

## General Introduction

### *General background*

'Successful reproduction by seeds' is indispensable to long-term maintenance of a plant population, even in a clonally propagated perennial, of which genets inevitably suffer from mortality (Harper 1977). In natural habitats, seed production is affected by various biological and abiological factors acting independently or interrelatedly. These include the density, spatial pattern and frequency of mating groups (Mulcahy 1967; Platt et al. 1974; Wyatt & Hellwig 1979; Antonovics & Levin 1980; Barrett & Thomson 1982), the composition, abundance, and behavior of the pollinator fauna (Levin & Berube 1972; Waser 1978; Barrett 1980a; Bertin 1982, Snow 1982; Motten 1986), physical environmental conditions during pollination, fertilization, and embryo development (Lewis 1943; Barrett 1980b), and antagonistic biological agents such as flower, fruit and seed consumers and pathogens (Inouye et al. 1980; Louda 1982a, b, 1989; Alexander & Antonovics 1988; Burdon et al. 1989; Louda & Potvin 1995), as well as the availability of resources for fruit and seed maturation in the plant (Brink & Cooper 1947; Lloyd et al. 1980; Udovic & Aker 1981; Stephenson 1981).

Pollination is a process consisting of a series of events related to transfer of pollen grains to the stigma by pollen vectors in angiosperms (Proctor et al. 1996). As sessile organisms, seed plants are passive recipients of pollen brought from conspecific plants including themselves. The plant population size, spatial pattern, and the density of mating groups (e.g., Antonovics & Levin 1980; Barrett & Thomson 1982; Feinsinger et al. 1991), and abundance and behaviors of the pollinator fauna (e.g., Bertin 1982; Snow 1982; Motten

1986) are expected to influence plant reproductive success through quantitative and qualitative patterns of pollination.

The contribution of pollination to plant reproductive success should be evaluated qualitatively by pollen donor diversity (mixed pollination) as well as quantitatively by stigmatic pollen load size (number of pollen grains on a stigma). These parameters influence the plant fitness through seed set quantity and/or progeny quality. Generally, seed set is higher in plants that receive pollen from genetically diverse pollen donors (Niesenbaum & Casper 1994; Quesada et al. 1996; Niesenbaum 1999), and combination of pollen and ovule parents can affect seed set considerably due to post-pollination rejection of apparently compatible pollen (Marshall & Ellstrand 1986; Marshall 1991). If such difference in reproductive success due to combination of pollen and ovule parents is present, it is likely that high pollen donor diversity as well as pollen load size is a required condition for full reproductive success of plant.

Floral evolution generally involves two classes of adaptations that promote mating success. The morphological adaptations that characterize floral design and display modify the actions of pollen vectors so as to enhance fertilizing as many ovules as plants can develop into seeds (Harder & Barrett 1996; Proctor et al. 1996). In contrast, physiological traits mitigate unsatisfactory pollen dispersal by rejecting unsuitable male gametophytes (Marshall & Ellstrand 1986; Seavey & Bawa 1986; Snow & Spira 1991; Walsh & Charlesworth 1992) or zygotes (Stephenson 1981; Casper 1988; Becerra & Lloyd 1992; Montalvo 1992). As a result of postpollination processes, the realized mating pattern does not simply mirror the pattern of pollination (e.g., Campbell 1991; Waser & Price 1993). However, these processes can only filter the incipient mating pattern established during pollination, so that pollination fundamentally determines the maximum frequency and diversity of mating opportunities.

Most evolutionary biologists would accept that floral design and display modify the actions of pollen vectors so as to enhance fertility and to minimize limitations related to both pollen load size and donor diversity (Harder & Barrett 1996). Nevertheless, the supposed merits of the adaptation and the effects of pollinator visitation on patterns of both stigmatic pollen load and donor diversity as well as their reproductive consequences remain to be understood based on the data. Information on the role of pollinators in pollination success in plant populations with various size and spatial structure are also required to evaluate the importance of pollination process to seed set. In order to demonstrate the effects of population size and pollinator visitation on stigmatic pollen load size, measuring the amount of pollen deposited per visit is needed.

Mixed pollination is an important and necessary condition of multiple paternity (Campbell 1998). Despite the important consequences of multiple paternity in plants, i.e., increase of fertility (Marshall 1991), reduction of genetic relatedness among offspring (Ritland 1989), and augmentation of maternal fitness (Marshall & Ellstrand 1986), the mechanisms by which it occurs remain to be clarified.

The following two mechanisms have the potential to produce mixed pollination. First, mixed pollination can result if pollen from different fathers is deposited sequentially in multiple pollinator visits to a flower (Dudash & Ritland 1991). Second, mixed pollination can result when a pollinator deposits a mixed pollen load simultaneously in a single visit (Marshall & Ellstrand 1985; Campbell 1998). The relative importance of these two mechanisms varies according to the plant and pollinator species and the condition surrounding them, and is expected to depend on the balance between pollinator visitation rate and pollen carryover.

Pollen carryover is defined as 'the proportion of the pollen grains picked up from the first flower, to those deposited on the second and subsequent flowers'. Pollen carryover

has long been also recognized as an important factor that affects the extent of gene flow in plant populations (Schaal 1980; Karron et al. 1995).

Direct observations of pollen carryover are rare because it is usually impossible to distinguish between self- and outcross pollen in stigmatic pollen loads. Despite the necessity of understanding real patterns of pollen carryover to estimate its effect on gene flow, most data in the previous studies were obtained from the measurements under rather artificial settings as follows.

