

Life-history monographs of Japanese plants. 2: *Trillium camschatcense* Ker-Gawl. (Trilliaceae)

MASASHI OHARA* and SHOICHI KAWANO†

*Graduate School of Environmental Sciences, Hokkaido University, Sapporo 060-0810, Japan and †Graduate School of Biological Science, Kyoto University, Sakyo-ku, Kyoto 611-0041, Japan

Abstract

The life-history characteristics and demography of *Trillium camschatcense* (Trilliaceae) were studied and are described here. *T. camschatcense*, a typical polycarpic perennial, is a representative of the spring plants growing in temperate broad-leaved deciduous forests in the lowlands of Hokkaido and northern Honshu, and adjacent Far East regions. Flowering takes place in early to late April. *T. camschatcense* typically has three white petals and three sepals, is primarily pollinated by bumblebees (*Bombus* and *Andrena* spp.) and beetles (*Carpophilus* and *Aphthonaltica* spp.), with pollen/ovule ratios of 2200, and is a conditional outbreeder. Occasional inbreeders are also known in the north-eastern populations of Hokkaido. Capsules mature in late summer, late July to August. Light-brown seeds, 2–3 mm long, with a soft juicy elaiosome containing sugars such as fructose, glucose and saccharose attracts ants and beetles, but only ants are effective dispersal agents for *Trillium* seeds. Our long-term monitoring study has continued for 25 years from 1980 to 2004, and most of the flowering individuals turned out to be extremely long-lived, at ages of well over 20 years. Notably, frequent switches in growth stages occur, not only proceeding from the single-leaf sterile stage to the three-leaved sterile-stage but also reverting to earlier growth stages.

Keywords: Arcto-Tertiary element, demography, life-history characteristics, myrmecochory, polycarpic perennial.

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Trillium camschatcense Ker-Gawl. (Trilliaceae)

Syn. *Trillium kamtschaticum* Pallas; *Trillium pallasii* Hulten; *Trillium obovatum* Kunth; *Trillium erectum* L. var. *japonicum* A. Gray.

Geographic and ecological distribution

Trillium camschatcense (*Trillium kamtschaticum*) is the only diploid perennial ($2n = 10$) of the genus *Trillium* (Trilliaceae) in north-eastern Asia among nine *Trillium* species known in eastern Asia (Haga 1937). Its geographic range extends from northern Honshu and Hokkaido to further north to the Kamtchatka Peninsula, throughout the Kurile

Islands (Hulten 1927; Tatewaki 1957; for distribution in Hokkaido and northern Honshu, see Fig. 1).

The genus *Trillium* includes nine Asian and 40 North American species of Arcto-Tertiary origin, of which *T. camschatcense* is the only diploid species in Asia. All 40 North American species are diploid with $2n = 10$ chromosomes, of which 25 species are sessile-flowered and are referred to the subgenus *Phyllantherum* (Freeman 1975).

Phenology and seasonal growth patterns

Trillium camschatcense is a representative of the spring plants in Hokkaido and northern Honshu. Right after the snowmelt in April, often showy populations with snow-white flowers of *T. camschatcense* cover the deciduous forest floor in the lowlands. Extensive populations of *T. camschatcense*, growing together with *Lysichitum camschatcense*, develop in the rather damp floor of the forests, mainly consisting of *Fraxinus mandshurica* var. *japonica*,

Correspondence: Shoichi Kawano

Email: kawano.shoichi@a0016656.mbox.media.kyoto-u.ac.jp

†Present address: 303-204 Greentown Makishima, 51-1 Moto-yashiki, Uji, Kyoto 611-0041, Japan.

