation. Increased resource availability could allow the plant to invest more in floral traits that increase pollination success (Campbell & Halama 1993), or augment the response to a high pollination success, leading to higher fruit and seed production (Mattila & Kuitunen 2000). Combined addition of both pollen and resources is thus necessary to reveal their relative role in maximising female fitness and governing selection on floral traits. Studies taking such a combined approach to study the factors regulating seed production have found evidence of resource limitation (Delph 1986; Caruso, Remington & Ostergren 2005), pollen limitation (Juenger & Bergelson 1997), or both factors (Vaughton 1991; Campbell & Halama 1993; Mattila & Kuitunen 2000; Asikainen & Mutikainen 2005; Brookes, Jesson & Burd 2008), but it is noteworthy that none of the latter found any significant interactive effects of pollen and resource addition.

In the case of simultaneous pollen and resource limitation, resource supplementation can alter natural selection on floral traits in two ways. First, it may change the functional relationship between trait expression and fitness, by altering the phenotypic trait distribution or by increasing the response to higher pollen receipt by allowing a higher fruit and seed production. Second, it may increase population mean fitness, and thus lower the opportunity

for selection (Rundle & Vamosi 1996). It is not clear how such effects would influence the strength of pollinator-mediated selection on floral traits. In natural populations, variation in plant size and floral traits is expected to be correlated with local resource availability, and it is likely that resource addition would diminish variation in both trait expression and female fitness, leading to weaker functional relationships and reduced opportunity for selection. For example, nutrient addition increased fruit production in small individuals of the orchid *Platanthera bifolia*, but had no effect in large individuals (Mattila & Kuitunen 2000).

Alternatively, it is possible that large plants respond more strongly to resource supplementation because they are more vigorous, thereby increasing variation in trait expression and fitness, and also the opportunity for selection. Finally, resource addition may increase trait values and/or fitness equally across phenotypes, with minor effects on selection.

To our knowledge, Caruso *et al.* (2005) is the only study that has quantified phenotypic selection on floral traits after manipulating both pollen and resource availability, although not in a crossed design. They found that resource supplementation in a natural population of *Asclepias syriaca* increased fruit production by 32%, but did not affect trait distributions or phenotypic selection on floral traits. There was no indication of pollen limitation. To test the hypothesis that selection on floral traits is influenced by variation in both pollen and resources, crossed manipulations should be informative.

The deceptive bumble bee-pollinated orchid *Dactylorhiza lapponica* is strongly pollen limited and experiences pollinator-mediated selection on floral display and spur length (Sletvold, Grindeland & Ågren 2010; 2013). In the studied population, individual fruit set rarely exceeds 50%, and fruit production varies widely among individuals. Supplemental hand-pollination regularly increases fruit set to 95-100%, indicating that pollen limitation dominates over resource limitation (Sletvold & Ågren 2014). Hand-pollination also increases number of seeds per fruit (Sletvold *et al.* 2010). However, a previous experiment documented

co-limitation by N and P of plant growth of several species in the habitat of the study population, and found increased nutrient concentrations in aboveground biomass after two years of NPK addition (Øien 2004). Here, we manipulated pollen and resource availability in a factorial design, to test the hypothesis that pollinator-mediated selection is affected by resource availability. Because nutrient addition was performed close to the start of flowering and corolla size and spur length was measured early in the flowering period, we do not expect nutrient effects on floral traits other than number of flowers. We ask whether 1) *D. lapponica* is resource limited, i.e., nutrient supplementation increases flower production and/or female fitness, 2) there is resource-mediated selection on floral traits, and 3) nutrient addition alters pollinator-mediated selection on floral traits, i.e., effects of resource and pollen supplementation are non-additive.

## Materials and Methods

### Study species and site

*Dactylorhiza lapponica* (Laest. ex Hartm.) Soó (Orchidaceae) is a long-lived, non-clonal terrestrial orchid, occurring 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-early June, and flowering individuals produce a single 7-30 cm tall inflorescence with ca 2-25 cerise, short-spurred flowers without nectar production. Flowers open from the base of the inflorescence and upward. The pollination system is generalized food deception.

