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Minireview

Toward predicting gene flow in plant populations

Izumi Washitani*, Fumiko Ishihama, Ayako Shimono, Miho Ajima Nishihiro

Institute of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo 113-8657, Japan
* E-mail: awashi@mail.ecc.u-tokyo.ac.jp Tel: +81-3-5841-8915 Fax: +81-3-5841-8916

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Abstract For comprehensive understanding of gene flow consisting of pollen flow and seed flow and the consequences, one of the promising approaches is the integration of ecological and genetic studies with a model plant species or related species group to disentangle complicated interactions of the ecological and genetic processes. We present a brief summary of research aiming to disentangle the factors affecting gene flow in wild populations of Japanese *Primula* species as a model of bumblebee-pollinated clonal herbs.

Key words: Conservation of biological diversity, gene flow, plant extinction process, pollen flow, seed flow.

Currently, under strong anthropogenic impact including habitat destruction, excessive exploitation, and biological invasion (McNeely et al. 1990; Primack 2000), biodiversity is declining on global, regional and local scales. Global climate change presumed to have started already is another major threat to biodiversity (Buse et al. 1999; Inouye et al. 2000; Schlaepfer et al. 2002; Thomas et al. 2004). With the rapid globalization of the world, the problems of biological invasion will become even more critical (Washitani et al. 2005).

In the context of this crisis caused by multiple interacting anthropogenic factors, the monitoring and assessing of population trends in relation to environmental changes in order to evaluate the ecological and/or genetic processes that underlie observed patterns are indispensable for effective conservation practices (Christensen et al. 1996; Yoccoz et al. 2001). The immediate strategic monitoring or assessment of plant populations should focus on the state of sexual reproduction, especially gene flow processes. Sexual reproduction creating diverse combinations of genes plays an important role in adaptation to a changing environment and in the acquisition of resistance to pests or pathogens (Maynard Smith 1998). Moreover, it is only in this stage of the life history that genes or individuals of plants as sessile organisms can move in space substantial distances. In addition, dormant seeds persistent in soil can assume temporal escape from various environmental risks (Harper 1977).

Generally, extinction is the consequence of a mutually accelerating demographic and genetic decline of a population (Frankham and Ralls 1998; Saccheri et al. 1998; Lennartsson 2002). Thus, understanding or evaluating gene flow mediated by pollen or seed movement is essential for the comprehension of the processes related to the extinction risks in plants. Moreover, gene flow from genetically modified crops into populations of wild or domesticated relatives has newly entered the research arena since late 1980's when social concern over the bio-security and risk assessment of genetically modified crops began to grow. Therefore, the current prediction of gene flow, especially over generations in wild plant populations, is an important issue in the study of biodiversity conservation and bio-security. However, it is never an easy task since the movements of pollen and genes are influenced by species-specific life history strategies such as the breeding system and the spatial structure of the population, as well as by a vast number of external factors including numerous stochastic ones that mutually interact.

For a comprehensive understanding of gene flow and its consequences such as the vices cycle of plant population extinction, one promising approach is the integration of ecological and genetic studies with a model plant species or related species group to disentangle complicated interactions of the ecological and genetic processes (Washitani et al. 2005). In the present paper, we present a brief summary of a part of such research aiming to disentangle the factors affecting gene flow in wild populations of Japanese *Primula* species as a model of bumblebee-pollinated clonal herbs.

This article can be found at http://www.jspcmb.jp/

Primula sieboldii and *Primula modesta* as models

Most *Primula* species are herbaceous perennials clonally growing in mountainous to alpine habitats, and have a distylous breeding system, i.e., heterostyly with two opposite morphs compatible each other (Richards 1986). *P. sieboldii* belonging to the section cortusoides and *P. modesta* of the section aleuritia are two representative Japanese *Primula* species (Richards 2003).

P. seiboldii was once common in various moist habitats on volcanic soils throughout the Japanese Archipelago, but in recent years has declined and is now listed as vulnerable on the national red list (Environment Agency of Japan 2000). Most remaining populations of the species are more or less isolated due to habitat fragmentation (Environment Agency of Japan 2000), but variously sized populations differing greatly with regarding to extinction probability and genetic diversity still remain. A relatively sufficient amount of data on the demographic, eco-physiological, and genetic traits of the species is available, most of which has been obtained from wild populations in Southern Hokkaido or central Honshu (Washitani et al. 1991, 1994a, 1994b, 1996; Kakishima et al. 1995; Washitani 1996; Nishihiro et al. 2000; Matsumura and Washitani 2000, 2002; Okayama et al. 2003; Ishihama et al. 2003, 2005; Watanabe et al. 2003; Noda et al. 2004; Honjo et al. 2004; Kitamoto et al. 2005).

