

# Pollination Effectiveness and Pollen Dispersal in a *Rhododendron ferrugineum* (Ericaceae) Population

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**Abstract:** Many alpine plants are predominantly outcrossing, thus plant reproductive success is highly dependent on effectiveness of pollinators. How pollinators transfer pollen from one flower to another is of great interest in understanding the genetic structure in plant populations. We studied (1) the role and effectiveness of insect visitors for pollination, and (2) their contribution as pollen vectors for gene dispersal in a *Rhododendron ferrugineum* population. Various insect visitors were recorded, including Hymenoptera, Diptera, Coleoptera, and Lepidoptera. The most frequent and effective insects were honey bees and bumblebees. Muscid flies were considered as important pollinators, particularly due to their relatively high visitation rate. Syrphid flies, Formicidae, and Coleoptera were ineffective in transporting pollen, while the effectiveness of Lepidoptera and Empididae was negligible. A fluorescence labelling experiment revealed that pollen dispersal was restricted (0–2 m) in a dense *R. ferrugineum* stand and decreased in a leptokurtic fashion. This might lead to geitonogamous self-pollination that could explain the close relationship between individuals found in genetic studies of *R. ferrugineum*. However, some pollen grains may travel 40–45 m, which implies the occurrence of cross-pollination through the foraging activities of bumblebees and honey bees.

**Key words:** Alpine plant, pollination effectiveness, pollen dispersal, pollen flow, *Rhododendron ferrugineum*.

## Introduction

In alpine environments, opportunities for plant growth are restricted to the spring and summer months. As a consequence, flowering must be limited to a very short period of the year (Kudo, 1993; Bergman et al., 1996). Though more than 90% of alpine plants can propagate vegetatively (Körner, 1999), sexual reproduction is important because the production of seeds is necessary for dispersal and colonization of new sites (Kevan, 1989). Harsh and unpredictable weather conditions (e.g., strong winds, frost, snow...) reduce pollinator activity, and

thus the opportunity for cross-pollination (Yumoto, 1986). Characters enabling self-pollination may be promoted as they assure seed production (Callaghan and Jonasson, 1995; Richards, 1997). However, many alpine plants are predominantly allogamous (Müller, 1881; Arroyo et al., 1982; Kudo, 1993; Bingham and Orthner, 1998).

The behaviour of pollinators can strongly influence reproductive success and gene flow in flowering plant populations (Barrett and Harder, 1996; Cartar and Real, 1997). Populations of many long-lived plants produce a large number of blossoms in a single flowering episode, which is likely to affect pollinator movement and foraging behaviour, and thus influence the breeding system of plants (Geber, 1985) and the genetic structure of plant populations (Richards, 1997). Flowers are often visited by many species of insects with different efficiencies in transporting pollen (Herrera, 1987; Young, 1988). Pollen load on an insect not only indicates the degree of floral constancy but is also useful in assessing the relative efficiency of different insect pollinators (Dorr, 1981). One insect species may be a more efficient pollinator than another when (1) it delivers pollen more often to the stigma of a visited flower (Motten et al., 1981; Motten, 1983), and (2) a greater number of pollen grains is left on the stigma (Bertin, 1982; Motten, 1983). Numerous studies have shown that in alpine and arctic environments Diptera and Hymenoptera are the main pollinators of entomophilous flowers. In some cases, the activity of Hymenoptera is limited and Diptera tend to predominate (Arroyo et al., 1982; Levesque and Burger, 1982; Mc Call and Primack, 1992; Totland, 1993), but in others bumblebees are the major visitors (Bergman et al., 1996; Jacquemart and Thompson, 1996; Mahy et al., 1998). More than one hundred years ago Müller (1881) stated that butterflies (Lepidoptera) were frequent visitors and pollinators above the timberline in the European Alps, and more recently other studies reached the same conclusion (Primack, 1978; Arroyo et al., 1982). Empididae have been considered as important flower visitors in the arctic (Downes, 1970; Kevan, 1973).

We studied the pollination of *Rhododendron ferrugineum* L. (Ericaceae), an evergreen shrub with a mean height of 70 cm, which grows in the subalpine zone in the Alps from about 1600 to 2200 m. It is able to dominate subalpine landscape on north-to-west-facing slopes, frequently reaching 90–100% of the cover by out-competing other species. This species reproduces both vegetatively (by layering) and sexually. Asexual

propagation occurs when the shrub is 50–60 years old (Pornon et al., 1997) and occurs preferentially downhill. The snow cover, during winter, pushes branches downslope to the ground where they can root and give rise to a new individual (Escaravage et al., 1998). Inflorescences consist of 5–22 bright red, nectariferous tubular flowers. *Rhododendron ferrugineum* produces a high number of flowers per square metre of heathland (more than 3000 flowers per m<sup>2</sup> in some places; Escaravage et al., 1997; Pornon et al., 1997). The species is a “steady state” strategist: it blooms asynchronously and flowering in the population is staggered over 20–30 days (Escaravage, 1997). Flowers last about 10 days, are protandrous (poricidal anthers mature before the stigma), and are initiated the year before anthesis. Each flower produces 480–600 ovules and about 400 000 pollen grains (Escaravage et al., 1997). *Rhododendron ferrugineum* is a self-compatible species, visited by many insect species not documented prior to this study. Emasculation and bagging experiments demonstrated that both selfing and entomophilous out-crossing occur (Escaravage et al., 1997). Previous genetic studies revealed high genotypic diversity and close relationships between genets in a closed population (Escaravage et al., 1998; Pornon et al., 2000), and so it is therefore of great interest to know how pollination activity contributes to selfing vs. outcrossing.

