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Commercialized European bumblebee can cause pollination disturbance: An experiment on seven native plant species in Japan

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ABSTRACT

Non-native pollinator species are now widely utilized to facilitate pollination of agricultural crops. Evaluation of the ecological risk of alien pollinators is necessary because they could have a large impact on native ecosystems through disturbing native plant–pollinator interactions. We conducted a greenhouse experiment to examine the impact of the non-native commercialized European bumblebees, *Bombus terrestris*, on the pollination success of seven Japanese bumblebee-pollinated plant species. Plants were exposed to three groups of bumblebees: native bumblebee(s) only (NATIVE treatment); the alien bee only (ALIEN) and a mix of the two (MIX). ALIEN treatment had negative effects on fruitset and/or fruit quality of five plants, including self-incompatible and compatible, herb and woody, and queen- and worker-pollinated species. The negative effects were caused by a decrease in legitimate flower visitation due to (1) physical inaccessibility to nectary in deep-corolla flowers by the alien bee with insufficient tongue length and, (2) biased flower preference between short-corolla flowers. Fruitset tended to decrease drastically for the self-incompatible species while fruit quality decreased moderately for the self-compatible species. Effects of MIX were not intermediate between NATIVE and ALIEN in most plant species, and caused pollination success to vary in an unpredictable manner amongst plant species, probably due to interaction between native and alien bees. This non-linear relationship between plants' pollination success and the relative density of the alien suggests that the alien bee can disturb pollination of a plant species even when only representing a small fraction of the total pollinator community.

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1. Introduction

Species invasion is one of the major factors in rapidly decreasing biodiversity. Ecological impacts caused by invasive species have so far been considered and verified mainly

through predation, competition and genetic introgression against the native species. However, the impact on species in different guilds or trophic levels through biological interactions and the cascade effect have to be focused on. Invasive pollinators possibly have a large effect on ecosystem because

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they affect native pollinators as well as native plants which rely on native pollinators for pollination. There have been many observational and experimental studies on the negative impact on native pollinators by two major worldwide alien pollinators, the honeybee (*Apis mellifera*; Kato et al., 1999; Roubik, 1978; Thomson, 2004) and the commercialized European bumblebee (*Bombus terrestris*; Dafni and Shmida, 1996; Hingston and McQuillan, 1999). However, there have been no studies of the impact of *B. terrestris* invasion on native plants (Goulson, 2003), although it has been shown to forage native plants (Hingston, 2005; Matsumura et al., 2004). It sometimes exploits floral rewards by a “nectar-robbing” habit, using its powerful mandibles to bite through the base of the corolla and thus has the potential to disturb the pollination of native plants (Goulson, 2003). Several studies have investigated *A. mellifera* and its effect on native plants, but the results vary depending on the plant species or environmental conditions. Some plant species appeared to be inefficiently pollinated by non-native honeybees (Gross and Mackay, 1998; Wilson and Thomson, 1991), while other plant species are pollinated as efficiently as they are by the native pollinators in terms of fruit or seed production (Freitas and Paxton, 1998; Roubik, 2002) or of pollen dispersal (Dick, 2001). Overall, the commonness of plants which are vulnerable to alien pollinators and the type of that plants have been scarcely understood.

To predict the impact of alien pollinators, the level of specialization by plants to native pollinators should be understood in an ecosystem which is subject to the invasion. In general, the relationship between pollinators and plants ranges from one-to-one to many-to-many, but the majority of plants utilize a certain range of plural pollinators (Johnson and Steiner, 2000; Waser et al., 1996). Plants specialized to particular pollinator(s) can be very inefficiently pollinated by an alien pollinator compared to those plants generalized to a wide range of pollinators. Although direct comparison of pollination efficiency amongst possible pollinators is a useful way to study such specialization (Kakutani et al., 1993; Sakai et al., 1999; Schemske and Horvitz, 1984), there has been a limited amount of work in this area.

B. terrestris L. has been exported from Europe to various countries to enhance crop pollination and is reported to be established in New Zealand (Donovan and Wier, 1978), Tasmania (Semmens et al., 1993), and Israel (Dafni and Shmida, 1996). It was first imported to Japan in 1991, followed by observations of wild colony establishment (Matsumura et al., 2004).

Since the first commercial import, Japanese ecologists have been concerned over its potential impact on native ecosystems (Kato, 1993).

We posed two questions regarding ecological impact of *B. terrestris*; whether it reduces the abundance of native bumblebees, and whether it reduces the pollination success of native plants. This study focused on the latter for seven bumblebee-pollinated plant species in Hokkaido, Japan, where a rich bumblebee and bumblebee-pollinated plant biota exists, by manipulating native and alien (non-native) pollinator composition in experimental greenhouses. In particular, we observed the visiting behavior of native and alien bees and the fruiting process of plants in order to determine (1) if any of the native plants cannot be sufficiently pollinated by *B. terrestris*, and if not, (2) by what mechanism does the unsuccessful pollination by *B. terrestris* happen and (3) how different proportions of *B. terrestris* affect the pollination success.

2. Material and methods

2.1. Site and the framework of the experiment

Three identical orchard greenhouses (18 × 7 m with 4.5 m height, Watanabe Pipes Inc., Tomakomai, Japan), constructed in the same direction and neighboring each other by 2 m distance, were used to enclose the study plants and bees in Tomakomai Experimental Forest, Hokkaido University (N42°40', E141°36', altitude 16 m) in Hokkaido Island in Japan. The site is dominated by cool temperate deciduous broad-leaved forest. Tomakomai Experimental Forest is uniformly covered with volcanic ash soil and, two years before the experiment, approximately 10 cm depth of black mold were overlaid where the greenhouses would be constructed. The greenhouses were located on an open field and not shaded throughout the day. The differences in daily average temperatures among greenhouses over the experimental period were 0.2 ± 0.1 °C (mean ± SD). We removed all weeds with flowers from the floors. To prevent bees from escaping, all greenhouses had double doors.

