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## Population size, pollinator visitation and fruit production in the deceptive orchid *Calypso bulbosa*

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**Abstract** It has been proposed that in non-rewarding animal-pollinated plants the pollination intensity should decrease with increasing population size and should increase with increasing local abundance of reward-producing plants. To test these hypotheses, we examined how population size, local abundance of *Salix caprea*, and tree cover were related to pollen removal and fruit production in 16 populations of the deceptive, early-flowering and bumblebee-pollinated orchid *Calypso bulbosa* in northern Sweden in 3 consecutive years. To determine whether fruit production was limited by pollinator visitation, supplemental hand-pollinations were performed in three populations in 3 years. Finally, to examine whether increased fruit production was associated with a reduction in future flower production, vegetative growth or survival, supplemental hand-pollination was repeated for 5 years in one population. The levels of pollen export, pollen deposition, and fruit set of *C. bulbosa* varied considerably among years and among populations. The proportion of plants exporting pollen was negatively related to population size, and positively related to density of *S. caprea* and to tree cover in 1 of the 3 years. In the other 2 years, no significant relationship was detected between proportion of plants exporting pollen and the latter three variables. In no year was there a significant relationship between fruit set and population size, density of *S. caprea* and tree cover. There was substantial among-year variation in the extent to which fruit production was limited by insufficient pollen deposition and in the amount of weather-induced damage to flowers and developing fruits. Fruit set was consistently higher in hand-pollinated than in open-pollinated plants, but this difference was statistically significant in only one of 3 years. Supplemental hand-pollination in 5 consecutive years increased cumulative fruit production 1.8 times, but did not affect flower production, plant size, or survival. Tree cover was negatively correlated with the inci-

dence of frost damage in 1 year. The results indicate that life-time seed production may be pollen limited in *C. bulbosa*, and that variation in population size and local abundance of the early-flowering, nectar-producing *S. caprea* can only partly explain the extensive variation in pollinator visitation among populations of this species.

**Key words** Fruit set · Pollen removal · Pollination by deceit · Pollinator limitation · Population size

### Introduction

In animal-pollinated plants, pollination intensity and plant fecundity are influenced by several factors including population size, plant community composition, and weather conditions during the reproductive period. Plant population size and degree of isolation can affect both the species composition and the local abundance of potential pollinators (e.g. Sowig 1989; Rathcke and Jules 1993; Aizen and Feinsinger 1994; Olesen and Jain 1994). Comparative and experimental studies of reward-producing plants (plants that offer nectar, pollen or some other reward to flower visitors) have shown that the rate of pollinator visitation may be positively correlated with population size (Sih and Baltus 1987) and plant density (Feinsinger et al. 1991; Kunin 1993). As a consequence, the likelihood of seed production being limited by insufficient pollen transfer should be higher in small than in large populations (Sih and Baltus 1987; Jennersten 1988), and this may result in a positive correlation between population size and plant reproductive output (Lamont et al. 1993; Widén 1993; Ågren 1996).

The functional relationship between population size and attractiveness to pollinators may depend on whether the plant offers the pollinator a reward or not. A large population of a non-rewarding species may be more conspicuous to pollinators, and therefore easier to detect at a distance, than a small population, but it does not provide more food. Stoutamire (1971) suggested that if pollinators learn to avoid the non-rewarding flowers after a few

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exploratory visits, this might result in lower pollination efficiency and plant fecundity in large than in small populations. There are very few empirical data available that could be used to evaluate this proposition. In a recent study of three non-rewarding orchids, Fritz and Nilsson (1994) found a negative relationship between population size and proportion of pollinia removed in two bumblebee-pollinated species of *Orchis*, and in the butterfly-pollinated *Anacamptis pyramidalis*, when data from 3 years of study were pooled. They also detected a corresponding negative relationship between population size and fruit set in *A. pyramidalis*, but not in the *Orchis* species.

It has been proposed that in non-rewarding plant species, pollinator activity and fruit production should be positively correlated with the local abundance of reward-producing plants (Lavery and Plowright 1988; Lavery 1992). There is some empirical support for this "magnet-species hypothesis". Dafni (1983) found that *Orchis caspia* had a markedly higher fruit set when growing together with two nectar-producing species (including one putative mimetic model) than when growing alone. In the nectarless *Podophyllum peltatum* L., fruit and seed production were negatively related to distance from the nearest *Pedicularis canadensis* L., which was heavily visited by bumblebees (Lavery and Plowright 1988; Lavery 1992). However, in contrast to the prediction of the magnet-species hypothesis, Lammi and Kuitunen (1995) found that experimental addition of a nectar-producing *Viola* cultivar decreased fruit set of neighbouring individuals of the nectarless orchid *Dactylorhiza incarnata*.