The present study was conducted in a population of approximately 1000 flowering individuals located at Sølendet, ca 750 m a.s.l. in central Norway (62°40'N, 11°50'E). The area consists of rich fens and moist grasslands that are mown extensively to maintain an open landscape. A nutrient addition experiment at Sølendet documented co-limitation by N and P

of plant growth at both the community level (above-ground biomass and shoot density) and in most of the dominant and sub-dominant species studied (Øien 2004). The population flowers during 3-4 weeks from mid-late June. The study population is bumblebee pollinated, and fruit production and fruit mass are strongly pollen limited (Sletvold & Ågren 2014).

#### Field experiment

To quantify resource- and pollinator-mediated selection, we manipulated resources and pollination regime in a factorial design (control vs. nutrient supplementation, open-pollinated control vs. supplemental hand-pollination, respectively).

In June 2014, a total of 360 plants with flower buds were haphazardly chosen and individually tagged. We randomly assigned 90 plants to each of the four treatment combinations in the factorial experiment. For each plant, we measured plant height to the nearest mm (distance from the ground to the topmost flower), and on one of the three 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 calipers. Corolla size was quantified as the product of width and height. We noted the total number of flowers at the end of the flowering period. We cut all the vegetation within 15 cm of all focal plants to about 2 cm above the ground to prevent indirect effects of resource addition via increased competition. In the nutrient-addition treatment, we added 10 NPK pellets (NPK Blå Korn 12-3-13, Bayer Environmental Science) within 15 cm of the focal plant. In the hand-pollination treatment, all flowers were cross-pollinated by rubbing one or two pollinia across each stigma, saturating the surface with pollen. We used pollinia from within the group of plants receiving supplemental hand-pollination, but also from plants not included in the experiment. During its flowering period, a hand-pollinated plant received pollen from multiple donors. At fruit maturation, we recorded the number of mature fruits produced and harvested up to three mature fruits 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; Sletvold *et al.* 2010). For each plant, we estimated female fitness as the product of number of fruits and mean fruit mass. We quantified pollen limitation as  $1 - (\text{mean female fitness of open-pollinated control plants} / \text{mean female fitness of hand-pollinated plants})$ . For each treatment, we calculated the opportunity for selection as the variance in relative female fitness. Due to a late incidence of frost, sample size was reduced to 50-60 individuals in each of the treatments.

The nutrient treatment was repeated in June 2015, prior to flowering. In July 2015, experimental plants were checked again, and floral traits were measured as in 2014 for all flowering plants. Fruit production was recorded at maturation, and we estimated female fitness as number of fruits.

#### Statistical analyses

We used two-way ANOVA to examine the effects of nutrient addition (control vs. NPK supplementation), pollination treatment (open-pollinated control vs. supplemental hand-pollination) and their interaction on floral traits and measures of reproductive success in both years. In these models, a significant interaction term between nutrient and pollination treatment demonstrates that the strength of pollen limitation depends on resource availability. In the second year, we included trait value in the first year as a covariate in the analyses of floral traits. Number of fruits and female fitness were square-root transformed to improve normality of residuals.

Selection was estimated following Lande & Arnold (1983), using multiple regression analyses with relative 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. To limit model complexity in analyses of treatment effects, we only included linear

selection gradients. Phenotypic correlations among traits were weak and mostly non-significant, with the strongest being observed between corolla area and spur length ( $r = 0.52$ ) and between plant height and number of flowers ( $r = 0.36$ ). Inspection of variance inflation factors (VIF) indicated no problems of collinearity in the selection models (all VIFs  $< 2.0$ ).

