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INVITED ARTICLE

Importance of life history studies for conservation of fragmented populations: A case study of the understory herb, *Trillium camschatcense*

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Abstract

Trillium camschatcense **is a common perennial woodland spring herb in Hokkaido, Japan. Anthropogenic land conversion (e.g. road construction and agricultural development) has resulted in a highly fragmented landscape with a large number of forest remnants and** ***T. camschatcense*** **is now often found in the understory of small remnant forests surrounded by pastureland or agricultural fields in the Hidaka region, Hokkaido. Accordingly,** ***T. camschatcense*** **in this region must have experienced dramatic changes in population size and spatial distribution, which may have significantly reduced seed production and genetic diversity. First, we summarize a series of life history studies on** ***T. camschatcense*****. Second, we introduce approaches to understand the effects of forest fragmentation on the understory herb,** ***T. camschatcense*****. Finally, we conclude that integration of data on demographic, reproductive biology and genetic approaches is essential for plant conservation, and we emphasize that it is important to have basic life history data, such as long-tem demographic data and variation in breeding system, on 'common species' before those species become 'new rares'.**

Keywords: demography, fragmentation, genetics, reproductive biology, *Trillium camschatcense*.

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Introduction

Since Soulé (1980, 1986, 1987), conservation biologists have attempted to test the effects of small population size on demography, reproductive biology and genetics. Various studies have investigated the relationships between population size and demographic stochasticity (Menges 1990, 1991), reproductive success (Jennersten 1988; Kwak & Jennersten 1991; Kwak *et al*. 1991; Aizen & Feinsinger 1994; Oostermeijer *et al*. 1998; Young & Brown 1998) and genetic variation (Ledig & Conkel 1983; Moran & Hopper 1983; McClenagham & Beauchamp 1986; Karron *et al*. 1988; Billlington 1991; van Treuren *et al*. 1991; Prober & Brown 1994; Raijmann *et al*. 1994). However, only rarely have attempts been made to incorporate all of these relationships in an assessment of the viability of small fragmented populations (Oostermeijer 2000; Washitani *et al*. 2005).

In 1996, the International Union for Conservation of Nature and Natural Resources (IUCN) proposed new categories for the Red list. In the new criteria defined by IUCN, 'threatened' species are classified into three categories: critically endangered, endangered and vulnerable (IUCN 1996, 1997). For example, a critically endangered species has a risk of extinction of 50% within 10 years or three generations, whichever is longer. These categories are based largely on population biology principles developed by Mace and Lande (1991). For defining endangerment, each category requires assessment of the

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probability of extinction and the generation time of focal species and populations. To assess the persistence of different plant populations, it is essential to understand integrated aspects of demography, reproductive biology and genetics, that is, life history features. As a matter of course, life history studies must cover various aspects of the life cycle of the species in question. Any characteristics that the species show have adaptive significance, both in connection with the survival or maintenance of the individual organism itself in the biotic communities with which it is associated and in relation to the maintenance of its future generations (Solbrig 1980; Kawano 1975, 1985; Silvertown 1987). Life history characteristics such as the timing to germination, survival of seedlings and adults, age at flowering, reproductive lifespan, and numbers of flowers and seeds refers to a set of responses that allows the organism to adapt to environmental conditions to ensure survival, reproduction and genetic variation (Oostermeijer & de Knegt 2004; Oostermeijer *et al*. 2003; Washitani *et al*. 2005).

For the species presented in this paper, *Trillium camschatcense* Ker Gawler, life history data were gathered on demography (Ohara & Kawano 1986a,b; Ohara *et al*. 2001), reproductive biology (Ohara & Higashi 1987; Ohara 1989; Ohara *et al*. 1990) and population genetics (Ohara *et al*. 1996). These basic life history studies on *T. camschatcense* enable us to face further conservation problems caused by habitat fragmentation (Tomimatsu & Ohara 2002, 2003a,b, 2004). In the present paper, we summarize our former studies on the life history of *T. camschatcense* and introduce our conservation studies integrating demography, reproductive biology and population genetic studies.

Life history studies on *Trillium camschatcense*

Demographic studies

The genus *Trillium* is an herbaceous member of the Liliaceae or Trilliaceae (*sensu*Dahlgren *et al*. 1985) and contains approximately 46 species, of which 11 species are known to occur in eastern Asia. The greatest proportion of the species, however, occurs in North America: 29 species occur in the East and seven in the West (Freeman 1975; Samejima & Samejima 1962, 1987).

Flowering of *T. camschatcense* occurs in May, along with that of other temperate woodland spring perennial herbs before forest canopy closure. As many woodland perennials normally require many years to become sexually mature, many different vegetative (non-flowering) individuals, as well as flowering ones, are found on the forest floor. The vegetative individuals of this genus comprise two morphologically distinct forms: one-leaf and three-leaf stages. Among the one-leaf individuals, seedlings have specific oblong-lanceolate leaves. Leaf shape progressively changes to an ovate shape with a round base in older one-leaf sterile individuals. After nearly 10 years in the one-leaf and subsequent three-leaf sterile stages with annual increases in leaf and rhizome sizes from accumulated photosynthate, they finally become flowering individuals. Accordingly, as with other species in this genus, *T. camschatcense* has four major growth stages: seedling, one-leaf, three-leaf and flowering (Fig. 1; also refer to Ohara & Kawano 2005). *Trillium camschatcense* is polycarpic and reproduces exclusively by seeds for the recruitment of offspring, and no clonal reproduction occurs (Ohara & Kawano 1986a).

For the genus *Trillium*, leaf size of an individual plant is a reliable criterion for determining the stage in the life history schedule in terms of reproductive potential (Kawano & Ohara 1986; Ohara & Kawano 1986b; Ohara & Utech 1988; Ohara 1989). Quadrats (2 m × 2 m) were established on the forest floor at flowering time at various localities, and all individuals within the quadrats were classified into leaf area categories (see Ohara & Kawano 1986b). The stage class structures (static population structure) based on leaf area categories at two different locali-

Fig. 1 Life history of *Trillium camschatcense*. Reproduced with permission from Masayoshi Umebayashi.

ties are shown in Figure 2. Population A (Urahoro) showed a sigmoid-type population structure characterized by a conspicuous decrease in the number of early juvenile stages, a subsequent leveling-off in the intermediate stages, and a decrease again in the larger stages of the life history process. In contrast, Population B (Tomakomai) showed a non-sigmoid, convex-shaped population structure characterized by extremely small numbers of plants in juvenile stages, suggesting that young juvenile plants are not recruited into this population. By using age structure pyramids for the prediction of human population dynamics, one can predict that although Population A is expanding or stable, Population B is shrinking and may not have flowers in the future.

Demographic studies can provide essential information on the most crucial stages in the life cycle, as well as on the effects of different types of habitat management (Oostermeijer *et al*. 1996; Silvertown *et al*. 1996; Lennartsson & Oostermeijer 2001). In particular, with long-term demographic monitoring data, we can make a realistic simulation model of the population dynamics, preferably including information on levels of environmental stochasticity. This can be obtained by studying as many sites for as many years as possible (Oostermeijer *et al*. 1996). We established a permanent plot (1 m × 1 m) in Nopporo Forest Park in 1980 to investigate the dynamic population structure of *T. camschatcense* in its natural habitat. All individuals within the plot, including the seedlings that emerged annually, were marked and mapped. The fates (survival and death) and successive changes in the growth stages of these plants were monitored continuously at anthesis in May when foliage leaves had fully expanded for a 12-year period (Fig. 3).

