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Floral distribution, clonal structure, and their effects on pollination success in a self-incompatible *Convallaria keiskei* population in northern Japan

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Abstract

In plant species, when clonal growth produces a patchy structure and flowering ramets are clustered, the amount of pollen contributing to reproductive success is often regulated by pollinator efficiency and geitonogamy. The spatial population structure may influence reproductive success. We examined the clonal structure, the spatial ramet distribution, and their combined effects on fruit set in a natural population of the insect-pollinated, self-incompatible clonal herb, *Convallaria keiskei*, in northern Japan. The number of shoots, flowers, and fruits in 1-m^2 quadrats were counted at every 5 m grid point in an established 100 × 90-m study plot. From all the quadrats where shoots existed, leaf samples were collected for allozyme analysis. Using the two spatial parameters of flowering ramet densities and genotypes, we then constructed individual-based fruit-set models. A total of 236 quadrats contained shoots, and 135 contained flowering ramets, which indicated expanded distribution of this plant throughout the study plot, while shoots, flowers and fruits all showed clustering

distributions. Allozyme analysis of 282 samples revealed 94 multilocus genotypes. The largest clone extended to more than 40 m, whereas 56 genotypes were detected in only one sample. Several large clones and many small clones were distributed close to each other. Fine-scale spatial modelling revealed that the neighbouring flower numbers of different genotypes, compared with local genet or flower diversity, more influenced fruit set, in which the range of the neighbour was 14.5 m. These findings indicate that the compatible pollen dispersed by insect pollinators has a significant effect on sexual reproduction, in this *C. keiskei* population. Consequently, the spatial structure, which includes both genet distribution and clonal expansion by ramets, had a significant effect on pollination success.

Introduction

In clonal plants, populations are maintained by two reproductive modes: sexual reproduction and clonal growth. Clonal growth can be advantageous in the establishment of offspring without pollination, whereas sexual reproduction with pollen and seed dispersal helps to maintain genetic diversity in plant populations. Depending on species and environments, the diverse modes of clonal growth include long or short spacers; vegetative organs such as rhizomes, stolons, or bulbs; or plant fragmentation (Harper 1977; Klimeš et al. 1997; Klimeš and Klimešová 1999). With regard to the growth form associated with spacer length, Lovett-Doust (1981) introduced the concepts of the "phalanx strategy" for growth via short spacers and the "guerrilla strategy" for growth via long spacers. These strategies result in the formation of different clonal structures. In principle, the phalanx type leads to clustering of genetically identical ramets and the guerrilla type leads to an intermingling of genets (Harper 1977; Wijesinghe and Hutchings 1997). Nevertheless, because spatial distribution by clonal growth often exhibits a nonuniform pattern owing to phenotypic plasticity under heterogeneous environmental conditions (Hutchings and de Kroon 1994; Price and Marshall 1999; Fischer and van Kleunen 2002), the clonal distribution (clumped or intermingled) is not necessarily uniform even within a population.

Clonal structure influences patterns of pollen dispersal and mating opportunities of individual plants (Charpentier 2002; Nuortila et al. 2002) and, thus, has an impact on reproductive success. For example, in the case of outcrossing entomophilous plants, large clone size and multiplication of flowering shoots from clonal growth may enhance geitonogamy (Handel 1985; Silander 1985; Wilcock and Jennings 1999; Eckert 2000). In a large clone, frequency of pollen deposit from the same clone must increase more than smaller clones. Then, high flowering density should attract pollinators, but thus pollinator movement may be restricted within a clone. Moreover, spatial scales of clonal spreading and intensities of gathered flowers may be different among and/or within clones, according to heterogeneous environment in a population (Evans and Cain 1995; Stuefer 1996; Price and Marshall 1999). Therefore, reproductive success should be affected by fine-scale spatial population structure, which includes both genet and ramet information, because the amount of compatible pollen depends on clonal structure as well as ramet spatial distribution. This impact also depends on the magnitude of pollen dispersal, which is affected by the distance pollinators travel between successive flowers (Nuortila et al. 2002; Somanathan et al. 2004); especially, self-incompatible rather than self-compatible species are exposed to the risks of a deficiency in compatible pollen (Beattie 1976; Feinsinger et al. 1991).

Convallaria keiskei Miq. (Convallariaceae) is a clonal perennial herb having an inflorescence of bell-shaped flowers with a mild fragrance. This species is self-incompatible, and its pollination requires receipt of cross pollen via insect pollinators (Araki et al. 2005). According to this previous study there was a higher percent of fruit and seed set in artificially outcrossed plants than in open pollination, indicating that pollen-limitation is occurring; because in *C. keiskei* the neighbouring floral environment (i.e., flowering ramet densities of the same or different genets) is likely to affect the amount of available and compatible pollen and ultimately the plant's reproductive success. Thus, sexual reproduction should be influenced by the genet diversity not only at the population level but at the individual level, with the response of pollinators reflecting the fine-scale spatial ramet distribution in a genet.

In this study, we measured the spatial distribution of ramets in a *C. keiskei* population and used allozyme electrophoresis to elucidate the clonal structure. We then examined the combined effect of these two spatial parameters (i.e., the flower distribution and the clonal structure) on fruit set, a measurement of female reproductive success. Specifically, by constructing an individual-based fruit-set model, we at first verified how the total number of neighbouring flowers with different genotypes influenced fruit-set ratios. We then compared this compatible flower model with models using flower or genet distribution of spatial

information to assess the efficiency of combining the two factors. We finally discuss the characteristics of the clonal structure of this *C. keiskei* population, some limitations of the model, the ecological implications of the "neighbour" deduced from the model, the role of local flower density for display effects, and its spatial population dynamics.

