Quantitative importance of staminodes for female reproductive success in *Parnassia palustris* under contrasting environmental conditions

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Abstract: The five sterile stamens, or staminodes, in *Parnassia palustris* act both as false and as true nectaries. They attract pollinators with their conspicuous, but non-rewarding tips, and also produce nectar at the base. We removed staminodes experimentally and compared pollinator visitation rate and duration and seed set in flowers with and without staminodes in two different populations. We also examined the relative importance of the staminode size to other plant traits. Finally, we bagged, emasculated, and supplementary cross-pollinated flowers to determine the pollination strategy and whether reproduction was limited by pollen availability. Flowers in both populations were highly dependent on pollinator visitation for maximum seed set. In one population pollinators primarily cross-pollinated flowers, whereas in the other the pollinators facilitated self-pollination. The staminodes caused increased pollinator visitation rate and duration to flowers in both populations. The staminodes increased female reproductive success, but only when pollen availability constrained female reproduction. Simple linear regression indicated a strong selection on staminode size, multiple regression suggested that selection on staminode size was mainly caused by correlation with other traits that affected female fitness.

Key words: staminodes, insect activity, seed set, spatial variation, *Parnassia palustris*.

Résumé : Chez le *Parnassia palustris*, les cinq étamines stériles, ou staminodes, agissent à la fois comme fausses et véritables nectaires. Elles attirent les pollinisateurs avec leurs extrémités bien visibles mais non-gratifiantes, et elle produisent également du nectar à la base. Les auteurs ont éliminé expérimentalement les staminodes, et ils ont comparé les taux et la durée des visites par les pollinisateurs, ainsi que la mise à graine, chez des fleurs avec ou sans staminodes, dans deux populations distinctes. Ils ont également examiné l'importance relative de la dimension des staminodes par rapport à d'autres caractères de la plante. Enfin, ils ont mis en sachet, émasculé et assisté la pollinisation croisée de fleurs afin de déterminer la stratégie de pollinisation et si la reproduction est limitée par l'abondance du pollen. Dans les deux populations, les fleurs dépendent fortement de la visite des pollinisateurs pour maximiser la mise à graine. Dans une population, les pollinisateurs ont surtout effectué la pollinisation croisée, alors que dans l'autre les pollinisateurs ont facilité l'auto-pollinisation. Les staminodes entraînent une augmentation du taux et de la durée des visites des fleurs dans les deux populations. Les staminodes augmentent les succès de la reproduction femelle, mais seulement lorsque la disponibilité du pollen limite la reproduction femelle. La régression linéaire simple indique une forte sélection sur la base de la dimension des staminodes, et la régression multiple suggère que la sélection basée sur la dimension des staminodes provient principalement d'une corrélation avec d'autres caractères qui affectent l'adaptation femelle.

Mots clés : staminodes, activité des insectes, mise à graine, variation spatiale, *Parnassia palustris*.

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Introduction

Insect-pollinated flowers exhibit diverse adaptations for attracting pollinators, including both desirable resources (e.g., food, mating sites) and attractive signals such as showy perianths and fragrance. (Plowright 1981; Cresswell and Galen 1991). Most research on the evolution of floral traits that function directly in pollination has focused on flower diameter, shape, colour, or nectar production (Schemske and Horwitz 1989; O'Connell and Johnston 1998; Totland and Sandvik 2000). The likelihood of selection on such floral traits has been pointed out in several studies (Campbell 1989, 1996; Galen 1989, 1996; Schemske and Horwitz 1989; Widén 1991; Herrera 1993; Mitchell 1994; Conner et al. 1996; Schemske et al. 1996; Mitchell et al. 1998; O'Connell and Johnston 1998; Totland et al. 1998; Armbruster et al. 2000; Maad 2000; Totland 2001). Few studies have, however, focused on the evolution of stamen morphology or function in relation to the quality and quantity of pollinator visitation (but see Harder and Barrett 1993;

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Harder and Barclay 1994; King and Buchman 1995, 1996; Walker-Larsen and Harder 2001).