A transition matrix model has been used to analyze the demography of populations of many perennial plants (Sarukhán & Gadgil 1974; Hartshorn 1975; Bierzychudek 1982; Burns & Ogden 1985; Harcombe 1986; Kawano *et al*. 1987; Nakashizuka 1991; Takada & Nakajima 1992; Nault & Gagnon 1993; Takada 1995; Ohara *et al*. 2001). This model provides a useful tool for the demographic analysis of plant populations because it describes the dynamics of a population with stage-structure, and valuable information about the dynamics of the population can be obtained from the matrix using simple mathematical cal-

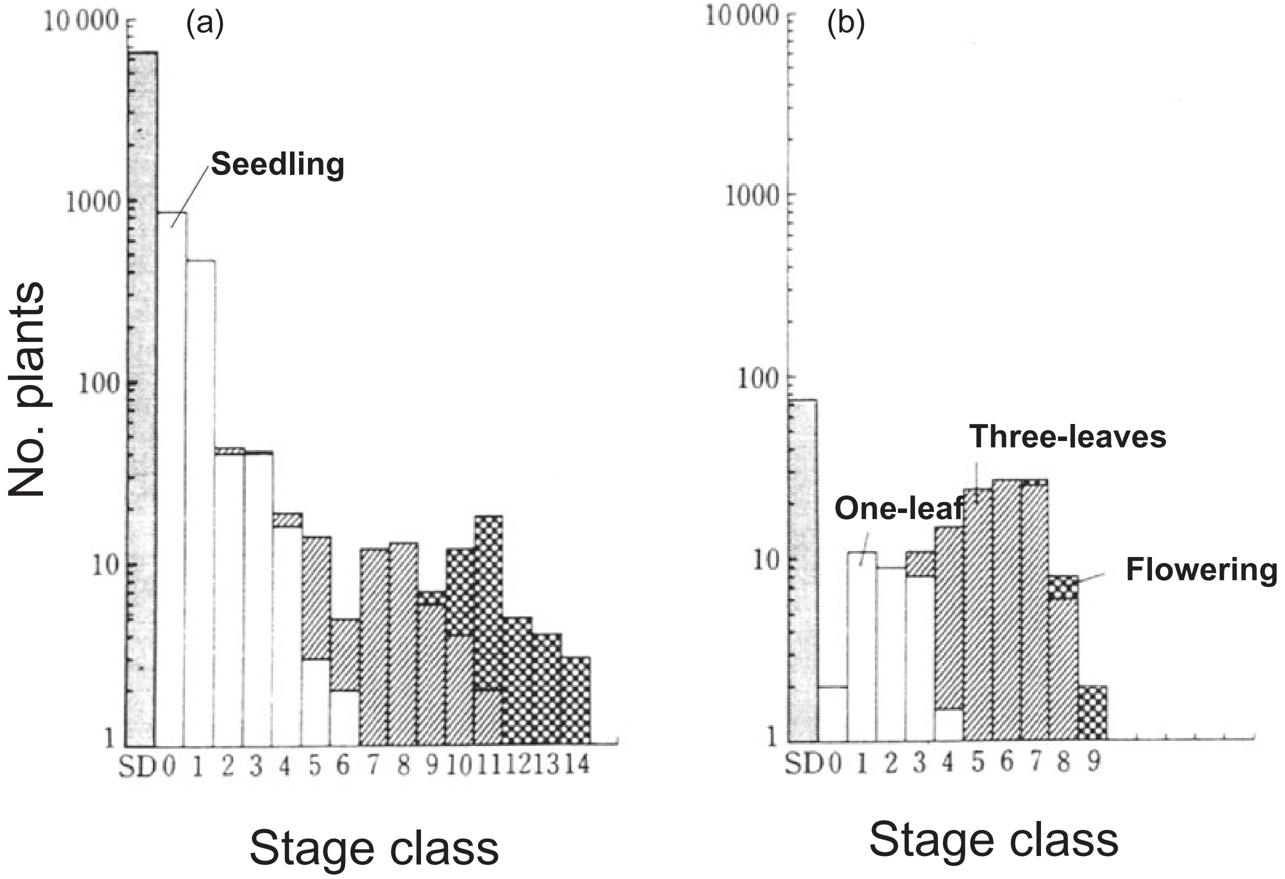

Fig. 2 Stage class structures from two natural populations ((a) Population A is Urahoro and (b) Population B is Tomakomai, Hokkaido) of *Trillium camschatcense.* The number of seeds produced (SD) was estimated by the number of flowering individuals present multiplied by the mean number of seeds produced per plant.

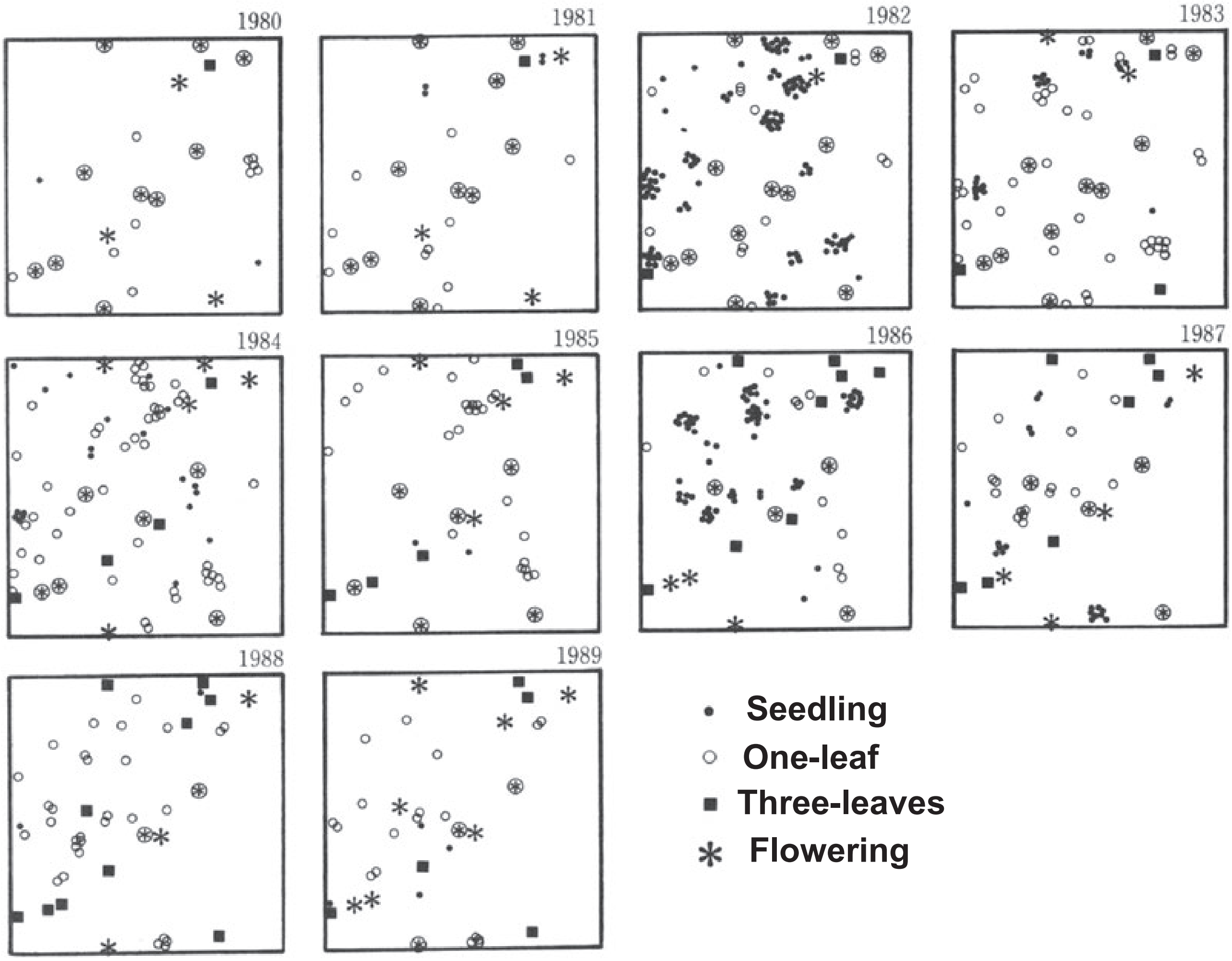

Fig. 3 Distribution and seasonal changes in *Trillium camschatcense* individuals in the permanent quadrat (1 m × 1 m, Nopporo Forest Park, Hokkaido) from 1980 to 1989.

