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Wind to insect pollination ratios and floral traits in five alpine *Salix* species

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Abstract: This study examines the ratio of wind to insect pollination of five alpine–arctic *Salix* species and possible correlations between plant traits and this ratio. The field work was done in an alpine area in southwest Norway, at an elevation of 1250 m. Female catkins were enclosed within mesh cloth to prevent insect visitation, and their fruit set was determined and compared with that of open-pollinated control catkins. Simultaneously, pollen production per anther, pollen dispersal, standing crop of nectar, flower length, number of flowers per catkin, and catkin length were measured. The amount of wind pollination of the species varied from 2 to 52%. All species spread pollen in the air. Nectar content, catkin length, flower number, and flower length showed a negative relationship with the percentage of wind pollination. Significant negative linear correlations between female catkin length and flower length and amount of wind pollination were detected. Pollen production per anther did not show any relationship to the pollination system. We suggest that the evolution to insect pollination in alpine *Salix* species may have resulted in a lengthening of anthers and pistils, and an increase in nectar production.

Key words: alpine, anemophily, entomophily, floral traits, pollination, *Salix*.

Résumé : Les auteurs ont étudié le rapport des pollinisations vent à insectes chez cinq espèces de *Salix* alpins–arctiques, pour déceler des corrélations entre les caractères de la plante et ce rapport. Ils ont effectué le travail de terrain dans une région alpine du sud-ouest de la Norvège, à 1250 m d'élévation. Ils ont enveloppé les châtons femelles dans des tissus ajourés pour empêcher les insectes de s'y rendre, puis ils ont déterminé la mise à fruit et l'ont comparée avec celle de châtons témoins soumis à la pollinisation ouverte. Ils ont également mesuré la poduction de pollen par anthère, la dispersion du pollen, la production de nectar sur pied, la longueur des fleurs, le nombre de fleurs par châton et la longueur des châtons. La quantité de pollinisation des espèces par le vent varie de 2 à 52%. Toutes les espèces répandent du pollen dans l'air. Le contenu en nectar, la longueur des châtons, le nombre de fleurs et la longueur des fleurs sont inversement proportionnels au pourcentage de pollinisation par le vent. On perçoit des corrélations négatives significatives entre la longueur du châton femelle et la longueur des fleurs avec la pollinisation par le vent observée. La production de pollen par anthère ne montre pas de relation avec le système de pollinisation. Les auteurs suggèrent que l'évolution vers la pollinisation par les insectes, chez les espèces de saules alpins, pourraient provenir d'une élongation des anthères et des pistils, ainsi que d'une augmentation de la production de nectar.

Mots clés : alpin, anémophilie, entomophilie, caractères floraux, pollinisation, *Salix*.

[Traduit par la Rédaction]

Introduction

The genus *Salix* (Salicaceae) has a mainly northern hemisphere distribution. It probably originated in the subtropics, then spread to the northern temperate and arctic regions. About 400 species and 200 fertile hybrids are known (Newsholme 1992). Willows are dioecious and bear catkins. The genus as a whole is widely accepted to be insect pollinated (Fægri and van der Pijl 1979), but several species are known to be at least partly anemophilous (Argus 1974; Vroege and Stelleman 1990; Fox 1992; Douglas 1997).

Morphologically, the flowers are intermediate between wind and insect pollination. They possess outreaching anthers or pistils, the perianth is absent, and they often flower before leafing (Fisher 1928). Some species release large quantities of pollen in the air when branches are shaken (e.g., *S. caprea*) (Vroege and Stelleman 1990), and *Salix* pollen are abundant in the atmosphere and in fossil peat deposits. These features suggest anemophily (Fægri and van der Pijl 1979; Whitehead 1983; Proctor et al. 1996). On the other hand, in many species both sexes produce nectar, release a scent, and the inflorescences are stiff, erect, and highly visible, suggesting that animals play an important role in pollination (Fisher 1928; Tollsten and Knudsen 1992). Most species are also frequently visited by insects, and certain species are known to be almost entirely entomophilous (Kevan 1972; Sacchi and Price 1988).

Two main explanations about the pollination history of *Salix* have been proposed (Stebbins 1970; Meeuse 1978; Hesse 1979; Cronquist 1988). Both theories agree that *Salix* changed to entomophily from an anemophilous ancestor and that the genus *Populus* (Salicaceae), being completely ane-

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mophilous, is more ancient than *Salix*. However, the opinions about the earlier evolutionary history of *Salix* species differ. Meeuse (1978) proposed that evolution of entomophily occurred directly from a diclinous, anemophilous ancestor, where anemophily is considered a primary feature among early angiosperms (Meeuse 1972). On the other hand, Stebbins (1970), Hesse (1979), and Cronquist (1988) proposed that entomophily was a primary feature and was re-acquired after passing through an anemophilous stage. The latter theory, with entomophily as the primary pollination system for angiosperms, is presently accepted by most pollination biologists and systematists (Crepet 1983). Despite these theoretical reflections, fairly little is known about the effective pollination mode of most *Salix* species. Less than 20 species have been examined, with varying results.

