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Causes and consequences of floral damage in *Aconitum lycoctonum* at high and low elevations in Switzerland

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Abstract The outcomes of interactions among plants and the insects that use their flowers are likely to vary among the physical environments and the communities in which they grow. In this study we quantified floral damage of *Aconitum lycoctonum* in high (>2000 m) and low (c. 500 m) elevation populations in Switzerland. At high elevation, floral damage was frequent and was caused by nectar-robbing short-tongued bumblebees. Nectar robbers make a hole in the flower when they collect nectar. A nectar robber exclusion experiment showed that nectar robbery by short-tongued bumblebees had no effect on the female reproductive success of plants; robbing bees rarely damaged the nectaries, and damage to the petals probably does not decrease flower longevity. In addition, nectar robbers tended to collect pollen during about 10% of their visits. Thus, these bees may act as low-efficiency pollinators and may, at times, be mutualistic associates. At low elevation, the holes in the flowers were caused by beetles (*Meligethes viridescens*) and not by short-tongued bumblebees. The beetles eat pollen and might also consume nectar. Since the beetles gain access to pollen and nectar by entering the flower through its opening, and later chew holes while foraging on floral tissue, the beetles are pollen eaters rather than nectar robbers. Overall, our results show that not all floral damage is caused by nectar robbers and that there can be strong altitudinal variation in the causes and consequences of floral damage.

Keywords Bumblebees · Beetles · Nectar robbery · Pollination · Mutualism

Introduction

Pollination systems can be influenced by non-pollinating but nectar- or pollen-eating visitors that can damage the flowers. Inouye (1980) defined various kinds of floral larceny and differentiated between robbery and thievery. Nectar robbing occurs when a hole is made in a flower, allowing the visitor to obtain nectar and thereby bypassing the opening used by pollinators. Similarly, pollen robbing occurs when a visitor collects pollen without pollinating the flower, and damages floral tissue. By contrast, nectar or pollen thieving occurs when the visitor collects nectar or pollen without pollinating, but without damaging floral tissue. Darwin (1878) listed a large number of plants, including the genus *Aconitum*, that were known to be visited by nectar-robbing bumblebees. He thought that nectar robbery was detrimental for all these plants because he assumed that pollinators did not visit robbed flowers, and thus that only selfed seeds, if any, could be produced. However, we now know that nectar robbers can have a range of effects on plant fitness, and that variation in the consequences of floral damage can be caused by a variety of factors.

Positive effects of nectar robbery on seed set have been found when the nectar robbers sometimes act as low-efficiency pollinators (Meidell 1944; Macior 1966; Koeman-Kwak 1973; Rust 1979; Higashi et al. 1988; Scott et al. 1993; Arizmendi et al. 1996; Morris 1996; Olesen 1996). Additional, positive effects may include longer-distance pollen movement (Zimmerman and Cook 1985), or even the attraction of more pollinators (Hawkins 1961).

There are a number of mechanisms by which nectar robbers reduce seed set. For example, pollinators may reduce the number of pollinating visits by taking advantage of the easy access to the nectar through the hole made by robbers (Kendall and Smith 1976; Reddy et al. 1992). Robbers may also reduce reproductive success by damaging the carpels (McDade and Kinsman 1980; Traveset et al. 1998). The presence of nectar robbers can also change pollinator behavior. Aggressive nectar rob-

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bers can even prevent hummingbirds from visiting the flower (Gill et al. 1982; Roubik 1982), and competition for nectar can lead to partitioning of the flowers between robbers and pollinators (Colwell et al. 1974). For example, hummingbird pollinators visited fewer plants with heavy nectar robbing (Irwin and Brody 1998), and might prefer the more rewarding flowers visited only by pollinators (Roubik et al. 1985). Irwin and Brody (1998) suggest that hummingbirds may avoid robbed flowers due to their extreme energy demands. Therefore, it is advantageous for the birds to make decisions about which plants and flowers to visit.

Most of these studies included only a single plant population. However, as pointed out by Thompson (1994), any analysis of evolving species interactions should include more than just a local population, and should also include geographic structure, since very few pairs of interacting species have identical geographic ranges. Moreover, the degree of specialization between pairs of species varies among physical environments, and they vary with the community context. The few studies that have included more than one population show that it is important to include different abiotic and biotic environments in studies on plant-nectar robber interactions. For example, Traveset et al. (1998) and Roubik et al. (1985) reported differences in the proportion of nectar robber visits, and thence also in the degree of their negative effects, when they studied plants in different populations. Morris (1996) showed that the outcome of robber exclusion experiments in two populations, one at lower and one at higher elevation, was the same in both populations, but he found differences among the populations in the timing of robbing.