culations (Lefkovitch 1965; Caswell 2001). We obtained a transition matrix for the population ssing the census data (Table 1). The matrix revealed the following:

1. Transition probability from 1L (one-leaf) to 3L (three-leaf) is only 0.9%, which means that only 1.6% (0.009/0.533) of survivors at 1L go on to the next stage over the course of 1 year. This in turn implies that it takes a long time for individuals to go through the 1L stage.
2. The survival rates increase gradually as individuals mature. The mortalities of 3L and F (flowering plant) are extremely low, only 1%. It is also interesting to note that even after a 3L sterile becomes a flowering plant, a regression from flowering to the 3L stage occurred in 16.3% of these plants.
3. A flowering individual contributes a mean of 2.7 seedlings to the population. Long-term census data allowed us to carry out a demographic analysis of our population and to investigate the reproductive strategies of *T. camschatcense* (Primack & Hall 1990; Primack & Stacy 1998).

Table 1 Transition matrix for *Trillium camschatcense* generated from the census data from 1980 to 1992

	Seedling	One-leaf	Three-leaves	Flowering
Seedling	0.000	0.000	0.000	2.706
One-leaf	0.485	0.525	0.000	0.000
Three-leaves	0.000	0.009	0.802	0.163
Flowering	0.000	0.000	0.197	0.836
Survival rate	0.485	0.533	0.999	0.999

The survival rate at each stage is the sum of the elements in each row with the exception of the first column.

Reproductive biology and population genetics

Trillium camschatcense flowers in May and fruits in July. Pollination experiments (bagging and emasculation before anthesis) were conducted in various populations in Hokkaido, Japan to characterize the breeding system (Ohara *et al*. 1996). *Trillium camschatcense* has an average of 225 ovules per flower and the average seed setting rates (S/O ratios) of naturally pollinated individuals varied among populations from 38% to 82% (Fig. 4; Ohara *et al*. 1996). Bagged individuals produced mature seeds in all of the northern and southern populations examined, suggesting that the plants in these populations were self-compatible and that substantial self-pollination had occurred. In contrast, in populations in the Hidaka and Tokachi regions, individuals with bagged flowers produced no seed at all. That is, self-incompatibility occurs in these populations (Fig. 4).

Plants emasculated prior to anthesis produced mature seeds in all populations examined, suggesting that substantial outbreeding occurs in *T. camschatcense.* Although the S/O ratios varied from 33% to 75%, the values did not differ significantly from the average S/O ratios of open-pollinated individuals except at NA, ER, YO, MN and TY, where S/O ratios were lower than those of open-pollinated plants. *Trillium camschatcense* does not produce nectar. However, the base petals have a sweet scent and a wide range of insects visit the flowers, primarily Coleoptera (e.g. *Epuraea* spp.) and Diptera (e.g. *Scatophaga stercoraria*) species (Fukuda 1961; Ohara *et al*. 1991; Tomimatsu & Ohara 2003a).

Breeding systems have been recognized to have a profound influence on the extent and structure of genetic variation in plant populations. In addition to the above-mentioned pollination experiments, an analysis of genetic structure was carried out based on allozyme variation (Ohara *et al*. 1996). As expected, self-incompatibility populations in the Hidaka and Tokachi regions were found to exhibit high genetic variability. In contrast, the northern and southern populations mostly exhibited very low genetic variability. These results suggest that the plants in the Hidaka and Tokachi regions are self-incompatible and that seed production and high genetic diversity result from predominant outbreeding (insect pollination). The

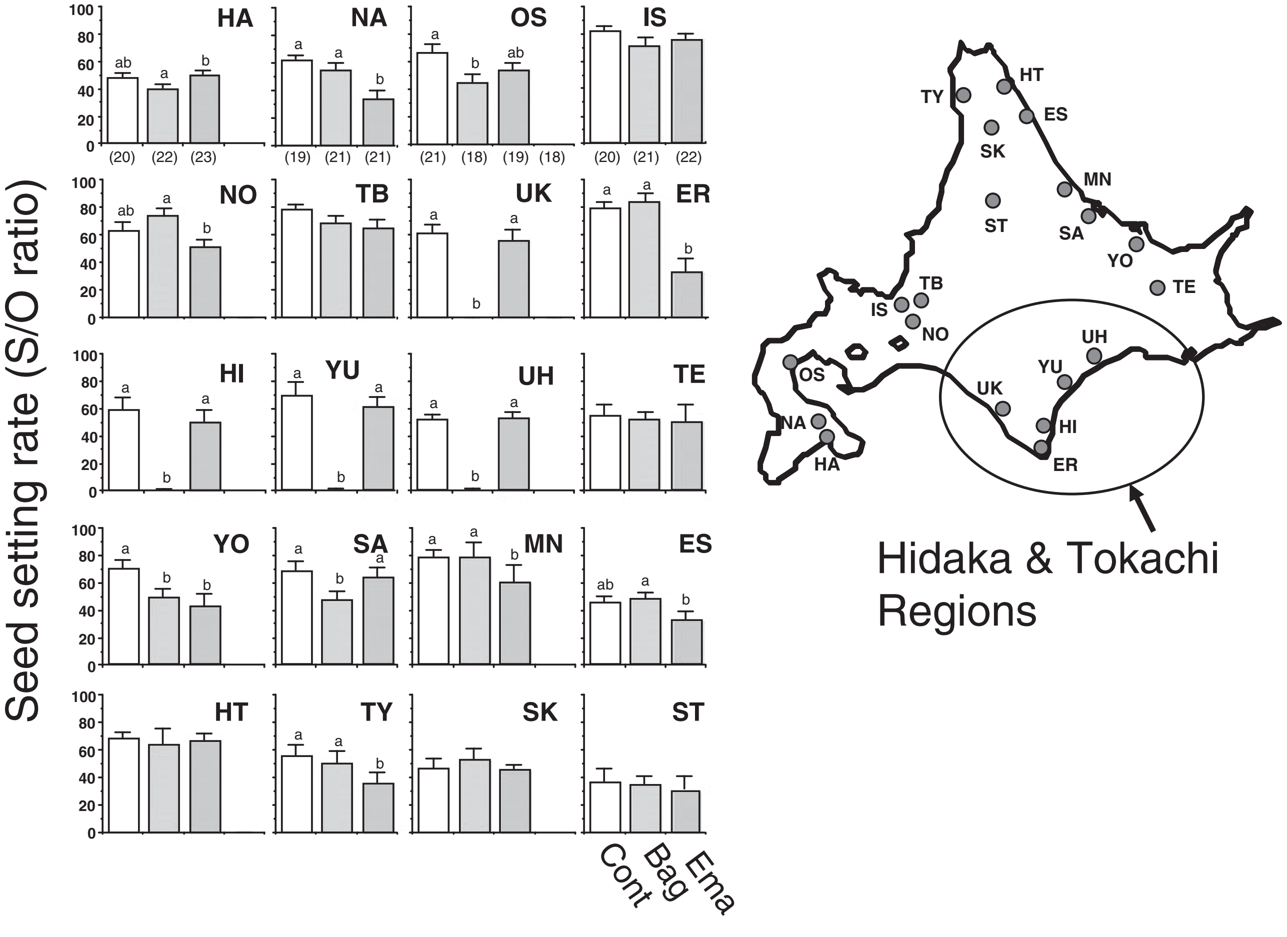

Fig. 4 Mean seed-setting rates (S/O ratios) obtained from the pollination experiments conducted with various populations of *Trillium camschatcense*. Bars represent standard errors of the mean. Results of treatments with different lower case letters were found to be significantly different (modified from Ohara *et al*. 1996). Cont, control; Bag, bagging; Ema, emasculation.

northern and southern populations with low genetic diversity potentially exploit a mixed system of outbreeding and inbreeding.