Salix arctica (Kevan 1972), *S. lasiolepis* (Sacchi and Price 1988), and *S. lucida* (Argus 1974) are entirely insect pollinated, whereas *S. petiolaris*, *S. discolor*, and *S. bebbiana* (Argus 1974), *S. repens* and *S. caprea* (Vroege and Stelleman 1990), and *S. setchelliana* (Douglas 1997) showed proportions of wind and insect pollination varying between 7 and 70%. The amount of wind pollination for *S. repens* varied from 20 to 70% depending on the location (Vroege and Stelleman 1990). Fox (1992) focused on pollen limitation on seed set in eight Alaskan willow species and found that seed set still occurred in the absence of insect visitation, but at a lower rate (Fox 1992).

No clear connection between the pollination mode and the habitat of a *Salix* species seems to exist. Although insect pollination is said to decrease with altitude and latitude (Bliss 1962; Regal 1982; Proctor et al. 1996), *S. arctica*, an arctic species, and *S. setchelliana*, an Alaskan alpine species, are fully insect pollinated. Moreover, in *S. caprea* and *S. repens*, both temperate lowland species, wind pollination plays an important role in determining fruit set.

None of the above-mentioned studies looked for a correlation between the flower morphology and the amount of wind pollination among species, although several floral traits are supposed to be related to the pollination mode of species (Fægri and van der Pijl 1979; Whitehead 1983; Proctor et al. 1996). Since wind pollination is believed to be more important at higher altitudes (Bliss 1962; Regal 1982; Proctor et al. 1996), we decided to investigate the amount of wind pollination in alpine *Salix* species. First, we examined the pollination mode of five arctic-alpine willows: *S. lanata* L., *S. lapponum* L., *S. myrsinites* L., *S. herbacea* L., and *S. reticulata* L. Second, we investigated the relationship between several flower features and the amount of wind pollination to try to detect evolutionary patterns.

This study addressed the following questions: (*i*) Do the species spread pollen in the air? (*ii*) What is the relative amount of wind and insect pollination, or the pollination ratio, of each species? (*iii*) Is there a correlation at the species level between the pollination ratio, flower and catkin morphology, and pollen and nectar production?

Methods

Study site

Finse is situated in the northern part of Hardangervidda in southern Norway. It has an alpine–oceanic climate, with high annual precipitation (1030 mm; Aune 1993a), not very cold winters

(average temperature December–February is –8.2°C; Aune 1993b), and cool summers (average temperature June–September is 5.5°C; Aune 1993b). The study site is situated in the low-alpine zone on a south-facing slope at 1200 to 1350 m elevation. Snow melted by mid-July in 1997. *Salix herbacea* grows all over the slope, except in waterlogged places. Bushes of *S. lanata* occur over almost the entire slope, but they are more abundant in the lower parts and not present in the highest region. A rather dense area of *S. lapponum* is situated in the lowest part. Both *S. reticulata* and *S. myrsinites* are found on a calcium-rich ridge at 1300 m.

Field methods

Pollen dispersal

We tested whether *Salix* pollen is truly dispersed by wind and whether our exclosure netting disrupts pollen flow by placing microscope slides with sticky tape near male plants, at ground level for the prostrate species *S. herbacea* and *S. reticulata* and just above bush level for the other species. At each plant, one open, uncovered slide was placed next to one slide enclosed within the same mesh cloth used for the insect exclosures. The slides were placed under dry and windy weather conditions, if possible. After 24 h in the field, the slides were collected, placed in a wind-proof container, and transported to the lab. We counted the pollen present on one stroke of tape. Seven pairs of slides were used for *S. herbacea* and *S. reticulata*, five pairs for *S. lapponum* and *S. lanata*, and two pairs for *S. myrsinites* (because flowering was already well advanced). Since it is nearly impossible to distinguish between pollen from different *Salix* species, the slides were placed in the immediate vicinity of a male plant to increase the possibility of the pollen deposited coming from the species under investigation. Sexes were highly mixed in the study sites and slides placed close to male plants are therefore close to female plants as well.