One or two simultaneously flowering plant species were introduced into enclosures for each experiment and the experiment was repeated for seven plant species of which pollination success was measured (Tables 1 and 2, detailed in Section 2.2). Three enclosures were used for different treatments, in which bee composition differed (Table 3, detailed in

Table 1 – Study plants

Scientific name	Family	Life form	Experimental period	Pollinator	Petal colour	Corolla tube width (mm)	Corolla tube length (mm)
<i>Corydalis ambigua</i> Cham. and Schlecht.	Papaveraceae	Perennial	11–16 May	Queen	Blue	3–5	15–19
<i>Primula japonica</i> A. Gray	Primulaceae	Perennial	18–19 June	Queen	Pink	3–4	20–25
<i>Polygonatum odoratum</i> Druce	Liliaceae	Perennial	18–19 June	Queen	White	6–8	22–27
<i>Cirsium kamtschaticum</i> Ledeb. ex DC.	Compositae	Perennial	16 July–7 August	Worker	Purple	2–3	7–10
<i>Ligustrum obtusifolium</i> Siebold and Zucc.	Oleaceae	Shrub	28 July–7 August	Worker	White	4–5	6–8
<i>Adenophora triphylla</i> A. DC.	Campanulaceae	Perennial	21–28 August	Worker	Purple	8–11	8–11
<i>Hosta sieboldii</i> (Paxton) J. Ingram var. <i>rectifolia</i> (Nakai) H. Hara	Liliaceae	Perennial	21–28 August	Worker	Purple	19–23	32–40

Plant species are ordered according to their dates of experiment.

Table 2 – Number of plants used for each experimental treatment

Species	Replication No.	Treatment	Number of plants			
			Introduced ^a		Fruitset measured ^b	
			Ramet	Flower	Ramet	Flower
<i>Co. ambigua</i>	1–3	Native	60	389	47	268
	1–3	Mix	58	382	44	295
	1–3	Alien	55	410	36	262
		Bag	–	–	65	335
<i>Pr. japonica</i>	1	Native	26	144	17	98
	1	Mix	24	127	19	122
	1	Alien	21	130	13	81
		Bag	–	–	44	466
<i>Po. odoratum</i>	1	Native	39	178	38	177
	1	Mix	36	124	36	124
	1	Alien	23	96	22	91
		Bag	–	–	188	1087
<i>Ci. kamschaticum</i>	1	Native	33	45	11	20
	1	Alien	29	55	16	24
	2	Native	10	13	7	9
	2	Mix	12	12	9	10
	2	Alien	12	14	10	15
	3	Native	5	7	2	2
	3	Mix	8	9	6	8
	3	Alien	7	7	3	4
		Bag	–	–	22	31
<i>L. obtusifolium</i>	1	Native	3	27,070 (1237)	3	746 (47)
	1	Mix	3	25,975 (1673)	3	899 (50)
	1	Alien	3	2322 (609)	3	482 (42)
	2	Native	3	1883 (695)	3	702 (48)
	2	Mix	3	6641 (610)	3	587 (56)
	2	Alien	3	16,382 (1412)	3	734 (42)
	3	Native	3	9332 (302)	3	604 (71)
	3	Mix	3	5430 (450)	3	670 (63)
	3	Alien	3	9989 (189)	3	795 (79)
		Bag	–	–	3	225 (20)
	<i>A. triphylla</i>	1	Native	53	398	23
1		Alien	47	268	20	144
2		Native	27	124	9	60
2		Alien	16	96	8	58
		Bag	–	–	44	388
<i>H. sieboldii</i>	1	Native	10	41	10	83
	1	Alien	11	38	6	71
	2	Native	6	9	5	26
	2	Alien	5	8	4	26
		Bag	–	–	31	573

a Numbers introduced to a enclosure. Number of branches (in parenthesis) were counted for *L. obtusifolium*, and total number of flowers were estimated using average number of flowers on branches used for fruitset measurement.

b Numbers used for fruitset measurement. Parenthesis indicate branch numbers. The number exceeds introduced flowers in *H. sieboldii* as it includes flowers which newly opened within a replication.

Table 3 – Number of bees, and plant species used for each bee release and treatment

Date	Plant (replication No.)	Treatment	Number of bumblebees ^a					
			Queen (colony)				Worker	
			A	D	H	T	H	T
11–12 May	<i>Co. ambigua</i> (1)	Native	6		6			
		Mix	4		4	4		
		Alien				12		
13–14 May	<i>Co. ambigua</i> (2)	Native	6		6			
		Mix	4		4	4		
		Alien				12		
15–16 May	<i>Co. ambigua</i> (3)	Native	6		6			
		Mix	4		4	4		
		Alien				12		
18–19 June	<i>Pr. japonica</i> (1), <i>Po. odoratum</i> (1)	Native		6	6			
		Mix		4	4	4		
		Alien				12		
16–17 July	<i>Ci. kamschaticum</i> (1)	Native			(4)		35	
		Alien				(4)	38	
28–29 July	<i>Ci. kamschaticum</i> (2), <i>L. obtusifolium</i> (1)	Native			(4)		34	
		Mix			(2)	(2)	17	
		Alien				(4)	34	
1–2 August	<i>L. obtusifolium</i> (2)	Native			(4)		24	
		Mix			(2)	(2)	12	
		Alien				(4)	24	
6–7 August	<i>Ci. kamschaticum</i> (3), <i>L. obtusifolium</i> (3)	Native			(4)		23	
		Mix			(2)	(2)	11	
		Alien				(4)	22	
21–23 August	<i>A. triphylla</i> (1), <i>H. sieboldii</i> (1)	Native			(4)		17	
		Alien				(4)	17	
26–28 August	<i>A. triphylla</i> (2), <i>H. sieboldii</i> (2)	Native			(4)		17	
		Alien				(4)	17	

a Abbreviation of bee species is; A: *B. ardens*, D: *B. diversus*, H: *B. hypocrita*, and T: *B. terrestris*.

Section 2.3). To cancel the effect of individual enclosure, we replicated the experiment and rotated treatments over enclosures over the course of the experiment as long as flowering period was long enough (Table 3, detailed in Section 2.4).

