

NOTES AND COMMENTS

# Floral visitors of *Trillium camschatcense* (Trilliaceae) in fragmented forests

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Received 18 November 2002; revision received 3 April 2003; accepted 23 April 2003

## Introduction

Plant–pollinator interactions are of particular interest in conservation biology because of their ecological and agricultural importance (Bachmann & Nabhan 1996; Allen-Wardell *et al.* 1998; Kearns *et al.* 1998). Pollinator deficits may result in shortfalls in fruit or seed production, and ultimately, plant extinction (Rathcke & Jules 1993; Olesen & Jain 1994; Kearns & Inouye 1997). Many recent studies have shown that seed production is reduced in small, fragmented plant populations due to pollen limitation (e.g. Jennersten 1988; Aizen & Feinsinger 1994a). However, the effects of habitat fragmentation on pollinator assemblages have been rarely examined directly (Didham *et al.* 1996; Kearns *et al.* 1998). Further understanding of the pollinator dynamics in a fragmented landscape, for example, what kinds of pollinators are likely to be lost from fragmented habitats, is required to obtain useful implications for conservation of plant–pollinator interactions.

In the Tokachi region of eastern Hokkaido, Japan, intensive agricultural development since the 1880s has resulted in a highly fragmented landscape with a large number of small forest remnants. *Trillium camschatcense* Ker Gawler (Trilliaceae), a representative woodland spring herb in this region, once often formed large (>5 ha), continuous populations, but is now mostly found in the understory of small remnant forests surrounded by pastureland or agricultural fields. In a previous study (Tomimatsu & Ohara 2002), we demonstrated that small, fragmented *T. camschatcense* populations generally produced fewer seeds than large, continuous populations and that the low stigmatic pollen load was largely responsible for the reduced seed production.

The aim of the present study was to describe floral visitors of *T. camschatcense* in fragmented forests, where the availability of pollinators was hypothesized to be

reduced (Tomimatsu & Ohara 2002). We observed floral visitors at five different-sized populations and examined the differences in species composition and visitation frequency among the populations. We also discuss the relationship between floral visitors and outcross pollen load, which was measured in our previous study (Tomimatsu & Ohara 2002).

## Materials and methods

*Trillium camschatcense*, the correct name for *T. kamtschaticum* Pall. (Fukuda *et al.* 1996), is a long-lived spring herb that is common to the understory of mesic, broad-leaved deciduous forests throughout Hokkaido, Japan. In the Tokachi region, *T. camschatcense* flowers during mid to late May, before the leaf expansion of canopy trees, and produces mature fruits by the end of July. Reproductive plants produce single or sometimes a few flowers and each flower has three large white petals. This species reproduces exclusively by seeds (Ohara & Kawano 1986). In the Tokachi region, populations show self-incompatibility and seed production results from obligatory outcrossing by insect pollination (Ohara *et al.* 1996). Although effective pollinators remain unknown, the flowers are generally visited by a wide range of insects mostly belonging to Diptera, Coleoptera and Hymenoptera (Fukuda 1961; Ohara *et al.* 1991).

Data were collected in May 1999, during the flowering peak of *T. camschatcense*. Out of the 14 populations investigated in our previous studies (Tomimatsu & Ohara 2002, 2003), five populations with different population sizes (SS, KK, RF, MB and HI; Table 1) were selected for this study. Although large populations tended to receive many pollen grains and produce many seeds, the large RF population exceptionally received few pollen grains and produced few seeds (Tomimatsu & Ohara 2002). At every observation, we established a plot (~10–20 m<sup>2</sup>) in the center of the population and the plants within the plot were observed for 3 h between 08:30 and 16:32 hours Japanese Summer Time (JST) (Table 1). The plots were designed to

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**Table 1** Study populations of *Trillium camschatcense*. In each population, we conducted 3-h observations twice (a and b)

