
Effects of Forest Fragmentation on Seed Production of the Understory Herb *Trillium camschatcense*

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Abstract: We investigated the effects of forest fragmentation on reproduction of a common understory perennial, *Trillium camschatcense*, in Hokkaido, Japan. We measured seed production of 12 (1998) and 14 (1999) populations, and we explored the relationships of seed production to the number of flowering plants (population size) and to the surrounding landscape condition (landscape type). We discriminated between two landscape types, isolated and continuous, to describe the quantity and distribution of forests around *T. camschatcense* populations. Population size may be correlated with attractiveness to pollinators and the availability of compatible mates, whereas landscape condition can affect the abundance of pollinators. The number of seeds per flower was related to population size in 1999, but not in 1998. In both years, small populations of <50 flowering plants produced few seeds. Although the effect of landscape type was not significant, continuous populations with abundant forest series in their neighborhood tended to produce a higher number of seeds than isolated populations located in small, isolated forest. We attribute the decrease in seed production to pollen limitation because the stigmatic pollen load had a positive relationship to seed production and addition of pollen by hand increased seed production. Variation in population structure (four stage classes) among populations was better explained by population size than number of seeds produced, suggesting the relative importance of population size for the demography of *T. camschatcense*. Larger populations had a higher proportion of seedlings, possibly because large populations had fewer edge effects, which reduce seedling recruitment and survival. Small populations with 220 flowering plants or less showed almost no seedling recruitment. The data on seed production and population structure suggest that continuous populations with at least 1000 flowering plants may be required to avoid the effects of fragmentation.

Efectos de la Fragmentación del Bosque en la Producción de Semillas de la Hierba de Sotobosque *Trillium camschatcense*

Resumen: Investigamos los efectos de la fragmentación del bosque en la reproducción de una perenne común en el sotobosque, *Trillium camschatcense*, en Hokkaido, Japón. La producción de semillas de 12 (1998) y 14 (1999) poblaciones fue medida, y exploramos las relaciones de la producción de semillas al número de las plantas florecientes (tamaño de la población) y a la condición del paisaje circundante (tipo del paisaje). Dos tipos de paisaje, aislados y continuos, fueron discriminados para describir la cantidad y la distribución de bosques alrededor de poblaciones del *T. camschatcense*. El tamaño de la población se puede correlacionar con la atracción de los polinizadores y con la disponibilidad de parejas compatibles, mientras que la condición del paisaje puede afectar la abundancia de polinizadores. Se relacionó el número de semillas/flor con el tamaño de la población en 1999, pero no en 1998. En ambos años, las poblaciones pequeñas de < 50 plantas florecientes produjeron escasas semillas. Aunque el efecto del tipo del paisaje no fue significativo, las poblaciones continuas con series abundantes de bosque a su alrededor tendieron a producir un mayor número de semillas que las poblaciones aisladas localizadas en bosques pequeños y aislados. Atribuimos la disminución de la producción de semillas a la limitación del polen porque la carga de polen estigmático tuvo una relación positiva con la producción de semillas y la adición manual de polen aumentó la

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Paper submitted October 2, 2000; revised manuscript accepted November 13, 2001.

producción de semillas. La variación en la estructura de la población (cuatro clases de etapa) entre poblaciones se explicó mejor por el tamaño de la población que por el número de semillas producidas, sugiriendo la importancia relativa del tamaño de la población para la demografía del T. camschatcense. Poblaciones más grandes tenían una mayor proporción de plántulas, posiblemente porque las poblaciones grandes tenían pocos efectos de borde que reducen el reclutamiento y la supervivencia de la plántula. Poblaciones pequeñas con 220 plantas florecientes o menos casi no mostraron reclutamiento de plántulas. Los datos sobre la producción de semillas y la estructura de la población sugieren que se pueden requerir poblaciones continuas con por lo menos 1000 plantas florecientes para evitar los efectos de la fragmentación.