Evolutionary modification of stamens often results in alternate morphological structures, which may function in many different ways. For example, sterile stamens, or staminodes, can serve many functions, which differ from those served by fertile stamens (Ronse Decraene and Smets 2001). They may provide nectar rewards (Hemsley 1903; Cronquist 1981; Endress 1984b, 1986), or enhance the visual display and thereby increase the attractiveness of flowers (Yeo 1992). Staminodes may also function as nectar guides and direct pollinators to the nectaries (Endress 1994) or act as levers that enhance pollinator contact with the stigma, and thereby increase pollen reception, as in *Penstemon palmeri* (Walker-Larsen and Harder 2001). In addition, staminodes may prevent self-pollination by shielding the receptive stigmas during pollen presentation, and therefore increase the opportunities for cross-pollination (Grant 1950; Torchio 1974; Endress 1984a, 1984b, 1994). On the other hand, staminodes may also have an important function in self-pollinating species, since pollinators may facilitate pollination by transferring pollen from the anthers to the stigmas within the same flower or between flowers within the same plant. Consequently, the impact of staminodes may be indirectly dependent upon factors such as pollinator abundance and activity, and pollen limitation.

Many species experience spatial and temporal variation in pollination success owing to the vagaries of pollinator activity (Burd 1994). Consequently, the importance of floral structures, such as staminodes, for reproductive success may also vary both in space and time. In the genus *Parnassia*, stamens have evolved into conspicuous false nectaries (Spongberg 1972; Richards 1986) for which the functional roles have been frequently discussed (Drude 1875; Daumann 1932, 1935; Kugler 1956; Proctor and Yeo 1973; Hultgård 1987). Although several studies have revealed different functional roles of staminodes, no study has, to our knowledge, examined the quantitative importance of staminodes for female reproduction.

Here, we assess the quantitative and qualitative importance of the staminodes of *Parnassia palustris* L. for seed set in two populations that experience different temperature regimes: one population is coastal and has a mean summer temperature 10°C higher than the second population situated in the alpine. Specifically, we address the following questions: (*i*) Does experimental removal of staminodes affect insect visitation rate and duration, and fruit mass? (*ii*) Does female reproductive success (fruit mass) differ among individuals freely exposed to pollinators, individuals restricted to self-pollination, and individuals prevented from self-pollination? (*iii*) Does pollinator activity limit female reproductive success? (*iv*) What is the importance of staminode size, relative to other floral traits, and vegetative size for fruit mass?

Material and methods

Study species and sites

Parnassia palustris is a perennial herb with a circumboreal distribution. According to Hultgård (1987), *P. palustris* is common in the north of Scandinavia and de-

creases in abundance towards the south. It grows in open, wet to moist, basic habitats from sea level to 1700 m altitude (Hultgård 1987). Each plant has basal leaves and 1 to 20 flowering stems, each with one terminal flower and usually a single cauline leaf.

The flower of *P. palustris* is protandrous and the five stamens usually discharge their pollen before the stigma begins maturation. The flower usually has five white petals and five staminodes. The staminodes are deeply divided into thread-like rays, with shiny, rounded knobs at the tips that resemble nectaries (Müller 1883; Richards 1986). The colour of the staminodes changes from green to yellow-green or yellow-brown as they age. Two exposed green nectaries are visible below the rays at the base of each staminode (Proctor and Yeo 1973; Hultgård 1987). The basal parts of the staminodes also produce a honey-like scent (Proctor and Yeo 1973). Several authors have studied the pollination of *P. palustris* (Drude 1875; Eichler 1875; Eichinger 1908; Martens 1936; Cunnell 1959). Martens (1936) and Hultgård (1987) found that it is usually cross-pollinated and rarely autogamous. Diptera, particulary hoverflies (Syrphidae) were the most common visitors in a study of *P. palustris* in south Sweden (Kullenberg 1953). Syrphidae and other Diptera (Muscidae and Calliphoridae) are also important pollinators of *P. palustris* on the British Isles (Proctor and Yeo 1973) and in the alpine regions of Norway (Totland 1993). In addition, many other insects (ants: Hultgård 1987; Tipulidae, Diptera: Proctor and Yeo 1973; short-tongued Hymenoptera: Daumann 1932, Kullenberg 1953; Proctor and Yeo 1973; Lepidoptera: Totland 1993) have been observed visiting the flowers of *P. palustris*.

