



Wind pollination and insect pollination of two temperate willow species, *Salix miyabeana* and *Salix sachalinensis*

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Abstract

Pollination systems of *Salix miyabeana* and *Salix sachalinensis* were studied at a riverside in northern Japan in order to measure the balance of wind pollination and insect pollination. In 1996, 19 clones of each species were selected, and seed-set success by a net-bagging (to exclude insect visitation) and an artificial pollination (to remove pollen limitation) were compared to by natural pollination. For *S. miyabeana*, the same experiment was repeated in two populations in 1997. Proportion of seed set through wind pollination dominated in both species in this study. Pollen limitation was common under natural conditions, and *S. sachalinensis* relied more on insect pollination for seed production than *S. miyabeana*. Meteorological factors such as precipitation and hours of sunshine during the flowering season influenced the potential reproductive activity of the willow between years. In the wet and cloudy spring of 1996, clones which obtained high seed set depended more on insect pollination for both species, whereas in the dry and sunny spring of 1997, such clones depended more on wind pollination for *S. miyabeana*. Because the efficiency of wind pollination seemed to be more sensitive to fluctuating weather conditions than insect pollination, insect pollination was considered to play an assurance role for seed production in these willows.

Introduction

Entomophily and anemophily are two main pollination systems in vascular plants. Entomophily is dominant in angiosperms, and it has evolved from anemophily, which is a common system in gymnosperms. Animal pollination depending on insects or birds is considered to be a more specialized and advanced system than anemophily because the foraging behavior of pollinators is often determined by floral characteristics of plants resulting in predictable pollen flow (Howe & Westley 1997). Anemophily could be effective when the density of compatible mates is high, filtration of aerial pollen by plant canopies is less, and activity of pollinators is low (Regal 1982; Whitehead 1983).

There are some reports indicating that willows have two pollination systems, anemophily and entomophily. Willows are often dominant on riversides, volcanoes, or alpine and subarctic regions.

Willow species are considered to have evolved from anemophilous ancestors and have secondarily reacquired the entomophilous system (Fisher 1928; Sacchi & Price 1988). Although many studies have demonstrated that entomophilous pollination dominated in northern willow species (Kevan 1972; Sacchi & Price 1988; Elmqvist et al. 1988; Douglas 1997), a few studies suggested that wind pollination plays a facultative role (Argus 1974; Vroege & Stelleman 1990; Fox 1992; Kevan et al. 1993). In spite of the wide distribution range in the Northern Hemisphere, the pollination system of willows has mainly been studied in arctic, subarctic, alpine and boreal regions. We need more information about temperate willows to evaluate widely the pollination systems in willows.

Why do willows need to maintain the two pollination systems? In some cases, a mixed-pollination system might allow plants to achieve stable seed production when unpredictable conditions or unstable

pollinator abundance causes variation in the pollination success of plants (Regal 1982; Paige & Whitman 1985; Gómez & Zamora 1996). If the quantity or quality of produced seeds varies depending on the pollination systems, parent fitness could be influenced directly by the balance of pollination modes fluctuating between sites or years (Berry & Calvo 1989; Gómez & Zamora 1996). Moreover, the importance of different pollination systems for reproductive success would change among species or populations growing in different conditions (Berry & Calvo 1989).

We tried to estimate the balance of the different pollination systems, anemophily and entomophily, by field experiments in two temperate willow species, *Salix miyabeana* and *Salix sachalinensis*. These species are common in disturbed wet sites on riversides in lowlands of northern Japan. The purpose of this study is to evaluate the relative importance of these pollination systems under natural conditions between species, years, or sites.

Materials and methods

Study sites and species

Salix miyabeana Seemen and *S. sachalinensis* Fr. Schm. are common willows in northern Japan, which often make mixed stands on riversides or wet places in lowlands. Both species are dioecious and deciduous like other willows. *Salix miyabeana* grows up to 3–5 m, and *S. sachalinensis* up to 10–15 m. Flowering of both species occurs from mid April to early May in Hokkaido. Female trees develop their infructescences during May, and disperse the seeds in mid June.