We chose the bee-pollinated *Aconitum lycoctonum* L. emend. Koelle (Ranunculaceae) (monkshood) to examine floral damage and its consequences. It has long been reported that short-tongued bumblebees, such as *Bombus wurflenii* Radosz. act as nectar robbers on *A. lycoctonum* at high elevation (Hegi 1974). *A. lycoctonum* occurs from the lowlands to the subalpine zone, whereas some of the presumed nectar-robbing bumblebees only occur at high elevation. Thus the nectar robbers and the plant species do not have identical ranges. We asked: how frequent is floral damage and what animals cause this damage at different altitudes? We quantified floral damage and nectar robbing in four populations at two different altitudes over a 2-year period. At higher elevation, where nectar robbery by short-tongued bumblebees was frequent, we studied the influence of nectar robbery on female success of the plant by performing experiments and making behavioral observations. At lower elevation, where we found holes in the helmet but never observed any short-tongued bumblebees as nectar robbers, we tested whether a common flower-inhabiting beetle, *Meligethes viridescens* Fabricius (Coleoptera: Nitidulidae) (C. Besuchet, personal communication) chewed the holes, and if so, what other effects the beetles had on the flower.

Materials and methods

Natural history of the study system

A. lycoctonum (Ranunculaceae) is a yellow-flowered perennial herb. It is widespread over central and southern Europe where it mostly occurs in shady habitats from the lowlands to the subalpine zone. Due to extreme morphological variability many taxa of different taxonomic rank have been described. However, we showed (Utelli et al. 1999; Utelli et al. 2000) that genetic differentiation among populations from central and southern Europe is low, indicating that *A. lycoctonum* in central and southern Europe is a single species.

The flowers of *Aconitum* have five petals and are zygomorphic. The posterior petal is helmet-shaped, covering the two nectaries. The tips of the nectaries of *A. lycoctonum* are strongly curled. There are numerous stamens and usually three (but up to five) free carpels, each of which develops into one follicular fruit. The flowers are protandrous (Hegi 1974), and the multiflowered racemes open from the bottom to the top.

Numerous insect species visit the flowers of *Aconitum*, including several bumblebee species. How nectar is collected depends on the length of the proboscis and on the ability to bite holes into the helmet. Short-tongued bumblebees act as (1) primary nectar robbers that make holes into the helmet of the blossom to "steal" the nectar, or as (2) secondary nectar robbers that use the hole made by a primary nectar robber to obtain nectar, or as (3) pollen collectors. Long-tongued bumblebees such as *B. gerstaeckeri* Morawitz, *B. hortorum* L., and *B. pascuorum* Scopoli (Utelli and Roy 2000) are nectar collectors or pollen/nectar collectors that pollinate the flower by passing the stamens and pistils when they forage for nectar. *B. wurflenii* has been described as the commonest nectar robber on *A. lycoctonum* in the Pyrenees (Bosch et al. 1997). *B. wurflenii* mainly occurs at high elevation; in the lowlands it is rarely found (Amiet 1996). Several other short-tongued bumblebees have similar colour variants to *B. wurflenii* and it is not possible to distinguish among them in the field. Other likely short-tongued species, in addition to *B. wurflenii* at high elevation, are *B. mendax* Gerst., *B. ruderarius* (Müller) and *B. soroensis* (Fabr.). At both low and high elevation, *B. lapidarius* (L.) and *B. pratorum* (L.) are found (Amiet 1996). *B. lapidarius* and *B. pratorum* have been described as nectar robbers of several plant species (von Hagen 1994).

Study sites

Field studies were performed at four sites during the summers of 1997 and 1998. Two populations at low elevation and two populations at high elevation were chosen to estimate the frequency of floral damage and nectar robbery: Grellingen, Swiss Jura mountains, 450 m; Zürich, Swiss Midland, 500 m; Arolla, southwestern Swiss Alps, 2050 m; and St. Moritz, southeastern Swiss Alps, 2020 m. Field experiments at high elevation were conducted in the population at St. Moritz, field experiments at lower elevation in the population at Zürich. The greenhouse experiment took place in Zürich, at 530 m.

Quantification of floral damage and activity of nectar robbers

First, since a helmet-shaped petal covers the nectaries and robbers have to bite holes in the helmet to get to the nectar, we estimated the percentage of open flowers that were damaged. We thus counted the open and pierced flowers of a subsample of plants in the population, or if the population was small (<100 plants), we made these counts on all open flowers.

Second, we observed nectar robber behavior under natural conditions. One day of observation consisted of eight 30-min observation periods by two observers, resulting in a total of 8 h of observation per day. Two or four plots were alternately observed by the two observers. Each plot contained at least two plants with several

open flowers; the number of plants and open flowers were counted for each plot. The plots were selected to be representative of the range of variation of environmental factors, such as shade, in the population. During the observations, the weather was mostly clear, and the temperature was always above 10°C. We counted the number of flowers visited by each robber and then calculated the number of visits per open flower per time (visitation rate). We did not distinguish between primary and secondary robbers. The insects were identified during the field observations, when possible.