Primula modesta Bisset et Moore is a common perennial herb that grows in a range of montane to alpine habitats throughout Japan from Hokkaido to Kyushu. Demographic and genetic data have obtained from the subalpine zone of Mt. Asama (36°24′12″N, 138°31′34″E, 2568 m a.s.l.) in Nagano Prefecture, central Japan (Shimono and Washitani 2004), where *P. modesta* is distributed in fenland, grassland, and rocky tracts at elevations from 1900 to 2300 m a.s.l.

Pollen flow measurements in experimental populations with different spatial structures

Merit of using an experimental population under a natural pollination service

Generally, since plants are sessile, the spatial structure is one of the major factors governing gene flow in the population. In some natural populations of *P. sieboldii*, a strong effect of opposite-morph density within a 5 m radius on the seed set was found (Nishihiro et al. 2000; Watanabe et al. 2003), implicating relatively short pollen-dispersal distance and the possible importance of the local arrangement of genetically compatible mates for successful seed reproduction.

Since the components of spatial structure and environmental factors often covary in a natural population, detection of independent effect(s) of spatial components in measurements is usually difficult (Rathcke 1983; Aizen 1997; Kunin 1997). The use of an experimental population set under natural conditions of pollination would be an effective approach to circumventing such drawbacks. In an experimental population, parameters of spatial structure such as the local and/or global density of plants or flowers of a compatible morph can be intentionally manipulated. The combined use of an experimental population with highly polymorphic molecular markers such as microsatellites is a highly effective means of evaluating the influences of spatial structures on plant gene flow (Smithson and Macnair 2003). Another advantage to using an experimental population is the feasibility of choosing parental genotypes that enable unambiguous determination of maternal and paternal individuals through paternal analysis (Karron et al. 1995; Richards et al. 1999).

The following are the major findings on pollen dispersal and seed set in two experimental populations of *P. sieboldii* differing in plant arrangement and density: a patchy population of low density at the whole-population level (global scale) (Figure 1A) and a high-density population with a regular linear arrangement of genets (Figure 1B).

Pollen flow in a population with a patchy structure having high local and low global densities

Using seven microsatellite markers (Ueno et al. 2003), the paternity of 94.9% of the seeds analyzed in the low-density patchy population (Figure 1A) was determined. Even though seed set was significantly affected by the presence-absence of opposite-morph plants within the same patch, considerable pollen flow was ascertained to occur between the patches, and the mean pollen dispersal distance within the experimental population was 7.32 m. Pollen flow between the patches separated by more than 30 m accounted for 43.2% of between-patch pollen flow. Such a distant pollen flow can be explained by the long carryover of pollen by *Bombus diversus*, the major pollinator of *P. sieboldii* (Matsumura and Washitani 2002), the small size of the patches (Ellstrand et al. 1989), and the absence of the compatible opposite morph for a large proportion of individuals in the same patch.

Pollen flow in a high-density experimental population

The high-density experimental population consisted of regularly arranged small patches of plants with a flowering ramet density about 15 times that of the low-density population, and the density of opposite morph differed greatly between the ends and the middle of the linear population (Figure 1B).

Seed set was significantly correlated with the number of opposite-morph flowers within a 2–3 m radius. Of