Materials and Methods

Study species

Convallaria keiskei is a rhizomatous perennial herb, distributed in Japan (Hokkaido, Honshu, and Kyushu), Sakhalin Island, Korea, China, and eastern Siberia (Utech and Kawano 1976). In Hokkaido of northern Japan, *C. keiskei* grows on relatively shaded forest floors, in open grasslands, and along the seacoast, occasionally forming large populations. New aerial shoots (sheath leaves) elongate and appear aboveground from late April to May, and flowering occurs from late May to June. On the forest floor, there are two distinct vegetative aerial shoots (a one-leaf shoot and a two-leaf shoot), as well as a two-leaf shoot that bears an inflorescence. An inflorescence typically has three to 15 mildly scented bell-shaped flowers

and produces fruits that each contains three to 17 seeds. The aerial shoots die between September and October. *C. keiskei* is highly self-incompatibility (at least in the study population described below), and outcrossing is mediated by insect pollinators of the orders Diptera, Coleoptera, and Hymenoptera (Araki et al. 2005). *Convallaria keiskei* also propagates clonally through stolons that elongate from the parent to a new offspring ramet, whereas it is unclear how long they grow per year and connect ramets. A ramet consists of a shoot bud and a cluster of fleshy storage roots borne at the tips of slender subterranean stolons.

Study site

The study was conducted in a windbreak forest (143°10'N, 42°39'E) in Nakasatsunai, Hokkaido, Japan. In this region, *C. keiskei* has grown widely before the agricultural reclamation of the 1880s. Currently, its highly fragmented populations are present in preserved areas, secondary forests and artificially planted forests. The study site is part of a windbreak forest that is several kilometres long and fragmented by roads and agricultural fields; planted *Larix leptolepis* is the most dominant tree, followed by naturally established

Quercus dentata and *Betula platyphylla*.

For long-term monitoring of this *C. keiskei* population, a 90 × 100-m study plot was established on the forest floor in 2001. The grid point at every 5 m was plotted (total 21 × 19 = 399 points) and characterized by *X*-*Y* coordinates. The northern and southern edges ($Y = 0$ and $Y = 90$) face agricultural lands, and the eastern side ($X = 0$) faces a road. The forest continues beyond the western edge ($X > 100$) but is covered with dense brush of *Sasa nipponica*, and very few *C. keiskei* flowering shoots exist there.

Field measurements

In order to explore the overall population structure of the 0.9 ha plot, we conducted the following field measurements in 1 × 1-m quadrats centred at the 399 5 × 5-m grid points in July 2004 (fruit maturing season). We counted the number of all shoots present and the number of flowers and fruits in their inflorescences. The number of flowers was measured by counting both the number of matured fruits and the number of pedicels with no fruit in each inflorescence. The total flower (fruit) number in each 1-m^2 quadrat was calculated as the sum of the number of flowers (fruits) over all the flowering ramets. The number of shoots (S_i),

flowers (N_i), and fruits (M_i) per 1-m^2 were assigned to the ith grid point (i = 1, 2, ..., 399).

Allozyme analysis

The clonal structure of the *C. keiskei* population extending to 0.9 ha whole was investigated by allozyme electrophoresis. In June 2001, fresh leaf tissue was sampled from a shoot nearest to each 5 m grid point, with no sample collected if there was no shoot within 2.5 m (in horizontal and vertical distance) of the focal point. Leaf tissues were kept at a low temperature before being brought to the laboratory and stored at –80 °C.

For electrophoresis, approximately 100 mg of leaf was homogenized in 1.0 ml of extraction buffer made up of 0.1 M Tris-HCl (pH 7.5), 0.2 g/ml glycerol, 63 mg/ml Tween 80, 8 mM dithiothreitol, 0.5% (v/v) β-mercaptoethanol, 0.40% (w/v) β-nicotinamide adenine dinucleotide, 0.45% (w/v) β-nicotinamide adenine dinucleotide phosphate, 0.3% (w/v) bovine serum albumin, and 7% (w/v) polyvinylpolypyrrolidone. After the homogenates were centrifuged (15 000 rpm for 30 min at 4 °C), 12 μl of the resulting supernatant was used for electrophoresis. Electrophoresis was performed using acrylamide gel according to the method of Davis (1964) and Orstein (1964) and was carried out at 4 °C. For each gel, enzymes were

stained during incubation using the method described by Shiraishi (1988).

Clear polymorphic band patterns were detected for six enzyme systems: phosphogluconate dehydrogenase (6Pgdh), phosphoglucomutase (Pgm), aspirate aminotransferase (Aat), acid phospatase (Acp), shikimate dehydrogenase (Skdh), and leucine aminopeptidase (Lap). Genetic interpretations of banding patterns were inferred from segregation patterns with reference to typical subunit structures (Gottlieb, 1981, 1982; Crawford 1983), and the genotypes of eight putative loci, 6*Pgdh*-1, 6*Pgdh*-2, *Pgm*, *Aat*-1, *Aat*-2, *Acp*, *Skdh*, and *Lap* were recorded.