The study was conducted in willow forests established on a riverbank of the Ishikari River in central Hokkaido, northern Japan (43°11' N, 141°24' E). We selected two sites for this study, hereafter called Site 1 and Site 2. Site 1 is located 4 km downstream of Site 2. Density of willows is higher at Site 2 than at Site 1, and the difference in density may influence the efficiencies of wind and insect pollinations. *Salix miyabeana* is the most abundant willow and *S. sachalinensis* is less abundant in the study sites. Other coexisting willows are *S. integra* Thunb., *S. subfragilis* Andersson, *S. bakko* Kimura, and *S. pet-susu* Kimura. They usually form mixed stands and the ground is covered with dense grass.

Methods

In the first year, 1996, we compared pollination systems of *S. miyabeana* and *S. sachalinensis* at Site 1. We randomly selected 19 female clones with many flower buds for both *S. miyabeana* and *S. sachalinensis* in early spring before flowering. All selected plants were included within about a 3-ha area. Because stems of the willows often branch off at ground level, discrimination of neighboring clones is sometimes difficult. Thus, we did not select adjacent clones for sample trees. For each clone, nine one-year-old twigs having several flower buds were selected randomly and marked with numbering tags.

Three pollination treatments were conducted for the marked twigs; intact control (natural pollination by both insects and wind), hand pollination (addition of pollen artificially), and net bagging (exclusion of insects by a net bag with 1 × 1-mm mesh size). Each treatment was replicated three times within each clone. Pollen for artificial pollination was collected from flowering catkins of six male trees nearby in early May, and mixed within a plastic tube. Immediately, the mixed pollen was fully deposited on stigmas of the recipient catkins by using a soft brush. The flowering period of each species was about 10–14 days. The hand pollination should increase the seed set only when seed production is restricted by pollen limitation under natural conditions. Inversely, no change in seed set by the hand pollination indicates an absence of pollen limitation under natural conditions, at least at the twig level. The net bagging treatment is expected to inhibit only insect pollination, and wind pollination is available. If the net bagging decreases seed production in comparison with control, it indicates that pollen transfer by insects is effective for seed production in the willows.

In the laboratory, we evaluated the blocking effect of wind by the net bagging by using an electric fan and a wind velocity meter (SA 415, Testo, Germany). It was proved that the net bag evenly decreased the wind speed about 30%, irrespective of any wind speed. This result indicates that some pollen grains delivered by wind may be trapped by the net bag which results in a decrease in the efficiency of wind pollination in the net bagged catkins than in control. However, we could not detect any significant difference in seed set between the net bagging and control. This means that a similar amount of pollen is delivered to stigmas by wind even in the net bagged catkins to control.

In a preliminary experiment, we confirmed that catkins covered with paper bags, which prevented both wind and insect pollination, did not produce any seeds. Thus, seed production by autonomy (autonomous form of apomixis) did not occur in both species. There was also the possibility of pseudogamy (pseudogamous form of apomixis) which needed pollinations to product seeds. Although it was not examined, finding no reports of pseudogamy in *Salix* species by now, we considered the possibility of them being pseudogamous small.

In the next year, 1997, the pollination experiment was conducted at Site 1 and Site 2 for only *S. miyabeana* because of the high abundance at both sites. At Site 1, we observed the same 19 clones between the years. At Site 2, we randomly selected 18 female clones, and marked nine one-year-old twigs having several flower buds in each clone as mentioned above. The same pollination treatments used in the first year were conducted, i.e. control, hand pollination, and net bagging. Each treatment included three replications within each clone. In 1997, snow disappeared more rapidly and flowering occurred earlier than in 1996, thus we conducted the pollination experiments in late April in 1997. In early June, we harvested all fruits (i.e. infructescences) from the marked twigs just before seed dispersal. The harvested fruits were stored at 4°C in an incubator not to be dried before the measurement.

We tried to estimate the efficiencies of wind and insect pollinations on seed set, i.e. ratio of mature seed number to total ovule number per catkin. Seed sets of randomly selected 20 catkins (infructescences) were measured in each treatment for each clone, and the mean values of each clone were used for analysis. In this study, we defined an index of pollen limitation as $1 - C/P$, where C and P meant the seed set of the control and the artificially pollinated catkins, respectively. In this index, P is considered to be a maximum seed set ability of a clone. Potential ability of seed set may be influenced by resource conditions of each clone or weather conditions in each year. By using this index, therefore, effects of environmental factors other than pollen limitation on seed set can be minimized. Larger values of the index mean the severer pollen limitation for seed set under natural conditions.