2.2. Plants

We used seven bumblebee-pollinated plants from six families (Table 1), all of which are native to the floors or edges of the forests around the study site. We transplanted all herbal plants from their natural habitat to plastic containers (50–60 cm long, 20–30 cm wide, and 20 cm deep), and when they flowered, containers of one or two species were introduced into the enclosure and arranged 0.5 m apart from each other. One shrub species was obtained from a commercial nursery (Ohji Gardenings Inc., Sapporo, Japan) and planted on the floor of the enclosures. Number of ramets and flowers introduced varied over species and replications (Table 2) but almost the same among treatments of the same replication. Some of introduced flowers were used for the measurement of fruitset and fruit quality (detailed in Section 2.5). *Corydalis ambigua* were kept below black nylon mesh throughout the experimental period to be protected from direct sunlight.

2.3. Treatments and bumblebees

Each enclosure was assigned to either NATIVE, MIX, or ALIEN treatment (Table 3). NATIVE treatment included one or two native bee(s) species. *Bombus hypocrita sapporoensis* Cockerell, the most common species around the study site, was used for all plant species. For plants that have another major flower visitor in the field, we used the bumblebee species *Bombus ardens sakagami* Tkalcu or *Bombus diversus tersatus* Smith with *B. hypocrita*. These three native bumblebee species are the commonest around the study site (Inari et al., 2005; Nakajima et al., 2004). MIX included *B. terrestris* added to the same native pollinator(s) of NATIVE to simulate the initial phase of invasion. ALIEN included only *B. terrestris* to simulate a supposed final phase of invasion. MIX treatment was not applied for *Adenophora triphylla* or *Hosta sieboldii* or the first replication of *Cirsium kamschaticum* due to insufficient *B. hypocrita* colony. The total number of bees was the same or almost the same amongst treatments and amongst different bee species in a treatment (Table 3). In this design, we simulated reduction or exclusion of native bees by the invasion of *B. terrestris*. This is a reasonable situation to study as Inari et al. (2005) reported that the dominance of *B. terrestris* has achieved 70–90% of

total bumblebees around agricultural area near our experimental site.

Early flowering plants are foraged by queens whilst late ones are foraged by workers (Table 1) therefore we used queens and colonies accordingly. Active seasons of used native bee in close fields is this order (Inari et al., 2005; Matsumura et al., 2004); *B. ardens* (queen: late April–mid/late June), *B. hypocrita* (queen: early May–late June; worker: early June–late August), *B. diversus* (early May to late June), and these periods are fully covered by the active period of *B. terrestris* (queen: late April to late June; worker: late June to early September). Active seasons are substantially overlapped among used bee species for each plant.

Queens of the native species were collected around the study site. To establish colonies of *B. hypocrita*, we fed queens separately on pollen (Api, Gifu, Japan) and 50% sugar solution separately in a small wooden box to allow them to found a colony. After the colony was established we moved each nest to the plastic nest box (Api, Gifu, Japan) used for the commercial *B. terrestris* colonies. Queens and colonies of *B. terrestris* were obtained from Api (Gifu, Japan). We removed some of worker bees just before each experiment if necessary to equalize the number of workers among treatments.

Bumblebees' tongue length is known to be correlated with their ability to forage deep-corolla flower (Inouye, 1980). The total length of prementum and glossa (mean \pm SD) of queens was 8.78 ± 0.24 , 9.76 ± 0.39 , and 14.40 ± 0.20 mm for *B. hypocrita*, *B. ardens sakagamii* and *B. diversus*, compared to 9.59 ± 0.26 mm for commercialized *B. terrestris* (T. Nagamitsu unpublished; these values represent species average obtained in a different site).

2.4. Bee release and replication

The experiments were designed around the order and date that each collected plant species began to flower, and hence were performed from May to August in 2003. The experiments were replicated up to three times where the flowering periods were long enough and treatments were rotated over the enclosures. New queens or colonies were used whenever possible, otherwise experienced bees were swapped over the treatments, for every replication. When we used experienced bees, a proportion of new and experienced bees was equalized among treatments. The only exception was *Co. ambigua*, where new queens were used for every replication but the same enclosure was used for the same treatment. We released queens or regularly spaced colonies by a long side wall of the enclosures on the morning of the first day of a replication. Bees were then collected in the evening of the second day. However, bees for *A. triphylla* and *H. sieboldii* were collected on the third day. Longer duration for them is because we used relatively few numbers of workers for them and flowering period was long enough to allow longer duration.

Plants stayed in the same enclosure throughout a replication. If a flower stayed open over two replications, it was bagged with nylon mesh at one of replications to allow a flower exposed only for one replication. For *L. obtusifolium*, we divided each tree into three parts, each of which was bagged prior to experiments, and we opened one for each replication.

2.5. Plant pollination success

We observed flower visitation by bees for all introduced ramets and recorded bee species, number of flower visited and types of visitation (legitimate or nectar-robbing). Legitimate flower visitation refers to bees' trying to suck nectar or collect pollen from the front of a flower. Nectar-robbing refers to foraging nectar via a hole made by the same or another bumblebee at the base of the corolla. Infrequently, bees quickly touched a part of a flower without any foraging behavior. This type of visitation was included in the legitimate visitation category as whilst they were clearly different from exploitation, we could not exclude the possibility of occasional pollination by that quick motion. We repeated 10–30 min observations between 9 AM and 6 PM, summing up 120–150 min for *Co. ambigua*, *Primura japonica*, *Polygonatum odoratum* and *Ci. kamschaticum*, and 540–590 min for *A. triphylla* and *H. sieboldii*. Every single tree was observed separately for 40–70 min for *L. obtusifolium* in each replication. Observation time was standardized when analyzing (detailed in Section 2.6).

We monitored flower maturation and measured fruitset. To examine the ability of autogamy, some flowers were bagged by nylon mesh and not exposed to bees ("BAG" treatment hereafter). The criterion for "mature" fruit was the existence of sound (i.e. fully shaped, swollen and filled and developed to the saturated mature size) seed(s). This criterion was directly applied to *Co. ambigua*, *Ci. kamschaticum*, *Po. odoratum*, *L. obtusifolium* and *H. sieboldii*. For *Pr. japonica* and *A. triphylla*, maturity was regarded as fruit with holes on the ovary wall base or on the top of ovary wall, respectively.