Data analysis

Ramet distribution. To assess the degree of clumping of shoots, flowers, and fruits, we calculated Morishita's index (Cressie 1991, pp. 590-591),

$$I_\delta = n\sum_{i=1}^{n} X_i(X_i - 1)/X(X-1)$$

where n (= 399) is the sample size; X_i refers to S_i, N_i, or M_i; and $X = \sum X_i$. I_δ/n expresses the probability that two randomly chosen shoots (or flowers or fruits) belong to the same quadrat. $I_\delta = 1$ if shoots are randomly distributed, and $I_\delta > 1$ means that they are clumped. Correlations

among shoot, flower, fruit densities in 1-m^2 and fruit set were tested.

Clonal diversity. The multilocus genotype, based on the eight polymorphic loci, was determined for each shoot. Separate ramets often possessed identical genotypes. To evaluate whether such ramets represented the same genet, the probability that identical genotypes could result from independent formation of zygotes was estimated. The probability that a zygote acquires a given diploid genotype was calculated as the following equation:

$$P_{gen} = \left(\prod_l p_l q_l \right) 2^h$$

where p_l and q_l are the frequencies of the two alleles in each genotype at the lth locus and h is the number of loci that are heterozygous (Parks and Werth 1993).

Clonal diversity over the population was assessed by two measures: G/n, the probability that the next plant sampled will be of different genotypes, where G represents the number of genotypes detected (Pleasants and Wendel 1989), and Simpson's index, $D = 1 - \sum q_g^2$, where q_g is the frequency of the g-th genotype. The clone size was estimated by multiplying the number of sample points with that genotype by 25 m^2, and for the diameter of a clone the maximum distance among the grid points exhibiting that genotype was used.

Modelling. Because fruit sets fall into from zero to one, we constructed fine-scale spatial fruit-set models based on logistic regression, in which explanatory variables reflect neighbouring flowering conditions and clonal information.

When the fruit-set ratio is p, the probability that m fruits mature from n flowers is given as the binomial distribution ${}_nC_m p^m (1-p)^{n-m}$. Thus, if $p(i)$ is the fruit set at grid point i of the model, the likelihood of data $\{N_i, M_i\}$ is

$$\prod_i {}_{N_i}C_{M_i} \cdot p(i)^{M_i} \left\{1 - p(i)\right\}^{N_i - M_i}$$

If the fruit-set rate is constant over the population, that is, if $p(i) = p_0$, then p_0 can be interpreted as the average fruit set over the population.

The total pollen production of a ramet is generally proportional to the number of flowers in that ramet, and in insect-pollinated species a stigma tends to receive pollen from neighbouring flowers. Because of the self-incompatibility of *C. keiskei*, effective pollen is restricted to that produced by different genets. Therefore, the amount of compatible pollen transferred is like to be proportional to the number of neighbouring flowers with different genotypes. We quantified this amount at the ith grid point (C_i) as a weighted sum of neighbouring flowers (N_j) and applied the following equation (*Compatible flower model*):

$$p(i) = a_1 / \{1 + \exp(a_2 + a_3 \cdot C_i)\}$$

where a_1, a_2, and a_3 represent the logistic coefficients. The weight to each N_j was given by a decreasing function of distance, and we again used a logistic function so that we could test various distance-effects:

$$C_i(c_1, c_2) = \sum_{k_i \neq k_j} \frac{1}{1 + e^{c_1(R_{i,j} - c_2)}} \cdot N_j$$

where $R_{i,j}$ is the distance between grid point i and j and k_i refers to the genotype detected at the ith point. Maximum-likelihood method was conducted for the logarithm of the likelihood in which not only the logistic coefficients (a_1, a_2, a_3) but also c_1 and c_2 were optimized to determine the best range of neighbours. We used the parameter a_1 in the numerator because fruit set does not necessarily approach 100%, and a_1 indicates the upper limit ($0 < a_1 \leq 1$). A logistic curve decreases around a reflection point (for the second case, at c_2) and more drastically decreases when c_1 is larger. If a greater c_1 shows a greater log-likelihood, the optimized weighting will be a step function (= 1 if $R_{ij} < c_2$ and = 0 if $R_{ij} > c_2$; Figure 1).

Because Y = 80–90 are outside of the windbreak forest and the different environmental conditions might affect the mode of reproduction differently (Kudoh et al. 1999), these points were eliminated in the fruit-set models. To examine the possible advantages of the above compatible flower model that combines the two factors of flowering

densities and genotypes, we constructed similar individual-based spatial fruit-set models using only flowering or clonal information. For the *all flower model*, we assessed the amount of any pollen in a neighbourhood as

$$A_i(c_1, c_2) = \sum \frac{1}{1 + e^{c_1(R_{i,j} - c_2)}} \cdot N_j$$

and we tested

$$p(i) = a_1 / \{1 + \exp(a_2 + a_3 \cdot A_i)\}.$$

For the *clonal diversity model*, two genet diversity indices were modified to their local versions and applied. One index is $G_i(r)/n_i(r)$, where $n_i(r)$ indicates the number of samples within r metres from the ith grid point with at least one flower in the 1-m^2 quadrate and $G_i(r)$ is the number of different genotypes detected in the $n_i(r)$ samples. The other is Simpson's index within r metres: $D_i(r) = 1 - \sum_g q_{i,g}^2$, where $q_{i,g}$ is the frequency of the gth genotype in the $n_i(r)$ samples. We tested r = 5–20 m to find the best range for each model.

The optimized models were evaluated according to the Akaike Information Criterion (AIC = [– 2 × maximized log-likelihood] + [2 × number of parameters]) to identify the model that most accurately explained the data; a model with a smaller AIC value is considered to be a better model. The goodness of fit of the models was checked by comparing the observed number of fruits (M_i) with the expected number with 95% confidence intervals derived from

1000 simulations.