The objectives of this study were (1) to determine what insect taxa may be important pollinators in *R. ferrugineum*, (2) to determine the effectiveness of various pollinators in carrying pollen grains, and (3) to estimate pollen flow within a dense *R. ferrugineum* population.

## Materials and Methods

### Study site

This study was carried out above the alpine tree line in the sub-alpine zone, at approximately 2000 m a.s.l. on Mt. Patscherkofel near Innsbruck (Tyrolean Central Alps, Austria; 47°13'N, 11°27'E). The study site is composed of *R. ferrugineum* mixed with other ericaceous shrubs, such as *Vaccinium myrtillus*, *V. uliginosum*, *V. vitis-idaea*, and *Loiseleuria procumbens*. In some places, *R. ferrugineum* forms dense stands, with cover reaching nearly 95%, while in other places individuals are more scattered and cover is 13–30%. The study population is located on a west-facing slope reaching on average 36.3% ± 10.9 SD.

### Experimental design

#### Flower visitors

Field studies were conducted over the entire flowering period of the population, from mid-June to the end of July in 1999. Observations were carried out on a total of 17 days, every 2 h from 9:00 h through 19:00 h, focusing on groups of flowers (on average 20 inflorescences on a ca. 2-m<sup>2</sup> surface) for 20 min at a time. We recorded the number and type of flower visitors that touched either the anthers or the stigma. Weather conditions were noted for each observation as sunny, cloudy, foggy, or rainy. Insects were classified as Hymenoptera (i.e., Apidae divided in bumblebee and honey bee, and Formicidae), Diptera (i.e., Muscidae, Syrphidae, Empididae), Coleoptera, and Lepidoptera.

### Visitor effectiveness

#### Visitor behaviour on flowers

The foraging behaviour of insects was observed throughout the flowering period. For each insect group we recorded the foraging time per flower, the part of the body in contact with the stigma, and the number of flowers visited per individual plant during 20 min.

#### Pollen load on insects

To determine the pollen load on insects, throughout the study period individuals of each insect group (except for Empididae and Lepidoptera which were negligible) were collected, following or during a visit, using an aspirator or a net, and killed in jars with acetyl acetate. The pollen load was estimated for 30 individuals from each insect group. To map the location of pollen grains, we divided the body of the insect into six parts (head, thorax, and abdomen, ventral and dorsal sides). We used clear plastic tape to remove pollen from the different parts of the insect body and then placed the tape directly onto a microscope slide for identification and counting of pollen grains. For both bumblebees and honey bees, pollen grains packed in the pollen basket of their hind legs (*corbiculae*) were not considered in the estimation of the pollen load since, once packed, this pollen does not further participate in pollination. The composition of pollen loads was examined in order to estimate the floral constancy of the insect visitors to *R. ferrugineum*.

#### Stigmatic pollen carry over

Experiments were performed to determine the number and the proportion of outcross pollen grains deposited on the stigmas of successively visited flowers. We only considered the insects with the most significant pollen load (i.e., honey bees and bumblebees). In *R. ferrugineum*, anthers are dehiscent before flowers open, so that in a single flower, self pollen grains on the stigma cannot be distinguished from outcross pollen grains. Moreover, it is difficult to remove the stamens without damaging the corolla, which becomes less attractive for pollinators. To overcome these difficulties, we chose to use a dead insect to approximate the effect of a live one, a method which proved to be efficient in *Delphinium virescens* using a dead bumblebee (Waddington, 1981). For both insect groups we performed the following experiment: just after inflorescence bud break, five closed flowers per inflorescence were emasculated to avoid contamination with self pollen. When the flowers had opened, a recently killed bee was artificially loaded with pollen by simulating its natural position during a live visit. Subsequently, the bee was inserted sequentially into five virgin flowers with receptive stigmas for 5 s in the same way. Each pollinated stigma was removed, mounted on a microscope slide and the pollen grains were counted. After the fifth visit we counted the number of pollen grains remaining on the body by using adhesive tape as described in the previous section. This experiment was replicated using 30 honey bees and 30 bumblebees, thus a total of 300 flowers were studied. We (1) estimated the number of pollen grains deposited on the stigmas of the five successively “visited” flowers for both bumblebees and honey bees, and (2) characterized the proportion of pollen deposited by dividing the number of pollen grains de-

posited on the five successively “visited” flowers by the number of pollen grains present on the bee’s body at the beginning of the experiment. Data were statistically analyzed using SPSS software (SPSS, 1999): for each flower rank, the mean number and proportion of pollen grains deposited on the stigma was compared between honey bees and bumblebees by performing Student’s *t*-test. Values for proportions were arcsine-transformed prior to analysis.