Plant reproductive success is generally determined not only by the fruitset but also fruit quality, such as number of seeds, seeds mass and fresh mass to attract a seed disperser. We chose one of variables, for each species, which represent seed number or seed mass and varied among fruits. These were seed number for *Co. ambigua* and *Ci. kamschaticum*; fruit diameter for *Pr. japonica* (linear regression to seed mass: $R^2 = 0.59$, $P < 0.001$) and *A. triphylla*; fruit mass for *Po. odoratum* (linear regression to seed mass: $R^2 = 0.65$, $P < 0.001$) and mass of fruit hull for *H. sieboldii* (linear regression to seed number: $R^2 = 0.65$, $P < 0.001$). Each flower of *Ci. kamschaticum* were enclosed by a plastic zipper bag to trap wind-dispersed seeds. We did not measure any fruit quality for *L. obtusifolium* because all of the fruit has one seed per fruit in the species and the fruit size appeared not to vary.

2.6. Data analysis

We examined the effect of treatments on (1) existence of mature fruit (i.e. fruitset) for an individual flower, (2) fruit quality of an individual mature fruit, (3) number of legitimately visited flowers on a ramet and (4) number of nectar-robbled flowers on a ramet, using model selection (Johnson and Omland, 2004) with generalized linear mixed models (GLMMs). GLMMs can treat our "replication" and/or individual difference among plants as random effects. GLMMs also provide a solution to overdispersion (Allen and Weale, 2005), which was ob-

served for our fruitset and flower visit data when they were fitted to binomial or Poisson distribution. Parameters of the models were estimated based on the maximum likelihood method using the statistical software R 2.1.0 (R Development Core Team, 2005). lmer function (Bates and Sarkar, 2005) was used for fruit quality and glmmML function (Broström, 2005) for fruitset, legitimate flower visitation and nectar-robbing. Fruitset and fruit quality of BAG were only compared to NATIVE to examine the ability of autogamy.

The four dependent variables were fitted to a linear predictor:

$$\mu \sim \beta_0 + \beta_1 T + R + \text{offset}(O) \quad (1)$$

where μ was either of four dependent values for each plant, β_0 was an intercept; β_1 was an estimator of coefficient of T; T was the categorical variable of treatments; R was a random effect of combination of a replication and a ramet; O was log observation time for each plant, which was offset from the linear predictor and applied only for the two types of flower visitation. The link function of the linear predictor μ was $\log \mu$. Error distribution of μ was binomial, Gaussian and Poisson distribution for fruitset, fruit quality and the two types of flower visitation, respectively. The random effect includes uncontrolled factors such as individual difference among bees, individual difference of plants, balance between bee number and plant number, and phenological stage of plants. To examine which treatment had an effect on which factor, we reconstructed all the possible groups of treatments combinations and β_1 was estimated for each group. Then fitness of the constructed models was evaluated based on Akaike's information criterion (AIC) and a model with the lowest AIC was selected. For example, three treatments of NATIVE (N), MIX (M), and ALIEN (A) can be regrouped following five ways; $N = M = A$, $(N = A) \neq M$, $(N = M) \neq A$, $N \neq (M = A)$, $N \neq M \neq A$. If AIC of the third model was the lowest, we regarded NATIVE and MIX to be the same as each other and different from ALIEN.

GLMMs could not be applied for nectar-robbing of *Pr. japonica* because two of three treatments had no nectar-robbing. We instead calculated the probability at which the observed data is derived from a single Poisson distribution whose average is set as the average number of nectar-robbing visits per plant in a couple of treatments. We repeated this procedure for the three possible pairs of treatments. We used Bonferroni-corrected P value (0.017) instead of 0.05.

Flower visitation stated above included those by both native and alien bees, and the bee composition was manipulated by us. To examine whether the alien bee invasion decreases flower visitation of native bees, we compared legitimate visitation by native bees between NATIVE and MIX by the basically similar structure of GLMM:

$$\mu \sim \beta_0 + \beta_1 T + R + \text{offset}(O) + \text{offset}(N), \quad (2)$$

where μ was number of legitimately visited flower on a ramet. Other variables and coefficients were the same as Eq. (1), except for N, which is log number of native queens or colonies in an enclosure. This new variable was set to compare per capita visitation for number of native bees varied among enclosures. The link function and the error distribution of μ were $\log \mu$ and Poisson. The AIC were compared between two models assuming $N = M$ and $N \neq M$. Replication 1 of *Ci.*

kamtschaticum was removed from the data set as it lacked MIX treatment.

3. Results

3.1. Overall pattern of flower visitation

Nectar-robbing was the commonest in *Co. ambigua* (Figs. 1 and 2), in which *B. hypocrita* and *B. terrestris* visited the flowers only by the nectar-robbing manner, while *B. ardens* exhibited only legitimate visiting. Nectar-robbing for *Co. ambigua* was ALIEN > NATIVE > MIX (Fig. 2; the difference was supported by the model selection, see Table 4). Flowers of *Pr. japonica* and *Po. odoratum* were legitimately visited by *B. diversus*. *Primula japonica* was not visited by *B. hypocrita* and only scarcely visited by *B. terrestris*, while *Po. odoratum* was visited infrequently by *B. hypocrita* and scarcely by *B. terrestris*. Visitation by *B. hypocrita* and *B. terrestris* to these two plants consisted of both legitimate and nectar-robbing. However, most of their legitimate visitations were unsuccessful attempts at nectar-robbing or just momentarily touching petals. Nectar-robbing for these two plants was not higher in ALIEN. The other four species (*Ci. kamtschaticum*, *L. obtusifolium*, *A. triphylla* and *H. sieboldii*), which were exposed to workers of *B. hypocrita* and *B. terrestris*, were legitimately visited by both bee species at considerable frequencies (Fig. 2).

The legitimate flower visitations in ALIEN was the lowest among treatments for *Co. ambigua* and *Po. odoratum* (Fig. 2, Table 4), and tended to be the lowest in *L. obtusifolium* (<10.3% of other treatments) although this difference was not supported by the model selection. In contrast, the legitimate flower visitation for *Ci. kamtschaticum* and *A. triphylla* was highest in ALIEN. Treatments did not affect the visitation for *H. sieboldii*. For *Po. odoratum*, the legitimate visitation in MIX was lower than NATIVE, while that in MIX was the highest for *Pr. japonica* and *L. obtusifolium*.