Results

Spatial shoot distribution

The spatial distributions of shoots (S_i) and flowers (N_i) showed aggregated patterns (Figure 2). Among the 399 1-m^2 quadrats centred at the grid points, shoots existed at 236 points, and 135 of them contained at least one flowering ramet. The maximum number of shoots per 1 m^2 was 163 with an average of 29.4 ± 28.0 (± SD). There were 3.1 ± 5.9 flowering ramets on average (maximum, 40), resulting in a mean of 40.1 ± 49.8 flowers per 1 m^2, extending up to 246. The maximum number of fruits (M_i) was 134, with an average of 17.2 ± 20.3 (Figure 2). The degree of clustering was the highest for flowers (I_δ = 7.4), followed by fruits (I_δ = 6.9) and shoots (I_δ = 3.2). The numbers of flowering ramets and flowers (N_i) in 1-m^2 plot were significantly correlated with the number of shoots (S_i; R^2 = 0.645, p < 0.001; R^2 = 0.607, p < 0.001, respectively). Fruit number (M_i) was positively correlated with flower number (N_i; R^2 = 0.518, p < 0.001), and fruit set (M_i/N_i) had a negative relationship with flower number (R^2 = 0.075, p = 0.003).

Clonal structure

The allozyme analyses of 282 shoots identified 94 genotypes (i.e., g-1, g-2, …, g-94). The observed number of alleles per locus averaged 2.75, ranging from two to five. Consequently, the P_{gen} values were sufficiently low to reject the null hypothesis ($P_{gen} < 0.001$ in all cases), indicating that ramets with the same multilocus genotype were members of the same clone. For clonal diversity, $G/n = 94/282 = 0.333$, and the Simpson's index was 0.970.

Figure 3 illustrates the spatial distribution of multilocus genotypes. Individuals belonging to the same clone tended to be close to each other, particularly in large clones. Of the 94 genotypes, g-1 and g-2 were observed in 28 and 17 samples, respectively; five other genotypes (g-3, …, g-7) expanded further than 25 m. The largest clone (g-1) covered 700 m^2 area with a diameter of 40.3 m. Fifty-six samples showed a unique genotype, and 15 genotypes were detected from two or three points, some of which were dispersed rather than clustered (e.g., g-27, -30, -34; Figure 3). Genotype g-19 was more widely dispersed (51.5 m), the exceedingly scattering three points seem to be different clones but with the same genotype by chance.

Fruit-set model

The optimized models are summarized in Table 1. The average fruit set over the population was $p_0 = 53\%$. When fruit set was predicted by the number of neighbouring compatible flowers (C_i), the model exhibited much better log-likelihood and a smaller AIC. In contrast, the models using only neighbouring flower conditions, A_i, or neighbouring genet diversity, $G_i(r)/n_i(r)$ and $D_i(r)$ (the best range was $r = 14.5$ or 14.0 m, respectively), showed weaker log-likelihood (Table 1), suggesting the combined effects of flowering distribution and clonal structure on fruit set in this population.

The optimized weighting converged to a step function: the same weight was used within 14.5 m of the focal plant (more precisely, any distance between $10\sqrt{2} = 14.14$ and 15 m) and none for further points (Figure 1). Thus, the neighbourhood in the model contains the surrounding $5 \times 5 - 1 = 24$ grid points and is strictly smaller than 30×30 m.

Figure 4 illustrates the expected fruit-set curve of the optimized compatible pollen model, together with the scatter plot of the number of compatible flowers within 14.5 m (C_i) and observed fruit sets. The logistic curve initially increased and gradually became saturated at about 67%. Although the expected curve seems to not fit the data sufficiently, this is because the fruit-set ratio tends to have large variance when the sample size (N_i) is small. In

fact, when we excluded the points with small N_i and optimized the compatible pollen models, a logistic relationship arose and the observed fruit sets were spread around the expected curve. When points with high N_i (> 70) were further separately optimized, the fruit set increased more rapidly from smaller C_i (the optimum weighting of the three cases was the same as the step function, 14.5 m). In contrast, we found no relationship between fruit set and the number of compatible flowers for N_i < 20 (a constant function showed a better AIC than the logistic form when data were limited to these points). These results mean that fluctuating between 0% and 100%, points with small flower numbers (open circle in Figure 4) masked the true relationship due to their large stochastic errors in fruit-set estimations. The logistic curve also suggested that predictions based on the compatible flower model are especially efficient when C_i is small (< 100), in which case this model shows much lower fruit sets than the constant model, while its predicted ratios are similar to the constant when C_i is larger than 150 (50–70%).

These effects can be seen, for example, in genotypes g-3 and g-7, whose clones and flower densities are both sufficiently large (Figures 2 and 3). In g-3, except (X, Y) = (45, 55) that has a close neighbour with a different genotype at (40, 50), the observed fruit sets are much lower (12.1–32.7%) than the average over the population (p_0), and the model

adequately predicted lower fruit sets. Similarly, g-7 showed lower-than-average fruit sets (5.1–52.5%). Moreover, despite similar C_i scores (almost zero), point ((50, 65), 31%) showed better fruit set than ((55, 65), 12%), and the former had higher local flower density (N_i = 101 vs. 24).

Based on the last model *three classes model*, we conducted 1000 simulations. The observed fruit numbers in 68 of 106 grid points fell within the 95% confidence envelopes.