We used ANCOVA to determine whether nutrient and pollination treatments influenced linear selection gradients. The model included relative fitness as the dependent variable and the four standardized traits (plant height, number of flowers, corolla size and spur length), nutrient treatment (control vs. supplementation), pollination treatment (open-pollinated control vs. hand-pollination), and trait  $\times$  nutrient treatment, trait  $\times$  pollination treatment and trait  $\times$  nutrient  $\times$  pollination treatment interactions as independent variables. In this model, a significant trait  $\times$  pollination interaction indicates pollinator-mediated selection, a significant trait  $\times$  nutrient interaction indicates nutrient-mediated selection, while a significant three-way interaction term demonstrates that the strength of pollinator-mediated selection depends on resource availability. For each nutrient treatment, we quantified pollinator-mediated selection by subtracting for each trait the estimated selection gradient for plants receiving supplemental hand-pollination ( $\beta_{HP}$ ) from the estimate obtained for open-pollinated controls ( $\beta_C$ ),  $\Delta\beta_{poll} = \beta_C - \beta_{HP}$  (cf. Sletvold & Ågren 2010). For each pollination treatment, we quantified resource-mediated selection by subtracting for each trait the estimated selection gradient for plants receiving nutrient addition ( $\beta_{NPK}$ ) from the estimate obtained for plants experiencing natural nutrient levels ( $\beta_{nat}$ ),  $\Delta\beta_{res} = \beta_{nat} - \beta_{NPK}$ .

In the second year, too few individuals flowered to allow estimation of selection gradients, and we instead used univariate regressions to calculate selection differentials for plant height, number of flowers, corolla size and spur length. We used ANCOVA to determine whether nutrient and pollination treatments in the previous year influenced linear



selection differentials. Analyses were performed in SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

## Results

### Pollen- and resource-limitation in 2014

Supplemental hand-pollination significantly increased number of fruits, fruit mass and female fitness (Table 1). Nutrient addition tended to increase fruit mass (by 22% in C and 6% in HP,  $P = 0.074$ ), but did not significantly increase number of flowers, number of fruits or female fitness. No interactions between pollen and nutrient addition were significant (Table 1), and pollen limitation of female fitness was identical (0.74) in both nutrient treatments. The opportunity for selection was strongly influenced by pollination treatment, but was almost identical across resource treatments (control vs. nutrient addition: 2.32 vs. 2.18 in open-pollinated plants and 0.96 vs. 0.80 in hand-pollinated plants).

### Pollinator-mediated and resource-mediated selection

There was selection for more flowers and longer spurs in both open-pollinated treatments, and selection on spur length was significantly pollinator-mediated (significant spur  $\times$  pollination interaction; Table 2, Figure 1). The strength of pollinator-mediated selection was similar in the two nutrient treatments ( $\Delta\beta_{\text{poll}} = 0.54$  and  $\Delta\beta_{\text{poll\_NPK}} = 0.59$ ), and accounted for all observed selection on spur length. There was selection for larger flowers in the hand-pollination treatment without added nutrients (Table 2). There was no statistically significant resource-mediated selection (no significant two- or three-way interactions, Table 2, Figure 1). There was no statistically significant selection on plant height in any treatment.

Floral traits and selection in the second year

Pollen and resource addition had limited effects on floral traits, fruit production and selection the following year. Nutrient addition in 2014 increased flower size in 2015 (Table 3). Pollen- or resource addition in 2014 did not significantly influence the selection differential on any trait in 2015 (Table 4).

## Discussion

In the presence of concurrent pollen and resource limitation, resource addition could affect natural selection on floral traits by affecting trait expression or by influencing variation in relative fitness. In *Dactylorhiza lapponica*, nutrient addition did not increase flower production in the treatment year, and female fitness was not resource limited. No statistically significant resource-mediated selection was detected, and effects of increasing pollination intensity on estimates of selection strength were considerably stronger than effects of increasing resource availability.

The results show that female fitness is largely pollen limited in *D. lapponica*. This is similar to results for the congener *D. incarnata*, where nutrient addition had no effect on reproductive output in the treatment year (Mattila & Kuitunen 2000). Several other studies have also found evidence of only a single limiting factor of seed production (e.g., Corbet 1998; Caruso *et al.* 2005; Ne'eman, Ne'eman & Ellison 2006; Brookes & Jesson 2007), which can be explained by stochastic variation in pollen and/or resource limitation (Burd 2008; Rosenheim *et al.* 2014). No interactive effects of pollen and resources appeared, and conclusions based on studies manipulating one factor at a time should thus be valid, at least at the present levels of resource and pollen limitation.