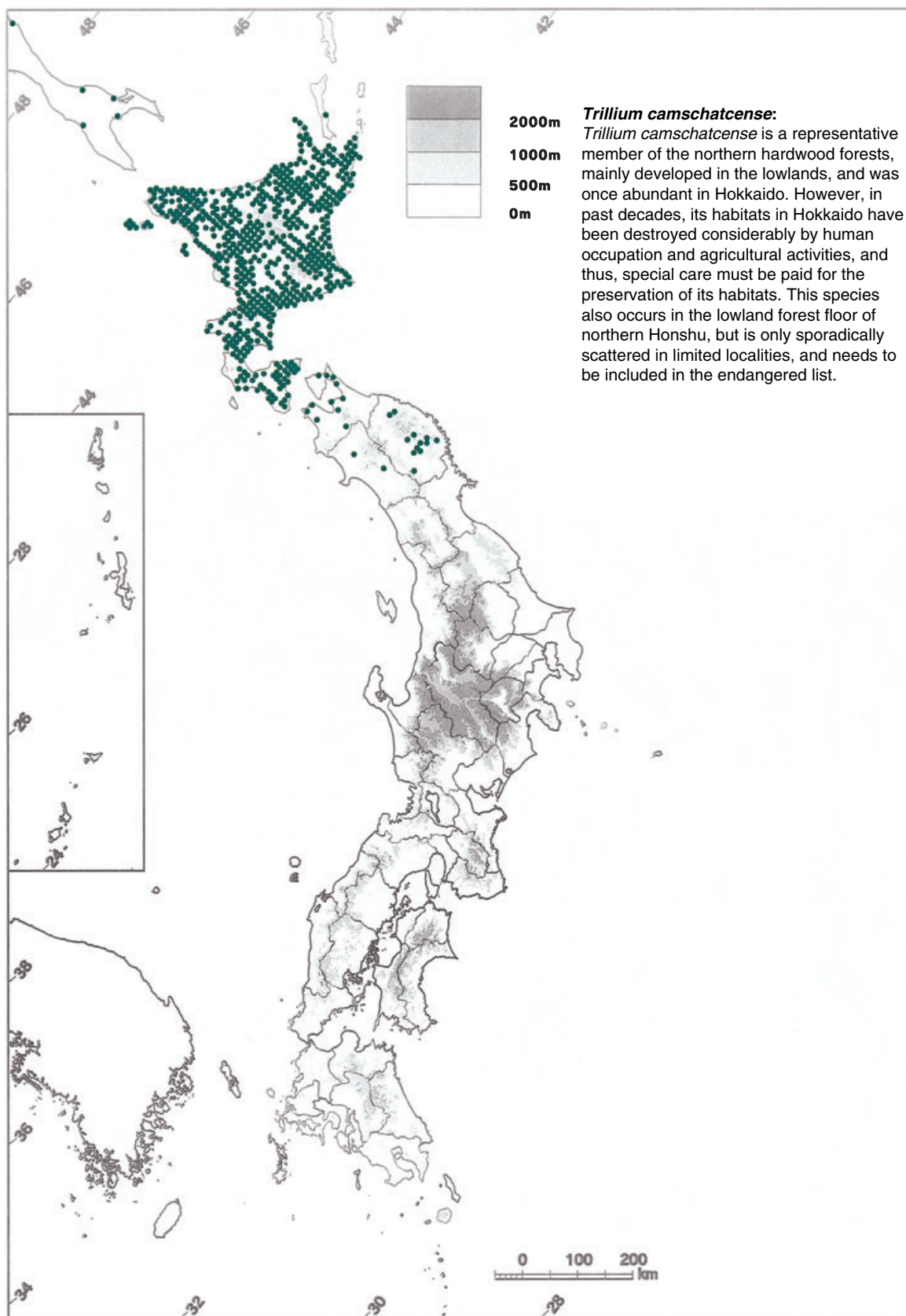


Fig. 1 Map showing geographic distributions of *Trillium camschatcense* in the Japanese Islands.

Ulmus davidiana var. *japonica* and others, in northern as well as eastern Hokkaido (e.g. a huge *Trillium* population exists in the wetland at the margin of Lake Abashiri).

In response to the development of the upper canopy layer in deciduous forests in late May to June, the understory becomes much darker, casting heavy shade over the forest floor, and then *Trillium* fruits gradually become mature. Eventually, *Trillium* plants bearing heavy mature fruits droop and fall on the forest floor. A rather long dormant stage continues for several months, but at the beginning of November roots first begin to elongate, and subsequently leaf and flower buds are formed in the underground soil layer. Just before snowfall, leaf and flower buds are completed and meiosis for producing both male and female gametes begins deep in the underground layer.