Discussion

In this *C. keiskei* population, shoots spread widely on the forest floor, forming a patchy structure that included very dense areas as well as sparse ones (Figure 2). The spatial distribution of multilocus genotypes revealed that ramets belonging to the same clone were likely to be near each other, resulting in the formation of a clumped genet structure (Figure 3). Allozyme analysis also detected clones expanding more than 30 m in the population (Figure 3), which is consistent with a previous report that clonal growth of *C. keiskei* can cover large spaces (Komarov 1949). At the same time, many unique genotypes and genotypes only detected at a few sampling points were present throughout the population. Hence, our first

genetic survey at the 5 m sampling clarified the over all clonal structure and genet diversity at this *C. keiskei* population in the 0.9 ha plot, even though in general this scale is not sufficient for intimate investigation of the complex local structure.

It is widely accepted that clonal populations are genotypically almost as diverse as sexually reproducing populations (Ellstrand and Roose 1987; Bayer 1990; Eckert 1999). For long-lived clonal plants, a small number of annual seedling recruits is sufficient to maintain clonal diversity in populations (Watkinson and Powell 1993; Widén et al. 1994). It is noteworthy that the *C. keiskei* population showed relatively high clonal diversity ($D = 0.97$, $G/n = 0.33$) compared with the mean values for 21 plant species ($D = 0.62$, $G/n = 0.17$) reported by Ellstrand and Roose (1987) and for 45 species ($D = 0.75$, $G/n = 0.27$) reported by Widén et al. (1994). Unfortunately, the processes of seed dispersal and clonal migration (i.e., rhizome growth) in *C. keiskei* are not well understood. In the case of *Convallaria majalis*, European lily-of-the-valley, mice were observed to be attracted to fruits (Eriksson 1999), and in *C. keiskei*, Choung (1994) reported that a genet was formed by two to 16 ramets connected by stolons. Demographic study would clarify not only mechanisms of both modes of reproduction but also the degree of success of subsequent establishment in various local environments.

In this study of the *C. keiskei* population, we found the combined effect of spatial ramet distribution and clonal structure on the fruit set and it was more significant than those when the two were separately considered. Thus we could demonstrated the importance of the number of neighbouring flowers having different genotypes (C_i), whose value was positively correlated to the amount of available compatible pollen transferred. In particular, the better predictability of our model at small *Ci* suggested that the limitation of compatible pollen reduced fruits production. The effects of clonal structure on reproduction have been investigated for several species. For example, in the self-incompatible *Calystegia collina*, Wolf et al. (2000) reported that ramets from the most abundant genotypes were less likely to produce seeds than less abundant genotypes. In the self-incompatible *Rubus saxatilis*, fruit set of isolated patches of clones were negatively correlated with distance to the nearest flowering patch (Eriksson and Bremer 1993). In contrast, we applied the fine-scale model and clarified the spatial heterogeneity in sexual reproduction within the population rather than at the whole population level. The fit of our model was not perfect, because of our way to estimate the amount of neighbouring compatible pollen. Our method (using *Ci* for calculating compatible pollen) would be perfectly precise if all ramets within 5 × 5 m were a clone. In reality, however, clonal structure seems to be much more complex. Ziegenhagen et al. (2003)

reported that small-scale sampling detected new genotypes that had not been found in large-scale sampling. There are also similar reports of small-scale clonal diversity of the seagrass *Cymodocea nodosa* (Ruggiero et al. 2005) and clonal woodland species, such as *Anemone nemorosa* (Holderegger et al. 1998; Stehlik and Holderegger 2000) and *Uvularia perfoliata* (Kudoh et al. 1999). Intermingled parts in the *C. keiskei* population might include additionally unidentified genotypes than detected at the 5-m sampling scale. Although it is physically difficult to genotype all ramets in the whole 0.9 ha plot, some improved sampling strategies could diminish such errors, and the compatible pollen model may then exhibit a better fit to observed fruit set.

The optimized logistic curves resulted in drastic reductions at the inflection point 14.5 m, whose distance might reflect something like the flying distance pollinators dispersed pollen. We presently have limited information about how far opportunistic pollinators, such as beetles of Coleoptera and hover flies and flies of Diptera (Kunin 1993; Gómez and Zamora 1999; Ashworth and Galetto 2001), visiting this plant, can carry pollen, in addition the fact that pollination effectiveness vary to different pollinator species(Schemske and Horvitz 1984; Herrera 1996; Thompson 2001). Hence, we have few evidences relating that scale directly with pollinator behaviours. Even though, our approach might become an operative estimation

upon starting studies about pollination capacity of those insects and 14.5 m could be used as the rough standard for subsequent studies. Finally, results of our three classes model imply another effect of the local flower density: the display efficiency to pollinators varied according to flower numbers. Namely, as shown in Figure 4 (the horizontal line), if fertilization at low flower density was determined by chance, regardless of the amount of compatible neighbouring flowers, which is probably because small sparse clumps of reward-producing plants are not attractive to pollinators (Sih and Baltus 1987; Kunin 1993). Only at middle and high floral densities were fruit sets positively related to the potential amount of compatible pollen transferred, implying that many flowers may operate as an efficient display for attracting pollinators (Klinkhamer et al. 1989; Brody and Mitchell 1997; Murren 2002) and thus influence fecundity (Harder and Barrett 1995). Because of the limited results, especially regarding what constitutes high density, we are uncertain how the local floral density combined with the potential amount of compatible pollen transferred affects the success of sexual reproduction. Our analysis, however, suggests that the floral environment reflecting the spatial genetic structure and other spatially heterogeneous factors through the intermediary of pollinator activities affect reproductive success.