Pollen supplementation also affected selection strength more strongly than did resource supplementation. In line with previous studies in the same population (Sletvold &

Ågren 2014), there was strong pollinator-mediated selection for longer spurs, and the present manipulations showed that the strength of selection on spur length was not altered by resource addition. In contrast to previous years, we did not detect any statistically significant pollinator-mediated selection on plant height. The absence of selection on plant height is likely caused by the clipping treatment. In another *D. lapponica* population, a reduction of vegetation height around individuals caused pollinator-mediated selection on plant height to disappear (Sletvold *et al.* 2013), demonstrating that the adaptive significance of visual display is context-dependent (cf. O'Connell & Johnston 1998; Ågren, Fortunel & Ehrlén 2006). Although effects of resources on estimates of selection strength were minor compared to those of supplemental pollination, estimates of resource-mediated selection on floral display traits were always in the predicted direction, i.e. positive ( $\Delta\beta_{\text{res}} = 0.12\text{-}0.37$ ). Selection for more flowers tended to be weaker in the nutrient-addition treatments compared to the controls, indicating that resource addition increased female fitness more in few-flowered individuals than in many-flowered individuals. This is similar to the pattern documented in the orchid *Platanthera bifolia*, where fertilization increased fruit production only in small individuals (Mattila & Kuitunen 2000). Also in *Asclepias syriaca*, selection on number of flowers was weaker after resource supplementation (Caruso *et al.* 2005). Likewise, estimates of resource-mediated selection on corolla size were positive, indicating that resource supplementation increased fitness most in individuals with small flowers. Non-pollinator mediated selection for larger flowers has previously been interpreted to reflect a correlation with ovule number (Fenster & Carr 1997; Sandring & Ågren 2009; Sletvold & Ågren 2010), and the present results suggest that this pattern can be driven by resource-mediated selection.

Nutrient addition did not increase flower or fruit production in any of the two years. The absence of effects on flower production the first year suggests that number of flowers is largely fixed at the time that flowering is initiated. In a previous experiment in the same

population, flower removal did not lead to higher flower production in the treatment year (Sletvold & Ågren 2015), indicating that plants are unable to rapidly reallocate resources to additional flower production. Flower primordia are formed in the autumn before flowering, and maximum flower production is likely set already at this time (Øien & Pedersen 2005). Resource availability during the flowering season could still affect the number of flowers that successfully develop, but no such effect was observed. Moreover, a higher resource availability during the growing season should allow individuals to grow a larger replacement tuber, and allocate more resources to reproduction next year. However, plants with added nutrients did not produce more flowers or fruits the following year, nor did they have a higher flowering probability. It may be that individuals instead allocated more to growth or storage, and that potential effects on reproduction will appear after a further delay (cf. Ehrlén & van Groenendaal 2001). Alternatively, two bouts of nutrient additions may be too little to produce an effect. For example, resource limitation was detectable only after two years of nutrient manipulations in a species of *Stylidium* (Brookes *et al.* 2008). This illustrates a general problem of short-term studies of reproductive allocation in long-lived perennials, where storage and allocation patterns involving multiple competing functions may produce complex and delayed responses (van Noordwijk & de Jong 1986). A final possibility is that the NPK addition did not increase nutrient status of the plants. However, we consider this unlikely for two reasons. First, we observed an increase in flower size the second year, indicating that plants did utilize the increased resource availability. Second, plant growth is usually limited by nitrogen, phosphorus, or their combination in fens and wet grasslands (DiTommaso & Aarssen 1989), and above-ground biomass, shoot density and nutrient concentration all increased after NPK addition in a previous experiment at the study site, where *D. lapponica* was one of the herbs included (Øien 2004).

In *D. lapponica*, nutrient addition increased flower size in the second year, but no statistically significant selection on flower size was detected by univariate analyses in any treatment. The limited number of individuals that flowered for two consecutive years prevented us from repeating the full selection analysis the second year, so without more long-term studies, we cannot rigorously evaluate whether there may also be delayed effects on selection.

