

## Chapter 2.

### Effects of population size and pollinator availability on pollination and seed set of a heterostylous plant, *Primula sieboldii*

#### Introduction

In outbreeding plant populations many factors are expected to influence seed set through pollination. These include the population size and spatial pattern of mating groups (Chapter 1, Matsumura & Washitani 2000; Antonovics & Levin 1980; Barrett & Thomson 1982; Kunin 1992, 1993, 1997; Aizen & Feinsinger 1994; Ågren 1996; Groom 1998; Aizen 2001) and the composition, abundance, and behaviors of the pollinator fauna (Chapter 1, Matsumura & Washitani 2000; Bertin 1982; Snow 1982; Motten 1986; Stone 1996; Herrera 2000). While positive effects of population size (e.g., Ågren 1996; Groom 1998) or plant density (e.g., Feinsinger et al. 1991; Kunin 1992, 1993, 1997) on fruit or seed set has been reported for a number of plant populations, few studies could demonstrate with certainty whether or not the effects are ascribed to pollination success, or other factors such as resource availability or genetic compatibilities.

In order to evaluate the importance of pollen limitation to seed set the quantitative relationships between the number of compatible pollen grains deposited on a stigma and the level of fertilization should be analyzed. However, until now the analysis of the relationships under field conditions have been performed for relatively few species (Shore & Barrett 1984; Nishihira et al. 2000).

A heterostylous plant species, *Primula sieboldii* E. Morren (Primulaceae) has following merits for the studies to understand how size and structure of natural populations as well as pollinator availability are related to pollination success and seed set. 1) The number of compatible pollen grains deposited on a stigma is easily countable due to marked difference in pollen grain size between the heterostylous morphs (Chapter 3, Matsumura & Washitani 2002). 2) Individual genets can be easily identified due to large variation in floral morphology (i.e., size, color, and shape) (Washitani et al. 1991). 3) Possible mating counterpart for each genet can be specified to some degree, since self- and intra- morph pollination are largely incompatible in the species (Washitani et al. 1994b). 4) Species of effective pollinators, i.e., nectar-feeding queen bumblebees (especially *Bombus diversus tersatus*), affecting the reproductive success of *P. sieboldii* has been well understood (Washitani et al. 1994a).

The previous study has demonstrated that natural populations of *P. sieboldii* were vulnerable to the detrimental effects of pollinator loss or population isolation, resulting in seed set failure (Chapter 1, Matsumura & Washitani 2000). In the isolated small populations (genets  $\leq 3$ ), fertility was constantly negligible, probably because of lack of compatible mating partners. In larger populations (genets  $\geq 7$ ), seed set of the individual populations was strongly limited by yearly variable pollinator availability, which was assessed by the proportion of flowering inflorescences with claw marks left by queen bumblebees on the visit. Seed set in the long-styled morph of *P. sieboldii* populations was generally higher than in the short-styled morph under the naturally pollen-limited condition (Chapter 1, Matsumura & Washitani 2000). Between-morph asymmetry in pollination may cause this difference in seed set between the morphs, i.e., flowers in the long-styled morph of *P. sieboldii* might have received more legitimate pollen from the short-styled morph flowers, as demonstrated in other distylous species (reviewed by Lloyd & Webb 1992). Also,

partial or cryptic self-compatibility of the long-styled morph (Matsumura & Washitani 2000) could partly explain the difference as discussed in Chapter 1.

The first aim of the present study is to reveal the effects of population size as well as pollinator availability on pollination and seed set of *P. sieboldii* populations. The second aim is to demonstrate the asymmetry in pollination between the morphs. First, I analyzed the effects of population size on seed set of *P. sieboldii* populations. Secondly, I measured stigmatic legitimate and illegitimate pollen load in each population and analyzed the effects of population size and pollinator availability on pollination.

## Materials and Methods

### PLANT SPECIES AND STUDY SITE

*Primula sieboldii* E. Morren (Primulaceae) is a clonally growing geophyte that occurs in a range of moist habitats throughout Japan. Each genet is composed of various numbers of physiologically independent ramets. The mean number of ovules per flower is approximately 120 (Nishihiro et al. 2000). In the southern Hokkaido, *P. sieboldii* is associated with maritime deciduous forests dominated by *Quercus dentata*.

The present study was carried out in natural populations of *P. sieboldii* in the Hidaka region of the southern Hokkaido. These are the same sites in my earlier study (Chapter 1, Matsumura & Washitani 2000).