3.2. Per capita legitimate flower visitation by native bees

The model selection (Table 5) suggested that the legitimate flower visitation by native bees in MIX was higher than NATIVE in *Pr. japonica* and the same between the treatments in *Co. ambigua* and *L. obtusifolium*, although the difference between treatments was considerable in *L. obtusifolium*. However, the visitation in MIX was lower in *Po. odoratum* and *Ci. kamtschaticum*, although the difference was small in *Ci. kamtschaticum*.

3.3. Fruitset

The fruitset of *Co. ambigua* and *L. obtusifolium* was lower in ALIEN than others (Fig. 2; the differences were supported by the model selection, see Table 4). The difference in the observed average fruitset between NATIVE and ALIEN were 4.1 and 1.7 times for these species, respectively. The fruitset of *Po. odoratum* was 4.1–11.2 times lower in both MIX and ALIEN than NATIVE. The fruitset of *Pr. japonica* was 1.1–1.2 times higher in MIX than others. Fruitsets for *Ci. kamtschaticum*, *A. triphylla* and *H. sieboldii* did not differ among NATIVE, MIX and ALIEN, according to the selected model.

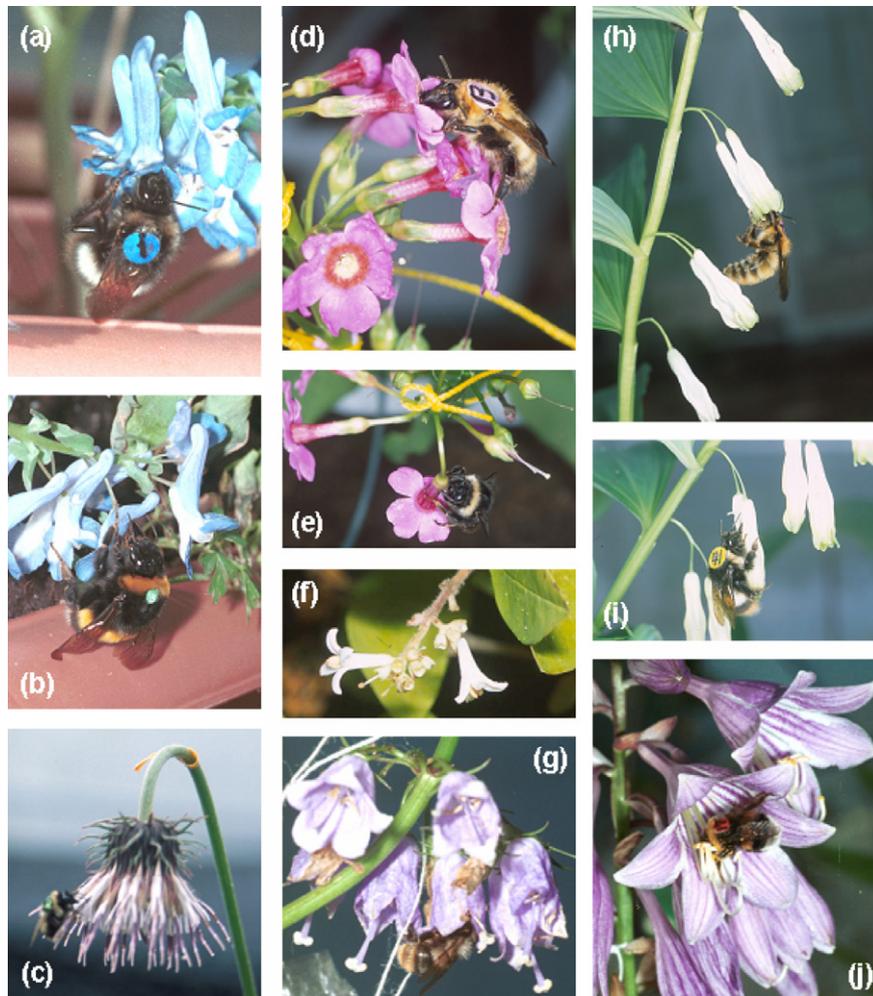


Fig. 1 – Flower shape for each plant species and visiting manners by used bumblebees. Flower of *Co. ambigua* visited by *B. ardens* legitimately (a), and nectar-robbled by *B. terrestris* (b). Flower of *Pr. japonica* visited by *B. diversus* legitimately (d), and nectar-robbled by *B. hypocrita* (e). Flower of *Po. odoratum* visited by *B. diversus* legitimately (h), and nectar-robbled by *B. hypocrita* (i). Flower(s) of *Ci. kamtschaticum* (c), *A. triphylla* (g), and *H. sieboldii* (j) legitimately visited by *B. hypocrita* (the former two) and by *B. terrestris* (the latter). Flowers of *Li. obtusifolium* (f), observed to be visited legitimately both by *B. hypocrita* and *B. terrestris*.

3.4. Fruit quality

The fruit diameter of *Pr. japonica* was only 1.04–1.08 times smaller in both MIX and ALIEN than NATIVE (Fig. 2) but the differences were supported by the model (Table 4). The fruit weight of *Po. odoratum* was also 2.6–7.4 times lower in both MIX and ALIEN than NATIVE. The fruit diameter of *A. triphylla* was 1.2 times smaller in ALIEN than NATIVE. In contrast, the weight of the fruit hull of *H. sieboldii* was 1.2 times greater in ALIEN than NATIVE but the difference in AIC was only 0.8. Treatments had no effects on fruit quality for *Co. ambigua* and *Ci. kamtschaticum*.