We studied two populations of *P. palustris*: one at sea level at Lista, south Norway (58°03′N, 6°47′E) and the other at Finse, southwestern Norway (60°36′N, 7°32′E) at 1550 m elevation. At Lista, *P. palustris* occurred in dune-slacks. The study site at Finse was a dry calcareous heath.

The climate at Finse (1222 m.a.s.l.) is alpine-oceanic, with mean precipitation during July–September of ca. 109 mm per month (precipitation normals from the period 1961–1990; Aune 1993a) and a mean temperature of 5.6°C (air temperature normals from the period 1961–1990; Aune 1993b). At Lista the corresponding values are 106 mm per month and 13.6°C (Aune 1993a, 1993b).

Field experiments and statistical analysis

Fieldwork was done from early August until late September 2001. We marked 206 randomly selected plants at Lista and 150 plants at Finse and randomly assigned one of five treatments to each of the plants. Only one flower per plant was used. We regarded any negative side-effect, such as altered resource allocation, of manipulating only one flower of a multi-flowered plant to be of minor importance for three reasons: (*i*) most plants at Finse had only one flower; (*ii*) the mean number of flowers per plant at Lista was low (only 3.5 flowers when four statistical outliers with exceptionally high number of flowers were omitted from the calculation); and (*iii*) flowers on individual plants opened sequentially, which may reduce the differences in resource allocation patterns between the populations.

To examine seed production after spontaneous self-pollination (autogamy), we bagged 32 flowers at Lista and

30 at Finse during the bud stage with white mesh (1 mm mesh-width) wrapped around a small wire cage. To test for the presence and amount of outcrossing, we emasculated 38 flowers at Lista and 30 at Finse during the bud stage with fine forceps and left the flower open to natural pollination. To examine whether pollen receipt constrained seed production, we experimentally hand-pollinated stigmas of 38 flowers at Lista and 30 at Finse with an excess of cross-pollen. We used fine forceps to clip stamens of donor flowers and brushed the mature anther across the stigmas of experimental flowers when they were receptive. These flowers were also exposed to natural pollination. To quantify the significance of the staminodes for reproductive output (fruit set and fruit mass), we removed the staminodes from 38 flowers in bud stage at Lista and 30 flowers at Finse. For controls, we marked 60 open-pollinated flowers at Lista and 30 at Finse. All flowers from the same population used in the experiment reached anthesis simultaneously. Mature capsules from all manipulated and all open-pollinated flowers were collected as soon as they matured and were oven-dried at 37°C for 24 h.

In the analyses of the plants at Lista, we omitted the individuals on which fruits were eaten by animals by the time of the harvest. We assessed the effect of each pollination treatment on female reproduction by fruit mass. This variable was used because it is simple to measure and because it is a good surrogate for seed number per fruit. To confirm that fruit mass is a good predictor of seed number per fruit, we randomly chose five fruits from each experimental group from Lista, weighed each of them (to the nearest 0.1 mg) and counted their seeds. A linear regression analysis showed that fruit mass explained 82% of the variance in seed number per fruit. We used one-factor ANOVA followed by Bonferroni multiple comparisons to examine whether fruit mass differed among the five treatments at Lista during 2001. The analysis on fruit mass was done only on those flowers that produced mature fruit. Many flowers did not set fruit at Finse during 2001, and fruits were unfortunately collected before seeds were mature. Therefore, we could not obtain a reliable estimate of seed number per fruit from this population. Consequently, we simply noted whether carpels had developed into fruits, and used separate chi-square analyses to test if the proportion of maturing fruits differed between the open-pollinated control group and each of the experimental groups. For comparison, we performed similar chi-square analyses on the Lista data set.

We compared the number of insect visitations (visitation rate) and the duration of each visit to 10 flowers with intact staminodes and 10 flowers from which staminodes had been removed. The corresponding number of observed flowers per treatment was 12 at Finse in 1995. We measured visitation rate and visit duration between 13:00 and 15:00 during 14, 17, and 18 periods of 10 min at Lista, Finse during 1995, and Finse during 2001, respectively. Visitation duration was unfortunately not measured at Finse in 2001. We used randomized block ANOVA, with period as the blocking factor to examine the effects of staminode removal and observation period on the number of flower visits and on visit duration. To avoid pseudoreplication, we used the average visit number and duration to observed flowers during each observation period. Separate analyses were conducted for the Lista

and Finse populations, and for the 2 years at Finse because of differences in the number of plants observed during the periods in the two treatment groups between sites and years. We log-transformed average visit duration per flower per period at Lista and at Finse in 1995 to meet the assumptions of homogeneity of variance and normal residuals of ANOVA.