Introduction

Habitat fragmentation caused by human activities, such as road construction and development of agricultural fields, dramatically reduces plant population sizes and increases their spatial isolation (Wilcove et al. 1986; Saunders et al. 1991; Rathcke & Jules 1993). Because plants are sessile, it is generally accepted that habitat fragmentation will alter both the ecological and genetic properties of plant populations (e.g., Ellstrand & Elam 1993; Rathcke & Jules 1993; Schemske et al. 1994; Young et al. 1996). In the case of outcrossing of entomophilous plants, for instance, population size is closely associated with the attraction to pollinators and should have a significant influence on reproductive success. Because small populations may be less attractive to pollinators than large populations (Sih & Baltus 1987), the reduction in population size results in decreased fruit or seed production due to insufficient pollen transfer (e.g., Jennersten 1988; Ågren 1996). Reduction in seed production is one of the major threats to plant life-history processes and may increase the probability of extinction of populations and species (Rathcke & Jules 1993; Olesen & Jain 1994).

The availability of pollinators may be affected not only by population size itself but also by the landscape that surrounds a focal population. Even if two populations of a plant species are the same size and have the same extent of floral display, pollinator availability necessarily will be different depending on the potential pollinator abundance, which may be related to the amount of available resources for pollinator species. Because pollinators continuously need flowering plant species over their entire life and often have specific nesting requirements (Kearns & Inouye 1997), a limited quantity of habitat available for pollinators may decrease pollinator abundance and activity and may consequently result in low reproductive output in plants. Moreover, when the spatial isolation among habitats by fragmentation becomes greater than the foraging range of pollinators, pollination services also will decrease considerably.

Trillium camschatcense is a common understory spring herb distributed mainly in Hokkaido and in north-

ern Honshu, Japan. In the Tokachi region of eastern Hokkaido, populations of *T. camschatcense* have been highly fragmented as a result of recent agricultural development. Populations in this region are self-incompatible, and seed production results from obligatory outcrossing by insect pollination (Ohara et al. 1996). We examined the effects of population size and surrounding landscape conditions on the reproduction and demography of *T. camschatcense* populations. We addressed the following questions: (1) Do large populations produce a significantly higher number of seeds than small populations? (2) Do populations located in a forest-abundant landscape produce a higher number of seeds? (3) What is the relative importance of the effects of population size and surrounding landscape conditions? (4) How does seed production relate to stage-class population structure?

Methods

Study Species and Study Sites

Trillium camschatcense Ker Gawler (Trilliaceae), a corrected name for *T. kamschaticum* Pall. (Fukuda et al. 1996), has four major life-history stages, as do other *Trillium* species: seedling, one-leaf sterile, three-leaved sterile, and flowering (Ohara & Kawano 1986b). It takes more than 10 years for a seedling to go through the stages to become a flowering plant. When the stems or leaves of the flowering plant are damaged, however, the plant often returns to its three-leaved sterile stage in the next year, possibly because of impaired photosynthesis (Ohara & Kawano 1986b). *Trillium camschatcense* reproduces exclusively by seeds (Ohara & Kawano 1986a). Most reproductive plants have one or two flowering stems, although some have three or four. In the study area, *T. camschatcense* flowers in mid or late May, and the flowers remain in anthesis for about 1 week. Flowers are visited by a wide range of insect species, mostly belonging to Diptera, Coleoptera, and Hymenoptera (Ohara et al. 1991; H. T. et al., unpublished data). In study populations, members of Nitidulidae, Melandryidae, Oedemeridae (Coleoptera), Scatophagidae,

Table 1. Study sites of *Trillium camschatcense* populations.

Population (abbreviation)	Latitude	Longitude	Population area (m ²)	Forest area (ha)	Flowering-plant density ^a	Population size	Landscape type ^b
Shibusan (SS)	42°52'55"N	142°59'13"E	550	0.19	10	220	C
Obihiro (OH)	42°53'23"N	143°8'41"E	1050	0.39	13	550	C
Motoimatsu (MM)	42°48'18"N	143°6'43"E	18700	1.9	24	18000	I
Toyonishi (TN)	42°48'11"N	143°9'9"E	3400	0.34	16	2200	I
Kiyokawa (KK)	42°45'22"N	143°6'52"E	7700	1.0	33	10200	I
Hirono-kita (HK)	42°45'54"N	143°2'7"E	1400	1.7	15	900	C
Hirono-minami (HM)	42°45'44"N	143°0'36"E	5500	1.2	25	5500	I
Pepekiki (PK)	42°42'17"N	143°6'5"E	1450	0.23	20	1200	C
Churui (CR)	42°33'55"N	143°20'34"E	400	0.80	0.25	4	C
Moewa (MW)	42°30'46"N	143°18'43"E	2600	240	0.07	7	C
Toberi (TB)	42°30'18"N	143°25'23"E	190	0.019	6	46	I
Rekifune (RF)	42°27'56"N	143°21'37"E	60000	31	64	153600	C
Monbetsu (MB)	42°26'35"N	143°19'41"E	480	0.048	18	350	I
Hiroo (HI)	42°18'57"N	143°19'53"E	50000	30	63	126000	C