In general, resource- and pollinator-mediated selection on flower production can be expected to be parallel, because pollinators prefer large displays. For other floral traits, resource variation can both oppose and reinforce selection exerted by pollinators. However, in *D. lapponica* such effects were weak, and selection on floral traits was to a large extent driven by interactions with pollinators, which may be common in strongly pollen limited populations.

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### **Data accessibility**

The data associated with this paper are archived in the Dryad Digital Repository)  
<http://dx.doi.org/10.5061/dryad.7g8q2> (Sletvold et al 2016)

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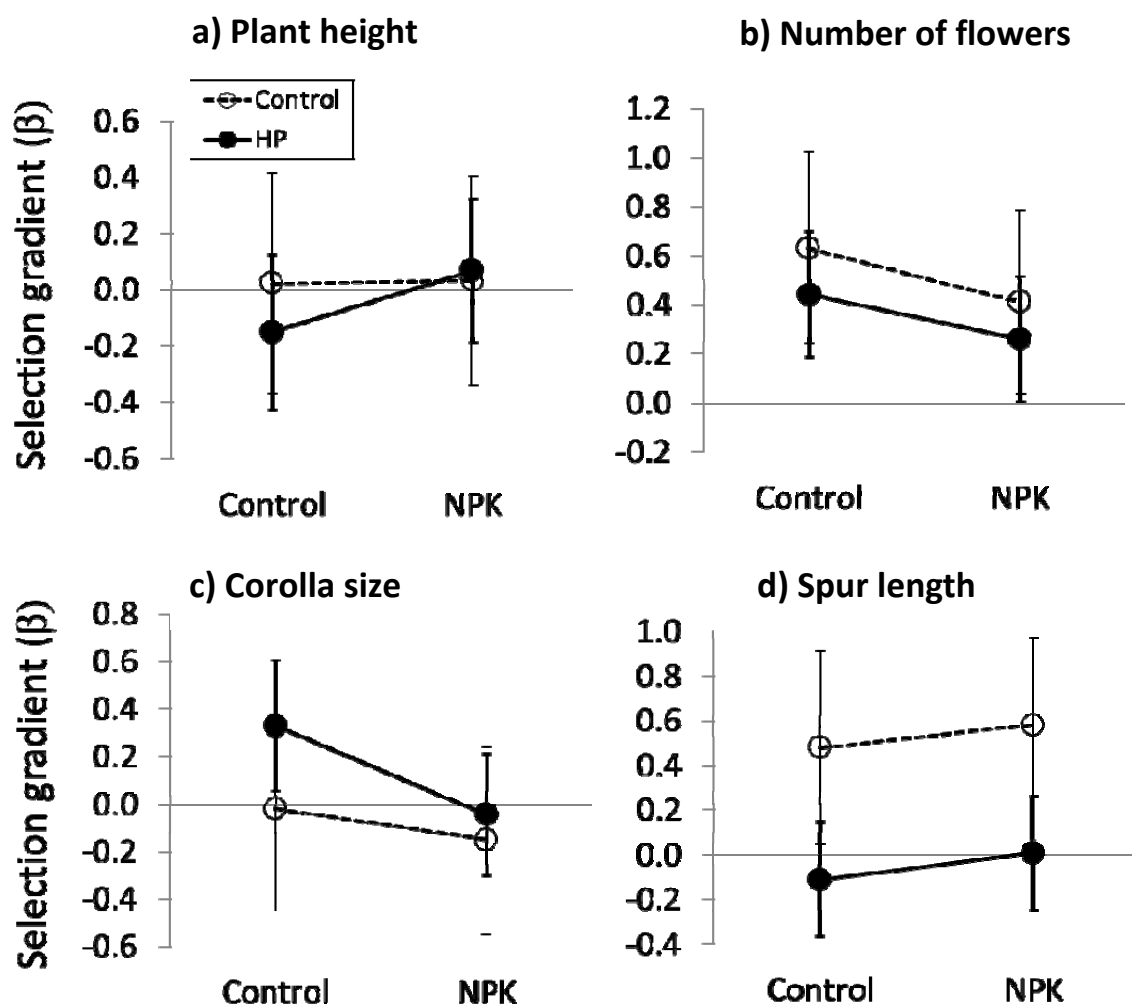


Figure 1. The effect of hand-pollination and nutrient addition on linear selection ( $\beta \pm 1.96$  SE) on a) plant height, b) number of flowers, c) corolla size, and d) spur length. Note the different scale on the y-axis.