Pollinator activity and fruit production may also be affected by the weather conditions during flowering and fruit development (e.g. Schemske et al. 1978; McCall and Primack 1992). In the temperate and boreal zones, cold weather at the beginning of the growing season can disrupt pollinator activity and may kill developing flowers and fruits of early-flowering species (Schemske et al. 1978; Ågren 1988; Elmqvist et al. 1988). In woodland herbs, the probability of lethal damage to flowers and developing fruits from frost (Ågren 1988) and drought should decrease with increasing tree cover.

In this study, we analyse processes affecting pollinator visitation and fruiting success in the deceptive orchid *Calypso bulbosa* L. In *C. bulbosa*, the pollen is packaged in pollinia that remain intact during transfer. Pollen removal and deposition are therefore readily scored in the field and can be used as indices of pollinator activity. *C. bulbosa* flowers early in the season and is pollinated mainly by bumblebee queens (Mosquin 1970; Ackerman 1981; Boyden 1982). At the sites used in this study, *Salix caprea* L. and *Vaccinium myrtillus* L. are likely to be the most important food plants for the emerging bumblebee queens. *S. caprea* begins flowering before *C. bulbosa*, at a time when there are few other sources of nectar and pollen available at the study sites. *V. myrtillus* starts flowering a few days after *C. bulbosa*.

To examine the effects of population size, local abundance of *S. caprea*, and tree cover on pollinator visitation

and fruiting success in *C. bulbosa*, we scored pollen removal and fruit production in 16 populations representing a wide size range, in 3 consecutive years. In particular we tested the hypotheses that in this non-rewarding species, pollination intensity (proportion of plants visited) (1) should decrease with increasing population size, and (2) should increase with increasing local abundance of *S. caprea*. To determine whether fruit production was pollinator-limited, we performed supplemental hand-pollinations in three populations in each of 3 years. If there is a cost associated with fruit production, the benefit of increased reproduction in one year may be partly or fully balanced by a reduction in future survival or fecundity (Primack and Hall 1990; Calvo and Horvitz 1990; Calvo 1993; Ågren and Willson 1994; Ehrlén and Eriksson 1995). Furthermore, if the demographic cost of fruit production is low, it may be detectable only when it has been allowed to accumulate over several years (Primack and Hall 1990). To examine the demographic consequences of increased pollination intensity, supplemental hand-pollinations were repeated for 5 years in one population, and the effects on survival, growth, and cumulative flower and fruit production were quantified. Finally, to determine the importance of other causes of fruiting failure, we quantified damage from adverse weather conditions (including late frosts) and floral herbivory in the study populations.

## Materials and methods

### Natural history

*C. bulbosa* (Orchidaceae) is a self-compatible, non-autogamous, perennial herb (Mosquin 1970; Ackerman 1981) with a circumboreal distribution (Hultén and Fries 1986). *C. bulbosa* is rare in Sweden (Ingelög et al. 1993). It is restricted to the northern part of the country, where it grows in relatively nutrient-rich, mesic to moist spruce [*Picea abies* (L.) Karst.] and spruce-pine (*P. abies*-*Pinus sylvestris* L.) forests.

The plant forms a single leaf from an underground corm in late summer. In spring, flowering plants develop an inflorescence 4–15 cm tall with a single flower with pink petals and sepals. The flower has a pouch-like lip and a bicornute spur. In northern Sweden, *C. bulbosa* flowers for 3–4 weeks beginning in late May or early June. The flower is nectarless but often produces a sweet odour. The pollen is packaged in four pollinia, arranged in two pairs on the viscidium. The whole pollinarium (the four pollinia and the viscidium) is usually removed as a unit. Pollinia may be deposited individually, or in groups of two, three or four together (Proctor and Harder 1994; R. Alexandersson and J. Ågren, personal observations).

Several *Bombus* species may pollinate *C. bulbosa* in Sweden. During this study, we have caught queens of *B. hypnorum* (L.), *B. jonellus* (Kirby) and *B. pratorum* (L.), with pollinia attached to the rear part of the thorax, indicating that they may act as pollinators. Wollin (1975) reported *B. pascuorum* (Scop) ssp. *sparreanus* as a pollinator of *C. bulbosa* in northern Sweden.

The flower may stay open for about 2 weeks if not pollinated. After pollination, the flower usually withers within 4 days (Proctor and Harder 1995). The fruit matures in late July and contains thousands of seeds. The old leaf senesces during fruit development. In August, a new corm is formed, and from this corm a new leaf, and sometimes a flower bud, is produced towards the end of the growing season. Vegetative propagation may result from the

production of two new corms on the same plant, but is infrequent in the study populations.

#### Study sites

The study populations were located in the province of Västerbotten and in the southern part of the province of Norrbotten, in northern Sweden (63–66°N). They were all situated in mesic-moist spruce and spruce-pine forest with a ground vegetation that included *Vaccinium myrtillus*, *V. vitis-idaea* L., *Gymnocarpium dryopteris* (L.) Newm., and frequently also *Geranium sylvaticum* L. and other herbs. The density of the forest varied considerably among study sites. To quantify the degree of shading from trees, tree canopy closure was estimated by eye to the nearest 10% in 20 evenly spaced 1-m<sup>2</sup> plots in each population in 1994.