We conducted several measurements to determine the relationships between fruit mass and various floral and vegetative traits and the size of staminodes. When the flowers were in full bloom, we used a digital electronic caliper to measure flower diameter (in millimetres) and a ruler to measure the length of the flower stem (in centimetres) on all open-pollinated flowers. We also counted the number of leaves and stems per individual plant, and the number of rays (staminode size) on all the five staminodes in each flower. We used both simple and multiple linear regression analyses to examine the relationships between female reproductive success (fruit mass) and floral and vegetative traits and staminode size. Each predictor variable (staminode size, flower diameter, flower stem height, number of leaves) was log-transformed to meet the assumptions of regression analysis. To avoid multicollinearity, the number of stems per individual plant was excluded from the multiple regression, since this variable was highly correlated to the number of leaves. Owing to the very low fruit set of open-pollinated flowers at Finse during 2001, only the data from Lista were analysed. Furthermore, we did not conduct a nonlinear analysis because sample size was too low. We used SYSTAT for Windows (version 10.1) for all the statistical analyses.

Results

Effects of staminodes on insect activity and reproduction

Different species of Diptera, especially Tephritidae (particularly *Euleia heraclei*) and Syrphidae were the most common flower visitors observed pollinating *P. palustris* at Lista. Diptera, especially species of Muscidae, Dolichopodidae, Anthomyiidae, and Empididae, were the most common flower visitors of *P. palustris* at Finse (see Totland 1993). Removal of staminodes greatly influenced insect activity to *P. palustris* flowers at both study sites. Visitation rate to intact flowers was 40% and 46% higher than to flowers without staminodes at Lista in 2001 and Finse in 1995, respectively, (Table 1, Fig. 1). At Finse in 2001, however, visitation rate to flowers was overall very low and staminode removal had no detectable effect on visitation rate (Table 1, Fig. 1). Visitation rate differed significantly between the observation periods both at Lista and Finse (Table 1).

Intact flowers received significantly longer visits compared with flowers that had their staminodes removed both at Lista in 2001 (48% longer) and at Finse in 1995 (56% longer) (Fig. 1, Table 1). The duration of the visits did not differ significantly between observation periods (Table 1).

One-factor ANOVA showed that there were significant differences among the five treatment groups (control, staminode removal, emasculation, supplemental pollination, and bagging) at Lista in 2001 ($F_{4,190} = 8.07$, $P < 0.001$). However, removal of staminodes had no effect on fruit mass

Table 1. Randomized block ANOVA on the effects of staminode removal (treatment) and observation period on rate and duration of visits to flowers of *Parnassia palustris* at Lista and Finse.

Source	df	MS	F	P
Rate (No. of visits)				
Finse 1995				
Treatment	1	2.38	15.30	<0.01
Period	17	0.35	2.26	>0.05
Error	17	0.16		
Finse 2001				
Treatment	1	0.03	0.03	>0.5
Period	18	2.03	2.22	0.05
Error	18	0.92		
Lista 2001				
Treatment	1	2.16	13.89	<0.01
Period	14	0.40	2.59	<0.05
Error	14	0.16		
Duration (s)				
Finse 1995				
Treatment	1	12.63	27.94	<0.001
Period	17	0.64	1.41	0.244
Error	17	0.45		
Lista 2001				
Treatment	1	5.80	14.74	<0.01
Period	14	0.68	1.72	>0.1
Error	14	0.39		

Note: Each observation period lasted 10 min. Visitation rate to flowers equals the average number of visits to individual flowers during 10 min. Duration of flower visits was log-transformed and measured in seconds.

of individual flowers at Lista (Fig. 2, Bonferroni multiple comparisons; staminode removal versus control: $P > 0.5$). Moreover, staminode removal had no effect on the percentage of flowers developing a fruit at Lista ($\chi^2 = 0.33$, df = 1, $P > 0.5$), where 94.7% of experimental and 91.7% of open-pollinated flowers developed a fruit. At Finse on the other hand, 30% of open-pollinated flowers developed a fruit, whereas only 6.7% of experimental flowers without staminodes did so, a significant difference ($\chi^2 = 5.45$, df = 1, $P < 0.05$).