^a Average number of flowering plants in 25 m² was calculated by randomly placing a 5 × 5 m quadrat five times within each population.

^b Two landscape types, isolated (I) and continuous (C), were discriminated according to the abundance and distribution of the forest around the populations. See text for details.

and Bibionidae (Diptera) were often observed (H.T. et al., unpublished data).

We conducted field studies on 14 populations located in the Tokachi region of eastern Hokkaido in 1998 and 1999 (Table 1). The overstories of the study populations were mostly broad-leaved deciduous trees, dominated mainly by *Ulmus davidiana* var. *japonica*, *Quercus mongolica* var. *grosseserrata*, and *Fraxinus mandshurica* var. *japonica*, except in the HK and HI populations, which included *Larix leptrepis* plantations. (Definitions for population abbreviations are provided in Table 1.)

Fruit and Seed Production

To assess the level of seed production, we randomly selected flowers (4–35) from each population before anthesis in early May and left them in an open-pollinated condition (266 and 277 flowers in total for 1998 and 1999, respectively). Individuals with only one flowering stem were sampled for this study, except when we could not obtain sufficient sample size in small populations. No significant differences in the number of seeds produced per flower were found, however, between plants with different numbers of flowering stems (one-way analysis of variance [ANOVA], $R^2 = 0.008$, $n = 41$, $F = 0.03$, $p = 0.86$; H. T. & M. O., unpublished data). All fruits were collected in July, and the number of seeds was counted for each fruit under a stereomicroscope. Three indices were calculated to characterize fruit and seed production for each population: (1) fruit set, a population measure described by the percentage of flowers that fruit; (2) number of seeds per mature fruit, a population measure described by the mean of the number of seeds that mature fruits contained; and (3) number of seeds per flower, a population measure calculated by

multiplying fruit set (1) and number of seeds per mature fruit (2). We examined 12 populations (excluding OH and CR) in 1998 and all 14 populations in 1999. In 1998 we conducted supplemental hand-pollination experiments in 11 populations (excluding OH, CR, and MW) to assess the possibility of pollen limitation. In each population, flowers (3–37; total $n = 244$) were artificially pollinated with pollen from at least three distant individuals. We measured the number of seeds produced per flower for each population after the fruits were collected in July and compared them with open-pollinated controls established at each site (6–35; total $n = 259$).

Stigmatic Pollen Load

To estimate the outcross pollen load of the study populations, flowers (4–10) of each population were emasculated before anthesis and the stigmas were collected late in the flowering period (52 and 80 flowers in total for 1998 and 1999, respectively). The samples were stored in 70% ethanol until examination, and we used the glycerin-jelly method to count the number of outcross pollen grains on the stigmas (Beattie 1971; Kearns & Inouye 1993). Although we cannot ignore the possibility of underestimating the number of pollen grains that may have already grown down the style during the experiment, this procedure can provide a good estimation of the outcross pollen load. The experiment was conducted for 11 populations (excluding OH, CR, and MW) in 1998 and for 12 populations (excluding CR and MW) in 1999.

Factors Affecting Seed Production

Population size and surrounding landscape type were evaluated as factors affecting seed production of the