#### Pollination effectiveness estimates

We used observations of active foraging behaviour to estimate the basic pollination effectiveness (PE) of all major visiting groups (Table 1). Estimates of pollination effectiveness were based on individual analyses of insect size, foraging behaviour, and analyses of the consistency of movement within or between inflorescences, and between plants. The values assigned ranged between 0 and 1 (Table 2). The scale had 11 categories at intervals of 0.1. A score of 0 was given to an insect for which no foraging and movement were observed, while a value of 1 was assigned to an active foraging insect with much movement between flowers, inflorescences, and plants. Intermediate scores were assigned based on evaluation of foraging behaviours, body size, and body aspect (hairy or not). For example a score of 0.1 was assigned if insects entered the corolla without contacting either anthers or stamens and only weak movement inside the flower. These created values, though highly subjective, represent the probability that foraging activity results in pollination (Lindsey, 1984).

#### Pollination importance

The relative pollination importance of each insect group was calculated according to Lindsey (1984) as follows: PIV (Pollination Importance Value) =  $VR \times PCC \times C \times PE$ , where VR = visitation rate of each insect group (%), PCC = pollen carrying capacity expressed as the relative proportion of total *R. ferrugineum* pollen sampled from all visitor groups which was carried by the insect, C = constancy expressed as the average proportion of *R. ferrugineum* pollen contained in each visitor group pollen load, and PE = pollination effectiveness previously estimated. The PIV gave a relative pollination importance for each visitor group. For ease of expression, each insect species was given a Pollination Importance Index (PII), representing a relative proportion of pollination importance, according to the formula:

$$PII \text{ (Pollination Importance Index)} = PIV / \sum PIV \times 100$$

#### Pollen travel distance

This experiment was carried out in a closed *R. ferrugineum* stand (95% of cover). We estimated the pollen flow between *R. ferrugineum* inflorescences by using fluorescent dye powder as a pollen analogue (Kearns and Inouye, 1993). The use of fluorescent dye powder has been seen as an efficient method to reveal pollen movements in *Ipomopsis aggregata* (Waser and Price, 1982). Even though dye particles may differ from pollen grains in their specific weight, size, and shape, and thus in their adhesive capacity, this method has proved to be efficient, especially in studies with sticky pollen such as that of *R. ferrugineum* to which the powdered dye easily adheres (Dafni, 1992). We selected individual plants with a surface of 4 m<sup>2</sup> and the dye was deposited on the pollen of freshly dehisced an-

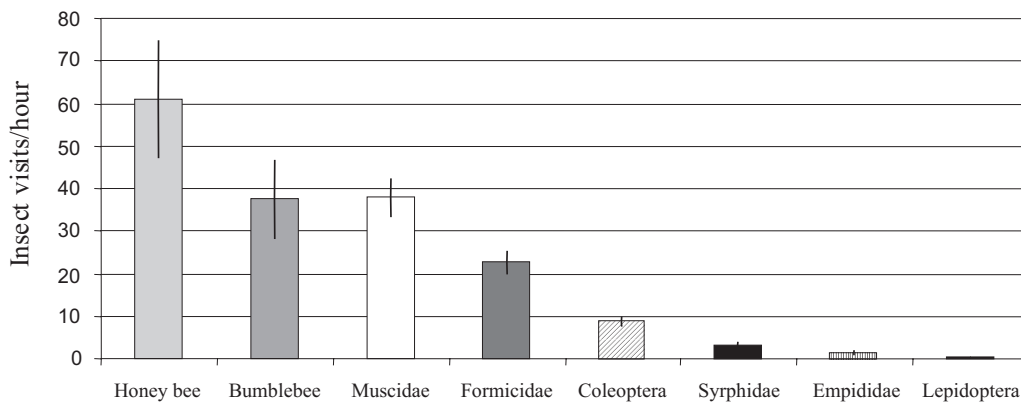
**Table 1** Visitors to flowers of *Rhododendron ferrugineum*

Order	Family	Species
Hymenoptera	Apidae	<i>Apis mellifera</i>
		<i>Bombus pyrenaicus</i>
		<i>Bombus lucorum</i>
	Formicidae	<i>Bombus sichelii</i> ssp. <i>alticola</i>
		<i>Bombus terrestris</i>
		<i>Formica lugubris</i>
		<i>Formica lemani</i>
Diptera	Muscidae	<i>Muscia</i> sp.
	Syrphidae	<i>Syrphus</i> sp.
	Empididae	<i>Empis</i> sp.
Coleoptera	Cantharidae	<i>Cantharis</i> sp.
	Staphylinidae	<i>Amphicroum canaliculatum</i>
		<i>Antophagus alpinus alpinus</i>
		<i>Eusphalerum anale</i>
		<i>Eusphalerum stramineum</i>
Lepidoptera	Nymphalidae	<i>Aglais urticae</i>