Breeding system of *Parnassia*

After one-factor ANOVA (see above), Bonferroni multiple comparison showed that bagged flowers produced significantly smaller fruits than both open-pollinated ($P < 0.001$) and emasculated ($P < 0.001$) flowers, whereas there was no significant difference in fruit mass between open-pollinated and emasculated flowers ($P > 0.5$). A one-factor ANOVA on the difference in seed to ovule ratio (S:O) of fruits of open-pollinated, bagged, and emasculated flowers at Lista ($n = 5$ in each) was highly significant ($F_{2,12} = 17.68$, $P < 0.001$). Bonferroni multiple comparisons showed that the S:O of open-pollinated flowers (mean S:O = 0.40, SE = 0.3) was significantly higher ($P < 0.05$) than that of the bagged flowers (mean S:O = 0.02, SE = 0.007), whereas S:O of emasculated flowers (mean S:O = 0.78, SE = 0.08) was significant higher than that of open-pollinated flowers ($P < 0.05$).

Fruit production in open-pollinated (outcrossed), auto-

Fig. 1. Mean (± SE) Visitation rate (A) and visitation duration (B) to *Parnassia palustris* flowers with (open squares) and without staminodes (filled squares). See Table 1 for statistical details.
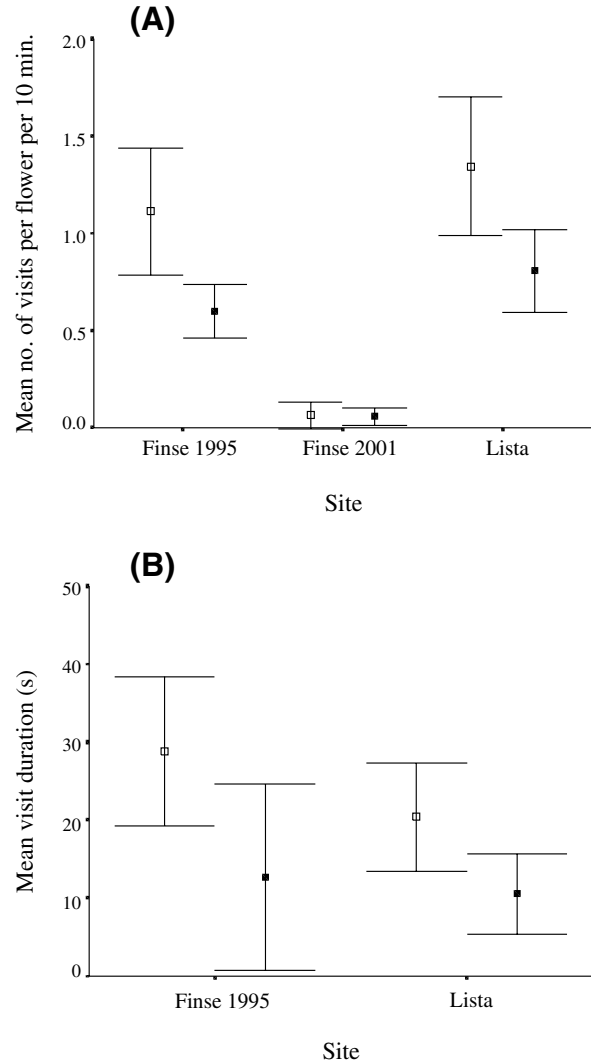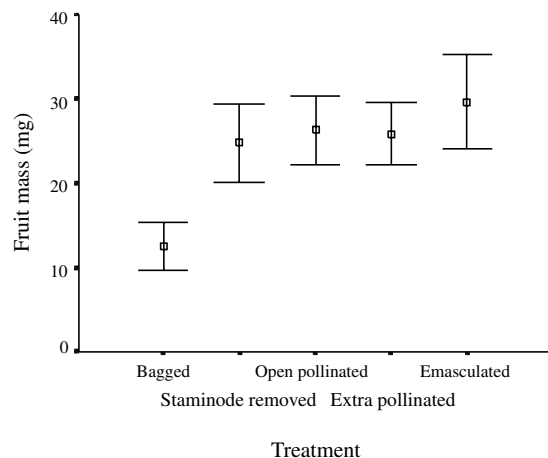