**Table 1.** Trait means  $\pm$  SD for open-pollinated plants (C) and plants receiving supplemental hand-pollination (HP) in absence and presence (+ NPK) of nutrient supplementation in the *Dactylorhiza lapponica* population at Sølendet in 2014. *P*-values associated with the effects of pollination treatment (Poll), nutrient treatment (Nutr) and their interactions in ANOVA are indicated.

Trait	C n = 54	C + NPK n = 59	HP n = 50	HP + NPK n = 54	<i>P</i> Poll	<i>P</i> Nutr	<i>P</i> Poll $\times$ Nutr
Plant height (cm)	13.8 $\pm$ 2.6	13.5 $\pm$ 2.9	13.7 $\pm$ 3.1	13.3 $\pm$ 2.8	0.82	0.39	0.95
Number of flowers	9.9 $\pm$ 3.2	9.5 $\pm$ 3.1	9.9 $\pm$ 2.5	9.3 $\pm$ 2.7	0.99	0.22	0.85
Corolla size (mm <sup>2</sup> )	68.0 $\pm$ 19.4	68.7 $\pm$ 20.1	77.1 $\pm$ 20.4	76.6 $\pm$ 22.0	<b>0.024</b>	0.96	0.82
Spur length (mm)	7.4 $\pm$ 1.2	7.6 $\pm$ 1.2	7.9 $\pm$ 1.1	7.8 $\pm$ 1.0	<b>0.025</b>	0.66	0.26
Number of fruits*	1.6 $\pm$ 2.2	1.7 $\pm$ 2.4	4.2 $\pm$ 3.2	4.6 $\pm$ 3.5	<b>&lt;0.0001</b>	0.57	0.77
Fruit mass (mg)	8.6 $\pm$ 3.8	10.4 $\pm$ 4.1	11.6 $\pm$ 4.3	12.3 $\pm$ 4.2	<b>0.0033</b>	0.074	0.41
Female fitness*	13.3 $\pm$ 20.2	14.9 $\pm$ 22.2	52.1 $\pm$ 50.9	58.2 $\pm$ 52.0	<b>&lt;0.0001</b>	0.46	0.95

\*square-root transformed in ANOVA

**Table 2.** Phenotypic linear selection gradients ( $\pm$  SE) for open-pollinated plants (C) and plants receiving supplemental hand-pollination (HP) in absence (nat) and presence (NPK) of nutrient supplementation in the *Dactylorhiza lapponica* population at Sølendet in 2014. P-values associated with trait by treatment interactions are indicated.

Trait	$\beta_{C\_nat}$	$\beta_{C\_NP}$	$\beta_{HP\_nat}$	$\beta_{HP\_NPK}$	$\Delta\beta_{poll\_nat}$	$\Delta\beta_{poll\_NPK}$	$\Delta\beta_{res\_nat}$	$\Delta\beta_{res\_NPK}$	<i>P</i>	<i>P</i>	<i>P</i>
									Poll	Nut	Pol
										r	l ×
											Nut
Height	0.057 $\pm$ 0.20	0.046 $\pm$ 0.19	-0.10 $\pm$ 0.14	0.092 $\pm$ 0.13	0.16	-0.046	0.01 1	-0.19	0.53	0.59	0.55
No. flowers	0.57 $\pm$ 0.20*	0.39 $\pm$ 0.19*	0.45 $\pm$ 0.13*	0.23 $\pm$ 0.13	0.12		0.18	0.22	0.63	0.23	0.90
Corolla size	- 0.019 $\pm$ 0.22	-0.14 $\pm$ 0.20	0.30 $\pm$ 0.14*	-0.015 $\pm$ 0.13	- 0.32	-0.13	0.12	0.32	0.22	0.20	0.58
Spur length	0.47 $\pm$ 0.22*	0.57 $\pm$ 0.20*	- $\pm$ 0.13	-0.018 $\pm$ 0.13	0.54 *	0.59*	- 0.10	-0.05	<b>0.03</b> 7	0.67	0.90