Similarly, we defined indexes of efficiency of wind pollination and insect pollination as N/P and $(C - N)/P$, respectively, where N meant the seed of the net-bagged catkins. N/P and $(C - N)/P$ reveal the contribution of insect pollination and wind pollina-

tion to the potential seed production, respectively. We compared these indexes between the species at Site 1 in 1996, between the years (1996 and 1997) for *S. miyabeana* at Site 1, and between the sites (Sites 1 and 2) for *S. miyabeana* in 1997.

To examine whether pollination success influenced the reproductive effort, individual fruit weight and total fruit mass per twig were measured. Because there is a significant correlation between seed number per fruit and individual fruit weight in both species ($N = 57$, $R = 0.649$, $P < 0.001$ for *S. miyabeana*, $N = 53$, $R = 0.541$, $P < 0.001$ for *S. sachalinensis*), fruit mass could be a good index representing total seed number per fruit or twig. Each fruit was weighed in fresh conditions because seeds were used for a germination experiment.

Finally, we assessed effects of pollination treatments on seed qualities, i.e. seed weight and germination ability. Comparisons of seed quality between wind-pollinated and insect-pollinated seeds are useful for adequate assessment of reproductive success between the pollinations systems as mentioned above. Seeds were stored under 20°C in an incubator for 24 hours in order to keep their moisture uniform before weighing. Each 50 seed grains were weighed in fresh conditions, then mean values of individual seed weight was calculated. After that, we sowed 50 seeds per petri dish and replicated four times per treatment (control, hand pollination, and net bagging treatments) in each clone. Each set of seeds was sowed on a moist filter paper, and germinated under the lighting condition in an incubator at 25°C. We defined the germination that a seed has a cotyledon recognized as separated two leaves. We checked the number of germinated seeds every 24 hours. The germination took place soon after watering without any prior treatment, and all viable seeds germinated within 3–4 days.

Statistic analysis

For each species, each year, and each site, we compared seed set, individual fruit weight, total fruit mass, individual seed weight, and germination rate among three pollination treatments by one-way ANOVA. As a post hoc test, Tukey's HSD test was used for the comparison between each paired treatments. Mean values of sampled twigs per clone were used for analyses. To meet the assumption of the homoscedasticity of the data, some variables were log-, root-, or arcsin-transformed. Each transformation was cited in results.

We used a multivariate analysis of variance (MANOVA) to conservatively test the effects of the pollination treatments and each comparative subject (species, years, or sites) on reproductive variables. The MANOVA considered simultaneously the five reproductive variables: seed set, individual fruit weight, total fruit mass, individual seed weight, and germination rate. After detection of a significant treatment effect, each MANOVA was followed by univariate ANOVAs to explore whole variables which contribute to significant MANOVA effects.

For each index of pollination efficiency defined in this study, i.e. pollen limitation, wind pollination and insect pollination, Mann-Whitney *U*-test was applied to detect the differences between species, years, or sites. In addition, we examined correlation between the index of wind pollination or insect pollination and seed set under natural conditions in each population of each year by a Pearson's correlation coefficient.

Results

The flowering phenologies of two species overlapped although *S. sachalinensis* started and ended flowering earlier than *S. miyabeana* by several days. The flowering periods of those populations were about two weeks, from late April to early May in 1996, and from mid to end of April in 1997. Mean daily temperature, wind speed, sunshine hours, and precipitation during the flowering periods in 1996 and 1997 were $9.3 \pm 2.6^\circ\text{C}$ and $9.8 \pm 3.9^\circ\text{C}$, 3.3 ± 1.3 m/s and 3.5 ± 1.3 m/s, 4.5 ± 4.2 h and 7.4 ± 4.1 h, and 3.2 ± 7.1 mm and 0.6 ± 1.1 mm, respectively (means \pm SD of 20 days) (data from Japan Meteorological Agency). Sunshine hours were shorter and precipitation was greater in 1996 than in 1997, significantly ($P < 0.05$, Mann-Whitney *U*-test, respectively). Although pollinator visitation on both male and female catkins was not frequent, flies and small bees (Andrenidae and Tenthredinidae) were observed on sunny and calm days. These insects seemed to be attracted by nectar of both male and female catkins and pollen of male catkins.