#### Population size, pollinator visitation and fruit production

To quantify the relationship between population size (number of flowering plants), pollinator visitation and fruit set, we scored pollen removal, pollen deposition and fruit production in 16 *C. bulbosa* populations representing a wide size range, in 3 consecutive years (1992–1994). A population was operationally defined as a group of plants separated from their closest conspecific by at least 100 m. All populations chosen for study were at least 500 m apart. In each year, all flowering plants in the study populations were individually marked, except in the largest population in which 250 flowering plants were marked. Each population was visited at least once during early flowering, once at the end of flowering, and once close to fruit maturation (late July). For each population, we recorded the total number of flowering plants, the number of plants that exported pollen, the number of plants that received pollen, the number of plants that produced a mature fruit, and any damage to flowers and developing fruits.

Damage to floral parts from herbivores, frost, and drought were observed. Flowers damaged by frost soon turned black, while flowers damaged by drought shrivelled and turned brown. Periods of frost and drought did not overlap. If there was any doubt about the source of the damage, it was classified as unknown.

#### Abundance of food plants early in the season

To quantify the local density of food-plants available for bumblebee queens during the early part of the growing season, we counted the number of flowering *S. caprea* within the *C. bulbosa* populations and within a 200-m-wide zone surrounding each population. The density of *S. caprea* was expressed as the number of flowering *S. caprea* ha<sup>-1</sup>. The local density of flowering *S. caprea* did not differ among years during the 3-year study.

The effects of population size, local density of *S. caprea*, and tree cover on the proportion of plants exporting pollen and the proportion of plants producing a fruit were evaluated by multiple regression. All proportions were arcsine square-root transformed prior to analysis.

#### Supplemental hand-pollination

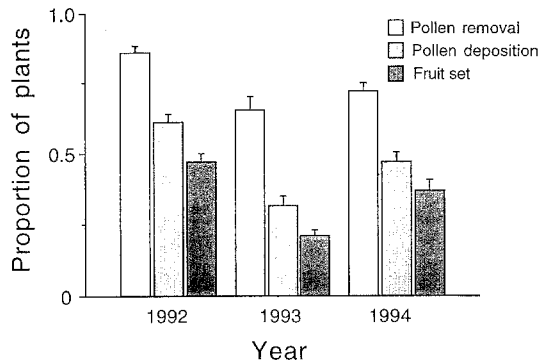
To determine whether fruit-production was limited by pollinator visitation, we conducted two experiments. In the first experiment, we performed supplemental hand-pollination in three populations that differed considerably in size. Up to 30 pairs of floral plants (nearest neighbours) were marked for this experiment prior to flowering (a maximum of two-thirds of the flowering plants in the population were included in the experiment). In each pair, one plant served as a control, and the second plant received supplemental pollination by hand. The number of flowering plants in the three populations varied among years (the number of plants receiving supplemental pollination in parentheses): 1992 population

A, 52 (14); population B, 149 (30); population C, 300 (30); 1993 population A, 22 (7); population B, 106 (23); population C, 217 (28); 1994 population A, 69 (21); population B, 244 (24); and population C 350 (26). The populations were visited every 3rd day, and the plants in the experimental group were pollinated as they opened. At pollination, two or four pollinia were transferred to the receptive stigma from another plant in the hand-pollinated group. The number of plants that had produced a mature fruit was scored in late July. The experiment was conducted for 3 years (1992–1994) with new pairs of floral plants randomly selected for study each spring. Log-linear models were used to analyse the effects of supplemental hand-pollination, population and year on fruit production.

To test whether there was a demographic cost associated with an increased level of pollination and fruit production in *C. bulbosa*, we conducted a second, long-term experiment in population C, from 1991 to 1995. In this 5-year experiment, we quantified the effects of repeated supplemental pollination on survival, growth and cumulative flower and fruit production. In 1991, 210 plants with flower buds were haphazardly chosen, mapped and marked with small, sequentially numbered plastic sticks. Plants with odd numbers served as controls and plants with even numbers received supplemental pollination by hand. Plants that did not flower in the 1st year were excluded from the experiment, which reduced the total number of plants to 194 (95 receiving supplemental hand-pollination and 99 control plants). Each year, the number of above-ground shoots, the status (flowering, vegetative, dormant or dead) and total leaf size of all experimental plants were scored about 1 week before flowering. Plants were scored as dormant if no aboveground parts were found, following a year when they had been scored as flowering or vegetative. Plants were scored as dead if no aboveground parts were found for 2 consecutive years. The width and length of leaves produced by the experimental plants were determined to the nearest mm with a ruler, and leaf area (leaf width×leaf length) was used as a measure of plant size. Leaf area is strongly correlated with corm-mass ( $r=0.72$ ,  $P<0.0001$ ,  $n=57$ ), which suggests that leaf area is a good indicator of plant vigour. In the first year leaf dimensions were measured on the day of flower opening for each individual plant, but in the following years all plants were measured on the same day. At the start of the experiment, there was no significant difference in leaf area between plants receiving supplemental pollination (mean±SE, 757±37 mm<sup>2</sup>,  $n=67$ ) and controls (698±35 mm<sup>2</sup>,  $n=71$ ,  $t=1.2$ ,  $P=0.25$ ). Each year, all flowering plants in the group receiving supplemental pollination were pollinated by hand. The pollination procedure was the same as in the hand-pollination experiment described above, and fruit set was scored in late July. In winter 1993–1994, part of the experiment was vandalized, which reduced the number of experimental plants to 145 (68 receiving supplemental hand-pollination and 77 control plants). Analyses of plant performance during the first 2 years after the initial treatment gave qualitatively similar results whether based on the initial 194 plants or on the 145 plants remaining in 1994, and only the results based on the latter sample will be presented below.