We quantified floral damage and observed nectar robber activity in 1997 in four *A. lycoctonum* populations in Switzerland, two at low elevations (Zürich and Grellingen), and two at high elevations (St. Moritz and Arolla) in the middle and towards the end of the flowering season, and additionally early in the season at St. Moritz and Zürich, at Zürich we only counted holes. In 1998, both methods were used three times (early, middle, and late in the flowering season) in two populations, Zürich and St. Moritz.

We compared the number of pierced flowers in 1997 in four populations (Zürich, Grellingen, St. Moritz and Arolla) at two times during flowering season. We also compared the data of 1997 and 1998 in two populations (Zürich and St. Moritz) at three different times during the flowering season.

For the behavioural data (visitation rate), we could only compare Arolla and St. Moritz as we never observed nectar robbers flying at the two low elevation populations. Visitation rate was square-root-transformed for all analyses. Preliminary analyses showed no significant effect of either time of day or observer on visitation rate, so these terms were dropped from the model. The ANOVA could not be computed for the 0.5-h observation period because there were too many observation periods with no observed nectar robbers. Therefore, we combined four 0.5-h observation periods to give 2-h periods for ANOVA.

Effects of nectar-robbing bumblebees on plant fitness components

A nectar robber exclusion experiment was conducted in the population at St. Moritz in both 1997 and 1998. We chose two neighbouring flower buds on 60 randomly chosen plants and marked them for pairwise comparisons. The helmet of one flower was covered with a piece of dialysis tubing, the other one was left open. Observations suggested that the bees were not disturbed by the dialysis tubing because some flowers were robbed at the edge of it and pollinators visited normally. All flowers were checked about 1 week later to make sure that the covered flower was not robbed and that the other one was. If this was not so, the plant was excluded from the experiment. To prevent loss of seeds as the fruits dried, the fruits were bagged with nylon mesh bags and harvested after ripening. Fruits and seeds were stored in paper bags. We then counted the total number of seeds per flower, weighed the seeds, and calculated the mean weight per seed. The effect of robbing on number of seeds per flower and weight per seed was tested by an ANOVA.

We tested the germination of seeds from robbed and unrobbed flowers from the 1997 experiment. A total of 200 seeds per treatment were used. We put 20 seeds on blotting-paper in each of ten petri dishes, and wetted them with 2 ml 0.05% gibberelic acid solution. The dishes were put into two climatic chambers, on two shelves in each chamber, with a daily cycle of 10 h dark at 2°C, and 14 h full light at 10°C; humidity was always 80%. The experiment was stopped after 4 months. For statistical analysis, all factors (chamber, shelf, dish and treatment) were treated as fixed.

We checked for any visible damage of the nectaries due to nectar robbers by opening robbed flowers. We opened one robbed flower from each of 50 plants in the population at St. Moritz in 1998. To determine whether the nectaries were still producing nectar, the tip of the nectary was pressed between two fingers. This procedure made the standing crop visible.

Since we also observed robbers collecting pollen on *A. lycoctonum* at St. Moritz, we estimated how often these bees collect pollen on *A. lycoctonum*. We followed 24 nectar-robbing bumblebees for as long as possible as they foraged at St. Moritz in 1998. We counted the number of visited flowers, and recorded whether pollen was collected, or nectar robbed.

Autofertilisation in *A. lycoctonum*

If autofertilisation of the flower results in full seed set, provided there is no inbreeding depression, then no negative effect of robbery is expected. We thus tested the ability of *A. lycoctonum* to autofertilise in a paired comparison experiment at St. Moritz 1997 and 1998. We randomly choose 40 plants in 1997 and 60 plants in 1998. On each plant, one bud was bagged with a nylon mesh bag, and a neighbouring bud marked but not manipulated. Fruits and seeds were treated as in the experiment above. We also computed the number of seeds per flower and the weight per seed. Data were analysed as in the experiment above, but number of seeds per flower was log-transformed so that the residuals met the normality assumptions of the ANOVA.

Germinability of seeds of bagged and open-pollinated flowers was tested in 1997. Germination conditions were as described for the previous experiment; 40 seeds of each treatment, 20 in each of two growth chambers were used.