The hand pollination treatment significantly increased seed set in comparison with the control and the net bagging treatment in almost all *Salix* populations (Table 1), indicating that seed set was limited by failure in pollen delivery to stigmas. On the other hand, seed set of the net bagging treatment did not differ significantly from that of control in all populations,

indicating that insect visitation did not contribute to increase the seed set under natural conditions in years of this study. Similar trends were observed also in individual fruit weight and total fruit mass per twig in both species in 1996, but there were no significant differences among the treatments in 1997. Individual seed weight and seed germination rates were not affected by the pollination treatments within a population. The only exception was *S. miyabeana* in 1996, where seed weight of the net bagging treatment was significantly heavier than that of control and the hand pollination treatment.

Results of the MANOVA indicated a significant effect of species and the pollination treatments on reproductive variables in 1996. No significant interaction was detected (Table 2a). *Salix miyabeana* had significantly larger seed set, total fruit mass per twig, individual seed weight, and seed germination rate than *S. sachalinensis*, but there was no significant difference in individual fruit weight between the species. Both species indicated the same trends in all reproductive variables, and there were no significant species-treatment interactions in any variables. The trends were almost the same as the results from the intra-species analyses in 1996 (Table 1).

A significant effect of year and the treatments was detected for *S. miyabeana* by the MANOVA, but no significant interaction was detected (Table 2b). Seed set, individual fruit weight, total fruit mass, and germination rate were significantly higher in the sunny and dry year, 1997, than in 1996, but individual seed weight did not differ between the years. Significant year-treatment interactions were not observed in any reproductive variables. By inspection of data, the effects of the pollination treatments on each reproductive variable were similar between the years, i.e. reproductive output was higher in the hand pollination treatment than in the control and the net bagging treatment.

A significant effect of site and the treatments was detected for *S. miyabeana* in 1997 by the MANOVA, but no significant interaction was detected (Table 2c). Seed set was significantly larger at Site 1 than at Site 2. Total fruit mass was significantly larger at Site 1 than at Site 2 because of larger fruit number per shoot ($P < 0.001$), but individual fruit weight did not differ between the sites. Individual seed weight was not affected by the difference of sites or the types of the pollination treatments, but seeds from Site 2 germinated better than those from Site 1. The pollination treatments had significant effects only on seed set; the

Table 1. Comparisons of seed set, individual fruit weight, total fruit mass per twig, individual seed weight, and seed germination rate of *Salix miyabeana* and *S. sachalinensis* among three pollination treatments (control, hand pollination, and net bagging). Each value is mean \pm se, and sample size is noted in the parentheses. Different letters mean significant difference ($P < 0.05$) within three pollination treatments from the results of one-way ANOVA and Tukey's HSD test. To meet the assumption of the homoscedasticity of the data, seed set and germination rate were arcsin-transformed, individual fruit weight was log-transformed, and total fruit mass was root-transformed.