Pollination mode

To quantify the proportion of the fruit and seed set resulting from wind pollination, randomly selected female catkins of each species were enclosed with mesh cloth (mesh size approx. 1 × 1 mm) before their stigmas were receptive to prevent pollen deposition on the stigmas by insects. An equal number of freely pollinated (open treated) control catkins was marked. The enclosures were placed within a range of 20 m from flowering male plants.

The exact method of enclosing the catkins differed among the species because of differences in size and morphology. On *S. lanata*, *S. lapponum*, and *S. myrsinites*, bushes that are a maximum of 1 m high, a small mesh bag (approx. 15 × 8 cm) was placed around an unopened catkin and secured on the branch with a plastic strap. Since *S. herbacea* and *S. reticulata* are creeping at ground level, it is difficult to enclose single catkins. Therefore, small tents of metal wire (approx. 15 cm in diameter) were covered with mesh cloth and placed over a patch of female plants.

For *S. lanata*, *S. lapponum*, and *S. myrsinites*, all bags were randomly placed on different bushes within a single location. The control catkins were marked on the same plants as the insect-excluded ones to avoid different environmental influences. The tents for *S. herbacea* and *S. reticulata* were placed in one dense area of plants of each species. Three unopened female catkins were marked inside each tent, and three control catkins were marked close to each tent.

The enclosures were removed when the stigmas of the catkin(s) inside were unreceptive for pollen, and catkins were collected before seed dispersal. Nearly all marked catkins could be collected from *S. lanata*, *S. lapponum*, and *S. myrsinites*. The few catkins missing had probably withered and fallen off. One tent of *S. reticulata* was damaged by sheep and had to be discarded. A few marked catkins in the other tents were missing. Of the 10 tents of *S. herbacea*, only 6 remained: 2 were destroyed by sheep and a

Table 1. Summary of the pollination experiment on five *Salix* species at Finse in 1997.

Species	S. lanata	S. lapponum	S. myrsinites	S. herbacea	S. reticulata
Type of enclosure	Bag	Bag	Bag	Tent	Tent
No. of catkins	20	25	25	30	30
No. of catkins in one enclosure	2	1	1	3	3
Date of enclosure placement	5 July	18 June	19 June	21 July	5 July
Time in the flowering season	Late	Early	Very late	Late	Middle
Flowering males around	Sufficient	Sufficient	Few	Sufficient	Sufficient
Date of enclosure removal	22 July	10 July	10 July	7 August	22 July
Date of catkin collection	29, 31 July	20, 21, 23 July	29, 31 July	17, 21 August	8, 15, 19 August
No. of catkins collected	18	23	22	11	23

few male catkins grew inside 2 other tents, which rendered them useless for the experiment. From the remaining six tents, many enclosed and control catkins were withered and lost. An overview of this experiment is given in Table 1.

After collecting the catkins, the ripe, empty, or aborted fruits were counted and the number of mature seeds from four randomly chosen ripe fruits per catkin determined. These data were used to calculate the percentage of ripe fruits per catkin and the mean number of mature seeds per fruit for each catkin. When no ripe fruits were available on a catkin, the value for seeds per fruit was assumed to be zero. Since insects cannot pollinate the insect-excluded catkins, fruit and seed set must be due to wind pollination, provided that apomixis does not occur in the studied species. The study of Sacchi and Price (1988) did not detect apomixis in *Salix lasiolepis*.

The amount of wind pollination was estimated by the ratio of fruit set in insect-excluded catkins (wind pollination only) to fruit set in open catkins (wind plus insect pollination) for each pair or group of catkins. A similar ratio was constructed using seed set per fruit rather than fruit set. For either case, a ratio of zero represents 100% insect pollination, while a ratio of 1 represents 100% wind pollination. The distribution of these ratios was skewed, so we used the median instead of the mean to obtain an estimate of the amount of wind pollination within a species. The data on seed set per fruit are not presented (see Discussion).

Standing crop of nectar

To estimate the standing crop of nectar in male and female catkins, the nectar content was measured with 0.5-µL microcapillaries. One capillary was inserted at random into 10 flowers on a flowering catkin, and the length of the column of liquid recovered was measured with a digital calliper. The measurements were done on dry days on 20 randomly selected catkins of each species and sex.

Catkin and flower morphology

Twenty randomly selected catkins from 20 randomly chosen plants of each species and sex were collected, and the length of the catkin, the length of individual flowers, and the number of flowers were measured on the same day as collection. Three flowers on each catkin were used for flower size measurements. The catkin was visually divided into three zones (bottom, middle, and top), and a flower was randomly chosen from each zone. The aim of these measurements was to determine how exerted the pistils and anthers are, since this is an important feature of wind-pollinated species (Fægri and van der Pijl 1979; Whitehead 1983; Proctor et al. 1996). The flowers are so reduced, however, that the length of anther or gynoecium equals the length of the flower, so we simply measured flower length. For the measurements, a digital calliper to the nearest 0.01 mm was used.