Populations structures

In 1980, a permanent plot measuring 1 m × 1 m was established in Nopporo Forest Park near Sapporo for *T. camschatcense*. Positions of all individual plants within the 1 m × 1 m quadrat were marked, and their fate and successive changes in leaf size (leaf length × leaf width) have been continuously recorded at anthesis, when foliage leaves were fully expanded. Fourteen different size classes were discriminated based on the number of leaves as well as total leaf area: seedling stage (0), single-leaf sterile stages (S1–S5), three-leaved sterile stages (S3–S12), and three-leaved fertile stages (F10–F14). The positions and sizes of all individuals present and their changes in succeeding years were continuously recorded: (i) newly sprouted seedlings; (ii) single-leaf sterile stage to three-leaved sterile stage or vice versa; (iii) three-leaved sterile stage to three-leaved fertile stage (i.e. flowering) or vice versa; and (iv) three-leaved fertile stage with a single flowering stem to plants with double flowering stems or vice versa.

As expected, the fate of seedlings was very severe. Most seedlings sprout in several highly dense clumps near flowering individuals. This fact indicates that, although some seeds are dispersed by ants as described in the following section, most of the seeds are directly established together with others in the capsules at the positions where flowering scapes fall down. This situation is well illustrated in the mapping data—most of the seedlings sprouting in several highly dense clumps near flowering individuals (see fig. 3-C in Ohara & Kawano 1986a). The seedlings that have sprouted in the previous year become single-leaf juvenile individuals in the succeeding year. However, obviously because of intense intraspecific competition and high mortality among juvenile plants forming clumps as well as other environmental constraints, drastic fluctuations occur in juvenile classes. However, after the critical juvenile stages, mortality obviously

decreases, and a more or less constant number of three-leaved sterile and flowering individuals are maintained continuously within the population.

This situation is seen more clearly if we look at fluctuations in the number of individuals belonging to the four major growth stages—seedling, single-leaf, three-leaved sterile and flowering stages—and the total number of individuals in the permanent plots. Drastic decreases occur, especially in the number of seedlings. Such changes in the seedling stage are evidently influenced by the number of seeds produced, the mechanisms of seed dispersal, the environmental conditions of the forest floor where the sprouts were settled, and the inherent germination rate each year.

Our long-term monitoring study has continued for 25 years from 1980 to 2004, and some individuals have turned out to be extremely long-lived, at well over 20 years of age (e.g. most of the flowering individuals recorded in 1980 produced flowers continuously for several years, despite leaf-size changes). Another notable fact is that there are frequent switches in growth stages, not only proceeding from the single-leaf sterile stage to the three-leaved sterile-stage, and further from the three-leaved sterile stage to the three-leaved single-stemmed flowering stage, but also frequently reverse changes occur (e.g. three-leaved sterile-stage to single-leaved sterile stage, or three-leaved fertile stage to three-leaved sterile stage). This fact clearly indicates that, once established, individuals at certain stages are often exceedingly long-lived. Thus, in order to accurately grasp the fate of any single individual, a long-term monitoring study is essential, and indeed the value of this simple but long-term project is enormously high (also refer to the monitoring study on *Erythronium japonicum* in this life-history monograph series; Kawano 2005).

Life-history characteristics

The life-history process of *T. camschatcense* is illustrated in Figures 2 and 3, which is based on the long-term field studies described below.

Trillium camschatcense has been confirmed as a polycarpic perennial. Our long-term census study on *T. camschatcense* populations over the past 25 years has demonstrated that it takes more than 10 years for plants to reach the sexually mature stage, but, as indicated above, the individuals that reach the biomass size classes capable of flowering do not necessarily flower continuously every season. There are overlapping generations within a population, which thus enhances the possibility of breeding among different generations.

The exact life expectancy of *T. camschatcense* is yet unknown, but judging from the number of scars left on the rhizomes, which are traces of aerial shoots from past



Fig. 2 Life history of *Trillium camschatcense*, a typical polycarpic perennial. B, berry; F, flower; Fl, flowering individual; J₁–J₄, single leaf juveniles; J₅–J₈, three-leaved juveniles; S, seedling; Sd, seeds.

years, some flowering individuals are apparently 40–50 years old (Ohara & Kawano, unpubl. obs.). Typical sigmoid-shaped size-class (or stage-class) structures consisting of numerous single-leaved (S1–S5) as well as three-leaved sterile plants (S3–S12) and three-leaved fertile plants with a single flowering stem and double flowering stems (F10–F14) clearly indicate that *T. camschatcense* is a species of exclusively sexual reproduction (see fig. 3-A, in Ohara & Kawano 1986a; Ohara *et al.* 1990).