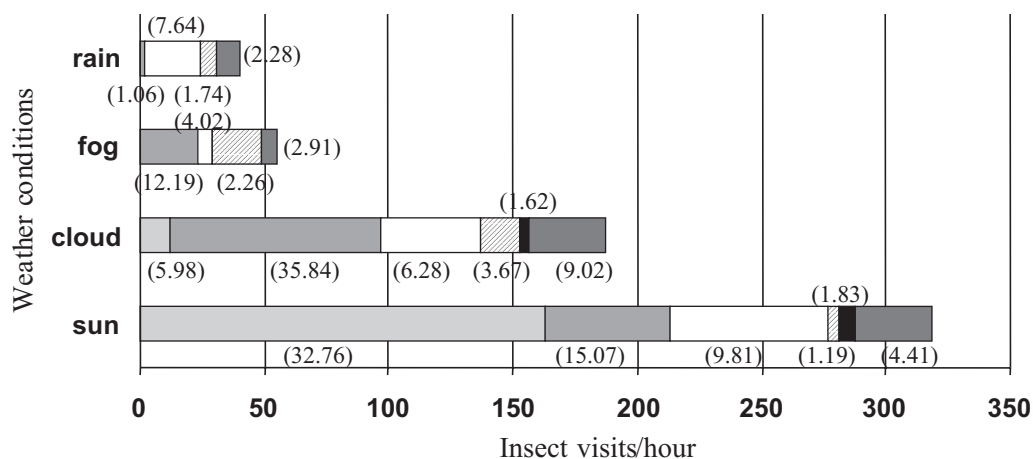
**Table 2** Pollination importance for all visitor groups. The PI Value (PIV) of each visitor group is calculated from the formula:  $PIV = VR \times PCC \times C \times PE$  (VR = visitation rate, PCC = pollen carrying capacity, C = constancy, PE = pollination effectiveness). The PI Index (PII) is expressed as a percentage of the total PI values for all visitor groups

Insect group	C	PCC	VR	PE	PIV	PII
Honey bee	0.99	0.4324	36.32	1	15.55	57.37
Bumblebee	0.92	0.5641	22.25	1	11.54	42.59
Muscidae	1	0.0006	21.60	0.6	0.0073	0.0269
Coleoptera	1	0.0026	4.88	0.1	0.0012	0.0046
Syrphidae	1	0.0001	1.91	0.3	0.0001	0.0004
Formicidae	1	0.0001	12.10	0.1	0.0001	0.0005

thers early in the morning before any pollinator activities had started. In the evening of the same day, all flowers within 45 m (between 0 and 45 m) from the dyed source individual, and over a width of about 1.5 m (45 × 1.5 m transect) were collected in four different directions and analyzed at the laboratory for a fluorescence signal using a UV lamp (Roth®: 6 V; 254–366 nm). We chose 12 distance intervals as shown on Fig. 5. Distances were chosen according to the geographical distribution of individual plants. Between 100 and 300 flowers were collected at each distance, depending on the dye. Directions were chosen in such a way that there were enough flowers to collect from every distance range. The angles between two directions ranged between 45 and 90 degrees. This experiment was repeated four times using 4 dyes (2 dyes per day; Radiant Color Corp.: red, blue, orange, and yellow) with different shrubs. For a given distance interval, the percentage of dyed flower was computed as the number of flowers with dye divided by the total number of investigated flowers in the distance interval × 100.



**Fig. 1** Insect visit frequencies to *Rhododendron ferrugineum* flowers.



**Fig. 2** Insect visit frequencies to flowers in relation to weather conditions during summer 1999. Values in parentheses represent standard error. For bar symbols see Fig. 1.

## Results

### Flower visitors

We identified 16 different insect species visiting the flowers of *R. ferrugineum* at the study site (Table 1). A total of 5820 visits were observed. Mean numbers of visits per hour, for the major insect groups, are presented in Fig. 1. Over the whole flowering period, the most frequent visitors were honey bees ( $60.95 \pm 13.82$  visits  $h^{-1}$ ), followed by bumblebees and Muscid flies ( $37.44 \pm 9.44$  and  $37.84 \pm 4.62$  visits  $h^{-1}$ , respectively) and Formicidae ( $22.5 \pm 2.73$  visits  $h^{-1}$ ). Other insects were much less frequent ( $< 10$  visits  $h^{-1}$ ) and Empididae and Lepidoptera were considered to be negligible visitors ( $< 0.5$  visit  $h^{-1}$ ).