In the present study, population size (number of flowering genets), pollinator availability, and population mean of seed set per undamaged (free from pathogens and herbivores) flower from the populations investigated in 1996 in Matsumura & Washitani (2000) were analyzed. In 1996, except for two populations, pollinator availability was more than 0.8 in populations investigated.

For the measurements of stigmatic pollen load, the eight populations of *P. sieboldii* (populations D, E, F, G, J, O, P, and Q) were selected from the 24 populations investigated in 1996 (Chapter 1).

The population traits concerning population size, pollinator availability, and seed set per undamaged flower in 1996 were: population P, number of genets > 100, pollinator availability > 0.8; populations J and Q, number of genets > 100, pollinator availability < 0.3; populations D, E, and O, number of genets < 50, pollinator availability > 0.8, high seed set among the small populations (Fig. 7 in Chapter 1); and populations F and G, number of

genets < 50, pollinator availability > 0.8, low seed set among the small populations (Fig. 7 in Chapter 1).

### **STIGMATIC POLLEN LOAD**

In the flowering season of 1996, stigmas were collected for pollen-load measurements using fine forceps when the corolla of the flowers would fall if lightly touched at the time of closure of individual flowers. At this stage fertilization of ovules is complete, but ungerminated pollen and exines of germinated pollen still remain on the stigma (Nishihiro et al 2000). Immediately after collecting, the stigmas were mounted on grass slides and sealed with transparent nail enamel. The stigmas were collected from 2 to 14 flowers without any symptoms of damage by predators or pathogens, from 2 to 12 arbitrarily chosen ramets of three to eight genets each of the long- and short-styled morphs.

For each stigma collected, all the pollen grains deposited on it were counted under a fluorescence microscope (BX50, Olympus, Tokyo) by morph being referred to their sizes; judgement criteria are given in Nishihiro et al. (2000).

For population P the data collected by Nishihiro et al. (2000) were used.

### **STATISTICAL ANALYSIS**

The significance of the relationship between population size (number of genets) and population means of seed set per undamaged flower was examined by product moment correlation coefficients.

Effects of population, morph, and their interaction on the number of pollen grains loaded on stigmas were analyzed by two-way ANOVA (Sokal & Rohlf 1995). The data

from the small populations, i.e., populations D, E, O and populations F, G, were pooled, because the sample size of each population was too small. Paired comparisons between the populations were made by *contrast*.

The data for number of genets and the number of pollen grains loaded on stigmas were normalized by  $\log_{10}(x + 1)$  transformation before parametric analyses (Sokal & Rohlf 1995). The data for pollinator availability were arcsine transformed before parametric statistical analyses (Sokal & Rohlf 1995).

## Results

### POPULATION SIZE AND SEED SET OF *PRIMULA SIEBOLDII* POPULATIONS

The quantity of seed set in the largest population P was the highest in the long-styled morph, and relatively high in the short-styled morph (Fig. 1). In the populations with less than 50 genets including populations D,E,F,G, and O, seed set per undamaged flower were varied widely among the populations (Fig. 1). In large populations J and Q of which pollinator availability was low, the mean value of seed set per undamaged flower was lower than population P and moderate relative to small populations (Fig. 1).

### STIGMATIC POLLEN LOAD

The patterns and the mean number of legitimate pollen grains loaded on stigmas varied significantly among the populations and between the morphs (Table 1, Fig. 2). Two-way ANOVA for the number of legitimate pollen grains loaded on stigmas revealed that both the effects of population and morph were significant and that the interaction of these factors was also significant (Table 1, Fig. 2).

In populations [D, E, O], [F, G], J, and Q, the flowers irrespective to the morph less than 10 accounted more than 60% (Fig. 2). Only a few flowers (4%) of the short-styled morph of populations [D, E, O] received a large quantity of legitimate pollen grains (Fig. 2).

In both morphs of population P, almost all the stigmas received legitimate pollen grains exceeding the ovule number (Fig. 2). The mean number of legitimate pollen grains deposited on the stigmas was significantly high compared with populations [D, E, O] (contrast  $F = 453.113$ ,  $P = 0.0001$  for the long-styled morph,  $F = 275.951$ ,  $P = 0.0001$  for

the short-styled morph), [F, G] (contrast  $F = 316.983$ ,  $p = 0.0001$  for the long-styled morph,  $F = 231.537$ ,  $P = 0.0001$  for the short-styled morph), J (contrast  $F = 299.281$ ,  $P = 0.0001$  for the long-styled morph,  $F = 212.901$ ,  $P = 0.0001$  for the short-styled morph), and Q (contrast  $F = 252.246$ ,  $P = 0.0001$  for the long-styled morph,  $F = 218.343$ ,  $P = 0.0001$  for the short-styled morph).