Reproductive resource allocation

For plants, one of the critical aspects is how much energy (photosynthate) of the net production must be invested in reproductive activities throughout its lifetime (Kawano 1975). A study of reproductive activities in *T. camschatcense* was based on the methods of dry matter economy and reproductive biology (Kawano 1970, 1975).

The seasonal variation in reproductive allocation (RA) at the flowering as well as fruiting stages was examined in *T. camschatcense*. The RA values were expressed as the ratio of the total dry weight of reproductive organs (flowers and peduncles at the flowering stage, and seeds, pericarp, sepals and peduncles at the fruiting stage) to the dry weight of total individual biomasses harvested at both flowering and fruiting stages. Very similar allocation patterns were found in *T. camschatcense*, *Trillium tschonoskii* and *Trillium smallii*, although the latter two species are a tetraploid and a hexaploid, respectively. The allocation to reproductive organs reached 5.2–13.0% at the fruiting stage (Ohara & Kawano 1986a).

Reproductive biology and pollinators

Trillium camschatcense is a typical insect-pollinated outbreeder, although occasional inbreeders have been known to exist in eastern Hokkaido (Kurabayashi 1958; Fukuda, pers. comm.). The ovule number per flower (158–341; mean 225.1 ± 39.8) and the number of pollen grains per flower ($4.2\text{--}5.7 \times 10^5$), with a pollen/ovule ratio of 2200 specify *T. camschatcense* as a conditional outbreeder. However, *T. camschatcense* maintains moderately high fecundity levels. The reproductive outputs per plant were 38–198 (mean 101.1 ± 39.4), with a seed/ovule (S/O) ratio of 44.91% in 1980, and 77–216 (mean 136.8 ± 32.7), with an S/O ratio of 66.77% in 1984 (both sampled at Hakodate-yama, Hokkaido; Ohara & Kawano 1986a).

The pollinators are represented by diverse insects, including *Bombus hypocrita*, *Andrena sublevigata* (Hymenoptera), *Scathophaga stercoraria*, *Eristalis cerealis* (Diptera), *Carpophilus chalybeus* and *Aphthonaltica angustata* (Coleoptera), among which large bumblebees, such as *Bombus hypocrita* and *Andrena sublevigata*, and beetles, such as *C. chalybeus* and *Aphthonaltica angustata*,

are known as the most effective pollinators for *T. camschatcense* (see Fig. 3b; Fukuda 1961; Ohara *et al.* 1991; Tomimatsu & Ohara 2003a).

Seed dispersal

The weight of a single seed is 2.93 ± 0.30 mg and the seed bears a soft, juicy elaiosome at the tip. Thus, the seed is susceptible to ant dispersal (see Fig. 3e; e.g. by *Myrmica kotokui* or *Aphaenogaster smythiesi japonica*; Ohara & Kawano 1986a).

The chemical components of the elaiosomes of *Trillium camschatcense* seeds are known to contain sugars (fructose, glucose, saccharose; Nishino & Kawano, unpubl. data), which play an important role, attracting not only various ant species to seeds but also various beetle species. However, only ants are efficient dispersal agents for *Trillium*. Beetles simply eat the elaiosomes, then abandon the seed bodies after feeding at sites near the mother plants.

Palynology

Takahashi (1983) studied the pollen morphology of the Japanese species of the genus *Trillium*. Pollen grains of *T. camschatcense* are characterized by spherical and inaperturate grains, as are those of the other Japanese species, but they are 28.3 ± 2.7 μm long, making *T. camschatcense* the smallest species, reflecting its ploidy level as a diploid species.

Geographic ranges and the members of the genus

The genus *Trillium* (Trilliaceae *s. str.*, formerly placed in Liliaceae) is one of the representative groups of Arcto-Tertiary origin, with disjunct geographic distributions in eastern and western North America, and also north-eastern Asia to the Himalayan Mountains. Today, the genus is known to include a total of 49 species, and all of the species are so showy and attractive that plant biologists have continuously focused keen attention on this genus (Samejima & Samejima 1962; Samejima & Samejima 1987; Ohara 1989; Kawano *et al.* 1992; Kawano & Kato 1995; Case & Case 1997).