3.5. Self-compatibility

Compared to NATIVE treatment, fruitsets in BAG treatment were 3.0–11.0 times lower for *Co. ambigua*, *Po. odoratum* and *H. sieboldii*; zero for *L. obtusifolium*; and moderately (1.7 times) lower for *Ci. kamtschaticum*. The differences were supported by the model selection (Table 6). Fruitset of these two treat-

ments were comparable for *Pr. japonica* and *A. triphylla*, although the model selection suggested higher fruitset in BAG for *Pr. japonica*. Fruit quality of BAG was lower compared to NATIVE for *Co. ambigua*, *Pr. japonica*, *Ci. kamtschaticum*, while that of *Po. odoratum* and *H. sieboldii* was not different from, or higher than NATIVE, respectively (Table 6).

4. Discussion

4.1. Which plants were affected?

Five plant species reduced their fruitset and/or fruit quality where only the alien bee was available. This is the first study that clarifies by experiment the negative impacts of alien pollinators on pollination success for native plants. Our results indicate that the alien bee of the same genus is a poor substitute for native pollinators. In contrast to the recent common view that the vast majority of plants utilize a range of plural pollinators (Waser et al., 1996), we showed that bumblebee-pol-

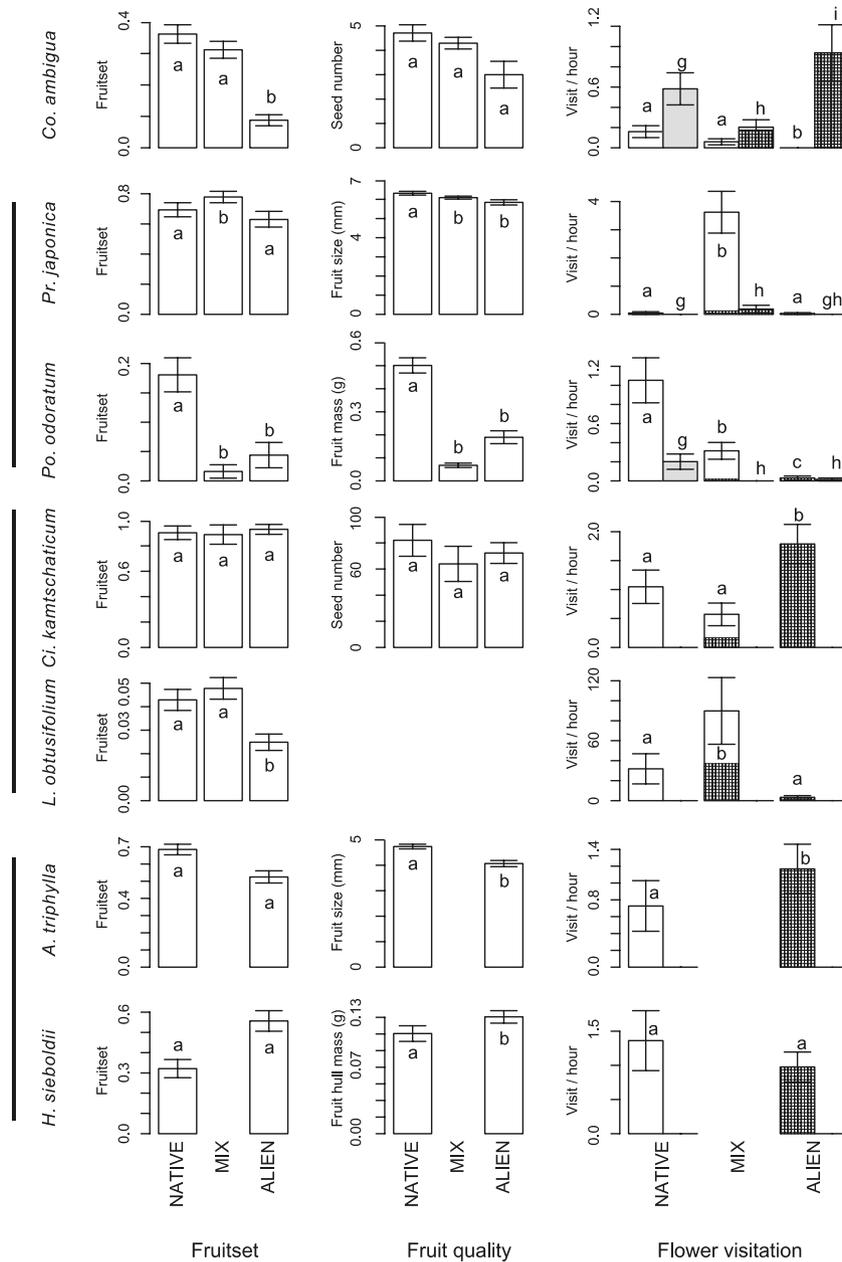


Fig. 2 – Average \pm SE of fruitset (the left column), fruit quality (the middle), and flower visitation frequency (the right) in each treatment for each plant species. A couple of plant sharing the same line on the left edge was used in the experiment together. Left (white) and right (gray) bars in each treatment in flower visitation graphs shows legitimate visitation and nectar-robbing, respectively. Open and hatched bars for flower visitation indicate visitation by native bees and the alien, respectively. Each bar of MIX treatments for flower visitation consist of bars of native bees and the alien with SE of the total value. Treatment(s) not sharing the same alphabet had different effect on each dependant values in the selected model. Legitimate visitation and nectar-robbing were compared separately among treatments. Only nectar-robbing in *Pr. japonica* was analyzed by calculating P value (see the text for detail).

linated plants are relatively specialized to native bumblebee species, implying that such partnerships are vulnerable to species invasion. The five plants that received the negative impact cover both herbal and woody, queen- and worker-pollinated, and self-incompatible and compatible species, implying a potentially wide impact of the alien bee on the plant community.