Generally, the mean number of legitimate pollen grains loaded on stigmas were higher in the long-styled morph than in the short-styled morph in all the populations (Fig. 2).

Although the mean number of illegitimate pollen grains loaded on stigmas were far over the ovule number (120) for both morphs, the means were higher in the long-styled morph than in the short-styled morph in all the populations (Fig. 3). Two-way ANOVA for the number of illegitimate pollen grains loaded on stigmas revealed that both the effects of population and morph were significant and that the interaction of these factors was not significant (Table 1).



## Discussion

The population size and pollinator availability of a plant population are expected to influence reproductive success through quantitative and qualitative differences in pollination (e.g., Shore & Barrett 1984; Kunin 1992, 1993, 1997; Aizen & Feinsinger 1994; Ågren 1996; Groom 1998; Herrera 2000; Aizen 2001; Bosch & Waser 2001).

In *P. sieboldii*, the number of compatible pollen grains deposited on the stigmas was significantly lower in relatively large populations J and Q with low pollinator availability than that of population P with sufficient pollinator services. This was likely to be responsible for lower seed set in populations J and Q.

Seed sets in the small populations varied greatly among the populations, while those of populations J and Q were moderate. It is likely that quality of pollination, i.e., the number of pollen donors within the stigmatic compatible pollen load was higher in populations J and Q than the small populations, because not all compatible pollen grains produce same reproductive success (Niesenbaum & Casper 1994; Quesada et al. 1996; Niesenbaum 1999). Direct measurement of genetic diversity of stigmatic pollen load is technically difficult at present. The extent of pollen donor diversity in stigmatic pollen load could be estimated indirectly through evaluation of the extent of pollen carryover and pollinator visitation rates (Chapter 3, Matsumura & Washitani 2002).

As previously reported, seed set in the long-styled morph of *P. sieboldii* populations was generally higher than in the short-styled morph under the naturally pollen-limited condition (Chapter 1, Matsumura & Washitani 2000). This is supposed to be related to the fact that the stigmas of the long-styled morph received more compatible pollen grains, though partial or cryptic self-compatibility of the long-styled morph (Matsumura & Washitani

2000) could partly explain the difference.

Studies of pollen deposition on naturally pollinated stigmas of heterostylous species have often revealed asymmetries in pollen transfer between the floral morphs (reviewed by Lloyd & Webb 1992). In 13 of 17 distylous species of which pollen flow has been measured, significantly greater pollen transfer from the short-styled to the long-styled morph flowers than in the reverse direction was demonstrated (Stone & Thomson 1994).

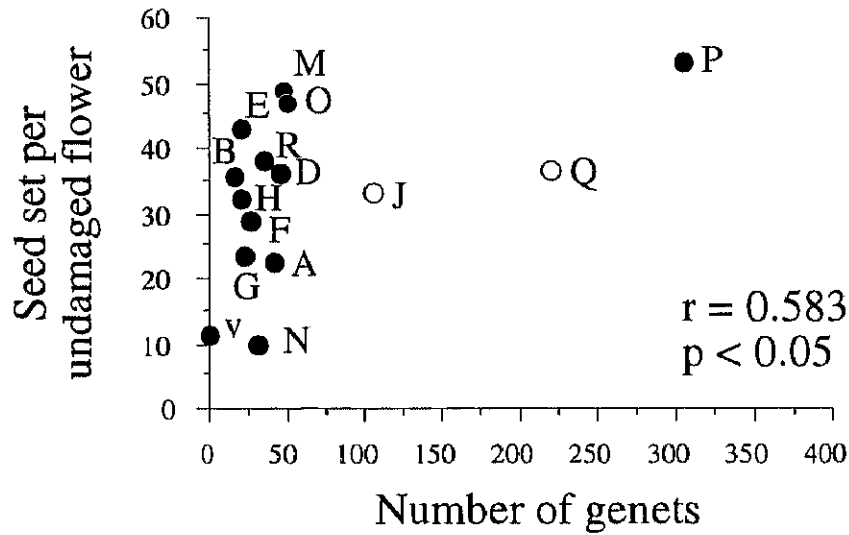
In the tubular flowers typical of distylous species (Ganders 1979), one of the most important factors causing this asymmetrical pollen flow is thought to be the way the pollinator's body contacts the stigmas or anthers, i.e., more protruded reproductive organs can make better contact with the pollinators' bodies (e.g. Ganders 1974, 1976; Stone & Thomson 1994).