Fig. 2. Mean (± SE) fruit mass of *Parnassia palustris* at Lista in 2001 in five treatment groups.

gamously pollinated (bagged), and emasculated flowers differed between the two studied populations. At Lista during 2001, 91.7% of open-pollinated flowers, 100% of bagged flowers, and 92.2% of emasculated flowers produced fruits, a nonsignificant difference ($\chi^2 = 2.79$, df = 2, $P > 0.1$). At Finse during 2001, 30% of open-pollinated flowers, 3.3% of bagged flowers, and 6.7% of emasculated flowers developed fruits, a significant difference ($\chi^2 = 10.96$, df = 2, $P < 0.01$).

Pollen limitation

Supplemental pollination of *Parnassia* flowers influenced fruit production at Lista and Finse differently. At Lista, 94.7% of supplementary pollinated flowers developed a fruit, whereas 91.7% of open-pollinated flowers did so, a nonsignificant difference ($\chi^2 = 0.33$, df = 1, $P > 0.5$). At Finse during 2001, on the other hand, 56.7% of supplementary pollinated flowers produced a fruit, whereas 30% of open-pollinated flowers did so, which represents a significant difference ($\chi^2 = 4.34$, df = 1, $P < 0.05$). Moreover, at Lista adding outcross pollen had no significant effect on fruit mass in the flowers that produced fruits, relative to open-pollinated flowers (Fig. 2, Bonferroni multiple comparisons: $P > 0.5$).

Relationship between staminode ray number and fruit mass

Linear regression showed that there were significant positive relationships between fruit mass and staminode size, flower diameter, and stem length (Table 2, Fig. 3). Multiple linear regression revealed a significant effect of flower diameter and stem length on fruit mass. Thus, once variation in flower diameter and stem length had been accounted for, neither the staminode size nor the number of leaves significantly affected fruit mass (Table 2). The overall regression model explained 57% of the variation in fruit mass and was highly significant (ANOVA; $F_{4,50} = 16.63$, $P < 0.001$).

Discussion

The significance of staminodes for pollinator activity and female reproductive success

The staminodes positively influenced insect visitation rate to *P. palustris* flowers. This is consistent with the findings of Proctor and Yeo (1973) that flies of genus *Phora* (Phoridae) and the hoverfly *Sphaerophoria* are attracted first to the staminodes in *Parnassia* before turning their attention to the nectar discs below. In an interesting experiment, Kugler (1956) demonstrated that inexperienced flies of *Lucilia* and Syrphidae were strongly attracted to the false nectaries and licked them when first introduced to the *Parnassia* flowers, but soon learned to find the real nectar and then overlooked the false nectaries. Daumann (1932, 1935), however, found that the insects ignored the false nectaries and that scent produced in the flower induced the insects to alight.

The importance of staminodes in *P. palustris* as attractive structures to pollinators did not differ between the two studied populations. However, at Finse intact flowers produced significantly more fruits than did flowers without staminodes, whereas at Lista there was no significant difference. These results suggest that the staminodes influence female reproductive success more at Finse than at Lista, and

Table 2. Simple (SRC) and partial (PRC) regression coefficients between standardized traits and fruit mass of *Parnassia palustris* at Lista in 2001 ($n = 55$).

Trait	SRC	P	PRC	P
Staminode size	0.53	<0.001	0.18	>0.20
Flower diameter	0.55	<0.001	0.33	<0.05
Flower stem length	0.61	<0.001	0.45	<0.001
No. of leaves	0.23	>0.05	0.09	>0.4

Note: Regression coefficients are from regressions with untransformed traits, whereas significance values are from regressions where all traits and fitness were log-transformed.