Application of life history studies to the conservation of fragmented populations of *Trillium camschatcense*

Habitat fragmentation caused by human activities, such as road construction and development of agricultural fields, dramatically reduces plant population size and increases 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. In the Tokachi region, Hokkaido, anthropogenic land conversion (e.g. road construction and agricultural development) since the 1880s has resulted in a highly fragmented landscape with a large number of forest remnants, many of which are smaller than 1 ha (Tan 1994). Thus, *T. camschatcense* is now often found in the understory of small remnant forests surrounded by pastureland or agricultural fields, and we can assume that forest fragmentation divided formerly huge *T. camschatcense* populations into small and isolated pieces. Accordingly, *T. camschatcense* in this region probably experienced dramatic changes in population size and spatial distribution, which may have significantly reduced seed production and genetic diversity. We investigated three processes of the effects of forest fragmentation on *T. camschatcense*: (i) effects on seed production and population structure; (ii) effects on genetic diversity; and (iii) edge effects.

Effects on seed production and population structure

For outcrossing of entomophilous plants, population size and plant density are closely associated with the attraction and activity of pollinators and probably 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 because of insufficient pollen transfer (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).

As shown previously, the populations of *T. camschatcense* in the Tokachi region show self-incompatibility and seed production results from obligatory outcrossing by insect pollination (Fig. 4). We investigated the effects of forest fragmentation on reproduction and measured seed production in 12 (1998) and 14 (1999) populations with various population sizes in the Tokachi region (Fig. 5). The numbers of seeds per flower was related to population size in 1998, and in both years small populations of <50 flowering plants produced few seeds. We attributed the decrease in seed production to pollen limitation because the stigmatic pollen load had a positive relationship to seed production and the addition of pollen by hand increased seed production (Tomimatsu & Ohara 2002).

In the study of population structure, we surveyed variation in population structure (four major growth stages: seedling, 1L, 3L and flowering) among six populations (Fig. 6). Although larger populations had a higher proportion of seedlings, no seedlings were observed in two pop-

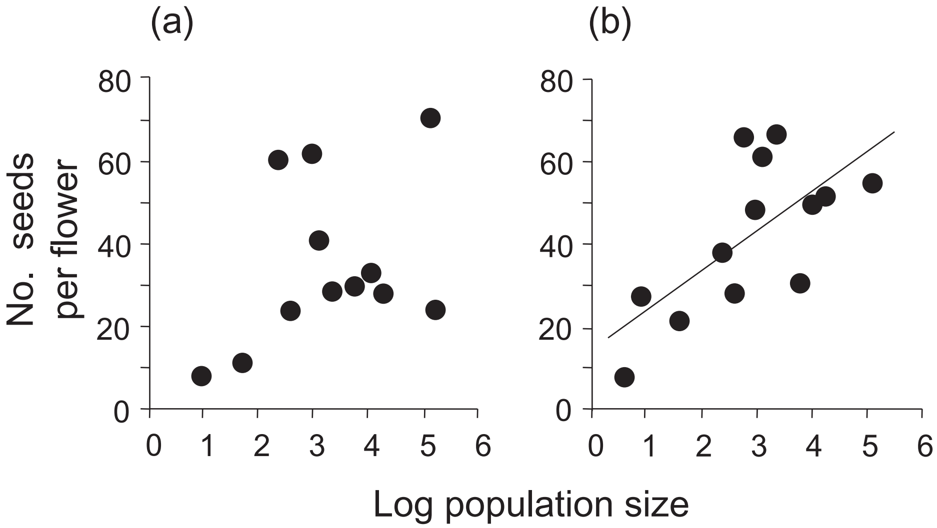

Fig. 5 Relationship between the log of population size (estimated number of flowering plants) and mean number of seeds produced per flower of *Trillium camschatcense* populations in (a) 1998 and (b) 1999 (modified from Tomimatsu & Ohara 2002).

ulations with extremely small population sizes of 7 and 46. We also found few seedlings in a relatively small population (population size 220). Reduced seed production in small fragmented populations must contribute to low seedling recruitment. In addition, environmental deterioration, such as 'edge effects' caused by fragmentation, may also have significant effects on population structure. As we will show and discuss below, forest fragments are exposed to drastic changes in microclimatic conditions, and the abiotic and biotic environments are greatly altered near the forest edges. 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 (particularly seedling establishment) of forest plant species growing in a relatively stable environment.

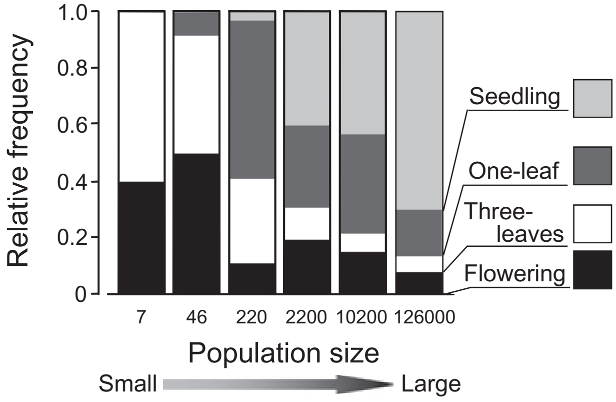

Fig. 6 Stage class population structure in six *Trillium camschatcense* populations. Proportions of each stage class within nine 1 m × 1 m quadrats established at each population are shown (modified from Tomimatsu & Ohara 2002).

Effects on genetic diversity

Theoretically, fragmentation is predicted to cause genetic erosion owing to founder effects, and subsequently to genetic drift and inbreeding (reviews by Barrett & Kohn 1991; Young *et al*. 1996). The genetic erosion induced by fragmentation may be harmful to population persistence because reduced heterozygosity may lead to decreased individual fitness (Raijmann *et al*. 1994; Oostermeijer *et al*. 1995) and the loss of allelic richness may eliminate the opportunities for future adaptation (Frankel *et al*. 1995). The theory predicts positive relationships between genetic diversity and heterozygosity and population size, and such relationships have been identified for a number of species that have recently experienced habitat loss (van Treuren *et al*. 1991; Prober & Brown 1994). However, because the responses to fragmentation may vary depending on habitat or life history characteristics (Young *et al*. 1996), more studies on genetic consequences of fragmentation need to be conducted.