In conclusion, in the self-incompatible, entomophilous, clonal herb *C. keiskei*, fruit set

is affected by the amount of neighbouring compatible pollen. The spatial population structure including both the genetic structure and ramet distribution, is a result from two reproductive modes, seed dispersal and clonal propagation, and this in turn has prominent effects on reproductive success.

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Figure 1. Variation of logistic curves. Dashed line: small c_1; solid line: large c_1. The bold line indicates the optimized weighting; logistic curves converged at a step function divided at 14.5 m.

Figure 2. The number of shoots (indicated by shading), flowers (upper numbers) and fruits (lower numbers) in 1 m^2 quadrates centred at 399 grid points in a population of *Convallaria keiskei* from Japan.

Figure 3. The spatial distribution of 94 multilocus genotypes centred at 282 grid points in the study plot. Numerals indicate each multilocus genotype, and open circles represent unique genotypes in a population of *Convallaria keiskei* from Japan.

Figure 4. Scatter plot of the number of neighbouring compatible flowers within 14.5 m (C_i) and observed fruit set (M_i/N_i) at 106 grid points in a population of *Convallaria keiskei*. Symbols differ according to the local flower density (N_i): open circles present low density ($N_i < 20$), closed circles intermediate ($20 \leq N_i \leq 70$), squares high ($70 < N_i$). The bold line indicates the predicted fruit sets for the compatible flower model, and the three solid lines

represent the predicted fruit sets optimized separately for the three N_i classes model. Note that the symbols on these curves correspond to the N_i classes for the observations.