Population	Population size†	Flowering plant density (25 m <sup>-2</sup> )†	Date, time	No. flowers observed
SS	Small	10	(a) 15 May, 11.17–14.17 hours	8
			(b) 22 May, 13.10–16.10 hours	11
KK	Medium	33	(a) 16 May, 10.15–13.15 hours	20
			(b) 22 May, 12.48–15.48 hours	16
RF	Large	64	(a) 22 May, 08.30–11.30 hours	35
			(b) 18 May, 13.32–16.32 hours	37
MB	Small	18	(a) 22 May, 08.34–11.34 hours	8
			(b) 29 May, 12.00–15.00 hours	9
HI	Large	63	(a) 30 May, 09.00–12.00 hours	17
			(b) 21 May, 13.12–16.12 hours	26

†Data from Tomimatsu & Ohara (2002), in which more detailed information is also available.

contain as many flowers as possible, as long as the observer could glance over them all at once. Any insects contacting anthers or stigmas were recorded. Where possible, the insects were identified in the field to minimize the sampling effects on the remaining pollinator fauna, otherwise they were collected and identified later in our laboratory. In each population, the observations were conducted twice at a different time and day to explore a temporal variation of flower visitation. During the study period, the time of sunrise was between 03:52 and 04:22 hours, solar noon was between 11:23 and 11:24 hours, and sunset was between 18:27 and 18:58 hours.

The order composition of flower-visiting insects was compared among populations by randomization test of independence of the contingency table using  $\chi^2$  statistic (Roff & Bentzen 1989). A conventional  $\chi^2$  test was not used because some expected frequencies were <5 (Zar 1999). The insect-visitation rate was determined as the number of visits/flower per h. In this analysis, we combined the data for all insect species, because the number of observed visits for each insect order or species was limited only a few (<5).

## Results

Most of the floral visitors came from two orders of insect, Coleoptera (60.0%) and Diptera (27.0%), both of which were observed in all five populations (Tables 2 and 3). The members of Hymenoptera (8.7%), Hemiptera (2.6%) and Lepidoptera (1.7%) were observed in low frequencies. At the family level, the members of Nitidulidae (Coleoptera) were the most common visitors (Table 2). During the course of this study, we observed *Carpophilus chalybeus* and *Epuraea japonica* (Nitidulidae: Coleoptera) visiting flowers one after another and walking around anthers and stigmas, their bodies being fully covered with plenty

of pollen grains. The members of Melandryidae, Oedeemeridae (Coleoptera), Scathophagidae and Bibionidae (Diptera) were also observed in relatively high frequencies (Table 2). As with the Nitidulidae species, *Oedemerina concolor* (Oedeemeridae) visited flowers successively. Some of these species were observed visiting other co-flowering species, *Anemone flaccida* and *Glaucidium palmatum*.

Among the five populations examined, the order composition of insects exhibited a significant difference ( $\chi^2 = 69.4$ ,  $P < 0.001$ ). In the large HI population, flowers were visited by the most diverse insects which consisted of 16 species and four orders (Table 2). In small populations (SS, KK and MB), Coleoptera species were the most frequent visitors, whereas Diptera species were more abundant in large populations (RF and HI; Tables 2 and 3). Hemiptera and Lepidoptera were rare and only observed in the HI and KK populations, respectively.

The insect visitation rates were generally low (Table 3). Flowers were visited 0.28 times/flower per h on average for all populations. In the smallest SS population, flowers received the highest number of pollinator visits (0.73 times/flower/h) and most of them (76%) were contributed by the members of Nitidulidae (Table 2). In the large RF and HI populations, the flowers received only 0.10 and 0.21 times/flower per h on average, respectively.

## Discussion

Flowers were predominantly visited by Coleoptera and Diptera (Table 3). In particular, the members of Nitidulidae (Coleoptera) were the most common visitors (Table 2). These results were consistent with Fukuda (1961) and Ohara *et al.* (1991), who previously described floral visitors of *T. camschatcense* at different localities in Hokkaido. Because of their foraging behavior and relatively high visitation rates, Nitidulidae species were putative effective pollinators. Other anthophilous beetles (e.g.