Pollen production

To measure the pollen production of the species, 10 branches with male catkins from different plants of each species were collected just before anthesis and placed indoors at room temperature until they opened, usually the next morning. An anther was then carefully picked off a catkin with a pair of tweezers and placed in a tube. The pollen was suspended in 150 µL 96% ethanol, 3 µL of the solution was placed on a hemacytometer four times, and the pollen was counted.

Statistical analyses

All analyses were performed using the statistical program SYSTAT 5.0 for Windows (Wilkinson et al. 1992). We used one-way analysis of variance (ANOVA) to examine if the catkin and flower measurements, pollen production of anthers, and standing crop of nectar differed among the species. Separate analyses were done for males and females. To meet the assumptions of normality and homogeneity of variance in ANOVA, the data on nectar content were square-root transformed. A two-factor fixed model ANOVA was initially applied to test if fruit set differed between the two treatments, i.e., enclosed catkins and control (open-treated) catkins, and between species. This ANOVA revealed a strong interaction between treatment and species ($P = 0.005$), suggesting that the bagging effect differed between species. Therefore we used a paired t-test to test for differences in bagging effect for each species separately. For *S. lanata*, *S. herbacea*, and *S. reticulata*, the mean values for each group of catkins inside and outside enclosures were used. This gave 10 groups for *S. lanata*, 6 for *S. herbacea*, and 9 for *S. reticulata*.

Two-factor fixed model ANOVA was used to examine if the amount of airborne pollen differed between open and enclosed slides and between species. To meet the assumptions of normality and homogeneity of variance in ANOVA, the data on airborne pollen were square-root transformed. After each significant ANOVA, Bonferroni's multiple comparison test was used to compare the differences in means between all possible combinations of groups.

Spearman rank correlations were used to examine relationships between the mean values of the catkin and flower traits and the amount of wind pollination for each species, and relationships between the nectar content, pollen production per anther, and flower size.

Results

Pollen dispersal

All species released pollen in the air (Table 2). Two-factor ANOVA showed that the number of pollen grains captured on the slides did not differ significantly among the five species ($F_{[4,42]} = 2.52$, $P = 0.055$), or between slides inside and outside bags ($F_{[1,42]} = 0.59$, $P = 0.45$). No significant interaction was found between species and treatment ($F_{[4,42]} =$

Table 2. The mean number (± SE) of pollen deposited on open and enclosed slides.

	Open slides (mean ± SE)	Enclosed slides (mean ± SE)
S. lanata	59±21	54±20
S. lapponum	169±54	66±10
S. myrsinites	4±2	20±5
S. herbacea	41±24	67±48
S. reticulata	147±55	39±14
Total*	94±22	53±14

*Mean deposition per slide for all species together.

1.11, $P = 0.36$), suggesting that any possible reduction of pollen deposition by the insect exclosures is similar for all species. A paired t test for the pairs of open and enclosed slides for all species together did not show a significant difference in means between the open and enclosed slides ($P = 0.086$). The mean pollen deposition on the enclosed slides for all species together was, however, only 56% of the mean deposition on the free slides (Table 2).

Pollination mode

Paired t tests showed that the mean fruit set in the open-treated catkins was significantly higher than in the enclosed catkins for each species except *S. herbacea* (Table 3). However, the power of the t test on *S. herbacea* was relatively low (0.27), suggesting that the ability to detect a true difference was reduced. The mean fruit set for the open catkins ranges from 39.6% for *S. myrsinites* to 75.7% for *S. reticulata* (Table 3), whereas the mean fruit set for the enclosed catkins ranges from 2.7% for *S. myrsinites* to 41.2% for *S. reticulata* (Table 3).

The pollination ratio between insect-excluded and open-treated catkins was higher than zero for all species, and they are thus at least partly wind pollinated (Table 3). *Salix myrsinites* has the lowest pollination ratio of the species (0.02) (Table 3). *Salix lanata* also has a low ratio (0.08). *Salix lapponum*, *S. herbacea*, and *S. reticulata* show the highest pollination ratios (0.47–0.57) (Table 3). The reduction in fruit set due to the insect exclosures in *S. herbacea* is not significant, suggesting 100% wind pollination for that species.