**Table 1** Characteristics of the studied *Calypso bulbosa* populations ( $N=16$ )

	Average ±SD	Median (Range)
Number of flowering plants 1992	136±130	83 (14–500)
Number of flowering plants 1993	101±129	49 (6–500)
Number of flowering plants 1994	178±238	90 (15–1000)
Tree cover (%)	48±20	45 (16–78)
Number of flowering <i>Salix caprea</i> ha <sup>-1</sup>	0.21±0.25	0.12 (0–0.79)



**Fig. 1** Proportion of plants that had their pollinia removed, received pollen, and that produced a mature fruit (mean ± SE) in 16 populations of *Calypso bulbosa* in northern Sweden

## Results

### Population characteristics

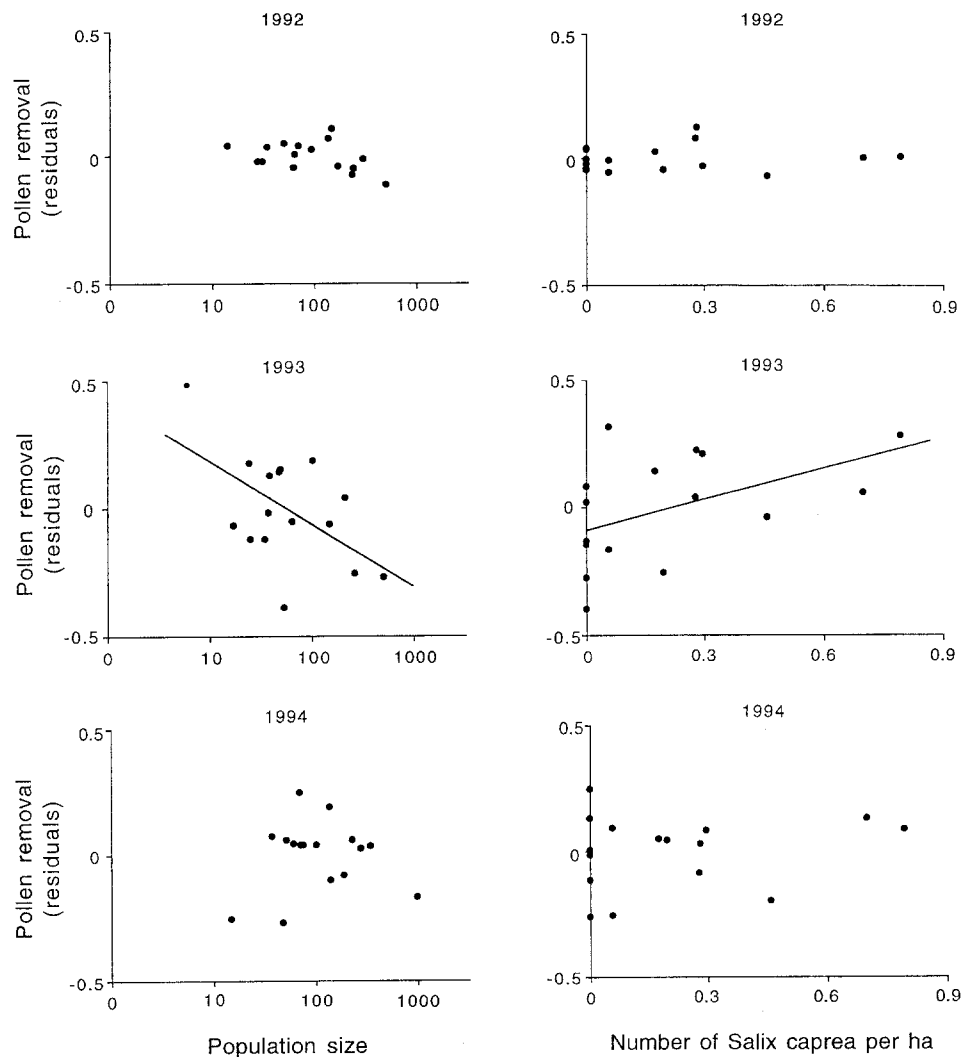
The number of flowering plants in the study populations ranged from fewer than 20 to several hundred in all 3 years of study (Table 1). Tree cover ranged from 16%