Species	Year	Site	Pollination treatment	Seed set (%)	Individual fruit wt. (g)	Total fruit mass (g)	Individual seed wt. ($\times 10^{-2}$ mg)	Germination rate (%)
<i>S. sachalinensis</i>	1996	1	Control	22.9 \pm 2.7(18) ^a	0.15 \pm 0.03(19) ^a	0.60 \pm 0.17(19) ^{ab}	16.4 \pm 0.6(16)	27.1 \pm 6.2(16)
			Hand pollination	42.6 \pm 4.2(18) ^b	0.28 \pm 0.05(19) ^b	1.13 \pm 0.20(19) ^b	15.1 \pm 0.6(17)	33.6 \pm 7.4(17)
			Net bagging	14.5 \pm 1.4(17) ^a	0.12 \pm 0.02(17) ^a	0.45 \pm 0.14(19) ^a	16.7 \pm 0.6(15)	22.0 \pm 7.1(15)
<i>S. miyabeana</i>	1996	1	Control	37.9 \pm 2.9(19) ^a	0.15 \pm 0.02(19) ^a	1.05 \pm 0.12(19) ^a	16.1 \pm 0.7(19) ^a	32.0 \pm 6.8(19)
			Hand pollination	53.2 \pm 2.9(19) ^b	0.23 \pm 0.03(19) ^b	1.85 \pm 0.21(19) ^b	15.2 \pm 0.5(19) ^a	45.2 \pm 6.6(19)
			Net bagging	33.0 \pm 2.4(19) ^a	0.12 \pm 0.02(19) ^a	0.85 \pm 0.09(19)	18.0 \pm 0.5(19) ^b	38.8 \pm 6.2(19)
<i>S. miyabeana</i>	1997	1	Control	59.7 \pm 4.2(19) ^a	0.37 \pm 0.05(19)	2.85 \pm 0.34(19)	15.6 \pm 0.7(19)	65.8 \pm 6.0(19)
			Hand pollination	74.5 \pm 2.9(19) ^b	0.44 \pm 0.04(19)	3.45 \pm 0.40(19)	15.7 \pm 0.6(19)	77.4 \pm 3.5(19)
			Net bagging	55.8 \pm 3.9(19) ^a	0.33 \pm 0.04(19)	2.60 \pm 0.33(19)	16.7 \pm 0.6(19)	75.8 \pm 5.4(19)
<i>S. miyabeana</i>	1997	2	Control	55.8 \pm 3.1(18) ^{ab}	0.32 \pm 0.04(18)	2.05 \pm 0.27(18)	16.3 \pm 0.6(18)	86.8 \pm 2.1(18)
			Hand pollination	64.0 \pm 2.9(18) ^b	0.33 \pm 0.04(18)	2.50 \pm 0.35(18)	14.9 \pm 0.5(18)	82.3 \pm 4.3(18)
			Net bagging	50.4 \pm 4.0(18) ^a	0.34 \pm 0.04(18)	2.32 \pm 0.26(18)	17.1 \pm 0.9(18)	86.8 \pm 2.1(18)

hand pollination increased the seed set compared to those of the control and the net bagging treatment. There were no significant interactions between the sites and the pollination treatments in any reproductive variables.

The extent of pollen limitation and the proportions of wind pollination and the insect pollination are shown in Table 3. Pollen limitation varied from 13% to 41%, but the difference between the species in 1996, between the years for *S. miyabeana*, or between the sites for *S. miyabeana* in 1997 was not significant, respectively due to the large variations. Wind pollination usually was the primary contribution to seed-set in both species. In 1996, *S. miyabeana* depended more on wind pollination than *S. sachalinensis*. Moreover, *S. miyabeana* at Site 1 was more dependent on wind pollination in 1997 than in 1996. About a quarter of maximal seed production per fruit was contributed by insect pollination in *S. sachalinensis* in 1996.

The index of insect pollination was positively correlated with the seed set under natural conditions (control) in both species in 1996 (Table 4). This indicates the importance of insect pollination in 1996. In contrast, in 1997, the index of wind pollination was positively correlated with the seed set of controls in *S. miyabeana* at both sites. This indicates that seed set depends on the success of wind pollination in 1997. Thus, the relative importance of pollen vectors (wind vs. insects) changed among years.

Discussion

Seed set in the observed *Salix* populations was basically restricted by pollen limitation. Insect pollination did not contribute to an increase in the seed set since the insect exclusion treatment resulted in similar seed production compared with the control. These results indicated that wind played an important role for pollination success, but it was insufficient to maximize the seed production in our study sites. Some studies reported the existence of pollen limitation in *Salix* species (Elmqvist et al. 1988; Fox 1992) with a few exceptions (Sacchi & Price 1988). On the other hand, almost all reports on *Salix* pollination indicated a lower efficiency of wind pollination (Argus 1974; Douglas 1997) or predominance of insect pollination (Kevan 1972; Elmqvist et al. 1988; Sacchi & Price 1988; Fox 1992) for seed production (but see also Vroege & Stelleman 1990). This is probably because many of those *Salix* species had shrubby growth forms and were growing in high elevations or high latitudes like Arctic or boreal regions. There are few studies focusing on the pollination system of temperate *Salix* species which often make tall and dense stands in lowlands. Early flowering before leaf emergence on such dense populations may increase the pollination success by wind because filtration of pollen by leaves is small and activity of pollinating insects is low in early spring due to the cool weather conditions.