study populations. Population size, defined here as the estimated number of flowering plants in each population, was calculated by multiplying the population area by the flowering plant density (Table 1). Population size was log-transformed for analysis. The surrounding landscape type was evaluated by classifying the study populations into two types, isolated and continuous (referred to as I and C, respectively), based on the abundance and the distribution of the forest around the focal *T. camschatcense* populations (Table 1). Populations classified as I were surrounded by pastures or agricultural fields in all directions and were distant (at least 200 m) from other large forests. In contrast, C populations were located within wind-break forests or surrounded by several adjacent patchy forests, both of which were much larger than the focal *Trillium* population itself. In either case, no other *Trillium* populations were found in the focal forest fragment. We used two variables to characterize the two landscape types: (1) a ratio of forest area to population area (log-transformed data, mean 0.08, range, 0–0.34 for I and 1.02, 0.20–2.97 for C, $t = 2.18$, $p < 0.05$) and (2) the proportion of the forest within a radius of 500 m from the center of the focal population (mean 0.06, range 0.02–0.11 for I and 0.20, 0.06–0.43 for C, $t = 2.18$, $p < 0.05$). Both variables represented the marked differences between the two landscape types. To determine forest areas and their related variables, we optically scanned aerial photographs on a Macintosh computer and analyzed them using the NIH Image Program (version 1.62, developed at the U.S. National Institutes of Health and available from <http://rsb.info.nih.gov/nih-image/>).

Trillium camschatcense is one of the dominant understory herbs in this region. It appears that formerly huge *T. camschatcense* populations were divided into small and isolated pieces with the fragmentation of the forests. Therefore, we presume that the remnant population sizes and their landscape types reflect the history of forest fragmentation. All study populations were distant from other *T. camschatcense* populations, and we did not consider the effect of population isolation.

Stage-Class Population Structure

In May 2000, we investigated population structures based on life-history stages in six populations (SS, TN, KK, MW, TB, and HI). We randomly established 10, 1×1 m quadrats on the forest floor of SS, TN, KK, and HI populations. We established one quadrat in the MW population and four quadrats in the TB population because of their small population size. All of the individuals in each quadrat were classified into one of the four stage classes (i.e., seedling, one-leaf sterile, three-leaved sterile, or flowering).

Statistical Analyses

Fruit set, number of seeds per mature fruit, and number of seeds per flower of each population were tested for differences between the 2 years by paired t test. This t test was conducted based on the mean values of each population studied over 2 years. We tested the effect of supplemental-hand pollination by nested analysis of variance by comparing the number of seeds of hand-pollinated flowers with open-pollinated controls. The dependent variable was the number of seeds produced, and the effect of hand pollination was nested in that of population to distinguish between the variations among populations from the effect of artificial pollen addition. Relationships between outcross pollen load and seed production were explored by least-square linear regressions. We used analysis of covariance (ANCOVA) to test the effects of population size and landscape type on the number of seeds produced per flower of each population. The dependent variable in this model was the number of seeds per flower. The main effects were the effect of population size (continuous variable) and of landscape type (categorical variable).

To analyze the population structure, we assessed the extent of heterogeneity of stage-class frequencies among the populations examined through a Monte Carlo simulation in which a chi-square value was compared with that estimated from 1000 randomizations by permuting the original matrix (6×4 matrix) with the constraint that row and column totals remain constant (Roff & Bentzen 1989). To explore the relationships between each stage-class frequency in each population and population size, or the number of seeds per flower, we used Kendall's coefficient of rank correlations. All statistical analyses, except for the randomization test in the study of population structure, were performed with the software SAS version 6.12 (SAS Institute 1997) or JMP version 4.0 (SAS Institute 2000).

Results

Fruit and Seed Production

Three indices were determined for each population to characterize fruit and seed production (i.e., fruit set, number of seeds per mature fruit, and number of seeds per flower) (Table 2). We could not obtain data for the RF population in 1999 because of the extremely low recovery of fruits from the marked individuals which resulted from intensive grazing, possibly by Yeso sika deer (*Cervus nippon* var. *yesoensis*). A mature fruit produced, on average, 77.8 seeds in 1998 and 81.3 seeds in 1999. Each flower had 169.7 ± 56.9 ovules (mean \pm SD; $n = 253$), and approximately half the ovules developed into seeds. Fruit set and mean number of seeds per ma-

Table 2. Fruit and seed production (mean \pm SD) for all *Trillium camschatcense* populations examined for each year of the study.*

	Year		Paired t test	
	1998	1999	t	p
Fruit set (%)	44.5 \pm 19.5 (14.3–72.4)	52.9 \pm 20.0 (22.2–79.2)	0.89	0.40
Number of seeds per mature fruit	77.8 \pm 28.5 (34.0–120.7)	81.3 \pm 27.8 (15.5–133.9)	0.79	0.45
Number of seeds per flower	34.8 \pm 19.9 (6.7–70.2)	41.8 \pm 19.1 (7.8–66.9)	1.20	0.26

*Each variable was tested for the difference between years by paired t test. Ranges are in parentheses.

ture fruit varied markedly among the populations examined. Therefore, mean number of seeds produced per flower, which was employed as an index of total seed production, differed greatly among populations, ranging from 6.7 (MW) to 70.2 (HI) in 1998 and from 7.8 (CR) to 66.9 (TN) in 1999. None of the three indices differed between years (Table 2).