We examined the genetic diversity and population genetic structure of 12 fragmented populations with different population sizes using allozyme electrophoresis (Table 2). Fragmented populations are predicted to experience stochastic loss of rare alleles first, because only a small portion of the original gene pool remains after fragmentation (Barrett & Kohn 1991; Ellstrand & Elam 1993; Young *et al*. 1996). Subsequently, random genetic drift will reduce allelic richness and increase interpopulation genetic divergence; however, it takes several generations

Table 2 Genetic diversity estimates and inbreeding coefficients (F_{IS}) for 12 *Trillium camschatcense* populations (after Tomimatsu & Ohara 2003a)

Population	Population size	N	P	Ap	H_o	H_e	F_{IS}
SS	220	42	18.2	1.18	0.037	0.046	0.211
OH	550	44	54.5	1.73	0.122	0.191	–0.043
MM	18 000	48	54.5	1.55	0.083	0.092	0.107
TN	2200	48	54.5	1.55	0.057	0.054	–0.056
KK	10 200	48	54.5	2.00	0.068	0.078	0.129*
HK	900	48	54.5	1.55	0.051	0.053	0.057
HM	5500	48	54.5	1.55	0.034	0.035	0.034
PK	1200	48	54.5	1.91	0.131	0.125	–0.037
TB	46	24	18.2	1.18	0.083	0.081	–0.004
RF	153 600	48	54.5	1.55	0.097	0.108	0.166
MB	350	31	18.2	1.27	0.067	0.080	0.257*
HI	126 000	40	81.8	2.09	0.132	0.133	0.072
Mean			47.7	1.59	0.080	0.090	0.074
Standard error			(5.6)	(0.09)	(0.010)	(0.013)	(0.030)
Overall		517	54.5	2.55	0.076	0.095	

*Significant deviations from Hardy–Weinberg expectations (randomization test; $P < 0.05$). Ap, mean number of alleles per polymorphic locus; Fis, inbreeding coefficient; He, expected heterozygosity; Ho, observed heterozygosity; N, sample size; P, percent polymorphic loci.

for drift to have a significant impact. In the study populations in the Tokachi region, only a few generations (probably one or two generations) have passed since fragmentation because of the long life and reproductive span of *T. camschatcense* (see Figs 1–3; Ohara & Kawano 1986b, 2005). The percentage of polymorphic loci and the mean number of alleles per locus were positively related to population size (Tomimatsu & Ohara 2003a). The heterozygosity and inbreeding coefficients were not related to population size, and a number of small populations showed relatively high inbreeding coefficients (Table 2; Tomimatsu & Ohara 2003a). Of 28 alleles detected, 13 were recognized as rare. Although the mean number of common alleles per locus was the same for all populations, all alleles that were not observed in small populations were rare, that is, rare alleles (frequency of $q < 0.1$) were lost from small populations (populations with 350 flowering plants or fewer). Because the effects of drift were expected to be minor (it takes several generations for drift to have significant impacts), the decrease in allelic richness in small populations was attributable to genetic bottlenecks at the time of fragmentation, probably as a result of the stochastic loss of rare alleles (frequency of $q < 0.1$) in small populations (Fig. 7). The results indicate that populations are expected to lose 0.17 allelic richness for each reduction in their population size by 1/10 of the original (Fig. 7). Several studies have shown a loss of rare alleles when populations were reduced in size in a number of perennial plant species including *Salvia pratensis* and *Scabiosa columbaria* (van Treuren *et al*. 1991), *Eucalyptus albens* (Prober & Brown 1994), *Gentiana pneumonanthe* (Rajimann *et al*. 1994), *Rutidosis leptorrhynchoides* (Young *et al*. 1999) and *Swainsona recta* (Buza *et al*. 2000).

Edge effects

Another important consequence of forest fragmentation is a dramatic increase in edges (Lovejoy *et al*. 1986; Saunders *et al*. 1991; Murcia 1995). Many physical conditions are often altered with fragmentation. For example, forest edges are characterized with higher air and soil temperatures and lower relative humidity and soil moisture than forest interiors. These changes in physical conditions could directly influence plant reproduction and recruitment (Powell & Powell 1987; Ferreira & Laurance 1997). Thus, we investigated the effect of forest edges on the recruitment of *T. camschatcense* in a small forest fragment (0.8 ha), and six transects (80–120 m) crossing at right angles were established. Along these six transects we examined the spatial distribution of four growth stages (seedling, IL sterile, 3L sterile and flowering), seed production and microclimatic conditions (air and soil temperature, and soil moisture) (Fig. 8; Tomimatsu & Ohara 2004).

Seedlings were most affected by edge effects. The density of seedlings was considerably lower in the forest edges than in the interiors and lower on the southern side of the forest. Three stages suffered edge and oriented effects and flowering plants experienced much more moderate edge effects than juvenile stages and showed relatively uniform distribution. The results suggest that the recruitment of juvenile stages was strongly limited near forest edges. Seedling density was correlated with microclimatic conditions and not with seed production. Edge and orientation effects on stage class structures were also observed in two other small populations, emphasizing the role of edge-related decreases in seed germination and subsequent survival for limiting the recruitment of a small, fragmented population of *T. camschatcense* (Tomimatsu & Ohara 2004).

We can conclude that the size of this fragmented population (0.8 h), which is typical for the Tokachi region, is insufficient to avoid significant edge effects. The size of this population (10 200 flowering plants) is much larger than that required to maintain high seed production (1000; Tomimatsu & Ohara 2002) and high allelic diversity (550; Tomimatsu & Ohara 2003a). Thus, the effects of edge

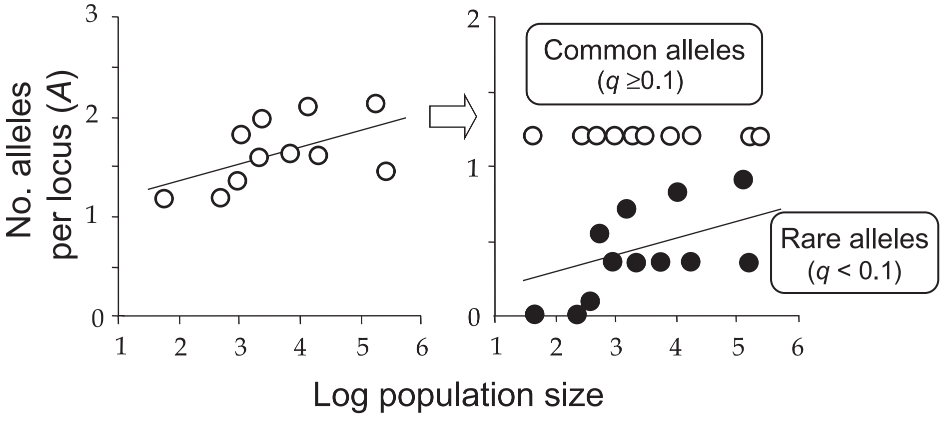

Fig. 7 Relationships between the log population size (estimated number of flowering plants) and mean number of alleles per locus. The relationship between population size and allelic richness for rare (●; frequency of $q < 0.1$) and common (○; frequency $q \geq 0.1$) alleles are shown (modified from Tomimatsu & Ohara 2003b).

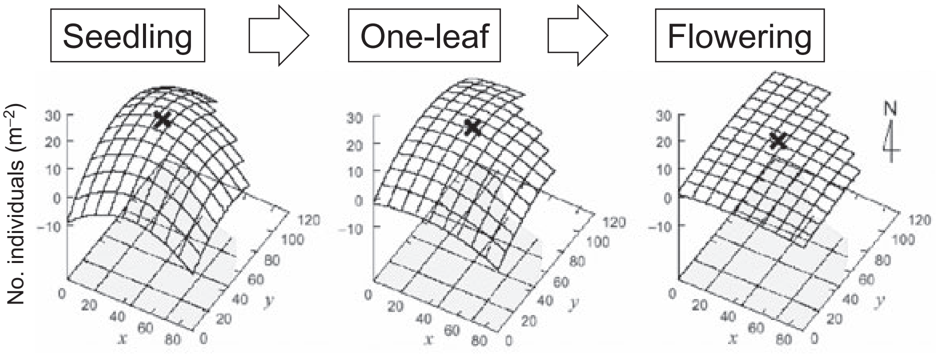

Fig. 8 Response surfaces of the number of *Trillium camschatcense* in each stage class. The point where the number of individuals was predicted to be highest by the model is indicated by a cross (x). Shaded areas indicate the forest fragment (Kiyokawa population, Tokachi, Hokkaido) (after Tomimatsu & Ohara 2004).