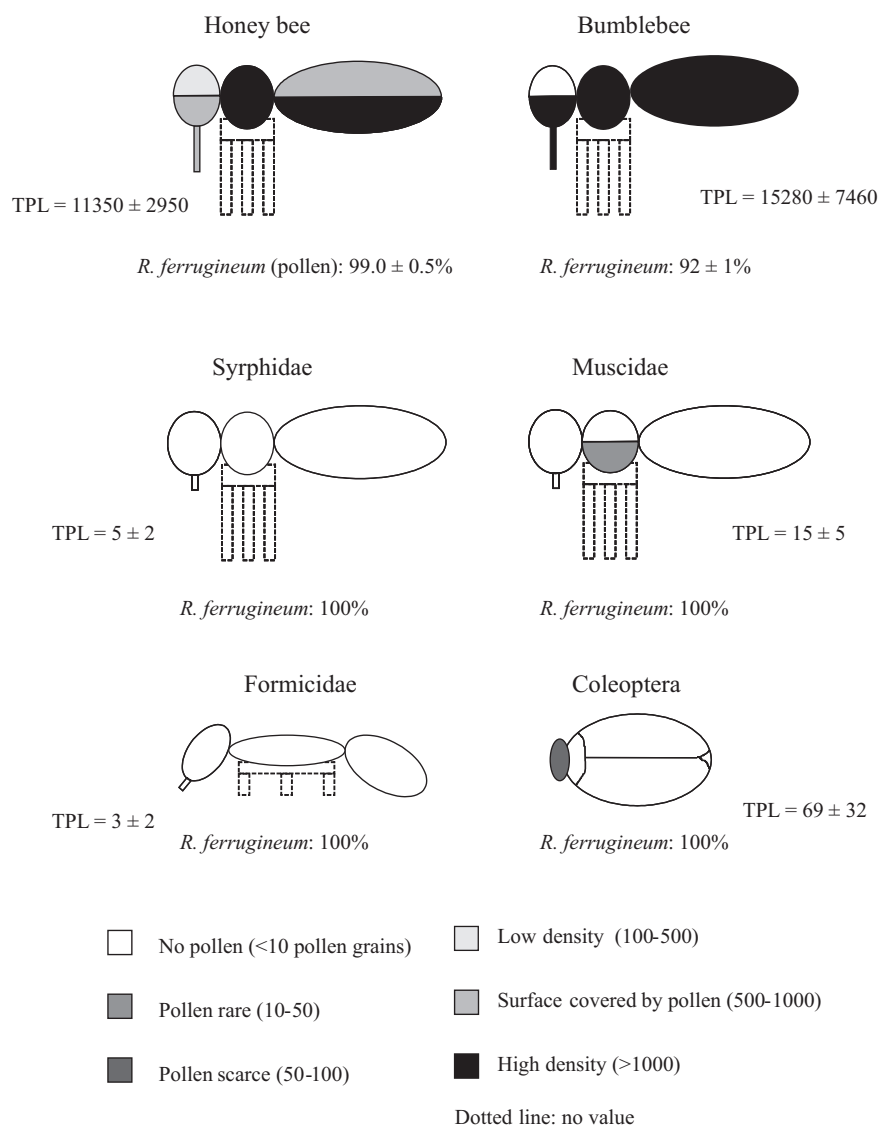
Of the one hundred and two 20-min observations, 41% were made in sunny weather conditions, 34.4% during rain, 17.7% with cloudy conditions, and 6.9% in fog. Insect visitation rates for each weather condition are presented in Fig. 2. Empididae and Lepidoptera were not considered. In sunny weather, honey bees were the most frequent flower visitors ( $162.93 \pm 32.76$  visits  $h^{-1}$ ), while bumblebees and Muscid flies reached respectively  $50.56 \pm 15.07$  and  $63.24 \pm 9.81$  visits  $h^{-1}$ . Formicidae appeared to be very active in visiting flowers ( $30.81 \pm 4.41$  visits  $h^{-1}$ ). When the weather deteriorated the total number of visits decreased. During cloudy weather the visit frequency of honey bees was reduced ( $11.85 \pm 5.98$  visits  $h^{-1}$ ) but the frequency of

bumblebees increased ( $84.61 \pm 34.85$  visits  $h^{-1}$ ). The occurrence of fog and rain was always accompanied by temperatures between 5 and 10°C (data not shown). Under these conditions no honey bee was observed and only a low amount of bumblebees were active ( $23.62 \pm 12.19$  and  $1.75 \pm 1.06$  in the fog and the rain, respectively), while Muscid flies and Coleoptera were observed visiting flowers. Formicidae foraged into corolla in all weather conditions.

### Visitor effectiveness

#### Visitor behaviour on flowers

Bumblebees and honey bees systematically foraged on many flowers in a single inflorescence or individual plant. The foraging time per flower for honey bees and bumblebees, respectively, was  $4.01 \pm 1.41$  s and  $1.71 \pm 1.39$  s (ranging from 1 to 6 s for both). During foraging, the stigmas of visited flowers were mainly touched by the ventral thorax and sometimes with the dorsal head. Among all insects examined, bumblebees and honey bees had the highest pollen load (Fig. 3). An estimate of the total pollen load (TPL) was about 11 350 and 15 270 pollen grains for honey bees and bumblebees, respectively. The pollen load of all insect groups examined was mainly composed of *R. ferrugineum* pollen. For bumblebees 92% of the pollen load were *R. ferrugineum* pollen, and the remaining 8% were pollen of different species that flower at the same time, such



**Fig. 3** The distribution of pollen on the bodies of insect visitors and the percentage of *Rhododendron ferrugineum* pollen found in pollen loads. Means ± SE. n = 30 for each insect group. Note: TPL (Total Pollen Load) was computed as the sum of pollen grains present on the different parts of the body, except for the legs.

as *Ranunculus* sp. For honey bees 99% of the pollen observed was *R. ferrugineum* pollen, and the other 1% was the pollen of anemophilous species (pine or spruce). With their high body pollen loads, their foraging behaviour that promotes frequent contact with stigmas and their high activity, bumblebees and honey bees were the most effective pollinators of *R. ferrugineum* in the study population.