Pollen Load and Effect of Hand Pollination

The mean number of outcross pollen grains on stigmas varied among populations from 22.8 \pm 12.6 (means \pm SD; TB, $n = 4$) to 148.5 \pm 60.5 (HI, $n = 5$) in 1998 and from 16.0 \pm 11.9 (TB, $n = 4$) to 209 \pm 93.4 (HI, $n = 8$) in 1999. Data from the SS population were excluded from the analyses in both years because the samples contained many pollen grains from several other species that could not easily be distinguished from those of *T.*

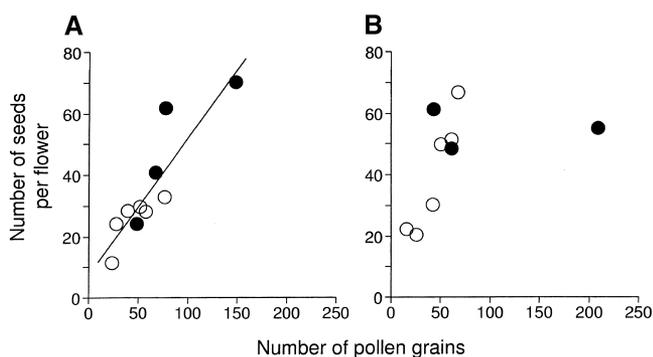


Figure 1. Relationship between mean outcross pollen load and mean number of seeds produced per flower of *Trillium camschatcense* populations in (a) 1998 and (b) 1999. Data are given for isolated (open circles) and continuous (closed circles) populations. Linear-regression lines are indicated in those cases where the slope of the line was significantly different from zero.

camschatcense. The mean number of seeds per flower was significantly related to the outcross pollen load in 1998 (Fig. 1a; regression coefficient, $b = 0.45$, $R^2 = 0.79$, $n = 10$, $t = 5.55$, $p < 0.001$), but not in 1999 (Fig. 1b; $R^2 = 0.21$, $n = 9$, $t = 1.34$, $p = 0.22$). The relationship in 1999, however, seemed to be similar to that in 1998 except for one outlier (HI; Fig. 1b). We therefore used the regression line obtained in 1998 ($y = 7.70 + 0.45x$, where x and y are the number of pollen grains and the number of seeds per flower) to predict the number of seeds per flower that we should have found in 1999. With this procedure, we found no significant difference between the predicted and observed number of seeds in 1999 (paired t test; $n = 9$, $t = 1.12$, $p = 0.30$), indicating a significant relationship between stigmatic pollen load and seed production in 1998 and the data obtained in 1999. The mean number of seeds per flower pollinated by hand was 57.9 \pm 21.8 (mean \pm SD), which was higher than open-pollinated controls (37.4 \pm 21.8). Artificial hand pollination significantly increased the number of seeds produced (nested analysis of variance [ANOVA]; $df = 11$, $MS = 6674.81$, $F = 2.08$, $p < 0.05$), whereas the difference among populations was highly significant ($df = 10$, $MS = 16647.02$, $F = 5.19$, $p < 0.0001$). The mean number of seeds per flower after hand pollination ranged from 25.3 (MB) to 104.3 (HI).

Effects of Population Size and Landscape Type on Seed Production

In 1999 the mean number of seeds per flower was significantly and positively related to the log of population size (regression coefficient, $b = 9.65$, $R^2 = 0.43$, $n = 13$, $t = 2.91$, $p = 0.01$; Fig. 2). In contrast, the mean number of seeds per flower in 1998 had no relationship to the log of population size. Analysis of covariance also detected a significant effect of population size in 1999, whereas no effect was observed in 1998 (Table 3). Populations smaller than 50 flowering plants consistently showed low seed production in both years (Fig. 2).