**Table 2** List of floral visitors of *Trillium camschatcense* in five populations in 1999. The number of each insect species visiting flowers during a total of 6 h of observations is indicated for each population

Order	Family	Species	Population					Total
			SS	KK	RF	MB	HI	
Coleoptera	Byturidae	<i>Byturus affinis</i> Reitter	1	–	–	–	–	1
	Chrysomelidae	<i>Basilepta balyi</i> (Harold)	–	–	–	–	1	1
	Melandryidae	<i>Anisoxya conicicollis</i> Champion	3	–	–	3	1	7
		<i>Microtonus</i> sp.	1	–	–	–	–	1
	Nitidulidae	<i>Carpophilus chalybeus</i> Murray	31	–	–	–	–	31
		<i>Eपुरaea japonica</i> (Motschulsky)	1	4	2	1	4	12
		<i>Eपुरaea mandibularis</i> Reitter	–	3	1	–	–	4
		<i>Haptoncurina paulula</i> (Reitter)	–	4	–	–	–	4
	Oedemeridae	<i>Oedemerina concolor</i> (Lewis)	1	–	–	5	–	6
	Staphylinidae	<i>Philonthus cunctator</i> Sharp	–	–	–	–	1	1
<i>Philonthus nakanei</i> Sawada		–	–	–	–	1	1	
Diptera	Agromyzidae	(Unidentified)	–	–	–	–	1	1
	Anthomyiidae	<i>Acrostilpna collini</i> (Ringdahl)	–	1	–	–	–	1
	Bibionidae	<i>Bibio aneuretus</i> Hardy et Takahashi	–	–	–	–	1	1
		<i>Bibio pseudoclavipes</i> Okada	–	1	–	–	–	1
		<i>Dilophus aquilonia</i> Hardy et Takahashi	–	–	8	–	2	10
	Calliphoridae	<i>Lucilia caesar</i> Linnaeus	–	–	–	–	2	2
	Ceratopogonidae	<i>Culicoides</i> sp.	–	–	2	–	–	2
	Muscidae	<i>Dichaetomyia japonica</i> Hori et Kurahashi	–	–	–	–	1	1
	Scathophagidae	<i>Scathophaga stercoraria</i> (Linnaeus)	1	2	1	1	4	9
	Syrphidae	<i>Cheilosia japonica</i> (Herve-Bazin)	–	–	–	1	–	1
<i>Metasyrphus corollae</i> (Fabricius)		1	–	–	–	1	2	
Hemiptera	Iassidae	<i>Batracomorphus mundus</i> (Matsumura)	–	–	–	–	2	2
	Lygaeidae	<i>Nysius expressus</i> Distant	–	–	–	–	1	1
Hymenoptera	Andrenidae	<i>Andrena pruniphora</i> Hirashima	–	–	–	–	1	1
	Braconidae	<i>Meteorus camptolomae</i> Watanabe	1	–	–	–	–	1
	Formicidae	<i>Lasius alienus</i> (Foerster)	–	–	1	–	–	1
		<i>Pheidole feroidea</i> F. Smith	1	–	4	1	–	6
	Ichneumonidae	(Unidentified)	–	–	–	–	1	1
Lepidoptera	Lycaenidae	<i>Celastrina argiolus ladonides</i> (de l'Orza)	–	1	–	–	–	1
	Pieridae	<i>Leptidea amurensis</i> (Ménétrières)	–	1	–	–	–	1

(–), no data

**Table 3** Comparison of floral visitors of *Trillium camschatcense* among the five populations. The number of insects visiting flowers, their visitation rates (flower<sup>-1</sup>.hour<sup>-1</sup>), and their relation to stigmatic pollen load (the number of outcross pollen grains received per flower) are shown. In each population, we conducted 3-h observations twice (a and b; cf. Table 1)

Order	SS		KK		RF		MB		HI		Total
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	
Coleoptera	13	25	6	5	1	2	5	4	4	4	69
Diptera	1	1	2	1	4	8	2	–	4	8	31
Hemiptera	–	–	–	–	–	–	–	–	3	–	3
Hymenoptera	2	–	–	–	1	4	–	1	1	1	10
Lepidoptera	–	–	1	1	–	–	–	–	–	–	2
Total no. insects	16	26	9	7	6	14	7	5	12	13	115
Visitation rate	0.67	0.79	0.15	0.15	0.06	0.13	0.29	0.19	0.24	0.17	
Mean	0.73		0.15		0.10		0.24		0.21		0.28
Stigmatic pollen load†	– ‡		50.6		23.8		26.0		209.1		

–: No observation.