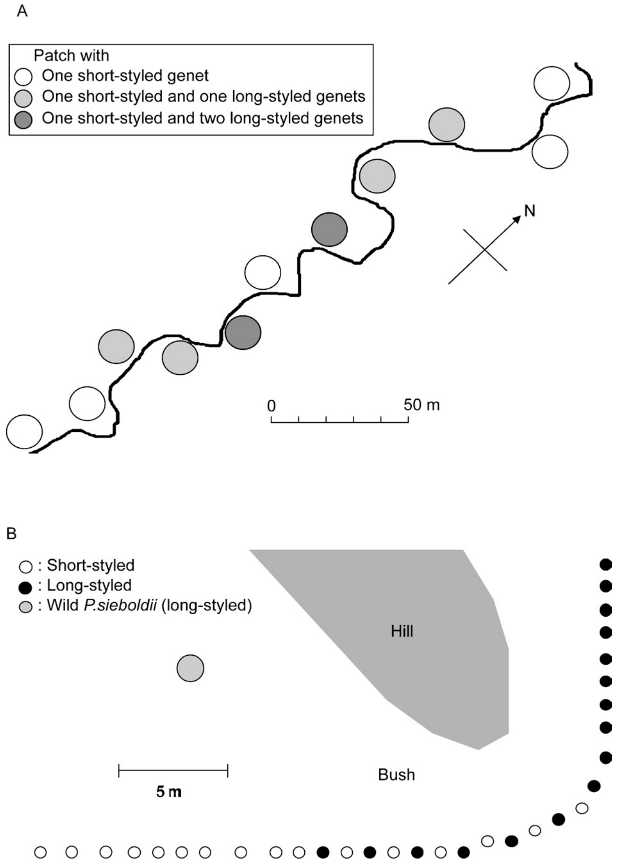

Figure 1. Experimental populations for the measurements of pollen flow in *P. sieboldii*. A: In the low density population, 11 patches were arranged along a stream, which is indicated as a curving line. Each patch was composed of one common short-styled clone and zero, one, or two long-styled clones, so that opposite-morph density differs among patches. (Modified from Figure 1 in Ishihama et al. 2003) B: The high-density experimental population was composed of 32 small patches arranged in a line with spacing of about 1.5 m. Each patch consists of a single clone differing among patches. The population was designed so that opposite-morph density is highest in the center of the population and lowest at both ends. The number of flowering ramets per patch was varied randomly.

336 seedlings obtained from the seeds, the paternity of 95.2% was successfully assigned. Of the assigned fathers, 68.4% were found within the experimental population. In the middle part of the experimental population, where the local opposite-morph density was high, irrespective of the morph, a large proportion of the seeds was sired by the opposite-morph genet within a 5 m radius. However, near the ends of the experimental population, where the opposite-morph density is low, most seeds born on the short-styled genets were sired by pollen from outside the experimental population. The mean pollen dispersal distance was 4.4 m, significantly shorter than that obtained for the low-density population (7.3 m). However, the distance is much longer than the expected value based on the reciprocal of the density (7.3/15 = 0.49 m), which can be expected for a linear population with the assumption of sequential visitation of the pollinator. The major mechanisms responsible for this deviation are mentioned in the next section.

There were remarkable differences between the morphs in the pollen donating and receiving patterns. The most conspicuous was greater self- and intra-morph fertilization in the long-styled morph. This tendency has been suggested by artificial pollination experiments and seed set patterns under pollinator limitation in some natural populations (Washitani et al. 1994b; Washitani 1996).

Major factors responsible for the difference in patterns between the experimental populations

The pollen dispersal distance of animal-pollinated plants largely depends on plant density. At a lower density, a longer flight distance of the pollinators can be expected (Fenster 1991; Morris 1993), and a longer pollen dispersal distance as well (Karron et al. 1995).

However, pollen dispersal was comparably restricted in the low-density population. The mean distance to the nearest potential mate in the lower-density experimental population, i.e., the distance from a short-styled flower to the nearest long-styled flower, was 10.9 m. Opposite-morph density at a very local scale (1–3 m) was shown to have the predominant effect on seed reproduction in both experimental populations.

The restricted dispersal distance in the low-density population is probably due to the complicated responses of pollinator behavior to population density. Visitation frequency per plant tends to be low in such a low density population (Kunin 1993,1997).

To further clarify the mechanisms of the effect of the demographic components, pollinator behavior in relation to the size and spacing of plant patches was investigated. Visitation frequency per patch was shown to be highly dependent on the average flower number per patch. Moreover, pollinators tend to visit more flowers per patch at larger patches, and a larger proportion of flowers per patch with longer spacing. This suggests a predominance of geitonogamous self-pollination in large patches consisting of the same genet, and less frequent pollen flow between patches with longer spacings. The tendency for a pollinator moving between nearby patches, which was demonstrated here, well explained the pollen flow pattern revealed by the paternity analysis, i.e. the predominance of pollen flow among nearby patches.

These results and observations are consistent with previous studies on pollinator behavior (Morris 1993; Cresswell 1997; Ohashi and Yahara 1998), and strongly indicate the particular importance of the proximity of compatible mates for successful reproduction by seeds at least to the stage of seed set. Generally, the effective population size may be much smaller than the actual population size due to restricted pollen dispersal in a plant population.