\*\*  $P < 0.01$ , \*  $P < 0.05$ , (\*)  $P = 0.086$

**Table 3.** Trait means  $\pm$  SD for open-pollinated control plants (C) and plants receiving supplemental hand-pollination (HP) in absence and presence (+ NPK) of nutrient supplementation in the *Dactylorhiza lapponica* population at Sølendet in 2015. *P*-values associated with the effects of the covariate (trait value in 2014), pollination treatment (Poll), nutrient treatment (Nutr), and their interaction are indicated.

Trait	C n = 31	C + NPK n = 29	HP n = 35	HP + NPK n = 32	<i>P</i> Covariate	<i>P</i> Poll	<i>P</i> Nutr	<i>P</i> Poll $\times$ Nutr
Plant height (cm)	14.9 $\pm$ 4.6	16.8 $\pm$ 3.4	14.6 $\pm$ 4.0	15.6 $\pm$ 3.7	<b>0.0005</b>	0.86	0.080	0.17
Number of flowers	11.9 $\pm$ 4.7	12.5 $\pm$ 4.0	10.7 $\pm$ 3.7	11.0 $\pm$ 3.6	<b>&lt;0.0001</b>	0.59	0.28	0.67
Corolla size (mm <sup>2</sup> )	83.2 $\pm$ 18.0	103.5 $\pm$ 31.8	88.12 $\pm$ 29.2	95.2 $\pm$ 23.1	<b>&lt;0.0001</b>	0.93	<b>0.0038</b>	0.094
Spur length (mm)	8.5 $\pm$ 1.3	8.8 $\pm$ 1.2	8.3 $\pm$ 1.6	8.7 $\pm$ 1.1	<b>0.0029</b>	0.66	0.28	0.97
Number of fruits*	1.7 $\pm$ 2.1	1.8 $\pm$ 2.9	0.91 $\pm$ 2.0	1.3 $\pm$ 1.8	NA	0.08	0.54	0.28

\*square-root transformed ( $n_C = 27$ ,  $n_{C+NPK} = 27$ ,  $n_{HP} = 32$ ,  $n_{HP+NPK} = 30$ )

**Table 4.** Selection differentials ( $\pm$  SE) quantified in 2015 for open-pollinated plants (C) and plants receiving supplemental hand-pollination (HP) in absence (nat) and presence (NPK) of nutrient supplementation in the *Dactylorhiza lapponica* population at Sølendet in 2014. *P*-values associated with trait by treatment interactions are indicated.

Trait	$S_{C\_nat}$ n = 27	$S_{C\_NPK}$ n = 27	$S_{HP\_nat}$ n = 32	$S_{HP\_NPK}$ n = 30	<i>P</i> Poll	<i>P</i> Nutr	<i>P</i> Poll $\times$
Height	0.34 $\pm$ 0.25	0.43 $\pm$ 0.32	-0.093 $\pm$ 0.44	0.32 $\pm$ 0.25	0.37	0.87	0.63
No. flowers	0.48 $\pm$ 0.25(*)	0.82 $\pm$ 0.28**	0.54 $\pm$ 0.42	0.61 $\pm$ 0.23*	0.88	0.48	0.66
Corolla size	0.33 $\pm$ 0.28	0.021 $\pm$ 0.33	-0.21 $\pm$ 0.46	0.23 $\pm$ 0.25	0.30	0.55	0.29
Spur length	0.29 $\pm$ 0.28	0.64 $\pm$ 0.30	0.13 $\pm$ 0.50	0.28 $\pm$ 0.25	0.77	0.50	0.76

\*\*  $P < 0.01$ , \*  $P < 0.05$ , (\*)  $P = 0.064$