Longevity of artificially pierced and unpierced flowers

Nectar robbery could decrease fitness by decreasing flower longevity and thus pollination opportunity. We thus investigated longevity of artificially pierced flowers versus unmanipulated flowers in a greenhouse experiment. Plants were collected in the field between May 1995 and June 1997 and then maintained in a greenhouse at Zürich. We did the piercing experiment twice in 1998. Both series included 20 plants from a total of 11 different populations originating from different regions in central and southern Europe and from 360 to 2050 m. Two neighbouring flowers were chosen on each plant for a pairwise comparison. The helmet of one flower was pierced every day with a fine needle (Hamilton no. 80427, 0.42 mm diameter), starting on the first day the flower was open. The holes made were similar in size and shape to those observed in the field. The other flower was not manipulated. We counted the number of days each of the flowers was open. Since the series showed no effect in the ANCOVA, this factor was eliminated from the model. To test whether the position of the flower influenced longevity, we calculated a "flower placement index" (FPI=number of the flower from the bottom/total flowers in the raceme) which was used as a covariate in an ANCOVA.

Effects of flower beetles on *A. lycoctonum*

We found holes in the flowers at low elevation but never observed flying nectar robbers. However, because small black beetles (*M. viridescens*) were found in many damaged flowers, we estimated the percentage of open flowers with beetles at Zürich 1997 (late) and 1998 (early, middle, late in the season) and at Grellingen in 1997 (middle and late in the season). The percentage of flowers with beetles was correlated with the percentage of open flowers that were damaged. The correlation coefficient, $r=0.85$, was highly significant ($P=0.031$). We thus suspected that the small beetles were likely to be the cause of the holes in the helmet of *A. lycoctonum* at lower elevations. Two experiments were then conducted in the population at Zürich in 1999. The first experiment was a flying-robber exclusion, in which single flower buds were bagged with nylon mesh bags that allowed small crawling insects, such as flower beetles, access to the flower, but not flying insects. We bagged a first set of 13 buds, and a second set of 17 buds; 4 days after bagging we calculated the percentage of flowers that showed a hole in the helmet and counted the number of beetles in the bag. In the second set we also checked for damaged nectaries.

The second experiment was designed to quantify pollen consumption by beetles. We chose two neighbouring flower buds on 11 randomly chosen plants and marked them for pairwise comparisons. One bud was slightly opened and a beetle (*M. viridescens*) was put into the flower. The buds were bagged separately with dialysis tubing. After 4 days we counted the number of pollen grains per flower. The anthers and the lateral petals were placed in a microfuge tube, air dried, preserved in 50 µl ethanol and sonicated for 30 min to separate grains. The samples were spun on a

vortex mixer, and the pollen grains counted on a hemacytometer. Ten replicates per individual sample were counted and the average per flower was used in the analysis. The effect of the beetle on the number of pollen grains in the flower was tested by ANOVA.

Results

Floral damage

Flowers at higher elevation populations experienced significantly more damage. The highest percentage of pierced flowers (84%) was found at St. Moritz (Fig. 1). In a comparison of two of the populations over two years at all three times during the season we found fewer holes in both years at Zürich (low elevation) than at St. Moritz (high elevation).

Activity of nectar robbers

A similar pattern was also found by direct observation of nectar robbers; we never observed any flying robbers in the populations at lower elevation.

The two populations at higher elevation did not differ in robber visitation rates (Table 1). However, the interaction between flowering season and population was significant (Fig. 1). The ANOVA of the data from St. Moritz (Table 2) showed no significant effect of the year on visitation rate, but the visitation rate varied significantly between seasons among and within years.

The nectar robbers observed at Arolla and St. Moritz were all bumblebees, and according to their colouring were “*B.-wurflenii*-like” bees. Later identification of insects collected at St. Moritz, showed that *B. wurflenii* was likely to have been the main nectar robber.

Table 1 ANOVA on the effect of population and flowering season (middle and late) on nectar robber visitation rate in two high-elevation populations (Arolla and St. Moritz) of *Aconitum lycoctonum*

Source of variation	df	SS	F	P
Population ^a	1	0.02	1.32	0.4560
Flowering season	1	0.00	0.0020	0.9716
Population×Flowering season ^a	1	0.02	6.48	0.0257
Error	12	0.03		

^a Considered as a random factor in the model

Table 2. ANOVA on the effect of year and flowering season (early, middle, and late) on nectar robber visitation rate in one high elevation population (St. Moritz) of *A. lycoctonum*

Source of variation	df	SS	F	P
Year	1	0.0012	2.63	0.1222
Flowering season	2	0.0626	70.29	<0.0001
Year×Flowering season	2	0.0056	6.29	0.0085
Error	18	0.0080		