Seed flow and spatiotemporal dynamics of soil seed banks

Attempts to measure spatial and temporal patterns of seeds and seedlings

A conspicuous feature of seed flow is its extended scale of time. Seed flow is determined by not only the spatial dispersal, but also mortality, dormancy, and germination processes of the seeds, as well as by seedling performances that can be strongly influenced by microsite environmental conditions and the genetic quality of the seeds, such as inbreeding depression. These phases and processes of spatial and temporal dispersal are inevitably subjected to various stochastic events. We can deduce the seed flow patterns from the patterns of spatial and temporal distribution of seeds or seedlings in these phases. However, sufficient attention should be focused on the highly stochastic processes.

Temporal dispersal patterns of seeds can be predicted from the seed physiology of dormancy and germination. The seed physiology of *P. sieboldii* (Washitani and Kabaya 1988) and *P. modesta* (Shimono and Washitani 2004) indicates the formation of persistent seed banks under ordinary habitat conditions. Physiological traits also suggest that seeds persistent in soil will be released from the dormancy when exposed to high light or large diurnal alternations of temperature that are environmental features of a denuded surface soil. Therefore, natural or artificial disturbances have a considerable influence on temporal seed flows.

Estimation in the natural habitat of **P. sieboldii**

Direct measurement of the seed dispersal of *P. sieboldii* using seed traps (Figure 2) revealed the dispersal pattern, which can be approximated by a log-normal curve. More than 85% of seeds were dispersed within 15 cm of the mother plant, and the mean distance of seed dispersal was 9.2 cm in the field experiment.

Safe-site availability is shown to strongly influence the spatial pattern of seedling emergence. Therefore, the spatial pattern of emerged seedlings, which is the immediate consequence of seed flow, can be predicted from the seed dispersal pattern and the distribution of safe sites for seedling emergence, which was characterized by the field experiments and observation of actual seedling emergence. In the natural habitat, the emergence of *P. sieboldii* seedlings was confined to a certain type of microsite that is characterized by denuded soil without litter accumulation.

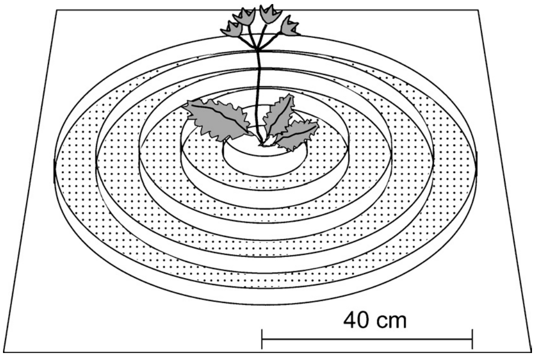

Figure 2. The seed trap for measuring primary seed dispersal distances from a mother plant. The plastic partition separates concentric circular zones (width, 5 cm) of sterilized sand with the fruiting plant as the focus. The radius of the entire trap is 40 cm.

Estimation with the experimental population of **P. sieboldii**

The relatively short seed flow suggested by the prediction mentioned above was ascertained in the experimental population. In the experimental population with a patchy structure and high local/low global densities, some seedlings were found to be clumped in a patch. The genotype of the seedlings found in the patch strongly suggested that all seeds were derived from the nearest long-styled genet, and the mean dispersal distance was estimated to be very short (10.4 cm) and well corresponded to the predicted seed flow mentioned above.

Population genetic structure

Factors potentially strengthening the genetic structure

Poor dispersal ability of pollen and seeds may inevitably lead to the formation of a marked genetic structure (Vekemans and Hardy 2004). Generally, seeds are more immobile than pollen as shown in the measurement of pollen and seed flow in the experimental population of *P. sieboldii* described above.

However, certain factors obscure the genetic structure. Inbreeding depression tends to negate the mating between closely located individuals resulting from short pollen flow, although spatial genetic structure within a population is expected to be rather obscure in preferentially outcrossing species compared to selfing species (Ennos 2001; Vekemans and Hardy 2004). In studies of outcrossing species, more significant genetic structure has tended to be detected in maternally inherited markers than in nuclear and paternally inherited markers (McCauley et al. 1996; Tarayre et al. 1997; Levy and Neal 1999; Caron et al. 2000). These results imply that seed dispersal is generally more limited than pollen transfer, and thus can result in a marked spatial structure.

The long persistency of seeds in soil seed banks is another important factor also lessening structuring. In plant species with limited seed dispersal, transient seed banks may develop a fine-scale spatial genetic structure based on that of the aboveground plant population. On the other hand, persistent seed bank may prevent the build-up and retention of a significant genetic structure by pooling the reproductive output of many generations and averaging out the effects of each generation's dispersal pattern. In addition, persistent seeds bank are likely to have more opportunities for secondary dispersal than transient seed banks, such as by water flow or soil disturbance, which may weaken genetic structure.