In order to reveal the factors causing variation in the number of pollen grains loaded on the stigmas between the morphs, measuring whole elements of the pollination process, i.e., pollen production, pollinator visitation, pollen removal from a flower by the pollinators, and pollen deposition on the stigmas of the flowers under conditions independent of influences from morph ratio should be needed (Chapter 3, Matsumura & Washitani 2002).

Table 1 Results of two-way ANOVA for the number of legitimate and illegitimate pollen grains loaded on the stigmas of *Primula sieboldii* populations.

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Number of legitimate pollen grains loaded					
Population	4	648.302	162.075	331.474	< 0.0001
Morph	1	13.657	13.657	27.931	< 0.0001
Population × morph	4	13.843	3.461	7.078	< 0.0001
Residual	912	445.925	0.489		
Number of illegitimate pollen grains loaded					
Population	4	109.447	27.362	32.882	< 0.0001
Morph	1	163.339	163.339	196.292	< 0.0001
Population × morph	4	4.675	1.169	1.405	0.2305
Residual	912	758.894	0.832		

## Long-styled morph



## Short-styled morph

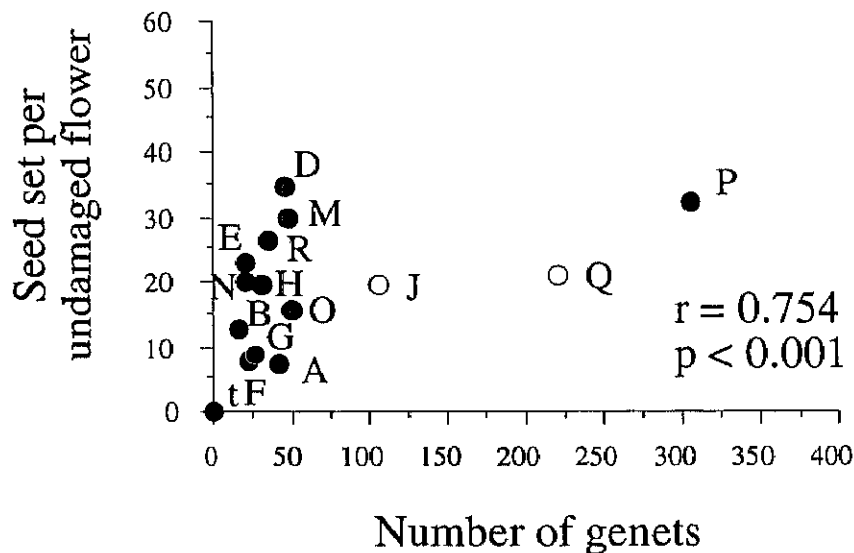


Fig. 1 Relationships between number of genets for the *Primula sieboldii* populations and mean seed set per undamaged flower in 1996. Uppercase letters indicate the populations with genets of both heterostylous morphs and lowercase letters indicate the populations with one genet. Filled circles and open circles represent the data for pollinator availability, i.e., proportion of inflorescences with claw marks more than 0.8 and less than 0.3, respectively. Correlation coefficient and its significance level are shown.