Standing crop of nectar

One-factor ANOVA showed significant differences in standing crop of nectar between the five *Salix* species for both sexes. Bonferroni multiple comparison showed that the species can be divided into two groups: *S. lanata*, *S. lapponum*, and *S. myrsinites* in one, and *S. herbacea* and *S. reticulata* in the other. Within the groups no significant differences were found between species and sexes, but the groups differed significantly from each other ($P = 0.0002$). The mean nectar content in 10 flowers in the first group ranged from 0.064 to 0.132 μL, while in the second group only *S. herbacea* males had a small amount of nectar (0.003 μL) (Table 4).

Catkin and flower morphology

One-way ANOVAs showed that all morphological characters (catkin length, flower number, and flower length) dif-

fered significantly among the five *Salix* species for both sexes (Table 4). *Salix lanata* produced the largest catkins and the most and longest flowers of all species, for both sexes (Table 4). *Salix herbacea* has the smallest catkins and the fewest flowers, whereas *S. reticulata* has the smallest flowers. The female catkins and flowers are consistently shorter and have fewer flowers than those of the male.

Pollen production

The pollen production per anther differed significantly between the five *Salix* species (one-way ANOVA, Table 4). The species can be divided into two groups: *S. lanata* and *S. lapponum* in one, and *S. myrsinites*, *S. herbacea*, and *S. reticulata* in the other. Within these groups no significant differences were found between species, but pollen production differed significantly between the two groups (Bonferroni, $P = 0.0007$). *Salix lanata* and *S. lapponum* both had nearly 5000 pollen grains per anther, while the number of pollen grains per anther in the other group was considerably lower (from 1600 to 2800 pollen per anther) (Table 4).

Correlation between plant traits and pollination ratios

Spearman rank correlation showed a significant negative relationship between the pollination ratio and female catkin length (Fig. 1a) and female flower length (Fig. 1c), whereas no other floral traits for any sex showed a significant correlation with the pollination ratio (Figs. 1a–1e). Nevertheless, some trends are apparent between the different floral traits and the pollination ratio. The mean catkin length, flower number, and flower length of male and female catkins seem to decrease with an increasing pollination ratio (Figs. 1a–1c). These trends are the clearest (and significant) for female flower and catkin length. There does not seem to be any relationship between the pollen production per anther and the pollination ratio of the five species (Fig. 1d). The nectar content appeared to increase with decreasing pollination ratio (Fig. 1e).

No significant relationship was found between the mean number of pollen grains per anther and the mean male flower size (Spearman, $r = 0.6$, $P = 0.29$). A significant positive relationship was found between the mean nectar content and mean flower size for male catkins ($r = 1.0$, $P = 0.01$) but not for female catkins ($r = 0.67$, $P = 0.22$).

Discussion

A problem in interpreting the results is the possible pollen reduction caused by the mesh bags. Although pollen deposition on slides inside mesh bags was 56% lower than in open air, this difference is not statistically significant ($P = 0.086$). The amount of pollen in the air varies greatly with the exact location, time, and weather conditions, and thus caused a great variance in the pollen deposition on the slides. More data are undoubtedly required for drawing clear conclusions about the reduction of pollen flow by the enclosures. Indeed, power analysis suggested that the ability to detect a true difference in pollen deposition was low. Sacchi and Price (1988) conducted a similar examination of pollen flow through mesh cloth (0.9 × 0.9 mm) for *S. lasiolepis*. They placed their slides outside for 4 to 6 days and found no significant reduction of pollen flow. Fox (1992), using slides

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Table 3. Fruit set of the five *Salix* species.

Species	Treatment	Mean ± SE	N	Pollination ratio	t values	P
S. lanata	Enclosed	5.4±1.5	10	0.08	−10.50	<0.001
	Open	75.4±5.2	10			
S. lapponum	Enclosed	30.0 ±3.3	23	0.47	−3.70	0.001
	Open	56.4±5.7	23			
S. myrsinites	Enclosed	2.7±0.8	22	0.02	−8.31	<0.001
	Open	39.6±5.8	22			
S. herbacea	Enclosed	18.7±7.4	6	0.57	−1.66	0.158
	Open	53.8±17.9	6			
S. reticulata	Enclosed	41.2±9.0	9	0.52	−4.84	0.001
	Open	75.7±5.2	9			

Note: The pollination ratio does not correspond to the ratio of the mean fruit set of the enclosed catkins to the mean fruit set of the control catkins for each species. We calculated a pollination ratio for each separate pair of enclosed and control catkins. Since the distribution of these pollination ratios for each species was skewed, we took the median to give an estimate of the average pollination ratio for each species. P, significance of the difference in means between enclosed and open treated catkins for each species (paired t test).