4.2. What is the mechanism of the negative impact?

Overall patterns in fruitset and/or fruit quality among treatments corresponded closely with that of legitimate visitation rate where legitimate visitation rate varied highly among treatments (i.e. in *Co. ambigua*, *Pr. japonica*, *Po. odoratum* and *L. obtusifolium*, Fig. 2). Legitimate visiting rate is therefore

Table 4 – AICs of evaluated models which incorporated different effects of treatments on each of three dependant values for each plant species

Plant species	Model ^a	Dependant values		
		Fruitset	Fruit quality	Legitimate visitation
<i>Co. ambigua</i>	N = M = A	622.1	660.8*	122.8
	(N = A) ≠ M	621.6	662.8	124.5
	(N = M) ≠ A	601.8*	661.9	116.1*
	N ≠ (M = A)	606.6	662.5	120.2
	N ≠ M ≠ A	606.1	663.8	116.4
<i>Pr. japonica</i>	N = M = A	359.1	560.0	188.8
	(N = A) ≠ M	358.1*	561.9	149.8*
	(N = M) ≠ A	359.3	557.7	179.7
	N ≠ (M = A)	360.9	557.4*	176.7
	N ≠ M ≠ A	359.7	557.6	151.7
<i>Po. odoratum</i>	N = M = A	230.6	-5.6	310.0
	(N = A) ≠ M	223.3	-8.6	309.1
	(N = M) ≠ A	230.2	-8.0	293.8
	N ≠ (M = A)	217.3*	-17.1*	295.5
	N ≠ M ≠ A	218.6	-15.7	286.8*
<i>Ci. kantschaticum</i>	N = M = A	57.5*	1048.0*	298.3
	(N = A) ≠ M	59.4	1049.3	298.9
	(N = M) ≠ A	59.4	1050.0	295.8*
	N ≠ (M = A)	59.2	1049.1	298.7
	N ≠ M ≠ A	61.2	1050.8	297.6
<i>L. obtusifolium</i>	N = M = A	1800.8	-	203.6
	(N = A) ≠ M	1797.2	-	202.2
	(N = M) ≠ A	1791.8*	-	200.6
	N ≠ (M = A)	1802.5	-	205.6
	N ≠ M ≠ A	1792.4	-	200.4*
<i>A. triphylla</i>	N = A	424.4*	713.8	372.1
	N ≠ A	426.4	708.5*	348.6*
<i>H. sieboldii</i>	N = A	224.5*	-255.9	218.2
	N ≠ A	226.4	-256.7*	217.7*

Model with the lowest AIC was selected and its AIC value is marked by “*”. Impact of the alien bee is the larger in a models in the lower row. a N: NATIVE, M: MIX, and A: ALIEN.

Table 5 – Comparison of flower visitation by native bees between NATIVE and MIX treatments

	Mean visitation (flower/h)		Mean per capita visitation (flower/h/bee)		AIC	
	NATIVE	MIX	NATIVE	MIX	N = M	N ≠ M
<i>Co. ambigua</i>	0.17	0.06	0.014	0.008	116.6*	118.1
<i>Pr. japonica</i>	0.05	3.45	0.004	0.432*	175.8	147.4*
<i>Po. odoratum</i>	1.05	0.31	0.088	0.039*	287.4	276.5*
<i>Ci. kantschaticum</i>	0.95	0.42	0.238	0.211*	92.4	91.2*
<i>L. obtusifolium</i>	31.83	57.79	7.958	28.89	184.4*	184.7

AICs of N = M and N ≠ M models for per capita visitation are showed and the lower one is marked with “*”. If N ≠ M model is selected, per capita visitation of MIX treatment is also marked by “*” and the value is in either bold or italic, which indicate decrease or increase compared to NATIVE, respectively.

considered as the primary factor determining the plants' reproductive success. All of these four plants received a negative effect in ALIEN. In contrast, where legitimate visitation rate did not vary highly among treatments (i.e. *Ci. kantschaticum*, *A. triphylla* and *H. sieboldii*) its correspondence to fruitset and/or fruit quality became unclear, and the effect of ALIEN fluctuated among negative, positive and no effect. Inconsis-

tent relationships between legitimate visitation rate and plants' reproductive success could be explained if there was a difference in pollination efficiency per visit between native and alien bees, but that is unclear in this study and further investigation will be required. Frequency of nectar-robbing itself did not correspond to reproductive success. So it is unlikely that reproductive organs were damaged during nectar-

Table 6 – Relative values of fruitset and fruit quality in ALIEN and BAG treatments compared to NATIVE

	BAG		ALIEN	
	Fruitset	Fruit quality	Fruitset	Fruit quality
<i>Co. ambigua</i>	0.09'	0.38'	0.24'	0.64
<i>Pr. japonica</i>	1.05'	0.92'	0.91	0.92'
<i>Po. odoratum</i>	0.17'	0.74	0.24'	0.38'
<i>Ci. kamtschaticum</i>	0.57'	0.42'	1.03	0.88
<i>L. obtusifolium</i>	0.00'	–	0.58'	–
<i>A. triphylla</i>	0.76	0.96	0.77	0.86'
<i>H. sieboldii</i>	0.33'	1.11'	1.73	1.17'

Values in NATIVE multiplied with the shown values makes values in BAG or ALIEN. Values for which decrease or increase from NATIVE were supported by the model selection are shown in bold or Italic, respectively, with "'". Indices of fruit quality for the seven species are seed number, fruit size, fruit mass, seed number, fruit size, and fruit hull mass, respectively and in the shown sequence. The cause to generate some higher values in BAG is unclear but might have been caused by the difference in average flowering date between NATIVE and BAG.

robbing (Inouye, 1983). Furthermore, frequency of nectar-robbing by *B. terrestris* was not high compared to native short-tongued *B. hypocrita*, which is consistent with their similar tongue length.

There are two mechanisms to cause the low legitimate visitation by *B. terrestris*. The first is physical inaccessibility to nectary by a deep corolla. *Bombus terrestris* was almost incapable of legitimate visits for plants with narrow and deep corolla such as *Co. ambigua*, *Pr. japonica* and *Po. odoratum*, because of their insufficient tongue length to suck nectar. It frequently nectar-robbed *Co. ambigua* using its powerful mandibles the way described by Inouye (1983) and Matsumura et al. (2004). In natural fields, *Co. ambigua* is nectar-robbed by *B. hypocrita* (Higashi et al., 1988) and legitimately visited by relatively long-tongued *B. ardens* or *B. hypnorum* (Kudo and Kasagi, 2004). Pollination efficiency is therefore higher by the latter (Kudo and Kasagi, 2004), which is consistent with the low pollination efficiency by *B. terrestris* in our experiment. *Bombus terrestris* made nearly no visitations to *Pr. japonica* and *Po. odoratum*, which have thicker petals than *Co. ambigua*, even when those plants were the only available resource. It is well known that bumblebees with different length tongue utilize floral resources with different depths of corolla (Inouye, 1980). In turn, plants with certain corolla depth are mainly pollinated by particular bumblebee species with corresponding tongue length (Inoue and Kato, 1992). Our result shows that such correspondence between bumblebees and plants is as tight as the degree in which the alien bumblebee does not forage uncorresponded flowers even when they are the only resource to survive in the absence of competitive native bumblebees.