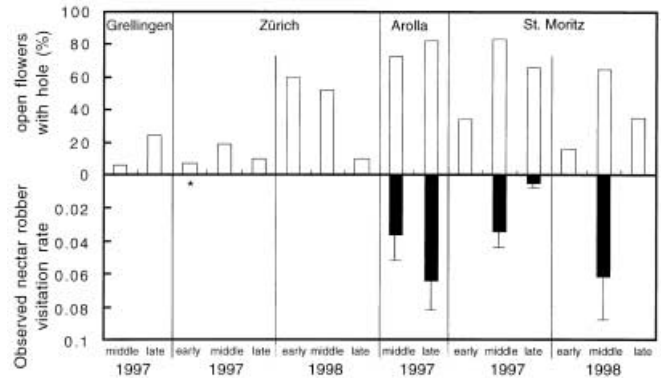


Fig. 1 Nectar robber activity in four populations of *Aconitum lycoctonum* in Switzerland in 1997 and 1998 at three times during the flowering season (early, middle and late). Grellingen (450 m) and Zürich (500 m) are low-elevation, Arolla (2050 m) and St. Moritz (2020 m) are high-elevation populations. Error bars indicate +1 SE. Visitation rate is calculated as number of nectar robber visits per open flower per 0.5 h, * indicates missing data

Effects of nectar-robbing bumblebees on plant fitness components

Nectar robbery did not affect female success of *A. lycoctonum*. Unrobbed flowers set similar numbers of seeds (1997: 7.52 ± 1.44 SE, $n=27$; 1998: 14.08 ± 1.48 , $n=24$) as robbed flowers (1997: 6.52 ± 1.31 , $n=27$; 1998: 16.08 ± 1.38 , $n=24$; Fig. 2a, Table 3). Weight per seed was also similar for the two treatments (unrobbed 1997: $3.1 \text{ mg} \pm 0.2$, $n=14$; unrobbed 1998: $2.6 \text{ mg} \pm 0.2$, $n=23$; robbed 1997: $3.2 \text{ mg} \pm 0.2$, $n=14$; robbed 1998: $2.5 \text{ mg} \pm 0.2$, $n=23$; Fig. 2b, Table 3). The ANOVAs showed that differences in female fertility of the plants were explained mainly by the year in which the experiment was conducted, and by the high variability among individual plants.

There was no difference in germinability between the seeds of robbed and unrobbed flowers: 79% of the “robbed” seeds and 81% of the “unrobbed” seeds had germinated when the experiment was stopped. The ANOVA showed no effect of robbery treatment ($F_{1,11}=0.1888$, $P=0.6723$) on germination.

Robbing bees rarely harmed the nectaries. The percentage of damaged nectaries was low; 16 of 50 flowers showed slight damage at the edge of the tube of both nectaries, and 8 of 50 showed one nectary slightly damaged at the edge of the tube. The slight damage did not appear to influence the productivity of the nectary, since in only four flowers was no nectar visible in either of the two nectaries. Pollinators or robbers, which we had not excluded from the flowers, might have recently visited these four flowers, and therefore the flowers did not show any standing crop. Only one flower had one nectary so badly destroyed that nectar production was impossible.

About 10% of the visits by short-tongued bees, which normally act as nectar robbers, could transfer pollen. When we observed how often these robbing bees collected pollen, we found that, of the total of 747 recorded visits, 68 were pollen-collecting and 679 were nectar-robbing.

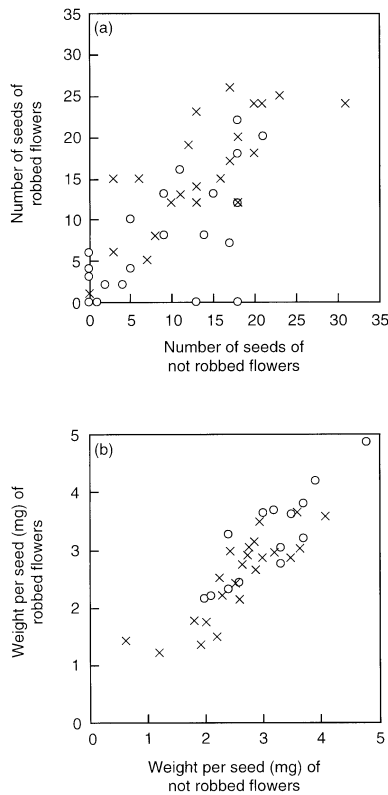


Fig. 2 Comparison of **a** seeds per flower and **b** weight per seed (mg) for the robbed and unrobbed flower of each plant in the nectar robber exclusion experiment in 1997 (○) and 1998 (×). If the treatment had no effect, the data points would fall on a 1:1 line

Autofertilisation in *A. lycoctonum*

Bagging of flowers reduced the number of seeds, but did not affect weight per seed. Bagged flowers set significantly fewer seeds (1997: 1.21 ± 0.53 , $n=29$; 1998: 1.03 ± 0.32 , $n=37$) than open-pollinated flowers did (1997: 5.21 ± 1.25 , $n=29$; 1998: 12.19 ± 1.30 , $n=37$; Fig. 3a, Table 4). However, weight per seed was similar for the two treatments (bagged 1997: $2.7 \text{ mg} \pm 0.3$, $n=6$; bagged 1998: $2.7 \text{ mg} \pm 0.3$, $n=11$; open-pollinated 1997: 3.0 ± 0.3 , $n=6$; open-pollinated 1998: $2.9 \text{ mg} \pm 0.2$, $n=11$; Fig. 3b, Table 4).