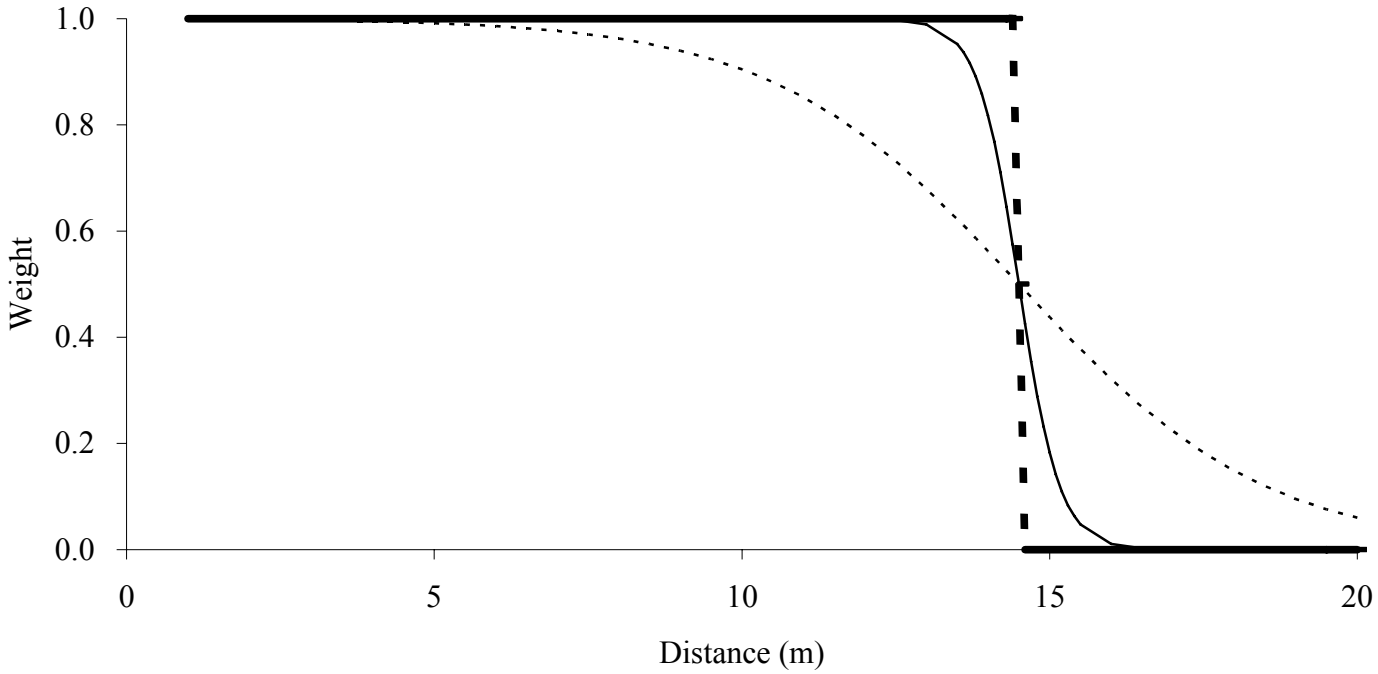

Figure 1

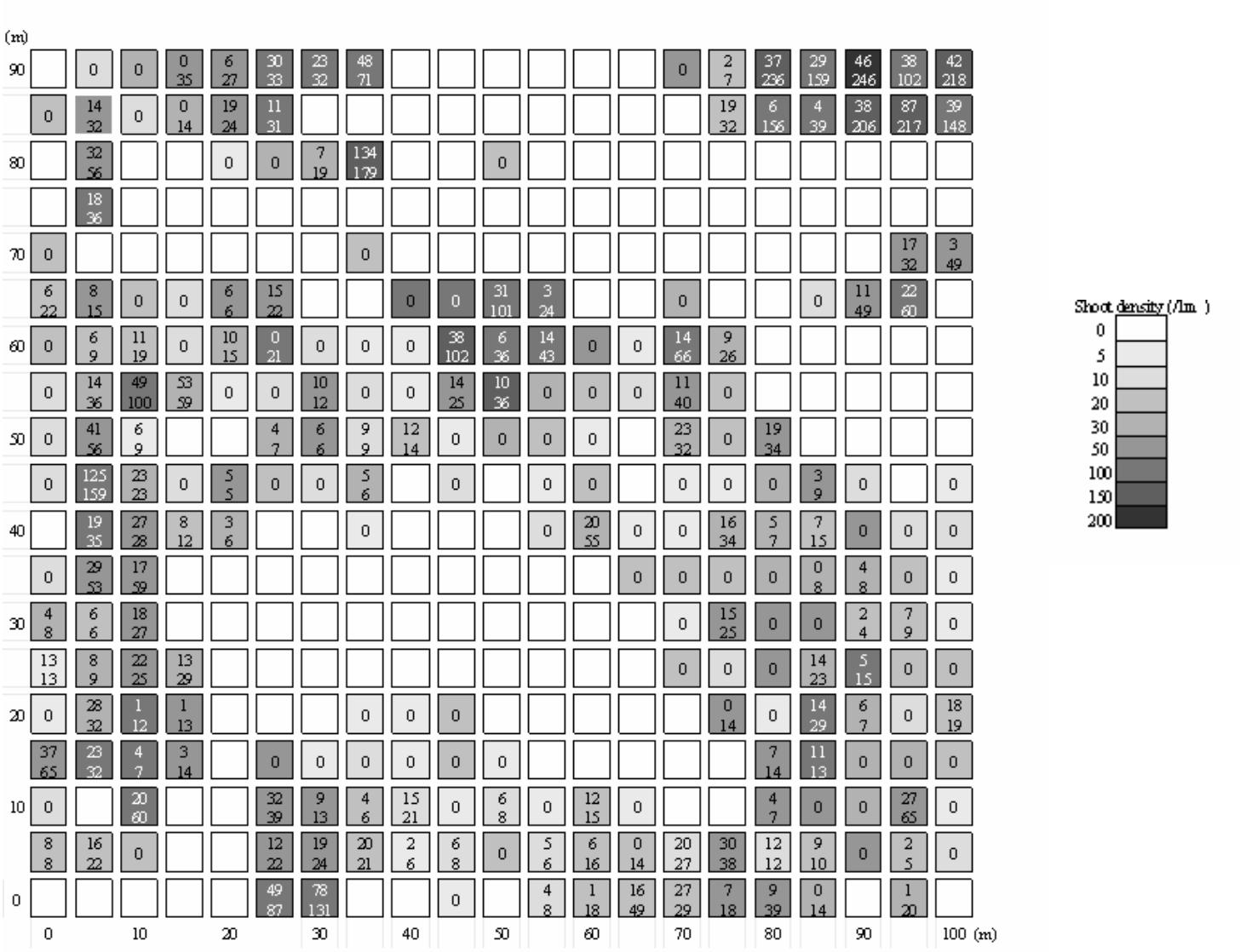
(m)
90
80
70
60
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100 (m)
Shoot density (/1m)
0
5
10
20
30
50
100
150
200