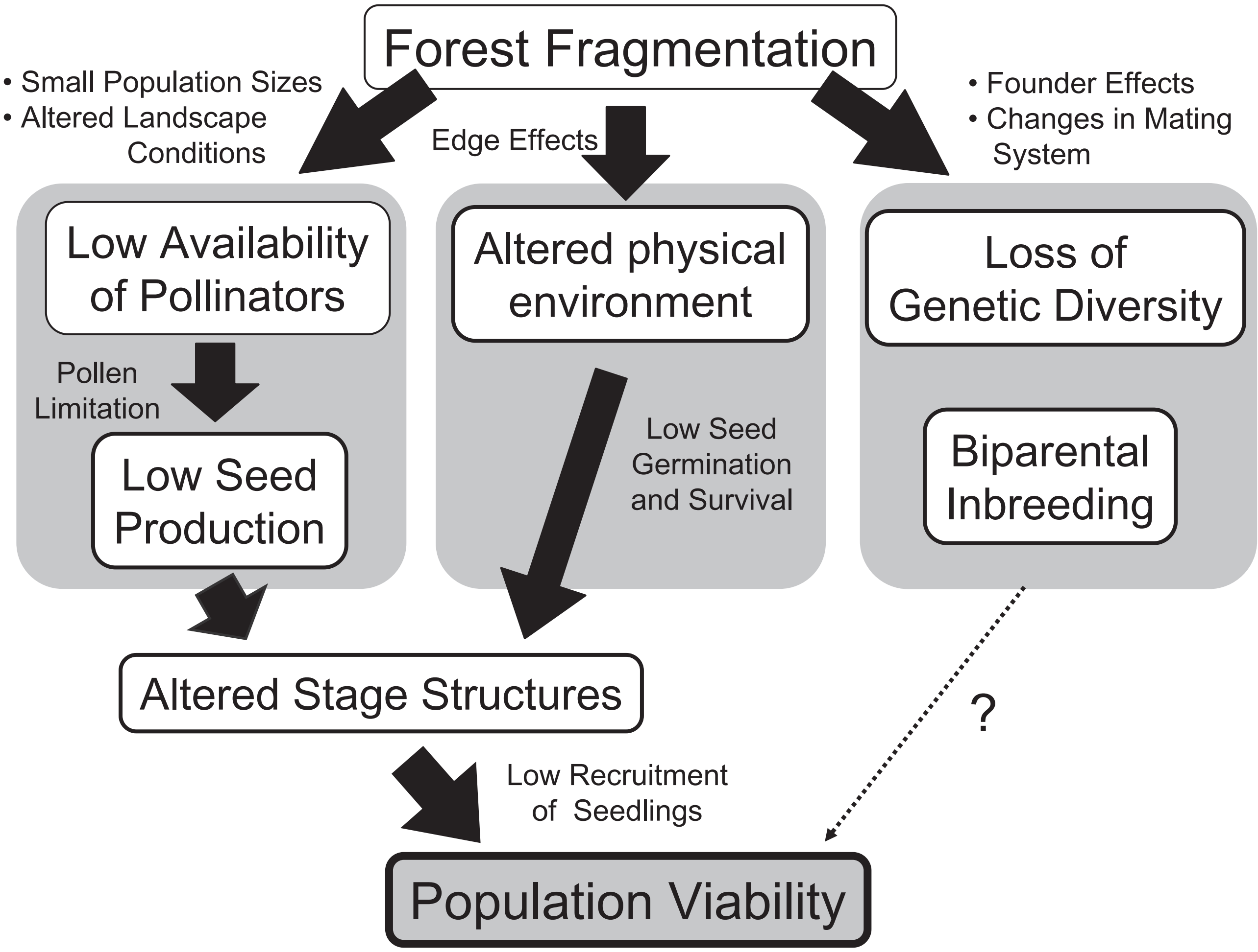

Fig. 9 Schematic representation of the effects of forest fragmentation on the population viability of plants.

orientation also provide management implications for reserve design. On the northern side of the forest, the density of young stages was high in the sample points, even at 10–20 m from the forest edges (Fig. 8). The results suggest that edge effects on recruitment can be greatly ameliorated if the shape and orientation of the remnant forests are taken into consideration.

Concluding remarks

The number of research papers on the conservation biology of rare plant species has increased enormously during the past 10 years. At the moment *Trillium camschatcense* is not an endangered or threatened species. However, human-induced habitat fragmentation may quickly transform formerly common plant species to endangered species called ‘new rares’ (Huenneke 1991; Oostermeijer *et al*. 2003). Although the amount of scientific information on the conservation of threatened plants with fragmented distribution has also increased enormously, we are still lacking essential data to enable us to assess the consequence of changing both genetic and demographic processes for population viability. This may be because the main aims of genetic studies have been to demonstrate that genetic variation is low or has been lost from small, isolated populations. In contrast, the studies on demographic viability focus on variation in fitness-related parameters, breeding systems and Allee-effects, but these studies rarely take genetics into consideration (Menges 2000). As emphasized in the book edited by Young and Clark (2000), the consequences of habitat fragmentation on plant populations have two aspects, a demographic aspect and a genetic aspect, and we need to evaluate the viabilities of focal plant populations based on both aspects. We need to focus on the impact of demographic vital rates to understand the importance of genetics and reproductive biology on population viability (Fig. 9; Lande 1998; Menges 2000; Oostermeijer *et al*. 2003 Washitani *et al*. 2005). For this purpose, we need to have good demographic data, both from natural populations and demographic genetic experiments. Long-term demographic studies can provide essential information on the most crucial stages in the life cycles, as well as on the effects of different types of habitat management (Silvertown *et al*. 1996; Lennartsson & Oostermeijer 2001; Oostermeijer *et al*. 1996, 2003). Long-term demographic monitoring data is essential for making a realistic simulation model of population dynamics, preferably including information on levels of environmental stochasticity (Ohara *et al*. 2001; T. Takada & M. Ohara, unpubl. data, 2005). To understand the viability of fragmented plant populations, it is clear that we need to integrate demography, reproductive biology and genetics, that is, all life history data for a focal species and/or population, under various biotic and abiotic environmental factors to determine a complete picture.

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References

Ågren J. (1996) Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* **77**: 1779–1990.

Aizen M. A. & Feinsinger P. (1994) Forest fragmentation, pollination, and plant reproduction in a chaco dry forest, Argentina. *Ecology* **75**: 330–351.

Barrett S. C. H. & Kohn J. R. (1991) Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk D. A. & Holsinger K. E. (eds). *Genetics and Conservation of Rare Plants*. Oxford University Press, New York, pp. 3–30.

Bierzychudek P. (1982) The demography of jack-in-the-pulpit. A forest perennial that changes sex. *Ecological Monograph* **52**: 335–351.

Billington H. L. (1991) Effect of population size on genetic variation in a dioecious conifer. *Conservation Biology* **5**: 115–119.

Burns B. R. & Ogden J. (1985) The demography of the temperate mangrove. [*Avicennia marina* (Forsk.) Vierch.] at its southern limit in New Zealand. *Australian Journal of Ecology* **10**: 125–133.

Buza L., Young A. & Thrall P. (2000) Genetic erosion, inbreeding and reduced fitness in fragmented populations of the endangered tetraploid pea *Swansona recta*. *Biological Conservation* **93**: 177–186.

Caswell H. (2001) *Matrix Population Models*, 2nd edn. Sinauer Associates, Sunderland.