to 78%, and the density of flowering *S. caprea* from 0 to 0.79 ha<sup>-1</sup> (Table 1). Population size [log(number of flowering plants)] was not significantly correlated with tree cover (Pearson product-moment correlation, 1992  $r=0.01$ ,  $P=0.98$ ; 1993  $r=-0.08$ ,  $P=0.79$ ; 1994  $r=0.02$ ,  $P=0.93$ ;  $n=16$ ), or with density of *S. caprea* (1992  $r=0.32$ ,  $P=0.23$ ; 1993  $r=0.43$ ,  $P=0.10$ ; 1994  $r=0.42$ ,  $P=0.11$ ;  $n=16$ ). Moreover, there was no significant correlation between tree cover and density of *S. caprea* ( $r=-0.44$ ,  $P=0.09$ ).

### Pollen removal and fruit production

The average level of pollen export, pollen deposition, and fruit set varied considerably among years (Fig. 1). Multiple regression indicated that the proportion of plants exporting pollen was negatively related to population size, and positively related to density of *S. caprea* and to tree cover in 1993 ( $n=16$ ,  $R^2=0.50$ ,  $P=0.04$ ; Table 2, Fig. 2). However, these relationships, in particular the relationship between population size and pollen removal, were strongly influenced by the result from the

**Fig. 2** Relation between *C. bulbosa* population size (number of flowering plants) and proportion of plants that exported pollen, and between density of flowering *Salix caprea* and proportion of *C. bulbosa* plants that exported pollen in 1992–1994 ( $n=16$ ). Population size is plotted against the residuals from the regression of pollen removal on density of flowering *S. caprea* and tree cover, while density of flowering *S. caprea* is plotted against the residuals from the regression of pollen removal on population size and tree cover. Least-square regression lines are indicated in those cases where the partial regression coefficient was significantly different from zero (see Table 2)





**Table 2** Results of multiple regressions performed to evaluate the effects of population size (number of flowering plants  $\log_{10}$ -transformed), number of flowering *Salix caprea* per ha, and tree canopy cover on the proportion of plants exporting pollen and the proportion of plants producing a fruit in 16 *Calypso bulbosa* populations in northern Sweden

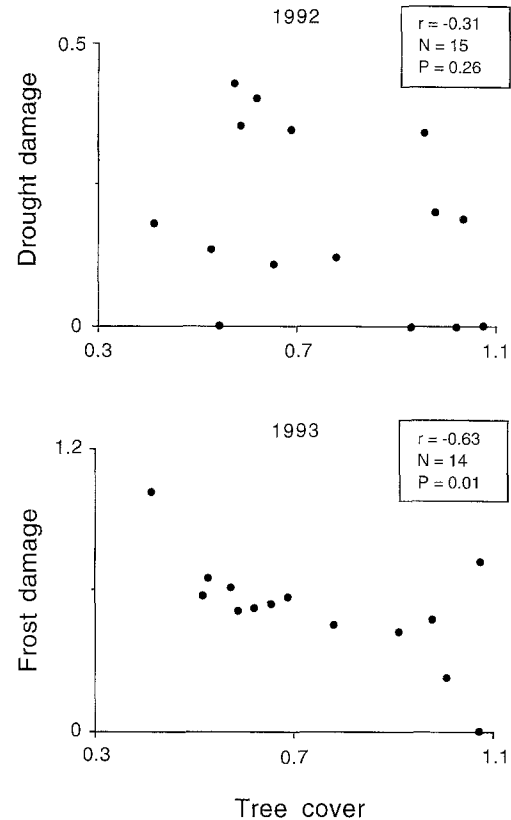
Dependent variable	Partial regression coefficient			$R^2$
	Population size	Number of flowering <i>S. caprea</i> per ha	Tree cover	
Pollen export 1992	-0.066	0.019	0.036	0.22
Pollen export 1993	-0.309*	0.587*	0.646*	0.50
Pollen export 1994	0.026	0.154	0.275	0.15
Fruit set 1992	-0.162	0.159	0.015	0.26
Fruit set 1993	-0.083	0.189	0.195	0.21
Fruit set 1994	0.067	-0.036	-0.331	0.09

\* $P < 0.05$ **Table 3** Factors preventing flowering *Calypso bulbosa* from producing a fruit (1992,  $n=15$ ; 1993,  $n=14$ ; 1994,  $n=16$  populations)