Genetic structure and inbreeding depression

In a natural population of *P. seiboldii*, a marked aggregation of closely related individuals within several meters was found. The effect of inbreeding depression on pollen dispersal was estimated by estimating kinship structure. A considerable inbreeding depression was suggested in mating within 5 m, according to predictions assuming a linear relationship between the exponential of the kinship coefficient and the magnitude of inbreeding depression, and 80–95% reductions in fitness of selfed progenies (Ishihama et al. 2005).

These results indicate the importance of considering not only the local opposite-morph density, but also kinship structure and consequent inbreeding depression in the conservation management of fragmented populations of *P. sieboldii*.

Genetic structures in soil seeds and relation to the above-ground population

The spatial genetic structure of the transient and persistent components of soil seed banks and its relation to that of aboveground plants was examined in an alpine fen habitat of *P. modesta*, where the soil bank with numerous seeds (1000–2700 seeds/m^2) was shown to be developed. A limited spatial dispersal of the seeds is presumed, since the seeds with no special adaptations for dispersal are released from a height of 10–20 cm. Stratified sampling from different soil depths allowed an analysis of the spatial genetic structure of somewhat different components of soil seeds, i.e., transient and persistent components, based on the assumption that most seeds that are even partially buried in the soil form persistent seed banks owing to a strict light requirement for germination (Shimono and Washitani 2004).

We analyzed the fine-scale spatial genetic autocorrelation between flowering plants and soil seed banks obtained by stratified sampling from different soil depths, using kinship coefficients (Shimono et al. in press). The spatial genetic association between the surface seed bank (0–1 cm depth) and the flowering genets was significantly positive over short distances. In contrast, a weak spatial genetic association between the deeper seed bank (1–5 cm depth) and flowering genets was detected.

These results suggest that the surface seed bank accounts for a large proportion of the previous season's seed dispersal, and the deeper seed bank pools the reproductive output of multiple generations and averages out the effects of each generation's dispersal pattern. The surface and deeper seed banks are likely to represent transient and persistent seed banks (Thompson and Grime 1979), respectively.

The directional kinship detected (Shimono et al. in press) indicated that secondary dispersal by running water modifies the spatial genetic structure and elongates dispersal distance. The persistent seed bank may have more opportunities for secondary dispersal than the transient seed bank, which is one possible reason for the absence of a spatial genetic structure in the deeper seed bank.

The stratifying sampling of the soil seed bank is likely to allow discrimination of the dynamics of transient from persistent components of the soil seed bank under natural conditions.

Toward modeling gene flow

Gene flow and its consequences in a plant population are highly idiosyncratic (Ellstrand et al. 1989), and affected or modified by a vast number of factors influencing demographic and genetic processes, which mutually interact. Thus, modeling gene flow requires a flexible combination of sub-models describing individual processes, some of which were considered in the present paper.

Modeling pollination processes constituting a major part of pollen flow requires a description of the pollinator movement among flowers within and between flower patches (Cresswell et al. 1995; Di Pasquale and Jacobi 1998; Cresswell et al. 2002) and the pollen carryover pattern (Morris et al. 1994). Modeling seed flow is likely to be a more difficult task, since the time span to be taken into account is much longer, and more stochastic events are involved. The modeling of the consequence of pollen flow at the viable seed production requires a comprehensive understanding of compatibility patterns between the potential mates, which is rather easy in a population of heterostylous plants.

Seed flow modeling should include both spatial and temporal processes and their interaction, which might be more complicated compared to pollen flow. A prerequisite for modeling temporal processes is an understanding of seed eco-physiology (Washitani 1987) based on the detailed germination/dormancy responses of seeds determined in laboratory experiments, and seed longevity or mortality patterns in the natural habitat, part of which can be revealed by seed placement/retrieval experiments. The immediate spatial pattern of the primary spatial dispersal of seeds can be modeled comparatively easily based on measurements with seed traps. The empirical clarification of later spatial patterns and their interaction with temporal patterns is not easy, but modeling of the pattern of seedling emergence which is the final result of seed flow and was attempted in *P. sieboldii* as described above would be rather promising. In the near future, with a more intensive study of the empirical modeling process, we should be able to predict the demographic/genetic fate of a population of *Primula* species with the integrated model approach.

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