Table 4. Mean ± SE of floral traits by species and sex.

|	Catkin length (mm)		Flower no. per catkin		Flower length (mm)		Nectar content (µL per 10 flowers)		Pollen per anther
	Female	Male	Female	Male	Female	Male	Female	Male	
S. lanata	26.6±1.2	37.6±1.3	154±5	200±9	5.5±0.1	7.6±0.2	0.091±0.013	0.132±0.021	4851±403
S. lapponum	20.2±0.7	23.5±1.3	119±6	147±7	4.4±0.1	7.3±0.1	0.065±0.011	0.096±0.025	4957±426
S. myrsinites	21.2±0.8	19.4±0.4	59±2	62±3	6.0±0.2	6.0±0.1	0.064±0.016	0.085±0.015	1666±168
S. herbacea	5.9±0.2	7.4±0.3	9±1	13±1	3.0±0.1	3.6±0.1	0	0.003±0.002	2696±170
S. reticulata	14.5±1.0	16.8±0.7	34±2	42±1	2.3±0.7	3.3±0.1	0	0	2515±215
N	20	20	20	20	20	20	20	20	10
F ratio	89.8	208.6	244.4	192.6	171.9	271.7	36.34	33.05	24.5
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
R^2	0.79	0.9	0.91	0.89	0.88	0.92	0.61	0.58	0.69

Note: F ratio, P (level of significance), and R^2 (coefficient of multiple determination) are calculated from a one-way ANOVA.

covered with 3 × 3 mm mesh cloth, examined reduction in pollen flow on *S. alaxensis*. He left the slides outside for 2 weeks and recorded a reduction of 18% ($P = 0.108$), concluding that a smaller sized mesh would reduce the pollen flow even more. Although we cannot make definite conclusions on the amount of reduction of the pollen flow, most experiments indicate that a reduction is common. The frequent presence of aborted fruits on control catkins for all species, hand-pollination experiments on *S. lanata* and *S. lapponum* (Ø. Totland and M. Sottocornola, unpublished data), and the occurrence of pollen limitation in the boreal species *S. myrsinifolia-phylicifolia* (Elmqvist et al. 1988) and in five Alaskan *Salix* species (Fox 1992) suggest that fruit and seed production is, in general, pollen limited in arctic-alpine willow species. Therefore any reduction of pollen flow through the enclosures should result in a corresponding reduction of fruit and seed set of the enclosed catkins. Given the possible reduction of pollen flow by the mesh bags, the actual ratios of wind to insect pollination are likely to be higher than those reported here. However, the nonsignificant interaction between treatment (open or enclosed slide) and species suggests that possible reductions in fruit and seed set will be similar for all species and that the relative differences in the amount of wind pollination between species will not change.

The amount of wind pollination differed considerably within a species, depending on which ratio (based on fruit set or seed set per fruit) was considered. The ratio of seed set per fruit is probably the least reliable as an indicator of the amount of wind pollination. First, it only represents the number of seeds per fruit when the fruit are ripe. Second, the number of seeds per fruit may depend on variables other than the amount of pollen that reached the stigmas. For example, plants may abort entire fruits with too few fertilized ovules and thus keep the number of seeds per fruit rather constant (Stephenson 1981; Burd 1994). In our case, a certain number of fruits were aborted, and the percentage of fruits actually ripening is likely to be a better estimator of the amount of pollination. We have no counts for the third option, the total number of seeds per catkin, and making an approximation by multiplying the number of fruits on a catkin by the mean number of seeds per fruit would allow for serious deviations from the real number. Therefore, we consider only the ratio based on fruit set as a representative indicator of the amount of wind pollination, and the data on seed set per fruit are not presented. The wind to insect pollination ratio of the five examined *Salix* species at Finse ranged from nearly complete insect pollination in *S. myrsinites* to about 50% wind pollination in *S. lapponum*, *S. herbacea*, and *S. reticulata*. *Salix lanata* is obviously mainly insect pollinated (8% wind pollination based on the