Eight species are known in Japan: *T. camschatcense* (2 \times), *T. tschonoskii* (4 \times), *Trillium apetalon* (4 \times), *T. smallii* (6 \times), *Trillium* \times *yezoense* (3 \times), *Trillium* \times *miyabeanum* (4 \times), *Trillium* \times *hagae* (3 \times , 6 \times) and *Trillium* \times *channellii* (4 \times), but *T. tschonoskii* (including var. *himalaicum*) (4 \times) extends its range to Taiwan, inland China and the Himalayan mountains. *Trillium govanianum* (4 \times) is the only species indigenous to the Himalayan mountains (Kurabayashi 1952; Kurabayashi *et al.* 1958; Fukuda *et al.* 1996). All the remaining 40 species are North American, of which 15 species are pedicellate, whereas 25 species are sessile,



Fig. 3 (a) Flowering individuals with three cauline leaves; (b) a pollinator Oedemeridae beetle visiting a flower of *Trillium camschatcense*; (c) a clump of seedlings sprouting by the mother plant; (d) fruiting stage; (e) ant species *Aphaenogaster smythiesi japonica* carrying a seed of *T. camschatcense* (note the large soft elaiosome containing rich sugars); (f) rhizome and flower bud enveloped with scaly sheath. Photos by Masashi Ohara.

lacking the pedicel, and thus are referred to as the subgenus *Phyllantherum* (Freeman 1975). Haga and Channell (1982) have attempted to cross-pollinate between *T. camschatcense* and some North American pedicellate species, and the results obtained showed that the compatible pedicellate-flowered species with *T. camschatcense* were *Trillium erectum*, *Trillium gleasonii* and *Trillium vaseyi*, all referred to the *Erectum* group.

A series of recent molecular phylogenetic studies have demonstrated that the genus *Trillium* consists of six major groups: (i) *Rivale* group (*Trillium rivale*); (ii) *Govianum–Undulatum* group (*T. govianum* and *Trillium undulatum*); (iii) *Erectum* group (*T. camschatcense*, *T. erectum* and *Trillium rugelii*); (iv) *Pusillum* group (*Trillium pusillum*); (v) *Grandiflorum* group (*Trillium grandiflorum* and *Trillium ovatum*); and (vi) *Sessile* group (all the sessile species) (Kato

et al. 1995a,b; Kazempour Osaloo & Kawano 1999; Kazempour Osaloo *et al.* 1999). Evolutionary backgrounds within the genus *Trillium* are very complex, but their origins, speciation and diversification are now being more rigorously clarified (Kawano & Kazempour Osaloo, unpubl. data).

Chromosome numbers and karyology

The chromosome numbers ($2n = 10$, K_1K_1 -genome constitution) and local variations in karyotypes of *T. camschatcense* were critically investigated based on the heterochromatin banding patterns after cold treatment in studies by Haga and Kurabayashi (1954); Kurabayashi *et al.* (1956, 1958); Kurabayashi (1958); Samejima (1958); and Fukuda *et al.* (1960).

Population genetics and conservation biology

Based on the differential heterochromatin banding patterns, three different population groups are known to have differentiated in Hokkaido—the northern, eastern and south-western Hokkaido population groups—but only one population group exists in northern Honshu, which clearly reflects past geologic history during and after the Pleistocene Ice Age (Kurabayashi *et al.* 1956, 1958; Kozuka & Kurabayashi 1960).

Ohara *et al.* (1996) analyzed genetic structure based on allozyme variation and also demonstrated that the populations in the eastern region of Hokkaido exhibit high genetic variability as a result of a self-incompatible breeding system. Tomimatsu and Ohara (2002, 2003b) recently studied populations in eastern Hokkaido, and demonstrated the consequences of fragmentation and isolation of local populations in rapidly decreasing their genetic diversity. Special attention needs to be paid to these fragmented populations for their future conservation.

Life-history voucher specimens

Life-history voucher specimens for *Trillium camschatcense*: Nopporo National Forests, Ishikari, Hokkaido, Masashi Ohara, 21 May 2003—flowering stage—SAPS; Masashi Ohara, 10 July 2004—fruiting stage—SAPS.

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