The foraging behaviour of Muscid flies differed from that of bumblebees and honey bees in several respects: (1) the mean foraging time (± SD) per flower was 52 ± 15 s (ranging from 1 to 203 s), (2) when visiting a flower these insects either behaved like real foragers by eating nectar or pollen (we found pollen grains in the digestive system after perforating the abdomen by accident), and regularly touching the stigma, or most of the time they stayed on flowers without foraging. (3) All individuals examined bore only a few isolated pollen grains. (4) They foraged on a few flowers on the same inflorescence or plant, and landed on flowers or leaves. Syrphid flies showed similar foraging behaviour to Muscid flies. The foraging time

per flower was shorter, 10.4 ± 9.8 s on average (ranging from 1 to 30 s) but, like Muscid flies, they bore few pollen grains. The foraging time per flower of Formicidae ranged from 10 s to more than the observation time (20 min); they often visited many flowers of the same inflorescence. For Coleoptera, the foraging time was difficult to estimate. Their size did not exceed 2 mm in length, and most of the time they stayed inside the corolla where they found food (pollen and nectar) and protection. The smooth body of both Formicidae and Coleoptera is poorly suited to pollen carry over.

**Stigmatic pollen carry over**

The number of pollen grains deposited on the stigma of the five successively "visited" flowers was significantly higher for bumblebees than for honey bees; the former could deposit on average 1039.0 ± 109.3 pollen grains on the stigma of the first flower, while this measure was 588.5 ± 88.7 for honey bees (Fig. 4). In the five successively "visited" flowers, both bumblebees and honey bees deposited on the receptive stigmas 41.7%

of the carried pollen on the first flower and about 80% on the first three flowers (Fig. 4). Little pollen remained on the bee's bodies after the fifth flower.

**Pollination importance**

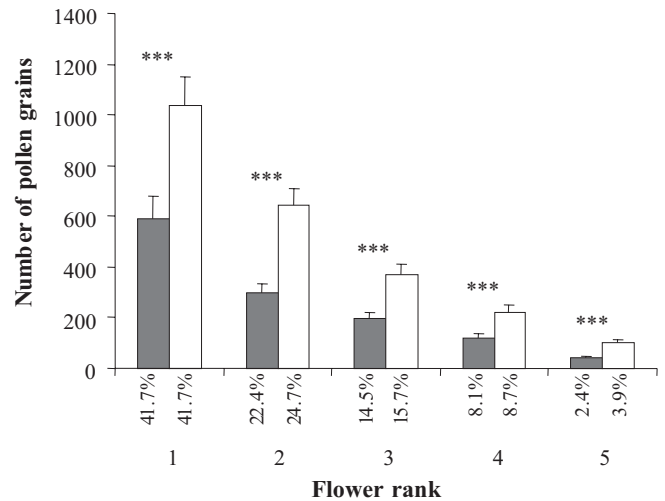
The Pollination Importance Value (PIV) was highest for honey bees (15.55) and bumblebees (11.54; Table 2). Pollinator Importance Index (PII) revealed that honey bees and bumblebees were, respectively, responsible for 57.37% and 42.59% of pollination. For Muscid flies PIV and PII were only 0.073 and 0.0027%, respectively. For other insects these values were much lower.

**Pollen travel distance**

About 61% of the dyed flowers occurred between 0 and 2 m from the dyed source individual, then the percentage rapidly decreased, but we found that some grains could travel at least 40 m (Fig. 5). This suggests only a short travel distance for most pollen grains.

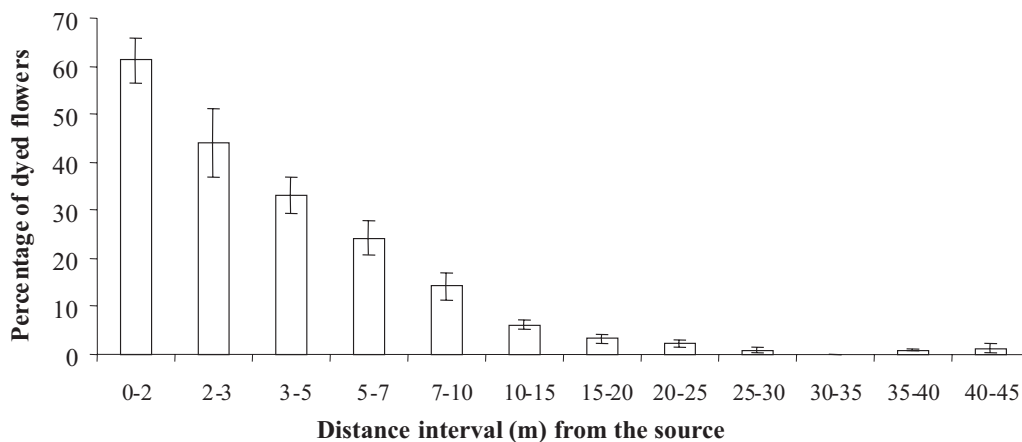
**Discussion**

Over the whole flowering period, the most frequent flower visitors to *R. ferrugineum* were honey bees, followed by Muscidae and bumblebees. Under sunny weather conditions, the number of honey bees visiting a flower was about 162.93 visits h<sup>-1</sup>, dramatically decreasing when weather conditions were bad. On the other hand, bumblebees were still active on flowers in rain and in fog. Honey bees and bumblebees had short foraging times (4.01 ± 1.41 and 1.71 ± 1.39, respectively) compared to flies, Formicidae and Coleoptera, but bore the highest number of pollen grains (11350 and 15270, respectively), with a high flower constancy for honey bees. Therefore, the PIIs were higher for honey bees and bumblebees (57.37% and 42.59%, respectively) than for the other insects. Together, they were responsible for nearly 100% of pollination. Stigmatic pollen carry over was very efficient for both honey bees and bumblebees since they could deposit, in a single visit, about 588 and 1000 pollen grains, respectively, which represented 41.7% of their total pollen load. In a closed stand, pollen travel distance through bee foraging activity was limited to a few metres from the dyed source individual.