Fig. 1. Relationship between the pollination ratio and floral traits for female and male catkins of the five *Salix* species. △, male catkins; ○, female catkins; lan, *Salix lanata*; lap, *S. lapponum*; myr, *S. myrsinites*; her, *S. herbacea*; ret, *S. reticulata*. (*a*) Relationship between the pollination ratio and mean catkin length. For females: $r = 0.90$, $P = 0.04$; for males: $r = 0.70$, $P = 0.19$. (*b*) Relationship between the pollination ratio and mean number of flowers per catkin. For females: $r = 0.70$, $P = 0.19$; for males: $r = 0.70$, $P = 0.19$. (*c*) Relationship between the pollination ratio and mean flower length. For females: $r = 0.90$, $P = 0.04$; for males: $r = 0.70$, $P = 0.29$. (*d*) Relationship between the pollination ratio and the mean number of pollen grains; $r = 0.20$, $P = 0.75$. (*e*) Relationship between the pollination ratio and the mean nectar content. For females: $r = 0.67$, $P = 0.22$; for males: $r = 0.60$, $P = 0.29$.
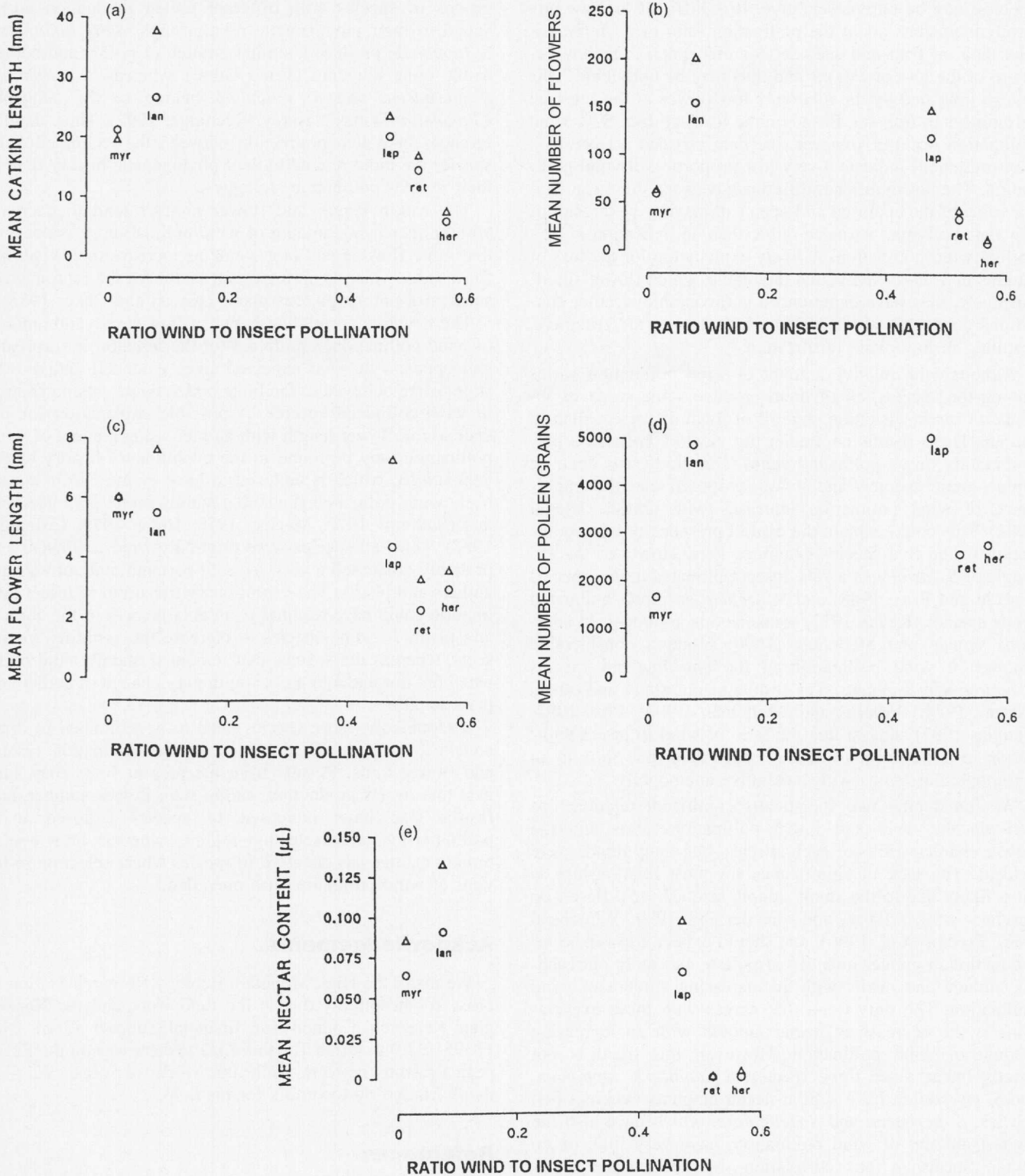
fruit set data). The pollination ratio for *S. lapponum* is 0.47, suggesting that a considerable amount of wind pollination (47%) occurs. *Salix myrsinites* seems to be mainly insect pollinated, since the fruit set data suggested that only 2% wind pollination occurred. However, *S. myrsinites* was already late in its flowering period when field work started and few males were still flowering, so the results for that species may be unrepresentative. It is difficult to draw any firm conclusions about the pollination ratio of *S. herbacea*. The data on fruit and seed set for this species are few because of the loss of catkins and thus may be unreliable. This is also indicated by the relatively low power of the *t* test on differences in fruit set. Based on the fruit set data, 57% wind pollination occurs. However, the data on seed set per fruit (not presented) indicate a very low proportion of wind pollination. The nonsignificant difference between the mean fruit set value of the enclosed and open catkins may be a result of few data and great variance rather than an indication of prevailing wind pollination. A likely explanation for the loss of catkins in *S. herbacea* is that the plants, which flower simultaneously, may have experienced unfavourable weather conditions during the short period when stigmas are receptive, resulting in low ovule fertilization.