Some investigators have estimated carryover of outcross pollen by counting the number of pollen grains deposited on stigmas in a sequence of visits to emasculated, previously unvisited recipient flowers (Waser 1988; reviewed by Robertson 1992; Rademaker et al. 1997). A major problem with this technique, however, is that there is no opportunity for outcross pollen to be interfered with the presence of self-pollen or anthers. In addition, pollen-collecting bees might visit emasculated flowers less often or for shorter time periods than normal flowers (Snow et al. 1996).

Researchers have also quantified pollen dispersal by using fluorescent dye powders as pollen analogue (e.g., Price & Waser 1979; Waser & Price 1984; Thomson et al. 1986; Hessing 1988; Waser 1988; Morris et al. 1994). Dispersal patterns of powders and pollen grains can be sufficiently different to preclude using dye particles for accurate estimates of pollen dispersal (Thomson et al. 1986; Waser 1988). However, in species with pollinia, this problem can be avoided by labeling the entire pollen dispersal unit (Peakall 1989; Pleasants 1991; Nilsson et al. 1992). For natural populations of *Erithronium grandiflorum*, a pollen color polymorphism was used to measure pollen carryover, but unfortunately, the color difference disappeared when pollen germinated on the stigma, introducing a good deal of error into estimates of pollen dispersal (Thomson 1986).

Theoretical quantification of pollen carryover has been also attempted by various

authors (Lertzman & Gass 1983; Morris et al. 1995; Morris et al. 1994; Harder & Barrett 1996; Harder & Wilson 1998). However, the results of such attempts should be tested by empirical data, before we can ascertain the validity.

### *The scope of the present study*

In its natural habitats, as mentioned above, seed production of the plant species is affected by various biological and abiological factors as well as pollinator availability and population size. Individual factors affecting seed production of one plant species vary through time and space (e.g. Alexander & Louda 1982a, b, 1989; Antonovics 1988; Horvitz & Schemske 1990; Herrera 1995; Louda & Potvin 1995; Waser et al. 1996), so that in order to know the contribution to plant reproductive success of pollinator availability or population size or structure, it is required to evaluate various factors potentially affecting fertility simultaneously as well as to compare among a large number of populations in a landscape for a number of seasons.

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 pollination 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 has been performed for relatively few species (Shore & Barrett 1984; Nishihira et al. 2000).

The purpose of the present study is to reveal the effects of pollinator visitation on both stigmatic pollen load size and donor diversity as well as their relation to seed set in natural populations with various number of genets in *Primula sieboldii* E. Morran (Primulaceae).

*Primula sieboldii* E. Morran is a perennial clonal herb that occurs in a range of moist habitats throughout Japan. Like many other *Primula* species, *P. sieboldii* is distylous, and, like most heterostylous species, it requires insect pollinators for legitimate pollination between mutually compatible morphs, i.e., long-styled morph and short-styled morph (Ganders 1979; Lloyd & Webb 1992). The importance of queens of bumblebees, *Bombus diversus diversus* Smith (Hymenoptera: Apidae) (in Hokkaido, subspecies *Bombus diversus tersatus* Smith) was strongly suggested as effective pollinators for legitimate (inter-morph) pollination of *P. sieboldii* by Washitani et al. (1994a). Phenological and morphological matches, i.e., early spring flowering during the queen bee emergence season and the similar lengths of the corolla tube and the bee proboscis, also support the inference that queen bumblebees play an important role in legitimate pollination of *P. sieboldii* (Washitani et al. 1994a). These coincidences between *P. sieboldii* and the queen bumblebee suggest that *P. sieboldii* adapts to the queen bumblebees in pollination (Washitani et al. 1994a).

*Primula sieboldii*, which is heterostylous, has the following merits for the study to understand how size and pollinator availability of natural populations are related to pollination and seed set and also for the measurement of pollen carryover. 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*, have been well understood (Washitani et al. 1994a).

Until now how pollination process affects seed set of wild plant species in natural habitats is poorly demonstrated because of the following reasons. First, in most wild plant species, limiting factors of seed set are poorly understood. Secondly, investigation of the effects of pollination on seed production for many populations is difficult, because monitoring pollinator visitation on thousands of flowers through their flowering season is actually impossible.

On visiting the flowers of *P. sieboldii*, the queen bumblebees cling to the floral petals to leave clear claw marks on the flower petals, which therefore, are useful indicators for pollinator services provided by the bumblebees (Washitani et al. 1994a). The use of the claw marks for indicators for pollinator services enables us to investigate many *P. sieboldii* populations simultaneously.

### *Outline of the thesis*

In Chapter 1, in order to confirm the importance of pollination process in seed production in field plant populations, spatial and temporal variations in fruit and seed sets and biological factors limiting them were studied for 24 *P. sieboldii* populations in a landscape of southern Hokkaido where many populations of the species still remain, though intensively fragmented and isolated.

In Chapter 2, the effects of population size and pollinator availability on pollination and its relation to seed set of *P. sieboldii* populations were analyzed. 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.

In Chapter 3, in order to know how pollinator availability and population size affect on both pollen load size and diversity, under a semi-natural setting, I quantified between-morph pollen exchange patterns in *P. sieboldii* flowers by measuring pollen removal from the anthers on a single visit by *Bombus diversus tersatus* queen and stigmatic pollen deposition along the sequence of the visitation of the opposite-morph flowers by the bee.