There was no significant difference in germinability between selfed and open-pollinated seeds: 73% of the selfed and 75% of the open-pollinated seeds had germinated when the experiment was stopped.

Longevity of pierced and unpierced flowers

Flower longevity was not affected by artificial piercing of the flower (Table 5). However, significant differences in flower longevity were found at the population level. These differences were mainly explained by the elevation the population originated from. Flowers from low elevation (<600 m) populations lived significantly less long (4.61 ± 0.14 days, $n=38$) than did flowers from high elevation populations (>1400 m) (5.05 ± 0.20 days, $n=42$).

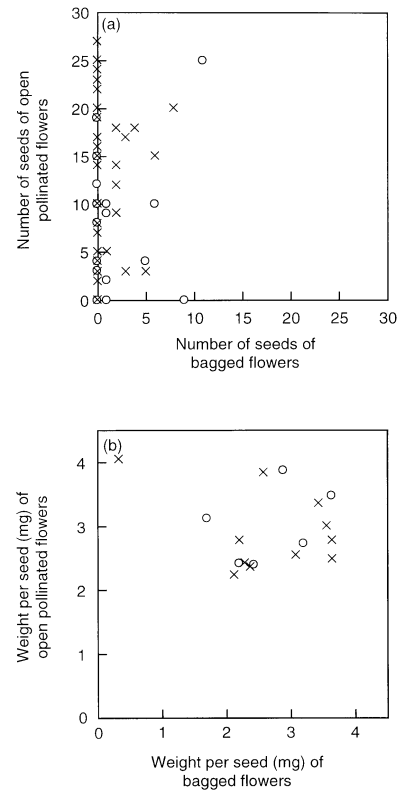


Fig. 3 Comparison of **a** seeds per flower and **b** weight per seed (mg) for the bagged and open-pollinated flower of each plant in the pollinator exclusion experiment in 1997 (○) and 1998 (×). If the treatment had no effect, the data points would fall on a 1:1 line

Table 3. ANOVAs on the effect of a robbed versus unrobbed treatment and year on the number of seeds per flower and weight per seed of *A. lycoctonum*

Source of variation	df	SS	F	P
Number of seeds per flower				
Treatment	1	6.35	0.46	0.5000
Year	1	1,652.81	19.06	<0.0001
Year×Treatment	1	57.18	4.16	0.0469
Plant number (Year) ^a	49	4,249.15	6.30	<0.0001
Error	49	674.00		
Weight per seed				
Treatment	1	1.56×10^{-9}	0.02	0.8937
Year	1	6.96×10^{-6}	6.22	0.0175
Year×Treatment	1	1.21×10^{-7}	1.40	0.2440
Plant number (Year) ^a	35	0.00004	12.97	<0.0001
Error	35	3.02×10^{-6}		

^a Considered as a random factor in the model

Effects of flower beetles on *A. lycoctonum*

Flower beetles caused the holes in *A. lycoctonum* flowers at low elevation: 27% of the flowers to which beetles had access, but flying insects did not, showed a hole in the helmet after the four day treatment. Up to six beetles were found per bag. None of the nectaries were damaged.

Table 4. ANOVAs on the effect of a bagged/open-pollinated treatment and year on the number of seeds per flower and weight per seed of *A. lycoctonum*

Source of variation	df	SS	F	P
Number of seeds per flower				
Treatment	1	10.48	78.54	<0.0001
Year	1	1.92	10.05	0.0023
Year×Treatment	1	1.74	13.06	0.0006
Plant number (Year) ^a	64	12.23	1.13	0.0766
Error	64	8.54		
Weight per seed				
Treatment	1	5.37×10 ⁻⁷	0.78	0.3917
Year	1	1.66×10 ⁻⁸	0.038	0.8604
Year×Treatment	1	2.25×10 ⁻⁸	0.038	0.8592
Plant number (Year) ^a	15	7.77×10 ⁻⁶	0.76	0.7072
Error	15	10.35×10 ⁻⁶		

^a Considered as a random factor in the model

Table 5. ANCOVA on flower longevity. The contrast between populations of low (below 600 m a.s.l.) and high elevation (above 1400 m a.s.l.) is included