	Proportion of non-fruiting plants (% $\pm$ SD)		
	1992	1993	1994
No pollen deposited	65.8 $\pm$ 18.0	52.0 $\pm$ 22.1	73.4 $\pm$ 16.2
Frost	0.0 $\pm$ 0.0	34.6 $\pm$ 20.3	4.0 $\pm$ 16.0
Drought	10.1 $\pm$ 11.5	0.9 $\pm$ 2.2	2.8 $\pm$ 2.9
Floral herbivory	4.5 $\pm$ 4.5	2.4 $\pm$ 0.6	5.3 $\pm$ 4.2
Floral shoot damaged (agent unknown)	4.7 $\pm$ 5.4	5.0 $\pm$ 10.0	5.1 $\pm$ 4.3
No visible damage (pollen deposited)	14.9 $\pm$ 8.1	4.9 $\pm$ 5.2	9.6 $\pm$ 6.5

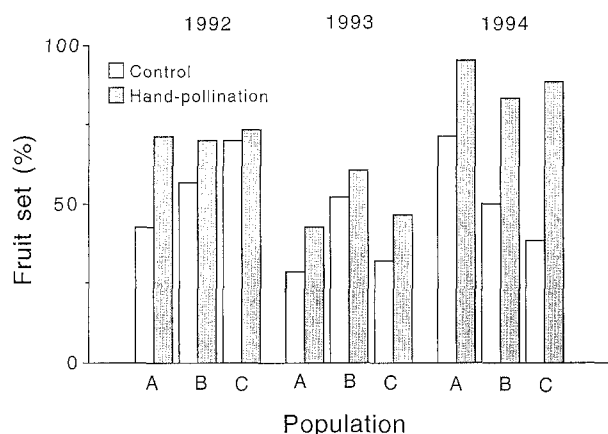
smallest population. If this population is excluded from the analysis, the multiple regression is not statistically significant (partial regression coefficient, population size -0.19, density of *S. caprea* 0.51, tree cover 0.54;  $n=15$ ,  $R^2=0.38$ ;  $P=0.14$ ). In 1992 and 1994, no significant relationship was detected between proportion of plants exporting pollen and the three environmental variables examined (Table 2). The proportion of plants that produced a fruit tended to be positively correlated with the proportion of plants that exported pollen (1992  $r=0.32$ ,  $P=0.23$ ; 1993  $r=0.62$ ,  $P=0.01$ ; 1994  $r=0.26$ ,  $P=0.35$ ,  $n=16$ ), but was not related to population size, density of *S. caprea*, or tree cover (Table 2).

Pollen limitation appeared to be an important factor preventing fruit production in all years. On average, 50–70% of the plants that failed to produce a fruit had not been pollinated (Table 3). The importance of late frosts and prolonged droughts for levels of fruit production varied among years. In 1993, 35% of the plants that failed to form a mature fruit had their inflorescence damaged by frost during the flowering period; in the other 2 years this proportion was <5% (Table 3). In 1992, the early part of the summer (including the *Calypso* flowering period and the subsequent 2 weeks) was warm and very dry, and desiccation accounted for 10% of the fruiting failure (Table 3). Tree cover was negatively correlated with the incidence of frost damage in 1993, and tended to be negatively related to the proportion of plants damaged by drought in 1992 (Fig. 3).

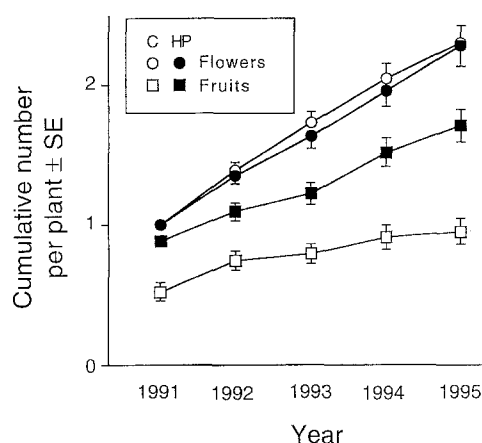
**Fig. 3** Relation between tree cover and the proportion of the flower-producing *C. bulbosa* that had their flowers or fruits killed by drought (1992) and frost (1993; all variables arcsine square-root transformed). The Pearson product-moment correlation coefficients are given

#### Supplemental hand-pollination

In the first experiment, fruit set varied considerably among the 3 years of study, but was consistently higher in hand-pollinated than in open-pollinated plants (Fig. 4). The initial analysis of the log-linear model indicated that there was no significant effect on fruit set of the interaction population $\times$ year $\times$ treatment (supplemental hand-pollination) ( $df=4$ ,  $\chi^2=1.4$ ,  $P=0.84$ ). Fruit set was significantly affected by the interaction between year and population ( $df=4$ ,  $\chi^2=10.3$ ,  $P=0.04$ ), and between year and treatment ( $df=2$ ,  $\chi^2=8.9$ ,  $P=0.01$ ), but not by the interaction between population and treatment ( $df=2$ ,  $\chi^2=0.2$ ,  $P=0.90$ ). In other words, the effects of popula-