Table 2. Results of MANOVA and ANOVA to test the effects of (a) species \times pollination experiment, (b) year \times pollination experiment, and (c) site \times pollination experiment on seed set, individual fruit weight, total fruit mass, individual seed weight, and seed germination rate. Wilks' λ is shown as a statistic value for MANOVA. To meet the assumption of the homoscedasticity of data, seed set and germination rate were arcsin-transformed, individual fruit weight was log-transformed, and total fruit mass was root-transformed.

(a) Between species		Species		Pollination treatment			S \times P		
Source of variation	df	F-value	P	df	F-value	P	df	F-value	P
Wilks' λ	5,95	15.07	< 0.001	10,190	8.08	< 0.001	10,190	1.40	0.18
Seed set	1	37.09	< 0.001	2	41.28	< 0.001	2	2.12	0.13
Individual fruit wt.	1	0.22	0.64	2	16.24	< 0.001	2	0.26	0.78
Total fruit mass	1	21.57	< 0.001	2	12.87	< 0.001	2	0.13	0.87
Individual seed wt.	1	6.30	< 0.05	2	6.55	< 0.010	2	0.84	0.43
Germination rate	1	22.09	< 0.001	2	1.29	0.28	2	0.38	0.68

(b) Between years		Year		Pollination treatment			Y \times P		
Source of variation	df	F-value	P	df	F-value	P	df	F-value	P
Wilks' λ	5,104	28.35	< 0.001	10,208	5.03	< 0.001	10,208	1.19	0.30
Seed set	1	66.68	< 0.001	2	19.11	< 0.001	2	0.01	0.99
Individual fruit wt.	1	93.15	< 0.001	2	11.57	< 0.001	2	1.57	0.21
Total fruit mass	1	63.59	< 0.001	2	7.22	< 0.010	2	0.59	0.56
Individual seed wt.	1	0.89	0.35	2	5.70	< 0.010	2	1.27	0.29
Germination rate	1	50.97	< 0.001	2	2.15	0.12	2	0.16	0.85

(c) Between sites		Site		Pollination treatment			S \times P		
Source of variation	df	F-value	P	df	F-value	P	df	F-value	P
Wilks' λ	5,101	5.88	< 0.001	10,202	2.20	< 0.05	10,202	0.79	0.64
Seed set	1	5.54	< 0.05	2	10.50	< 0.001	2	0.55	0.58
Individual fruit wt.	1	2.39	0.13	2	1.09	0.34	2	1.03	0.36
Total fruit mass	1	5.38	< 0.05	2	1.19	0.31	2	0.53	0.59
Individual seed wt.	1	0.04	0.84	2	2.95	0.06	2	0.73	0.49
Germination rate	1	12.88	< 0.010	2	0.72	0.49	2	1.53	0.22

Table 3. Summary of three pollination indexes; extent of pollen limitation, efficiency of wind pollination, and efficiency of insect pollination which are calculated from seed set in control (C), hand pollination (P), and net bagging (N) treatments. For detail, see text. Mean \pm sd. Sample size is noted in the parentheses. Each index is compared between species (in 1996), years (for *S. miyabeana* at Site 1), or sites (for *S. miyabeana* in 1997) by Mann-Whitney *U*-test. ns means $p > 0.05$.

Species	Year	Site	Pollen limitation 1 - C/P	Wind pollination N/P	Insect pollination (C - N)/P
<i>S. sachalinensis</i>	1996	1	0.41 \pm 0.29(18) ns	0.42 \pm 0.31(17) $P < 0.01$	0.23 \pm 0.25(17) ns
<i>S. miyabeana</i>	1996	1	0.29 \pm 0.16(19) ns	0.63 \pm 0.16(19) $P < 0.05$	0.10 \pm 0.10(19) ns
<i>S. miyabeana</i>	1997	1	0.21 \pm 0.16(19) ns	0.75 \pm 0.18(19) ns	0.07 \pm 0.07(19) ns
<i>S. miyabeana</i>	1997	2	0.13 \pm 0.12(18)	0.78 \pm 0.19(18)	0.11 \pm 0.10(18)

Table 4. Coefficients of determination (r^2) between the index of pollination efficiency (wind pollination or insect pollination) and seed set under natural conditions (control) in each *Salix* population in each year.