Figure 2

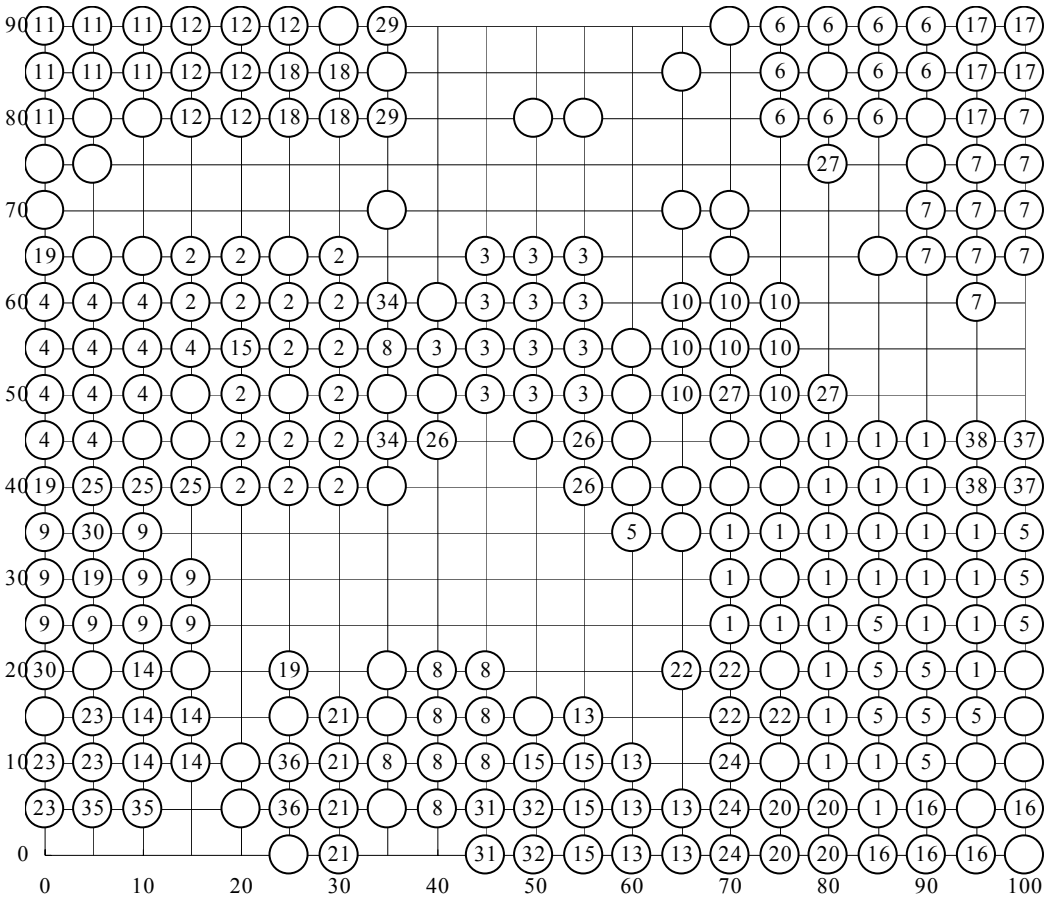

Figure 3

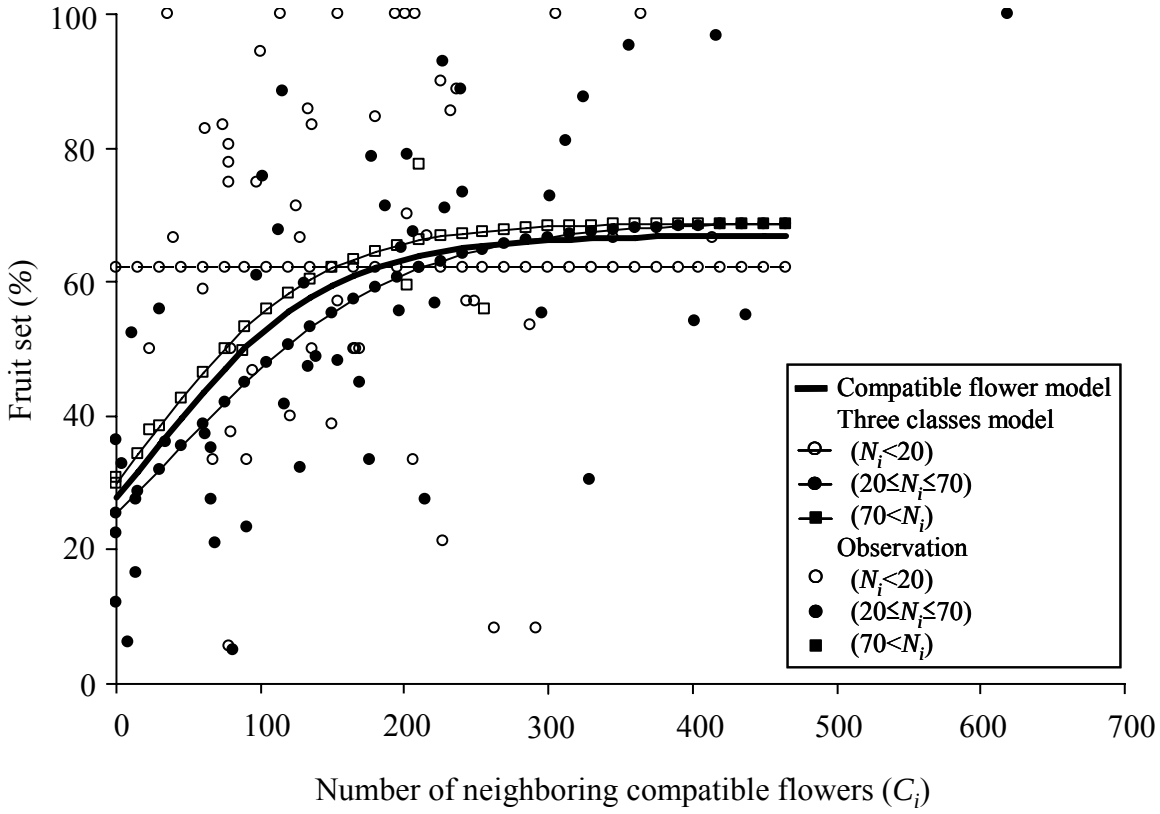
Fruit set (%)
100
80
60
40
20
0
0
100
200
300
400
500
600
700
Number of neighboring compatible flowers (C_i)
Compatible flower model
Three classes model
(N_i<20)
(20≤N_i≤70)
(70<N_i)
Observation
(N_i<20)
(20≤N_i≤70)
(70<N_i)

Figure 4

Table 1 Results of the fruit-set models. For each model, the maximum log-likelihood, the number of parameters, AIC (the Akaike Information Criterion), and resulting parameter values are shown (For explanation see text).

Model	Factors included in the model	Log-likelihood	No. of parameters	AIC	Parameter values			
					a_1 (p_0)	a_2	a_3	c_2 (r)
Constant	-	-550.22	1	1102.44	0.532	-	-	-
Compatible flower	C_i	-437.80	5	885.60	0.670	0.344	0.016	14.5
All flower	A_i	-514.56	5	1039.12	0.609	-0.002	0.002	14.5
Clonal diversity (G/n)	G_i/n_i	-497.00	5	1004.00	0.589	2.284	-13.160	14.0
Clonal diversity (Simpson's index)	D_i	-459.53	5	929.06	1.175	1.251	-1.789	14.5
Three classes	C_i. N_i	-422.58	-	-	0.691	0.262	-0.016	14.5
					0.689	0.583	-0.013	14.5
					0.622	0.000	0.000	14.5

1