Source of variation	df	SS	F	P
Main effects				
Piercing treatment	1	0.0006	0.002	0.9667
Population ^a	10	35.43	2.78	0.0148
Plant number (Population) ^a	29	38.66	3.71	<0.0001
Flower placement index ^b	1	0.84	2.34	0.1346
Error	38	13.65		
A priori contrast for population				
High altitude vs. low altitude	1	6.21	4.87	0.0351

^a Considered as a random factor in the model

^b Covariate

A single beetle bagged in a flower ate a significant amount of pollen ($F_{1,10}=5.3721$, $P<0.05$). Flowers without a beetle had about twice as many pollen grains ($10,018\pm 1,812$ grains, $n=11$) than flowers in which one single beetle was allowed to eat pollen over four days ($5,550\pm 1,026$ grains, $n=11$). Plant genotype did not affect the number of pollen grains available ($F=1.3343$, $P=0.3285$).

Discussion

Nectar robbing of *A. lycoctonum* in populations at high altitude

Nectar robbery can be quite severe in *A. lycoctonum*, but our estimated frequency of nectar robbery of up to 85% of open flowers was not exceptionally high. For example, Müller (1881) reported 100% robbery and mentioned the nectar robber *B. wurflenii* as the most frequent visitor, even more frequent than any pollinator,

on *A. lycoctonum*. Werth (1940) also reported that up to 100% of the flowers in a population of *A. lycoctonum* were robbed, but he also mentioned very large differences among years. Studies on other interactions between plants and nectar robbers have also shown high robbing frequencies (e.g., Morris 1996; Irwin and Brody 1998).

Negative effects of robbery on bee-pollinated plants are usually explained by a reduction in pollinating visits. Robbed flowers may become less attractive, or pollinators may become secondary nectar robbers by gaining access to the nectar through the hole made by primary robbers (Kendall and Smith 1976; Reddy et al. 1992). We found no negative effect of nectar-robbing bumblebees on fitness components of *A. lycoctonum*. Our data suggest that robbery does not prevent pollinators from visiting the flowers, since robbed and unrobbed *A. lycoctonum* flowers set the same number of seeds. Our pollinator-exclusion experiment showed that if pollinators did not visit the flowers, the flowers set no or very few seeds. Behavioural observations also showed that pollinators were just as likely to visit robbed flowers as unrobbed flowers (A.-B. Utelli and B.A. Roy, personal observations).

Robbing bees do not damage the nectaries or the ovaries of *A. lycoctonum*. Thus, nectar production is not reduced as a result of robbers. The ovaries are not damaged since the nectar in *A. lycoctonum* flowers is so spatially separated from the ovaries that the nectar robbers never bite into these reproductive structures while biting holes into the helmet. McDade and Kinsman (1980) and Traveset et al. (1998) both found that nectar robbers decrease reproductive success because the robbers caused severe damage to the carpels. Robbing could also decrease fitness if pierced flowers last less long, so that the probability of being visited by pollinators would also decrease. Our artificial piercing of the flower might not exactly mimic the bite of a nectar robber, but since we pierced the same flower several times, just as the insects do, the total damage was of similar size. Since this artificial piercing did not affect longevity of the flowers, we suspect that the floral damage by nectar robbers does not change the availability of a flower for pollinators.

Robbing might have a positive effect on fitness if pollinators have to visit more flowers to get the same amount of nectar, and thus increase outcrossing rates (Heinrich and Raven 1972). We have shown here, and in an experiment with daily self-pollination resulting in full seed set (Utelli and Roy 2000), that *A. lycoctonum* at St. Moritz is self-compatible. Thus, positive effects of higher outcrossing rates could only be shown by genetic investigations of seed-families of robbed and unrobbed flowers. However, our more circumstantial data do not support this hypothesis. We found neither a significant positive correlation between the percentage of flowers with holes in a plot and the percentage of pollinator movements among plants ($r=-0.31$, $P=0.1333$, $n=25$), nor a significant negative correlation between the percentage of flowers with holes in a plot and the percent-

age of pollinator movements within plants ($r=-0.16$, $P=0.4580$, $n=25$). Both correlations would be expected if nectar reward was limited for pollinators in robbed flowers, since pollinating bumblebees are expected to switch plants after probing a flower with a lower nectar reward (Hodges 1985; Cresswell 1990).