There was no consistent trend for the effect of landscape type on seed production (Table 3), but C populations produced more seeds than I populations in

Table 3. Effects of population size (size), landscape type (type), and their interaction (size \times type) on the mean number of seeds per flower observed for all *Trillium camschatcense* populations examined (analyzed with analyses of covariance).

Source	1998				1999			
	df	MS	F	p	df	MS	F	p
Size	1	582.87	1.76	0.22	1	1837.24	7.92	<0.05
Type	1	1004.31	3.03	0.11	1	377.10	1.62	0.23
Size \times type	1	5.24	0.02	0.90	1	8.32	0.04	0.85
Error	8	331.04			9	232.12		

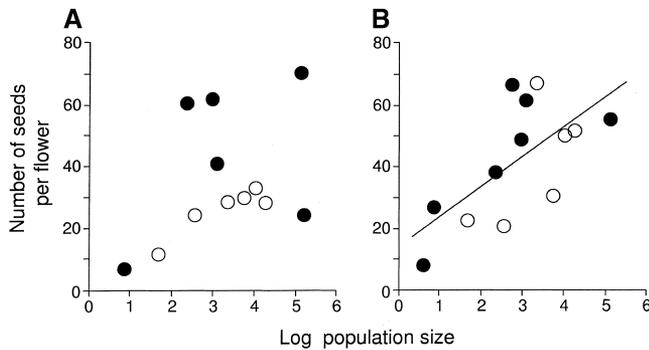


Figure 2. Relationship between the log of population size (estimated number of flowering plants) and mean number of seeds produced per flower of *Trillium camschatcense* populations in (a) 1998 and (b) 1999. Data are given for isolated (open circles) and continuous (closed circles) populations. Solid line in 1999 represents regression line for all 13 populations where regression coefficient was significantly different from zero.

both years (Fig. 2). In particular, only some C populations with over approximately 1000 flowering plants (PK, HK, and HI) were consistently successful in producing a certain number of seeds (e.g., 40 seeds) (nearly average numbers of seeds per flower, cf. Table 2) in both years.

Stage-Class Population Structure

In the study of population structure, we surveyed 1865 *T. camschatcense* individuals in total (Table 4). No seedlings were observed in MW and TB, which have extremely small population sizes. We also found few seedlings in the relatively small SS population (Table 4), although a substantial number of seeds were produced (the mean number of seeds per flower was 60.4 in 1998 and 38.0 in 1999). The relative frequency of each stage class differed significantly among popula-

tions ($\chi^2 = 478.3$, rank = 1/1000, $p = 0.001$). The proportion of seedlings had a higher correlation with population size ($\tau = 0.966$, $p < 0.01$) than with the number of seeds per flower in 1998 ($\tau = 0.690$, $p = 0.058$) and in 1999 ($\tau = 0.690$, $p = 0.058$). In large populations (TN, KK, and HI), the proportions of three-leaved steriles were slightly lower than those of flowerings, whereas small populations had 0.83 (TB) to 2.48 (SS) times more three-leaved steriles than flowerings. The proportion of three-leaved steriles was also significantly and negatively correlated with population size ($\tau = -1.000$, $p < 0.01$), but not with the number of seeds per flower (1998: $\tau = 0.600$, $p = 0.09$; 1999: $\tau = 0.200$, $p = 0.57$).

Discussion

We studied the effects of forest fragmentation on the pollination, seed production, and demography of *T. camschatcense* populations displaying different population sizes and occurring in different landscape conditions for 2 consecutive years. Seed production differed markedly among populations examined but not between years. The positive relationship between outcross pollen load and seed production suggested that reduced seed production could be attributed to pollen limitation. In 1999 but not 1998, we found a positive relationship between population size and seed production. Although no significant effect of landscape type was observed on seed production, C populations tended to produce a higher number of seeds than I populations. Stage-class population structure was better explained by population size rather than the number of seeds produced. The proportion of seedlings was significantly and positively related to population size. On the contrary, the proportion of three-leaved steriles was negatively related to population size but was uncorrelated with seed production. Collectively, these results point to the importance of population size in promoting consistent reproduction in this species.