that pollinator limitation on female reproduction is potentially more pronounced at Finse than at Lista. (It should be noted, however, that our study is not replicated in the alpine and coastal areas. Thus, any impacts of other factors than those that differ along an elevation gradient (e.g., temperature) cannot be fully assessed.)
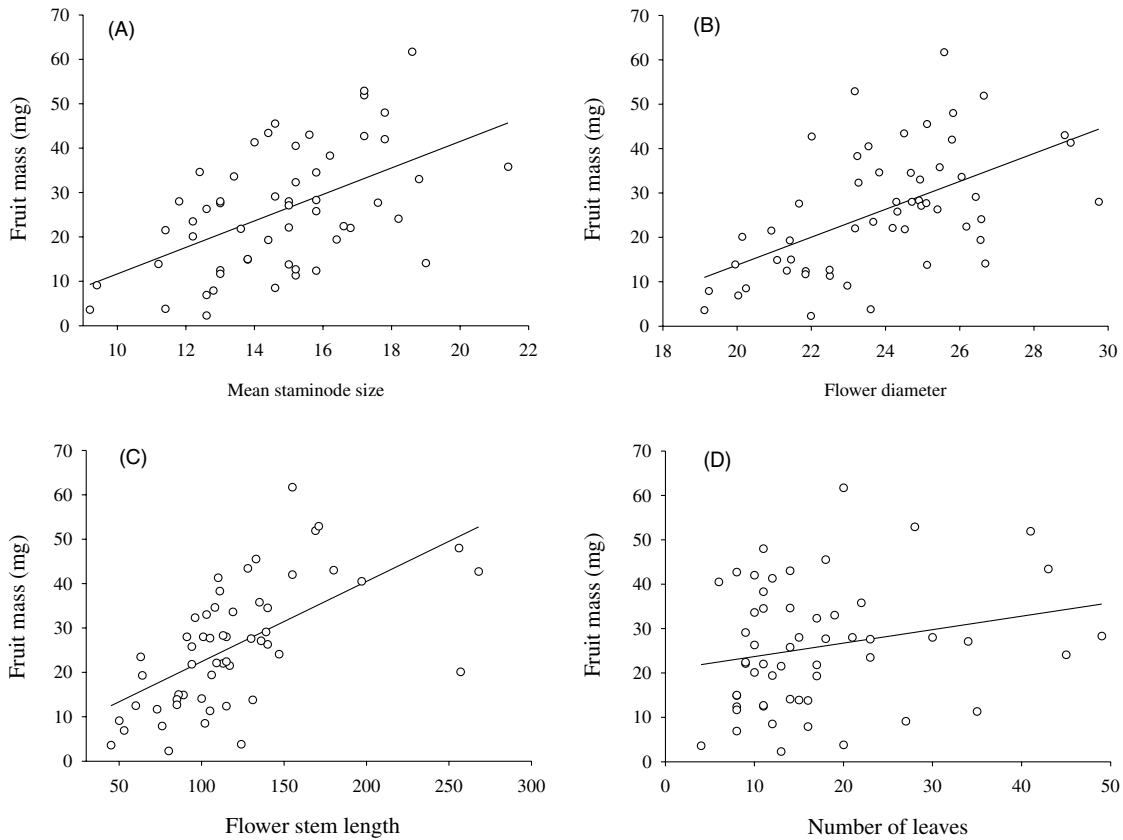
Pollinator visitation rates were affected differently by the staminodes at Finse in 1995 and 2001. It is possible that rapidly changing weather conditions may explain the variation in the impact of staminode on pollinator activity, both between short observation periods within a season and between years. Visit duration in flowers with staminodes was higher both at Lista and Finse compared with flowers without staminodes. A significantly higher temperature inside the flower of *P. palustris* compared with the outside (Lack 1976) suggests that pollinators visiting flowers are offered a warming place. These results show that the staminodes increase both the number and the duration of visits. Furthermore, it is likely that increased duration of visits may contribute to increasing both the male and female reproductive success in *P. palustris*.

Breeding system

All the bagged flowers at Lista and a few bagged at Finse produced fruits, showing that *P. palustris* reproduces by autogamous pollination in both studied populations. However, because of the drastically reduced seed set in bagged flowers (95% lower S:O) compared with open-pollinated flowers, and the insignificant difference in fruit production between the emasculated and open-pollinated flowers, we suggest that *P. palustris* flowers at Lista are mainly outcrossing and strongly dependent on pollinators for optimum pollination and maximum seed set.