Species	Year	Site	Wind pollination N/P	Insect pollination (C – N)/P
<i>S. sachalinensis</i>	1996	1	0.024	0.655*
<i>S. miyabeana</i>	1996	1	0.144	0.243†
<i>S. miyabeana</i>	1997	1	0.406‡	0.021
<i>S. miyabeana</i>	1997	2	0.332†	0.088

* $P < 0.001$, ‡ $P < 0.01$, † $P < 0.05$.

In 1996, reproductive output in every manipulation was lower than in 1997. This means the existence of other factors beside pollen limitation, like weather condition, constrained the potential seed production. In spring 1996, the flowering phenologies were delayed for about 10 days compared with 1997, because late snow melt in spring of 1996 postponed the thermal signal for willows to start flowering (Mosseler & Papadopol 1989). Also in 1996, mean daily sunshine hours were shorter, and mean daily precipitation was greater than in 1997 during the flowering season. Rainfall would decrease the efficiency of wind pollination by capturing pollen grains (Whitehead 1983). Seed-set success is influenced not only by pollination success but also by post-pollination processes such as pollen germination activity, pollen tube growth, and development of fertilized seeds (e.g. Stephenson 1981; Lee 1988). All such processes seem to be affected by temperature especially for early flowering species (Kudo 1995). In *Salix arctica*, temperature of female catkins was much warmer than that of ambient air or soil under sunny conditions because they were effective absorbers of solar radiation and temperatures, although male catkins did not show the tendency so strongly (Mølgaard 1982; Kevan 1990). Therefore, at least the yearly difference of precipitation and hours of sunshine during the flowering season should cause the yearly seed-set success of willows to vary. Also in an early flowering perennial herb, *Jeffersonia diphylla*, seed set and fruit set were decreased by freezing temperatures due to late spring frosts in some years (Smith et al. 1986). Alatalo & Totland (1997) reported that the two-year temperature enhancement by setting open-top-chambers in the field increased the seed set of a subarctic plant, *Silene acaulis*, which was one of the early-flowering species in their sites.

Seed set of *Salix sachalinensis* was relatively lower than that of *S. miyabeana* in this study site because

S. sachalinensis was less dependent on wind pollination and more on insect pollination. This would be due to the lower density of *S. sachalinensis* than *S. miyabeana* and its characters showing insect dependence, e.g. their pollen were stickier than that of *S. miyabeana* (personal observation). *Salix miyabeana* had higher seed set success by wind pollination in 1997 than in 1996. Seed set did not differ between the sites in 1997, partly because of similarity of environmental conditions between the sites.

The efficiency of wind pollination fluctuated between the years and between the species, while the efficiency of insect pollination was not influenced by the effects of species, year, or site, significantly. Seed-set success by insect pollination seemed to be less sensitive to the changes in the weather conditions than that of wind pollination. Although wind pollination always leads to a large proportion of total seed set, insect pollination would be a more reliable vector than wind pollination, and may have an assurance role in the year of low wind-pollination success. In this study, clones having high seed production within a population had high pollination success by wind in the warm and dry spring in 1997, while in the wet and cool spring in 1996, they had high pollination success mediated by insects. Thus, we could conclude that having two pollination systems was a kind of bet-hedging strategy to reduce the variance of seed production.

Salix species are common pioneer plants immigrating into disturbed open sites, so reproductive systems to ensure a stable seed output may be selected against in unpredictable and fluctuating environments (Fisher 1928; Regal 1982). Completion of reproductive events during a short period early in the season may be advantageous to assure seed production under conditions of short growing seasons in boreal, alpine or Arctic regions. In addition, as shown in this study, having two pollinating systems would be advantageous when

not only weather conditions but also availability of pollinating agents are unpredictable. These characters of *Salix* plants are considered to contribute to its distribution over a wide range of the Northern Hemisphere.

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