Table 4. Stage-class population structure in six *Trillium camschatcense* populations observed in 2000.*

Population	No. of quadrats examined	No. of individuals per 1 m ²			
		flowering	three-leaved sterile	one-leaf sterile	seedling
MW	1	2.0 (0.40)	3.0 (0.60)	0.0 (0.00)	0.0 (0.00)
TB	4	1.8 (0.50)	1.5 (0.42)	0.3 (0.08)	0.0 (0.00)
SS	10	2.7 (0.12)	6.7 (0.30)	12.7 (0.56)	0.6 (0.03)
TN	10	7.8 (0.19)	5.2 (0.12)	12.0 (0.29)	17.0 (0.40)
KK	10	5.5 (0.16)	2.5 (0.07)	12.1 (0.35)	14.9 (0.43)
HI	10	7.5 (0.09)	4.9 (0.06)	13.3 (0.16)	59.2 (0.70)

* The mean number of *T. camschatcense* individuals per 1-m² quadrat is listed for each stage class. Proportions of each stage class within populations are shown in parentheses.

Effects of Fragmentation on Plant Reproductive Output and Its Mechanisms

The results of many previous studies concerning the effects of fragmentation on plant reproductive success suggest that seed production is reduced in small, fragmented populations in both self-incompatible and self-compatible plant species (Jennersten 1988; Lamont et al. 1993; Aizen & Feinsinger 1994; Gigord et al. 1999; Molano-Flores & Hendrix 1999; Kéry et al. 2000). Although the consequences of fragmentation are potentially diverse, such as increased spatial isolation between populations and changes in the abiotic and biotic conditions of habitats suitable for plants and pollinators, studies on factors other than size are limited. For instance, Yasaka et al. (1994) found that surrounding green area positively correlates with fruit set of populations of *Corydalis ambigua*, a bumblebee-pollinated understory spring herb. In *Clarkia concinna*, population size and spatial isolation have an interaction effect on pollination and seed production (Groom 1998).

The effect of population size on seed production in *T. camschatcense* was observed in one of the study years, and small populations of <50 flowering plants produced few seeds in both years (Fig. 2). As in many studies (e.g., Jennersten 1988; Ågren 1996), the reduced number of seeds could be attributed to pollen limitation. The outcross pollen load had a positive relationship with seed production (Fig. 1), and hand pollination significantly increased seed production, indicating that the amount of outcross pollen could be a constraint for the seed production of self-incompatible populations of this plant. Thus, the reduced seed production in small populations must be a result of low quantity and quality of pollination services. Small populations may be less attractive to pollinators, resulting in low pollination success (Sih & Baltus 1987). In some small populations with low flowering plant density (Table 1), the density-related decline in pollination efficiency may be more important because pollinators will deposit other species' pollen through their long travel via nonconspecifics (Kunin 1993). Also, in populations of extremely small sizes, low genetic variation at the self-incompatibility locus (S locus) decreases mate availability (Byers & Meagher 1992; Byers 1995) and consequently seed production, regardless of how many pollinators and resources are available.

Differences in landscape conditions can also affect the availability of pollinators in various ways (see also Kearns & Inouye 1997). Although some C populations apparently produced a higher number of seeds than I populations, the effect of landscape type was not significant in either year. The result may have been complicated by an outlier because the largest C population (RF) received only a few pollen grains on stigmas in both years (27.5 for 1998 and 23.8 for 1999; the data for 1999 do not appear in Fig. 2 because of fruit predation). Flower visitors of *T.*

camschatcense, small insect species belonging to Diptera and Coleoptera, can travel only relatively short distances and are thought to forage within only one or between several closely linked forests. Because their foraging ranges are still unknown, however, scale of the landscape analysis in our study may not accurately reflect the scale of insect-visitor movements. Therefore, we attempted to design better models by adding other landscape variables (i.e., forest area and a proportion of forest within the radius of a certain distance [125 m, 250 m, 500 m, 1 km] from the center of the focal population) to the present ANCOVA model and incorporating them instead of landscape type; however, none of these models satisfactorily described the variations observed in the number of seeds per flower in 1998. It is also possible that landscape variables were not significant because each pollinator species may have different requirements for nesting and food for larvae. The interactions between population size and other variables illustrate complex phenomena (Kearns et al. 1998), and further studies are required to gain more knowledge about the effects of fragmentation.