**Fig. 4** Fruit set in control plants (open bars) and plants receiving supplemental hand-pollination (shaded bars) in three *C. bulbosa* populations from 1992 to 1994. See Materials and methods for sample sizes



**Fig. 5** Cumulative flower production (circles) and fruit production (squares) per plant, in control plants (open symbols;  $n=77$ ) and plants receiving supplemental hand-pollination (filled symbols;  $n=68$ ) in a large *C. bulbosa* population, from 1991 to 1995

**Table 4** Log-linear analysis of the effects of population and treatment (supplemental hand-pollination) on fruit set in three *Calypso bulbosa* populations in three different years. See Materials and methods for sample sizes

Source	df	1992		1993		1994	
		$\chi^2$	P	$\chi^2$	P	$\chi^2$	P
Population× treatment	2	1.1	0.57	0.1	0.95	0.8	0.67
Population	2	2.0	0.36	3.7	0.16	5.2	0.07
Treatment	1	2.5	0.12	1.8	0.18	24.2	0.0001

**Table 5** Leaf area of plants receiving supplemental hand-pollination and open-pollinated controls in a five-year experiment performed in a large *Calypso bulbosa* population 1991–1995

Year	Supplemental pollination		Control		t	P
	Mean±SE (mm <sup>2</sup> )	n	Mean±SE (mm <sup>2</sup> )	n		
1992	576±46	49	601±41	56	0.40	0.69
1993	547±33	52	547±35	62	0.002	1.00
1994	446±29	50	426±30	55	0.49	0.66
1995	510±30	49	515±39	53	0.11	0.91

tion and hand-pollination differed among years, but the effect of hand-pollination did not differ among populations. We therefore proceeded to analyse the effects of supplemental pollination for each year separately. The increase in fruit set following hand-pollination was statistically significant in 1994, but not in 1992 and 1993 (Table 4).

In the second, long-term experiment in population C, cumulative flower production by year 5 did not differ between plants receiving supplemental pollination and controls (mean±SE,  $2.3\pm0.15$ ,  $n=68$ , vs.  $2.3\pm0.13$ ,  $n=77$ ; Mann-Whitney *U*-test,  $P=0.80$ ), but hand-pollinated plants had produced 1.8 times as many fruits ( $1.7\pm0.12$ ,  $n=68$ , vs.  $0.95\pm0.09$ ,  $n=77$ ; Mann-Whitney *U*-test,  $P<0.0001$ ; Fig. 5). The higher fruit production of plants receiving supplemental pollination was not associated with a reduced survival or growth. Plant mortality over the 5-year period was 22% ( $n=68$ ) in the hand-pollinated group and 23% ( $n=77$ ) in the control group ( $G=0.04$ ,  $df=1$ ,  $P=0.85$ ). There was no difference in leaf area between plants of the two groups at the end of the experiment, but both hand-pollinated and control plants were smaller in 1995 than in 1991 (see Table 5 and above). Moreover, the proportion of plants producing two leaves did not differ between the hand-pollinated and the control group in the last year of the experiment (6%,  $n=68$  vs. 10%,  $n=77$ ;  $G=0.99$ ,  $df=1$ ,  $P=0.32$ ).

## Discussion

It has been suggested that several non-rewarding plant species depend for their pollination on exploratory visits by inexperienced pollinators foraging for nectar and pollen (e.g., Heinrich 1979; Little 1983; Nilsson 1992). It has been further hypothesised that in such species the proportion of plants visited by pollinators should be negatively related to population size (Stoutamire 1971) and positively related to local abundance of food plants (Lavery and Plowright 1988; Lavery 1992). The present study of *C. bulbosa* provided only limited support for these hypotheses. The predicted relationships between pollen removal and population size, and between pollen removal and local abundance of *S. caprea* (probably the most important food plant for the pollinators prior to the flowering of *C. bulbosa*), were detected in only 1 of 3 years (Table 2). Moreover, the statistical significance of these relationships depended on a high rate of pollen removal in the smallest population in that year.

The results further indicate that variation in fruit set is independent of population size in the non-rewarding *C. bulbosa*. In contrast, evidence for a positive correlation between population size and plant fecundity has been found in the reward-producing animal-pollinated plants *Banksia goodii* (Lamont et al. 1993), *Senecio integrifolius* (Widén 1993), and *Lythrum salicaria* (Ågren 1996). In these three studies, it was suggested that the positive correlation between population size and plant fecundity was due to insufficient pollen transfer in small populations, and this hypothesis was supported by the results of supplemental hand-pollinations performed in *L. salicaria* (Ågren 1996).