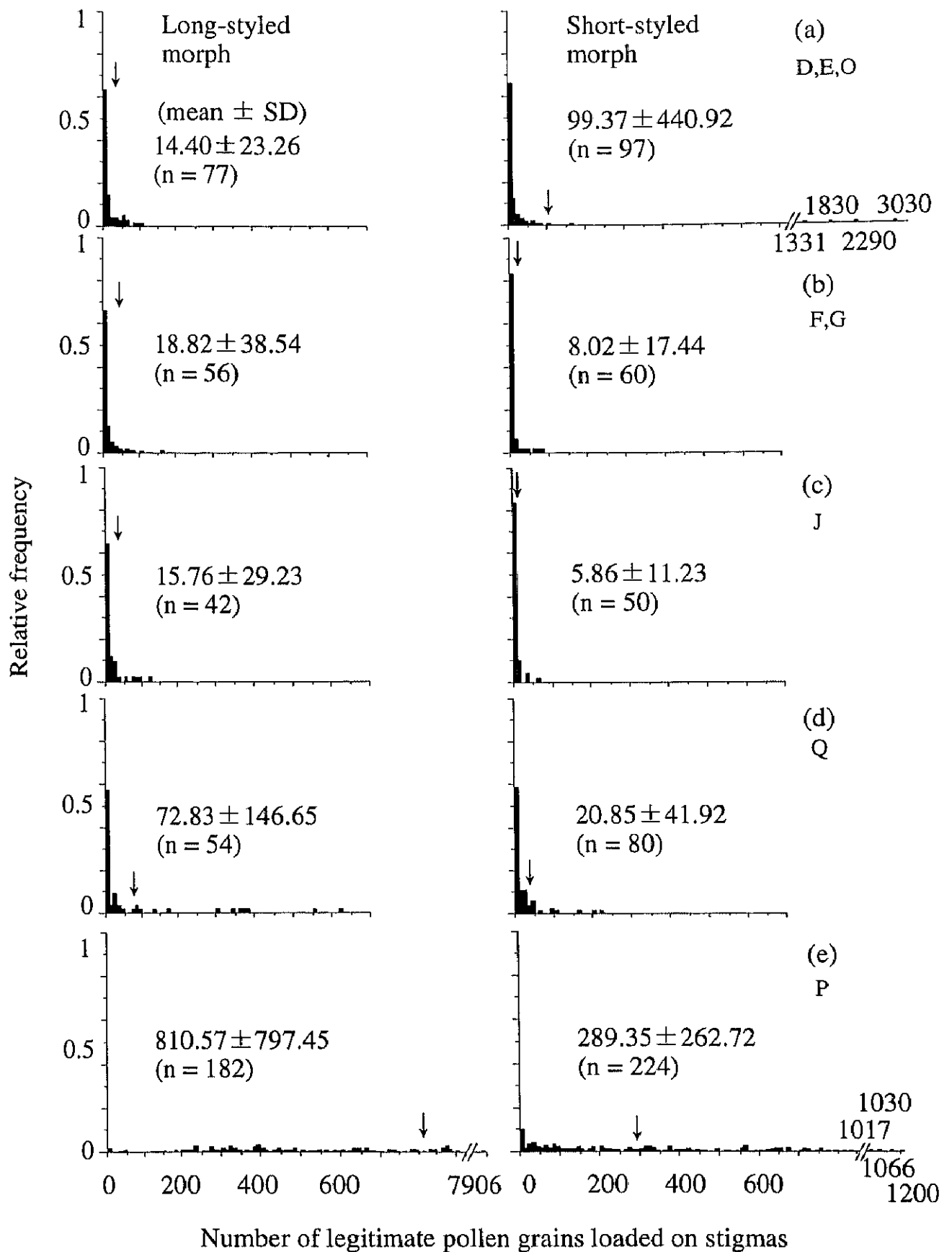


Fig. 2 Relative frequency distribution of number of legitimate pollen grains loaded on the stigmas of the long- and short-styled morph flowers of *Primula sieboldii* (populations; (a) D,E,O; (b) F,G; (c) J; (d) Q; (e) P ). An arrow in each histogram indicates the average. The number of stigmas sampled in each population is given in parentheses.

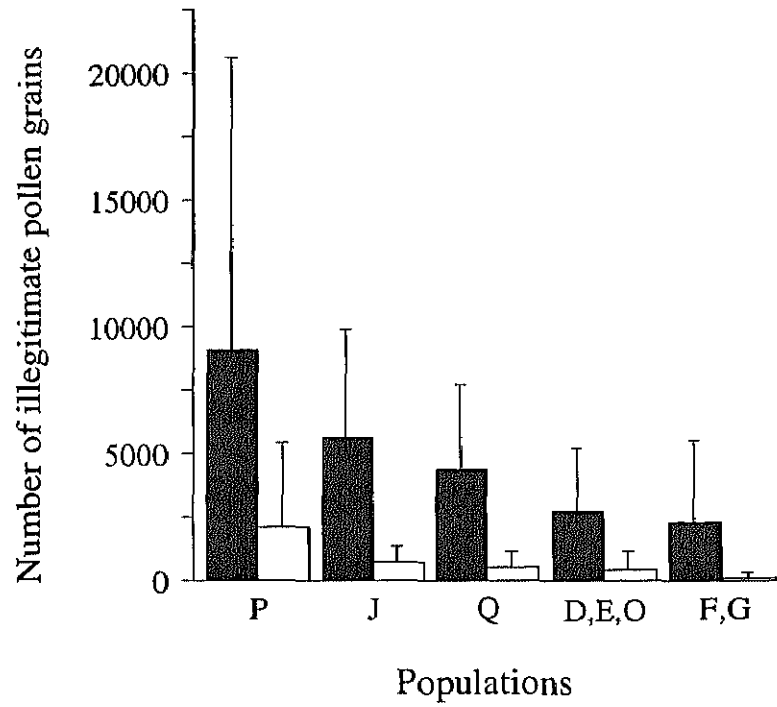


Fig. 3 Mean (column) and S.D. (bar) for the number of illegitimate pollen deposited on the stigmas of the long- (shaded column) and short- (unshaded column) styled morph flowers of *Primula sieboldii*.