Our study seems to suggest that pollen limitation is just one of several to many factors influencing reproductive output in fragmented populations. Seed production was inconsistent between years, and seed production after supplemental hand pollination varied among populations. In such a fragmented landscape, other factors, such as environmental deterioration (Oostermeijer et al. 1998), pesticide use (Batra 1982), and inbreeding depression (Gigord et al. 1998), may also be responsible for reduced pollination services or seed production. These factors may explain the high variance of seed production and the exceptionally low seed production of the large, continuous RF population.

Consequences of Reduced Seed Production

The study of stage-class population structure indicates that population size rather than number of seeds produced has a high correlation with the relative frequency of seedlings and three-leaved steriles, suggesting that pollen limitation is not the most important factor determining demographic processes. Few seedlings and many three-leaved steriles were observed in small populations. In this context, population size has more relative importance for demographic processes than does surrounding landscape condition. Although *T. camschatcense* shows continuous growth from seedling to flowering, with a gradual yearly increase in leaf size, flowering plants sometimes return to the three-leaved sterile stage in the subsequent year (Ohara & Kawano 1986b). This step back in life-history stage could be due to accidental damage to stems or leaves in the previous year (Ohara & Kawano 1986b) or as a result of deterioration in environmental conditions. Thus, the high number of three-leaved

steriles in small populations may be caused by an increased step-back change from flowering to three-leaved sterile.

Reduced seed production must in part contribute to low seedling recruitment in small populations, but environmental deterioration, such as "edge effects" caused by fragmentation, may also have significant effects on population structure. Our other study of edge effects on *T. camschatcense* showed similar results. The forest edges in the KK population had a lower number of seedlings and a higher number of three-leaved steriles than the forest interiors, and these effects of edge formation penetrated more than 70 m from the edges into the forest (H. T. & M. O., unpublished data). Forest fragments are exposed to drastic changes in microclimatic conditions, and abiotic and biotic environments are greatly altered near forest edges (Lovejoy et al. 1986; Murcia 1995; Laurance & Bierregaard 1997). The extent of edge effect should be correlated with the size and shape of the focal forest. In the study populations, the distance from the center of the population to the nearest forest edge was highly correlated with population size ($\tau = 0.729$, $p < 0.001$; H. T. & M. O., unpublished data). Therefore, small populations are expected to suffer from strong edge effects in addition to the effects of reduced seed production. Even small changes in light availability and soil-water conditions could affect the demography (especially seedling establishment) of forest plant species growing in a relatively stable environment.

Implications for Conservation

Our study does offer some information on the specific habitat size and landscape conditions that must be conserved. Small populations of <50 flowering plants consistently showed low seed production, whereas C populations with more than approximately 1000 flowering plants (PK, HK, and HI) were successful in producing constant and high seed production (Fig. 2). The demographic study suggested that C population with 220 flowering plants (SS) had almost no seedling recruitment (Table 4). It therefore appeared that C populations with more than 1000 flowering plants were essential to reproduction. However, seed production clearly was not the only factor determining subsequent demography. Further studies combined with long-term observations of demographic changes are required (e.g., Groom 1998; Jules 1998; Menges & Dolan 1998), and population viability analysis (Shaffer 1990; recently reviewed by Menges 2000), by means of matrix projections, can provide population-extinction probabilities and evaluate minimum viable populations (Shaffer 1981). Landscape conditions should be analyzed in detail and partitioned into some quantitative factors to lead to more concrete propositions for conservation.

Acknowledgments

We are grateful to T. Ishibuchi, A. Tazawa, K. Shiojiri, S. Maezaki, M. Imamura, M. Oda, and S. Shimura for assistance in the field; K. Hiei for technical advice on pollen counting; T. Yahara for comments on statistical analyses; and C. Kremen and three anonymous reviewers for critical comments on the manuscript. We also acknowledge the Hiroo town office for offering the use of their facility. This work was funded in part by the Sasakawa Scientific Research Grant from the Japan Science Society, the Hokscitec Foundation, Grants-in-Aid from the Japan Society for the Promotion of Science (JSPS) for Scientific Research (10440230, 13640637), and research fellowships of the JSPS for Young Scientists.

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