† Data from Tomimatsu & Ohara (2002). The number of pollen grains deposited on the stigmas of emasculated flowers were counted.

‡ Data are not available because the samples contained many other species' pollen grains (see Tomimatsu & Ohara 2002 for details).

Melandryidae and Oedemeridae) or flies (e.g. Scathophagidae and Bibionidae) could also pollinate flowers. The three large white petals, which also have a sweet scent, may attract these insect species. Although these species may feed on a variety of substrates including decaying fruits and fungi (Gazit *et al.* 1982), some of these insects would visit flowers of *T. camschatcense* for feeding on pollen or breeding within flowers. Because *T. camschatcense* does not produce nectar, flowers do not attract Hymenoptera and Lepidoptera.

Evidence showed that the Coleoptera species were the predominant insect visitors throughout the populations examined, although Diptera was more abundant in large populations (Table 3). This may reflect the habitat preferences of major visitors, such as Nitidulidae and Bibionidae. In small forest fragments, microclimatic conditions such as temperature and humidity are often altered (Kapos 1989; Didham & Lawton 1999). These abiotic changes (probably edge effects) may have differential effects on beetles and flies, changing the species composition of invertebrate communities.

Because knowledge of habitat preferences of floral visitors observed is, however, generally lacking, it is hard to discuss the direct causes of loss of Diptera species from small populations. The effects of forest fragmentation on insect populations are still poorly understood (Didham *et al.* 1996; Kearns *et al.* 1998). Among few studies, Didham *et al.* (1998), who studied the effects of forest fragmentation on beetles in Central Amazonia, found that species composition significantly changed with distance from forest edge and fragment area. Thus, different species may respond differently to fragmentation; some species may be edge or small-fragment specialists and some may not.

In spite of the significant difference among populations, the species composition of floral visitors was not related to the stigmatic pollen load, which was measured in the same populations and in the same year (Tomimatsu & Ohara 2002). In the large HI and RF populations, where Diptera species were more abundant, the stigmatic pollen load greatly differed between populations (Table 3). The visitation rates did not seem to be related to the stigmatic pollen load as well. In the large HI population, flowers received many pollen grains on stigmas; however, the flowers received only 0.21 times/flower per h on average (Table 3). The flowers in the MB population received insect visits as frequent as those in the HI population, but received fewer pollens.

Some studies have also shown the effects of fragmentation both on pollinator visitation and plant reproductive success (Jennersten 1988; Aizen & Feinsinger 1994a, 1994b). For example, Jennersten (1988) demonstrated that flowers of a butterfly-pollinated perennial herb, *Dianthus deltoideus*, in a fragmented habitat received fewer insect vis-

its and produced much lower seeds than in an undisturbed habitat. In our studies, the results gave no direct evidence of pollinator declines, although seed production of *T. camschatcense* was reduced in fragmented populations due to the paucity of outcross pollens (Tomimatsu & Ohara 2002). There are some possible explanations for this discrepancy. First, floral visitors are not always evenly effective pollinators (e.g. Fishbein & Venable 1996). Thus, the pollen load may be related to visitation rates of a particular species that effectively pollinates flowers. However, the pollen load was not simply related to the number of visits of Nitidulidae species, that is, the most common visitors (Table 2). Second, the abundance and visitation rates of pollinator species commonly fluctuate temporally and spatially (Herrera 1988; Kearns & Inouye 1994; Kearns 2001). If the variability was too large, our study may not give accurate estimates.

In summary, the pollinator composition of *T. camschatcense* differed among populations, and some common flies were not observed in small populations. Such changes of pollinator assemblages may have complex effects on plant reproductive success. Further studies are required to test this result and to clarify its consequences for plant-pollinator interactions in a fragmented landscape.

## Acknowledgements

We thank K. Okazaki for identifying flower visitors. This work was supported in part by the Sasakawa Scientific Research Grant from the Japan Science Society, Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology for 21st Century COE programs, and from the Japan Society for the Promotion of Science (JSPS) for Scientific Research (C) (13640637) and Research Fellowships of the JSPS for Young Scientists.

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