**Fig. 4** Distribution of the mean number of pollen grains deposited on the stigma of the 1st to the 5th flower "visited" by honey bees (■) and bumblebees (□). Vertical bars represent standard error, n = 30. For each flower rank, means are compared using Student's t-test (\*\*\*)  $p < 0.001$ ). Values below the bars represent the proportion of pollen grains deposited by each insect class. For each flower, rank values are not significantly different between honey bees and bumblebees.

In this study, the pollination importance index, observations of foraging behaviour, pollen load, stigmatic pollen carry over, and the relative abundance of insects clearly showed that honey bees and bumblebees were the most effective pollinators of *R. ferrugineum*. Our results on pollen load revealed that individual bees visiting *R. ferrugineum* exhibit considerable flower constancy. Honey bees are more constant to *R. ferrugineum* flowers than bumblebees since almost pure (99%) and mixed (92%) *R. ferrugineum* pollen load were found on the honey bees's and bumblebees's bodies, respectively. Bumblebees, although temporally specialized in one flower species, occasionally forage on other minor species (Cruden, 1971; Heinrich, 1979). Constancy leads to efficiency by minimizing the time wasted by the bee in switching between handling techniques (von Frisch, 1953; Waddington, 1983; Waser, 1986). In addition, it is beneficial for the plant because less pollen is misplaced by the bee onto heterospecific flowers, resulting in a higher rate of successful fertilisation (Wilson and Stine, 1996).



**Fig. 5** Pollen travel distance within a *Rhododendron ferrugineum* population indicated by fluorescent dye. Vertical bars represent standard error.

However, a limited amount of inconstancy from the pollinator should not interfere with pollination efficiency since a mixed pollen load will often contain more than enough compatible pollen grains (Faegri and van der Pijl, 1979).

Among all insects visiting *R. ferrugineum* flowers, honey bees and bumblebees have the highest pollen loads. This result is consistent with other studies which have found that bee species carry and deposit more pollen grains onto the stigma than butterfly and fly species (Ehrenfeld, 1979; Lindsey, 1984). Many authors describe bumblebees as the most important pollinators of diverse temperate plants with poricidally dehiscent anthers, such as Ericaceae species (Free, 1970; Haslerud, 1975; Reader, 1977; Dorr, 1981).

In our study, Lepidoptera and Empididae were rarely observed in *R. ferrugineum* flowers, and seem to be of little importance as pollinators. However, other studies have shown that in alpine and arctic environments these two insect groups were important pollinators (Arroyo et al., 1982; Downes, 1970; Kevan, 1973; Levesque and Burger, 1982; Mc Call and Primack, 1992; Totland, 1993). From our results, it is clear that Coleoptera, Formicidae, and Muscid flies were ineffective in transporting pollen compared to honey bees and bumblebees. However, in *R. ferrugineum*, the pollination effectiveness of Muscid flies was important. Even though individuals had a low PII and bore only a few pollen grains, because of their high relative abundance they may be considered as relatively important pollinators. Their number may compensate for lower individual pollination effectiveness. This is in accordance with Tikhmenev (1979), who asserted that some *Rhododendron* spp. of the Tundra are pollinated by bees and Diptera. Although Formicidae were relatively frequent visitors of *R. ferrugineum*, they could not be considered as pollinators even though some studies conducted on alpine plant species contradict this (see Peakall et al., 1991; Puterbaugh, 1998; Gomez, 2000). In this study, the Coleoptera found in *R. ferrugineum* flowers were mostly Staphylinidae and therefore very small in size (ca. 2 mm long). Most of the time they stayed inside the corolla where they found food and perhaps protection, warmth, or mating and brood sites. The same has been reported for other insect groups (e.g., thrips, flies) but examples with significance for pollination are rare (Kevan and Baker, 1983; Mahy et al., 1998). Finally, *R. ferrugineum* can be considered as moderately generalist because only a few of the diverse visitors have high potential pollination effectiveness.