Dahlgren R. M. T., Cliford H. T. & Yeo P. F. (1985) *The Families of the Monocotyledons*. Springer-Verlag, Berlin.

Ellstrand N. C. & Elam D. R. (1993) Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* **24**: 217–242.

Ferreira L. V. & Laurance W. F. (1997) Effects of forest fragmentation on mortality and damage of selected trees in central Amazonia. *Conservation Biololgy* **11**: 797–801.

Frankel O. H., Brown A. H. D. & Burdon J. J. (1995) *The Conservation of Plant Biodiversity*. Cambridge University Press, Cambridge.

Freeman J. D. (1975) Revision of *Trillium* subgenus *Phyllantherum* (Liliaceae). *Brittonia* **27**: 1–62.

Fukuda I. (1961) Pollinators of the genus *Trillium*. *Bulletin Tokyo Women's University* **12**: 23–34 (in Japanese).

Harcombe P. A. (1986) Stand development in a 130-year-old spruce-hemlock forest based on age structure and 50 years of mortality data. *Forest Ecological Management* **14**: 41–58.

Hartshorn G. S. (1975) A matrix model of tree population dynamics in tropical ecological systems. In: Golley F. B. & Medin E. (eds). *Trends in Terrestrial and Aquatic Research*. Springer-Verlag, New York, pp. 41–51.

Huenneke L. F. (1991) Ecological implications of genetic variation in plant populations. In: Falk D. A. & Holsinger K. E. (eds). *Genetics and Conservation of Rare Plants*. Oxford University Press, New York, pp. 31–44.

International Union for Conservation of Nature and Natural Resources (1996) *1996 IUCN Red List of Threatened Animals.* IUCN, Gland.

International Union for Conservation of Nature and Natural Resources (1997) *Red List of Threatened Plants.* IUCN, Gland.

Jennersten O. (1988) Pollination in Dianthus deltoids (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. *Conservation Biology* **2**: 359–366.

Karron J. D., Linhart Y. B., Chaulk C. A. & Robertoson C. A. (1988) The genetic structure of populations of geographically restricted and widespread species of *Astragalus* (Fabaceae). *American Journal of Botany* **75**: 1114–1119.

Kawano S. (1975) The productive and reproductive biology of flowering plants. II. The concept of life history strategy in plants. *Journal of the College of Liberal Arts, Toyama University* **8**: 51–86.

Kawano S. (1985) Life history characteristics of temperate woodland plants in Japan. In: White J. (ed.). *The Population Structure of Vegetation*. Dr. W. Junk Publisher, Dordrecht, pp. 515–549.

Kawano S. & Ohara M. (1986) Life history studies on the genus *Trillium* (Liliaceae) II. Reproductive biology and survivorship of four eastern North American species. *Plant Species Biology* **1**: 47–58.

Kawano S., Takada T., Nakayama S. & Hiratsuka A. (1987) Demographic differentiation and life-history evolution in temperate woodland plants. In: Urbanska K. M. (ed.). *Differentiation Patterns in Higher Plants*. Academic Press, New York, pp. 153–181.

Kwak M. M. & Jennersten O. (1991) Bumblebee visitation and seedset in *Melampyrum pratense* and *Viscaria vurugaris*: heterspecific pollen and pollen limitation. *Oecologia* **86**: 99–104.

Kwak M. M., van der Brand C., Kremer P. & Boerrigter E. J. M. (1991) Visitation, flight distances and seed set in populations of the rare species *Phyteuma nigrum* (Campanulaceae). *Acta Horticulturae* **288**: 303–307.

Lande R. (1998) Anthropogenic, ecological and genetic factors in extinction and conservation. *Researches on Population Ecology* **40**: 259–269.

Ledig F. T. & Conkel M. T. (1983) Gene diversity and genetic structures in a narrow endemic, Torrey pine (*Pinus torreyana* Parry ex Charr.). *Evolution* **37**: 79–85.

Lefkovitch L. P. (1965) The study of population growth in organisms grouped by stages. *Biometrics* **21**: 1–18.

Lennartsson T. & Oostermeijer J. G. B. (2001) Demographic variation and population viability in *Gentianella campestris*: effects of grassland management and environmental stochasticity. *Journal of Ecology* **89**: 451–463.

Lovejoy T. E., Bierregaaard R. O. Jr, Rylands A. B. *et al.* (1986) Edge and other effects of isolation on Amazon forest fragments. In: Soulé M. E. (ed.). *Conservation Biology: the Science of Scarcity and Diversity*. Sinauer Associates, Sunderland, pp. 257–285.

Mace G. M. & Lande R. (1991) Assessing extinction threats: towards a reevaluation of IUCN threatened species categories. *Conservation Biology* **5**: 148–157.

McClenagham L. R. & Beauchamp A. C. (1986) Low genetic differentiation among isolated populations of the californian fan palm (*Washingtonia filifera*). *Evolution* **40**: 315–322.

Menges E. S. (1990) Population viability analysis for a rare plant. *Conservation Biology* **4**: 52–62.

Menges E. S. (1991) The application of minimum viable population theory to plants. In: Falk D. A., & &. Holsinger K. E. (eds). *Genetics and Conservation of Rare Plants.* Oxford University Pres, New York, pp. 450–461.

Menges E. S. (2000) Population viability analyses in plants: challenges and opportunities. *Trends in Ecology and Evolution* **15**: 51–56.

Moran G. F. & Hopper S. D. (1983) Genetic diversity and the insular population structure of the rare granite rock species, *Eucalyptus caesia* Benth. *Australian Journal of Botany* **31**: 161–172.

Murcia C. (1995) Edge effects in fragmented forests: implications for conservation. *Trends in Ecology and Evolution* **10**: 58–62.

Nakashizuka T. (1991) Population dynamics of coniferous and broad-leaved trees in a Japanese temperate mixed forest. *Journal of Vegetation Science* **2**: 413–418.

Nault A. & Gagnon D. (1993) Ramet demography of *Allium tricoccum*, a spring ephemeral, perennial forest herb. *Journal of Ecology* **81**: 101–119.

Ohara M. (1989) Life history evolution in the genus *Trillium*. *Plant Species Biology* **4**: 1–28.

Ohara M. & Higashi S. (1987) Interference by ground beetles with the dispersal by ants of seeds of *Trillium* species (Liliaceae). *Journal of Ecology* **75**: 1091–1098.

Ohara M. & Kawano S. (1986a) Life history studies on the genus *Trillium* (Liliaceae) I. Reproductive biology of four Japanese species. *Plant Species Biology* **1**: 35–45.

Ohara M. & Kawano S. (1986b) Life history studies on the genus *Trillium* (Liliaceae) IV. Stage class structures and spatial distribution of four Japanese species. *Plant Species Biology* **1**: 147–161.

Ohara M. & Kawano S. (2005) Life history monographs of Japanese plants. *Trillium camshatcense* Ker-Gawl. (Trilliaceae). *Plant Species Biology* **20**: 75–82.

Ohara M., Kawano S. & Utech F. H. (1990) Differentiation patterns of reproductive systems in the genus *Trillium*. *Plant Species Biology* **5**: 73–81.

Ohara M., Okazaki K. & Kawano S. (1991) Predominant inbreeding and resource limitation in four Japanese *Trillium* species. *Miyabea* **1**: 21–30.

Ohara M., Takada T. & Kawano S. (2001) Demography and reproductive strategies of a polycarpic perennial, *Trillium apetalon* (Trilliaceae). *Plant Species Biology* **16**: 209–217.

Ohara M., Takeda H., Ohno Y. & Shimamoto Y. (1996) Variations in the breeding system and the population genetic structure of *Trillium kamtschaticum* (Liliaceae). *Heredity* **76**: 476–484.