In plants pollinated by deceit, pollinator visitation and plant reproductive output may be affected by the local abundance of reward-producing species (Dafni 1983; Lavery and Plowright 1988). However, in this study there was no strong correlation between the local density of *S. caprea* and pollen removal and fruit set of *C. bulbosa*. This result would be expected if the local abundance of pollinators (bumblebee queens) is limited by factors other than food availability early in the season. Alternatively, because the method used to quantify the availability of nectar early in the season did not include a quantification of the flower production of individual *S. caprea*, it may have been too crude to allow the detection of a correlation with pollinator activity on *C. bulbosa*. Moreover, it is possible that the area checked for the presence of flowering *S. caprea* (the area covered by the *C. bulbosa* population and a 200-m wide-zone surrounding the population) was too small in relation to the foraging range of the pollinating bumblebees. Finally, because pollination efficiency may differ among bumblebee species, the reproductive success of *C. bulbosa* may be affected not only by the overall local abundance of bumblebees, but also by which species are locally represented and their relative frequencies.

Several observations indicate that fruit production is commonly pollinator-limited in the studied *C. bulbosa* populations. First, a large proportion (>50%) of the non-fruiting plants had not been pollinated. Second, supplemental hand-pollination consistently increased fruit set in three populations that were studied over 3 years. In iteroparous species, pollen limitation of seed production within a single season need not imply that lifetime fecundity is limited by pollen deposition. In several orchid species, the increase in fruit production resulting from supplemental hand-pollination has been found to be associated with a demographic cost in terms of a reduction in survival, growth, or future flower production (Montalvo and Ackerman 1987; Snow and Whigham 1989; Zimmerman and Aide 1989; Ackerman and Montalvo 1990; Primack and Hall 1990). For these species, demographic modelling is required to determine if an increase in seed production in one year represents a net fitness gain, or whether the benefit of this increase is outweighed by the demographic costs associated with fruit production (Calvo and Horvitz 1990; Calvo 1993; Ehrlén and Eriksson 1995). However, in our long-term study of

the effects of supplemental hand-pollination in *C. bulbosa*, the hand-pollinated plants had produced almost twice as many fruits as the controls after 5 years, but did not differ from the controls in terms of survival, leaf size or cumulative flower production. Thus we found no indication that costs associated with fruit production would reduce the potential fitness gain resulting from increased seed production in this species. The results suggest that lifetime reproductive success may be pollinator-limited in *C. bulbosa*.

In this study, the average fruit set of *C. bulbosa* ranged from 21% to 48%, which is high compared with many other non-rewarding orchids (e.g., Montalvo and Ackerman 1987; Gill 1989; Zimmerman and Aide 1989; Calvo 1990, 1993). The average proportion of plants receiving pollen ranged from 32% to 63% (Fig. 1). These levels of pollination are higher than those recorded in North American *C. bulbosa* populations. Ackerman (1981) reported that 11–34% of the plants were pollinated in five populations of *C. bulbosa* var. *occidentalis* in California. Studies of *C. bulbosa* var. *americana* in Canada have documented pollination rates between 1 and 30% (Mosquin 1970; Boyden 1982; Proctor and Harder 1995). It should be noted, however, that at least part of the variation in the reported levels of pollination may be due to differences in when and how often pollen deposition was scored. While the data presented in this study were obtained by scoring marked plants during flowering and at the end of the flowering period, some estimates from North American populations appear to be based on observations made during single visits to study populations during flowering (see Mosquin 1970; Boyden 1982).

In *C. bulbosa*, as in many other early-flowering species (e.g., Ågren 1988; Elmqvist et al. 1988), conditions for fruit production vary considerably among years. There was substantial among-year variation in the extent to which fruit production was limited by insufficient pollen deposition (Fig. 4), and in the amount of weather-induced damage to flowers and developing fruits (Table 3). In 1993, the floral shoot was killed by frost on 35% of the non-fruiting plants, while in 1992 a prolonged period of drought caused the floral shoot to wither on about 10% of the non-fruiting plants. Across populations, the amount of weather-induced damage to floral parts tended to be negatively correlated with tree cover. Tree cover was negatively correlated with the proportion of flowers and developing fruits that were killed by frost in 1993, and tended to be negatively correlated with the proportion of plants that had their floral parts lethally damaged by drought in 1992. Shelter from frost is also of greatest importance for the reproductive success of *Rubus chamaemorus*, another early-flowering plant in northern Sweden (Ågren 1988). The positive relationship between tree cover and pollen export in 1993 (Table 2) can at least partly be explained by the fact that, in this year, a considerable proportion of the flowering shoots were damaged by frost early in the season in populations with a sparse tree cover.

This study has documented considerable variation, both spatial and temporal, in pollen removal and fruit set

in the deceptive orchid *C. bulbosa*. While fruit set appears commonly to be limited by pollen deposition, variation in population size and local abundance of the early-flowering, nectar-producing *S. caprea* can only partly explain variation in pollinator visitation among populations. Further studies are therefore required to identify the factors governing the among-population variation in pollination intensity. In particular, it would be of interest to quantify the relative pollination efficiency of the different bumblebee species visiting *C. bulbosa*, and to determine the extent to which differences in pollinator fauna contributes to the among-population variation in fruit production.

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