In the present study, bumblebees deposited significantly more pollen per visit than honey bees. This seems to be a common trend (Bingham and Orthner, 1998). *R. ferrugineum* pollen carry over for both bumblebees and honey bees supports the typical pattern in which up to 50% of pollen is deposited on the first flower visited and deposition on subsequent flowers decreases in a leptokurtic fashion (Morris et al., 1994; Richards, 1997). As honey bees and bumblebees systematically forage on many flowers of the same inflorescence or individual, only the first three visited flowers on each plant would receive substantial amounts of outcross pollen. As *R. ferrugineum* produces 480–600 ovules per ovary (Escaravage et al., 1997), a single visit is sufficient to fertilize all the ovules. In our study, stigmatic pollen carry over might be over-estimated: it is well known that bees regularly groom pollen from their bodies and pollen loss through grooming is considered very important

(Thomson, 1986; Harder and Thomson, 1989; Harder, 1990). As we used a dead insect, this natural grooming was not taken into account. This may have a strong effect on stigmatic pollen carry over as the pollen pool may be constantly reduced and therefore pollen carry over shortened (Thomson, 1986). In our study, considering the huge amount of pollen carried by bees, stigmatic pollen carry over is expected to be very efficient.

In the study of a dense *R. ferrugineum* population, pollen flow was restricted since most of the dyed flowers were located within the first 2 m of the dyed source individual plant. As the surface of the dyed individual was about 4 m<sup>2</sup>, this suggests that geitonogamous pollination was likely to be important. As in stigmatic pollen carry over, the leptokurtic pattern of pollen dispersal is almost universal (Zimmerman, 1979; Waddington, 1981; Kevan and Baker, 1983). In such an experiment under natural conditions, it is not possible to know which insect category carried the pollen from one flower to another, but according to our results from the pollen load experiment and observations of pollinator behaviours during foraging, it is likely that the dyed particles were mainly carried by honey bees and bumblebees. However it is possible that some dye deposited on the stigma by one insect is picked up and moved farther by a second visitor in such a way that the pollen travel distance could have been over-estimated. For bee pollinated plants, it is usual to find that at least 80% of flights are less than 1 m in distance, and 99% are less than 5 m (Richards, 1997). However in some species pollen travels much further (30 m in *Primula veris*, Richards and Ibrahim, 1978; 137 m in *Delphinium virescens*, Waddington, 1981; 5.8–14.2 km in some *Ficus* species, Nason et al., 1998). These results depend heavily on plant spacing patterns. Many authors have shown that pollen flow does not equal gene flow; on the contrary, pollinator flight data may underestimate the extent of gene movement within populations (Levin, 1981; Campbell, 1991; Fernster, 1991; Karron, 1995).

*Rhododendron ferrugineum* forms dense patches with many flowers (Escaravage et al., 1997; Pornon et al., 1997) and therefore can be locally very attractive to pollinators. Pollinator behaviour is influenced by flower and plant densities (Levin, 1979). In general, pollinators concentrate on more rewarding patches, and therefore travel less far to the next flower, inflorescence or plant (Klinkhamer and de Jong, 1990). Increasing floral attractiveness might prolong pollinator visitation sequences and this might represent a cost in terms of increased selfing and reduced pollen export to other plants (de Jong et al., 1993; Barrett and Harder, 1996).

*Rhododendron ferrugineum* can produce large clones, up to 20 m<sup>2</sup> with limited intermingling of genets (Escaravage et al., 1998). As geitonogamy increases with plant size (Geber, 1985; Hessian, 1988; Robertson, 1992; Harder and Barrett, 1995), large clones will tend to have more geitonogamous pollination than small clones. This may reduce the fitness of offspring by causing inbreeding depression and pollen discounting (loss of pollen grains that could have been exported to conspecifics, de Jong et al., 1993; Klinkhamer and de Jong, 1993; Barrett and Harder, 1996). Escaravage et al. (1998) and Pornon et al. (2000) have shown that *R. ferrugineum* populations have a high genotypic diversity, however, the similarity index between clone pairs revealed close relationships among genotypes (0.85 on average). Their study demonstrated that the

closer the genotypes are located in the population, the more closely they are related, suggesting that seedling recruitment occurs a few meters from the mother plant. The restricted pollen flow observed in the study population, together with the reproductive assurance mechanisms (stamen dimorphism; see Escaravage et al., 2001), could result in a high proportion of selfed seeds, explaining the close relationship between individuals. This assumption would imply that, in *R. ferrugineum* populations, the mean distance of gene dispersal might not exceed the mean distance of pollen movement, but further studies are needed. The maintenance of a high genetic variability could, on the other hand, be due to the fact that *R. ferrugineum* does not bloom synchronously within an individual plant; rather, it staggers flowering, thus promoting outcrossing. In alpine environments, this "steady state" strategy may be advantageous since it reduces the risk of insects failing to visit the flowers because of bad weather conditions (Bawa, 1983). "Steady state" strategies should be selected to limit the constraints of geitonogamous pollination (inbreeding depression) in multiple-flowered plants (Harder and Barrett, 1995). In *R. ferrugineum* inbreeding depression did not seem to occur at early developmental stages (fertilised ovules, seed set, germination rate; Escaravage, unpublished data). An individual plant reaches maturity at about 10 years old, and we suppose that inbreeding depression might occur at later developmental stages.

Our study provides evidence that bumblebees and honey bees are the most effective pollinators of *R. ferrugineum* in the study population. Their foraging behaviour may, however, lead to numerous self-pollination events, through geitonogamy in a dense population. However, they probably also transfer a small amount of outcross pollen in the population. Further investigations should focus on the pollen travel distance in relation to plant density and flower attractiveness (nectar reward) which is likely to affect the amount of pollen deposited on an individual flower.

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