Ohara M. & Utech F. H. (1988) Life history studies on the genus *Trillium* (Liliaceae) V. Reproductive biology and survivorship of three declinate-flowered species. *Plant Species Biology* **3**: 35–41.

Olesen J. M. & Jain S. K. (1994) Fragmented plant populations and their lost interactions. In: Loeschcke J. Tmiuk J. & Jain S. K. (eds). *Conservation Genetics*. Birkhauser, Basel, pp. 417–426.

Oostermeijer J. G. B. (2000) Population viability analysis of the rare *Gentiana pneumonanthe*: the importance of genetics, demography and reproductive biology. In: Young A. G. & Clarke G. M. (eds). *Genetics, Demography and Viability of Fragmented Populations*. Cambridge University Press, Cambridge, pp. 313–334.

Oostermeijer J. G. B., Brugman M. L., de Boer E. R. & den Nijs J. C. M. (1996) Temporal and spatial variation in the demography of *Gentiana pneumonanthe*, a rare perennial herb. *Journal of Ecology* **84**: 153–166.

Oostermeijer J. G. B. & de Knegt B. D. (2004) Genetic population structure of the wind-pollinated, dioecious shrub *Juniperus* *communis* in fragmented Dutch heathlands. *Plant Species Biology* **19**: 175–184.

Oostermeijer J. G. B., Luijten S. H. & den Nijs J. C. M. (2003) Integrating demographic and genetic approaches in plant conservation. *Biological Conservation* **113**: 389–398.

Oostermeijer J. G. B., Lujten S. H., Krenova Z. V. & den Nijs J. C. M. (1998) Relationships between population and habitat characteristics and reproduction of the rare *Gentiana pneumonanthe* L. *Conservation Biology* **12**: 1042–1053.

Oostermeijer J. G. B., van Eijck M. W., van Leeuwen N. C. & den Nijs J. C. M. (1995) Analysis of the relationship between allozyme heterozygosity and fitness in the rare *Gentiana pneumonanthe* L. *Journal of Evolutionary Ecology* **8**: 739–759.

Powell A. H. & Powell G. V. N. (1987) Population dynamics of male euglossine bees in Amazonian forest fragments. *Biotropica* **19**: 176–179.

Primack R. & Hall P. (1990) Cost of reproduction in the pink lady's slipper orchid: a four-year experimental study. *American Naturalist* **129**: 876–896.

Primack R. & Stacy E. (1998) Cost of reproduction in the pink lady's slipper orchid (Cypripedium acaule, Orchidaceae): an eleven-year experimental study of three populations. *American Journal of Botany* **85**: 1672–1679.

Prober S. M. & Brown A. H. D. (1994) Conservation of Grassy White woodlands: population genetics and fragmentation of *Eucalyptus albens*. *Conservation Biology* **8**: 1003–1013.

Raijmann L. E. L., van Leeuwen N. C., Kersten R., Oostermeijer J. G. B., den Nijs J. C. M. & Menken S. B. J. (1994) Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonanthe* L. *Conservation Biology* **8**: 1014–1025.

Rathcke B. J. & Jules E. S. (1993) Habitat fragmentation and plant–pollinator interactions. *Current Science* **65**: 273–277.

Samejima J. & Samejima K. (1962) Studies on the eastern Asiatic *Trillium* (Liliaceae). *Acta Horti Gotoburgensia* **25**: 157–257.

Samejima K. & Samejima J. (1987) *Trillium Genus.* Hokkaido University Press, Sapporo.

Sarukhán J. & Gadgil M. (1974) Studies on plant demography: *Ranunculus repens* L., *R. bulbosusu* L., *R. acris* L. III. A mathematical model incorporating multiple modes of reproduction. *Journal of Ecology* **62**: 921–936.

Saunders D. A., Hobbs R. J. & Margules C. R. (1991) Biological consequences of ecosystem fragmentation: a review. *Conservation Biology* **5**: 18–52.

Sih A. & Baltus M. S. (1987) Patch size, pollinator behavior, and pollinator limitation in catnip. *Ecology* **68**: 1679–1690.

Silvertown J. W. (1987) *Introduction to Plant Population Ecology*, 2nd edn. Longman, London.

Silvertown J. W., Franco M. & Menges E. S. (1996) Interpretation of elasticity matrices as an aid to the management of plant population for conservation. *Conservation Biology* **10**: 591–597.

Solbrig O. T. (1980) *Demography and Evolution in Plant Populations*. Blackwell, London.

Soulé M. E. (1980) Thresholds for survival: maintaining fitness and evolutionary potential. In: Soulé M. E. & Wilcox B. A. (eds). *Conservation Biology: an Evolutionary-Ecological Perspective*. Sinauer Associates, Sunderland, pp. 151–169.

Soulé M. E. (1986) The fitness and viability of populations. In: Soulé M. E. (ed.). *Conservation Biology*. Sinauer Associates, Sunderland, pp. 13–116.

Soulé M. E. (1987) *Viable Populations for Conservation*. Cambridge University Press, Cambridge.

Takada T. (1995) Evolution of semelparous and iteroparous perennial plants: comparison between the density-independent and density-dependent dynamics. *Journal of Theoretical Biology* **173**: 51–60.

Takada T. & Nakajima H. (1992) An analysis of life history evolution in terms of the density-dependent Lefkovitch matrix model. *Mathematical Biosciences* **112**: 155–176.

Tan Y. (1994) Present condition of the forest islands in Obihiro. *Report of the Obihiro Centennial City Museum* **12**: 1–8 (in Japanese with English summary).

Tomimatsu H. & Ohara M. (2002) Effects of forest fragmentation on seed production of the understory herb, *Trillium camschatcense* (Trilliaceae). *Conservation Biology* **16**: 1277–1285.

Tomimatsu H. & Ohara M. (2003a) Genetic diversity and local population structure of fragmented populations of *Trillium camschatcense* (Trilliaceae). *Biological Conservation* **109**: 249–258.

Tomimatsu H. & Ohara M. (2003b) Floral visitors of *Trillium camschatcense* (Trilliaceae) in fragmented forests. *Plant Species Biology* **18**: 123–127.

Tomimatsu H. & Ohara M. (2004) Edge effects on recruitment of *Trillium camschatcense* in small forest fragments. *Biological Conservation* **117**: 509–519.

van Treuren R., Bijlsma R., Ouborg N. J. & van Delden W. (1991) The significance of genetic erosion in the process of extinction. 1. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. *Heredity* **66**: 181–189.

Washitani I., Ishihama F., Matsumura C., Nagai M., Nishihiro J. & Nishihiro M. A. (2005) Conservation ecology of *Primula sieboldii*: Synthesis of information toward the prediction of the genetic/demographic fate of a population. *Plant Species Biology* **20**: 3–15.

Wilcove D. S. C., McLellan H. & Dobson A. P. (1986) Habitat fragmentation in the temperate zone. In: Soulé M. E. (ed.). *Conservation Biology: The Science of Scarcity and Diversity.* Sinauer Associates, Sunderland, pp. 237–257.

Young A., Boyle T. & Brown T. (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* **11**: 413–418.

Young A. G. & Brown A. H. D. (1998) Comparative analysis of the mating system of the rare woodland shrub *Daviesia suaveloens* and its common congener *D. mimosoides*. *Heredity* **80**: 374–381.

Young A. G. & Clark G. M. (2000) *Genetics, Demography and Viability of Fragmented Populations*. Cambridge University Press, New York.

Young A. G., Merriam H. G., Brown A. H. D. & Zich F. A. (1999) Genetic structure of fragmented populations of the endangered daisy *Rutidosis leptorrhynchoides*. *Conservation Biology* **13**: 256–265.