The second mechanism to cause low legitimate visitation by *B. terrestris* is most probably floral preference. *Bombus terrestris* was capable of legitimate flower visitation for *L. obtusifolium* and showed considerable visitation in terms of absolute value (Fig. 2), but the frequency tended to be much lower compared to *B. hypocrita*. In contrast, *B. terrestris* showed a higher visitation rate to *Ci. kamtschaticum*, which was available together with *L. obtusifolium*. The sugar mass of nectar per flower during the experiment was higher in *Ci. kamtschaticum* (0.366 ± 0.431 mg) than *L. obtusifolium* (0.173 ± 0.170 mg) (Nagamitsu et al., in press) while the number of flowers and then total reward in a ramet is much higher in *L. obtusifolium*.

A relatively longer tongue of *B. terrestris* than *B. hypocrita* could explain its preference to *Ci. kamtschaticum* as both bee species need to thrust their head into the deep floret to reach the nectar, although other factors such as pollen amount and quality might be relevant.

A negative effect of ALIEN was clearest in the fruitset of *Co. ambigua*, *Po. odoratum* and *L. obtusifolium*, which had no or very low levels of fruitset when any pollinators were excluded (self-incompatible), while it was moderate in fruit quality for *Pr. japonica* and *A. triphylla* (self-compatible), which exhibited high level of autogamy (Table 6). In self-incompatible plants, unsuccessful cross-pollination caused by lack of appropriate native pollinator leads to fertilization failure of entire ovules in a flower, resulting in flower abortion. Insufficient cross-pollination can be compensated by selfing in the self-compatible plants to some extent, preventing flower abortion. However, inbreeding depression often operates even in self-compatible plants (Husband and Schemske, 1996). This decreases the proportion of fertilized ovule in a flower, leading slight decrease in fruit quality. It is not negligible that a decrease in outcrossing rate will cause loss of genetic diversity and change in spatial genetic structure as pointed out by Goulson (2003). Therefore, to regard the negative impact on these self-compatible plants as small, as the observed difference in fruit quality initially indicates, will lead to an underestimation for the impact on population maintenance or genetic diversity by the invasion.

4.3. How different proportions of *B. terrestris* affect plants?

Although the proportion of the alien pollinator is intermediate in MIX, the plants' reproductive success varied highly from as low as ALIEN (*Po. odoratum*) to a degree higher than NATIVE (*Pr. japonica*), depending on plant species. The legitimate flower visitations in MIX also tended not to be intermediate between NATIVE and ALIEN, suggesting interaction between native and alien bees, which is most probably responsible for the variable response of plants. Furthermore, when two plant species existed at the same experiment (that is, the combination of *Pr. japonica* & *Po. odoratum*, and *Ci. kamtschaticum* & *L. obtusifolium*), NATIVE and MIX treatments had opposing effect on the visitation rate between the plants. The

mechanism of this phenomenon is not clear but it implies that the invasion of the alien pollinator will change which plants receive sufficient visitations through the interaction in foraging behavior between native and alien bumblebees. Such a non-linear relationship between the proportion of alien pollinator and the plant pollination success suggests two points relevant to conservation policy. First, we cannot predict the consequences at the late phase of invasion by the extrapolation from the observation at the early stage when the proportion of alien pollinator is moderate. Second, even small levels of invasion by the alien pollinator can significantly disturb the pollination of a species.

4.4. The impact of the alien bee in natural fields

In this experiment, bee species composition was manipulated by the researchers, and the observed effect in ALIEN can be attributed to not only the presence of the alien pollinator but also just experimental removal of native bees. Therefore, the important question in predicting the consequence of the *B. terrestris* invasion in fields is whether or not it reduces visitation rate or population density of native bees, and it most likely does by two reasons.

First, per capita flower visitation by the native bees decreased under the presence of the alien bee for two plant species (*Po. odoratum*, *Ci. kamtschaticum*) in our experiment. In contrast, one plant species (*Pr. japonica*) had higher visitation and the pattern was not consistent. Nectar-robbing decreased visitation rate of the legitimate forager in other studies (Irwin and Brody, 1998, 1999). In this study, however, the response in visitation rates of the native bees under the presence of *B. terrestris* fluctuated regardless of whether the alien behave as nectar-robber. Such opposing effects of the invasion observed in this study can be caused by the interaction between native and alien bees as discussed above. Although it is hard to predict the consequence of this interaction for a given plant species, a certain proportion of plant species would receive poorer visitation by native bees or at least the plant community will be somewhat disturbed by the invasion of *B. terrestris*.

Second, *B. terrestris* possibly decreases population density of native bumblebees through competition for overlapped floral resources and nest site (Matsumura et al., 2004), and this prediction is consistent with recent data. Observed *B. terrestris* queens increased more than 100–150 times from 1997 to 2003 in a study area in Monbetsu, Hokkaido, Japan, which is 5–6 km apart from the nearest greenhouse complex, which is in Bira-tori (Matsumura et al., 2004; Matsumura and Washitani, 2002). In another site called Mukawa, which is approximately 12 km apart from the same greenhouse complex, native *B. hypocrita* suffered a 100-fold decrease in abundance (observations per hour per observer) from 2003 to 2005 while *B. terrestris* queens in early spring increased twice (M. Inoue et al. unpublished). Also, in some sites near our study site, *B. terrestris* highly dominates and contributes to 70–90% of trapped bumblebees (Inari et al., 2005).

We showed the wide impact of *B. terrestris* on pollination of various plants with different flower morphology and different mating systems through several mechanisms. Great attention should be paid to the conservation of a wide range of native

plants as well as native bees, while the commercial use of *B. terrestris* is rapidly increasing worldwide.

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