Although the relative amount of wind pollination varies among the species, all of them produce some seeds in absence of insect visitation, and all of them disperse pollen in the air. These results contradict the view of *Salix* as a predominantly insect-pollinated genus. Our study was done on alpine–arctic species, and it was proposed that the importance of wind pollination increases with altitude (Regal 1982). This could explain the overall presence of wind pollination in the five species examined here. However, the example of *S. lasiolepis*, a 98% insect-pollinated alpine species (Sacchi and Price 1988) and *S. arctica*, an insect-pollinated arctic species (Kevan 1972) weakens this argument. In addition, Vroege and Stelleman (1990) found a considerable amount of wind pollination in the two lowland species *S. caprea* and *S. repens*. The results of our study and others (Argus 1974; Vroege and Stelleman 1990; Fox 1992; Douglas 1997) suggest that the ratio of wind to insect pollination in *Salix* is intermediate, rather than that *Salix* is an entomophilous genus with facultative anemophily.

We found only two statistically significant relationships between the wind and insect pollination ratios and the flower characteristics of each species, but many trends were evident. The lack of significance for most relationships is most likely due to the small sample size ($N = 5$). Based on previous works (Fægri and van der Pijl 1979; Whitehead 1983; Proctor et al. 1996), one should expect a lower nectar production, a greater quantity of pollen, and more outreaching anthers and pistils with an increasing amount of wind pollination. The only trend that agrees with these expectations is the decrease in nectar amount with an increasing amount of wind pollination. However, this trend is not strictly linear, since three species, *S. lanata*, *S. lapponum*, and *S. myrsinites*, have similar nectar amounts, whereas two species, *S. herbacea* and *S. reticulata*, which have also the highest amount of wind pollination, have very little or no nectar. Spearman rank correlation showed that the nectar content for male and female catkins is positively correlated to the flower size. Thus, given the negative relationship between flower size and the amount of wind pollination, the negative relationship between nectar content and the amount of wind pollination might be an artefact.

The pollen production per anther showed no relationship to the pollination ratio. This is particularly striking for *S. lanata* and *S. lapponum*, as they have a very similar pollen production but different pollination ratios. The two groups of species with different pollen production correspond to their phylogenetic relationships. *Salix lanata* and *S. lapponum* produce a similar amount of pollen and belong to the same subgenus (*Vetrix* Dum.), whereas *S. myrsinites*, *S. herbacea*, and *S. reticulata* belong to the subgenus *Chamaetia* (Dum.) Nasarov (Rechinger 1992). Thus, the differences in pollen production between the examined *Salix* species are more related to their phylogenetic history than to their present pollination strategies.

The catkin length and flower number tend to decrease with an increasing amount of wind pollination. It is possible that more flowers and larger catkins increase their visibility for insects, which is an important factor for the reproductive success of entomophilous plants (Kevan and Baker 1983).

The negative correlation between flower size and amount of wind pollination, significant for the females, is surprising and opposite to what expected since extended anthers and stigmas are believed to facilitate pollen dispersal and capture in wind-pollinated species. A possible explanation for the decrease in flower length with an increasing amount of wind pollination may be found in the evolutionary history of the genus *Salix*, which is believed to have evolved from an entirely wind-pollinated genus to a mainly insect pollinated genus (Stebbins 1970; Meeuse 1978; Hesse 1979; Cronquist 1988). Thus, all species were originally wind pollinated and probably possessed a very reduced perianth and outreaching anthers and pistils. The evolutionary transition to insect pollination could have resulted in an enlargement of the anthers and pistils in some species to increase the visibility for insects, whereas the species that remained mainly wind pollinated did not undergo any evolutionary change of anther and pistil length.

Undoubtedly, more species need to be examined to draw conclusions on the relationship between pollination system and flower traits. Results from the present study may suggest that nectar production, catkin size, flower number, and flower size have increased in species exposed to a pollinator-mediated selection regime, whereas little evolutionary change has occurred in species where selection in favour of wind pollination has prevailed.

Acknowledgements.

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