At Finse, significantly more open-pollinated than emasculated flowers produced fruits than at Lista. This indicates that self-pollination, aided by pollinators transferring pollen from anthers to stigmas within the same flower, occurs more frequently than outcrossing at Finse. Our results agree with the idea that alpine species are often self-pollinated (Savile 1972; Bell and Bliss 1980; Molau and Prentice 1992; Molau 1993) but contrast with the earlier statement that *P. palustris* normally reproduces by cross-pollination (Martens 1936). We conclude that female reproduction in *P. palustris* at Finse mainly results from self-pollination but that self-pollination is highly aided by pollinators, whereas cross-pollination is the most common reproductive strategy in *P. palustris* at Lista.

Fig. 3. Relationship between fruit mass and (A) staminode size, (B) flower diameter, (C) flower stem length, and (D) number of leaves in *Parnassia palustris*. Line shown is the linear regression line. See Table 2 for statistical details.

Thus, our results differentiate the view stated by Martens (1936) and Hultgård (1987) that *P. palustris* is usually cross-pollinated owing to protandry, and suggest instead that outcrossing may vary substantially across the species distribution range.

Spatial variation in pollen limitation

Pollen-supplemented flowers at Finse produced significantly more fruits (47% increase) than naturally pollinated flowers, whereas at Lista, supplemental pollination had a small effect (4% increase). This shows that fruit production in *P. palustris* is pollen-limited at Finse, but not at Lista. Since *P. palustris* is most commonly visited by various Diptera in both lowland (Kullenberg 1953; Proctor and Yeo 1973) and alpine areas (Totland 1993) we believe that the abundance of pollinators and their flight activity influence reproductive success more than differences in pollinator composition. In particular, it is conceivable that severe weather conditions in alpine regions constrain the abundance of pollinators and their flight activity more at Finse than at Lista.

Relationship between staminode size and reproductive success

Simple linear regression showed a significant positive relationship between staminode size and fruit mass. This could suggest that staminode size contributed to heavier fruits and, presumably, more seeds. However, the significance of stami-

node size for fruit mass was not evident in a multiple regression where the effects on fruit mass of flower diameter, flower stem length, and leaf number per plant were accounted for. This indicates that the relationship between staminode size and fruit mass is basically caused by its correlation with other traits that influence seed mass. Flower diameter and flower stem length, on the other hand, had significant impacts on fruit mass, even after accounting for any impact of other measured traits on fruit mass. These results can, with caution, be interpreted in terms of phenotypic selection (Arnold and Wade 1984a,1984b; Endler 1986) on floral and vegetative traits of *Parnassia palustris* at Lista. The regression analysis indicates that total selection (as judged by the simple linear regression) is operating on the design of the staminodes. However, multiple regression results (indicating direct selection on traits) suggest that there is no direct selection on staminode size, and thus no causal link between staminode size and female reproductive success. The direct selection apparently operating on flower diameter and flower stem length is in line with findings of several other studies (Campbell 1989, 1996; Galen 1989; Schemske and Horvitz 1989; Wilson 1995; Totland et al. 1998; Totland and Sandvik 2000; Totland 2001). According to Johnston (1991), selection through female function on traits engaged in pollinator attraction will only operate if pollen availability dispersal limits reproductive success and if pollinators discriminate between different floral phenotypes. This implies that since pollen limitation appeared to

be absent in the population at Lista, selection mediated by pollinators may not operate on floral traits of *P. palustris* in this population, at least not during our study season.

Ronse Decraene and Smets (2001) summarize the different functions of the staminodes as attractive, nutritional, and structural organs. The staminodes in *P. palustris* are multifunctional. The threadlike rays with shiny knobs on the staminodes in *P. palustris* act as false nectaries and produce scent to attract pollinators. Furthermore, the staminodes produce and offer nectar to the pollinators. Even if pollen availability did not limit reproductive success at Lista, pollinators discriminated between flowers with and without staminodes, showing that the staminodes have a functional importance. Attractiveness of the flowers of *P. palustris* vary both within and between populations (Hultgård 1987) and pollen limitation may vary in time and space, so it is likely that the selective forces will vary both in space and time. Therefore, we suggest that the staminodes in *P. palustris* will be maintained by selection as long as the staminodes are multifunctional and affect pollinator visitation. Furthermore, we suggest that the selection on the staminodes mediated by pollinators may be most intense at Finse because our results suggest that pollen limitation is most severe at this cold site.

Acknowledgements

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