We propose that the interaction of *A. lycoctonum* and its nectar-robbing bumblebees is a mutualism because the robbing bees sometimes act as pollen vectors, and thereby benefit the plant. Several other studies also suggested that nectar robbers can have a positive effect on seed set when they occasionally act as pollen collectors and thereby act as pollinators (Meidell 1944; Koeman-Kwak 1973; Rust 1979; Scott et al. 1993; Morris 1996). We found that 10% of the visits nectar robbers made to *A. lycoctonum* were pollen-collecting visits. Thøstesen and Olesen (1996) showed that *B. wurflenii*, our most common nectar robber, is an efficient pollen remover on *A. septentrionale*. Given that *A. septentrionale* flowers are not very different from *A. lycoctonum*, we assume that *B. wurflenii* can also act as pollinator on *A. lycoctonum*. We found (Utelli and Roy 2000) that pollen-collecting visits by short-tongued bumblebees sometimes contribute a large amount of the total pollinating visits to *A. lycoctonum* (about 34% at Arolla 1997, 30% and 4% at St. Moritz 1997 and 1998, respectively). Thus *B. wurflenii* might be an important pollinator when long-tongued bumblebees are scarce.

Fluctuations in pollinator and nectar robber visitation rates might be common and have also been documented for insects on *A. septentrionale* (Løken 1949, 1950). However, she found that *B. wurflenii* as pollen collector never visited flowers in female phase and thus that *B. wurflenii* does not pollinate *A. septentrionale*. But later Thøstesen and Olesen (1996) showed that *A. septentrionale* is not completely protandrous, as is also true for *A. lycoctonum* (this study). Thus pollination by *B. wurflenii* is likely.

Floral damage to *A. lycoctonum* in populations at low altitude

We showed that small black flower beetles (*M. viridescens*) are the insects that pierced the helmet of *Aconitum* flowers at low elevation. Moreover, the holes in the flowers of plants at low elevation look very different from the holes bitten by bumblebees at high elevation (A.-B. Utelli and B.A. Roy, personal observations). Holes at lower elevation are more variable in size and shape, and more random in placement along the helmet than holes in flowers at higher elevations.

The larval and adult stages of *Meligethes* are flower-inhabiting insects that mainly eat pollen, and sometimes also other parts of the flowers, such as pistils and carpels (Freude et al. 1967). The larvae of *M. viridescens* are oligophagous on Cruciferae. The adult stage is found on many different Cruciferae, often on *Brassica napus*, but also on other herbs and shrubs (Koch 1989). Our data

suggest that *M. viridescens* is an important pollen eater on *A. lycoctonum*. One single beetle can eat about half of the available pollen within 4 days. It is more usual to observe several beetles in a single flower, so much of the pollen is destroyed. Once inside the flower, the beetles also crawl up to the nectaries and probably bite the holes from the inside. The flowers are often filled with beetles before a hole is pierced (A.-B. Utelli and B.A. Roy, personal observations), so the beetles cannot strictly be called pollen or nectar robbers since the hole is not necessarily made to get pollen or nectar.

Conclusions

There was a strong altitudinal pattern in our data; in high-elevation populations floral damage was caused by short-tongued bumblebees acting as nectar robbers, whereas in low-elevation populations holes in the flowers were caused by pollen-eating beetles. However, the populations investigated might only be at the two extremes of an altitudinal gradient in this flower-insect interaction. To gain more insight into the evolutionary consequences of pollen-eating beetles and nectar-robbing bumblebees on *A. lycoctonum* one should seek out populations where they co-occur. We now know that the flower-inhabiting beetles consume a considerable amount of pollen. We did not test whether the beetles pollinate the flowers, but we suspect that the beetle, *M. viridescens*, has more detrimental effects on plant fitness components of *A. lycoctonum* than the nectar-robbing bumblebees. As pollen eaters they are likely the reason why so little pollen is available in the flowers of *A. lycoctonum* at low altitude, and why fruit set is so low in these populations (A.-B. Utelli, personal observations). The beetles might also damage the carpels of the flower. Further, it has been shown that pollinating bees avoid flowers when they are inhabited by beetles (Kirk et al. 1995). Thus, the ability of the plant to autofertilise would be a strategy to avoid at least some of these negative effects. An experiment showed (Utelli and Roy 2000) that there is variation in the ability to autofertilise in *A. lycoctonum*. However, we do not yet know whether this ability correlates with the occurrence of flower-inhabiting beetles.

Our survey of the literature suggests that positive effects of nectar robbers are often found in bee-pollinated plants (Meidell 1944; Hawkins 1961; Koeman-Kwak 1973; Rust 1979; Waser 1979; Zimmerman and Cook 1985; Higashi et al. 1988; Scott et al. 1993; Morris 1996; Olesen 1996; Navarro 2000; for negative effects see Kendall and Smith 1976; Reddy et al. 1992). Negative effects seem to be commoner in hummingbird-pollinated plants (Colwell et al. 1974; McDade and Kinsman 1980; Gill et al. 1982; Roubik 1982; Roubik et al. 1985; Irwin and Brody 1998; Traveset et al. 1998; for positive effects see Macior 1966; Arizmendi et al. 1996). However, further studies are necessary to determine whether this pattern is real, or whether the negative effects have more to do with traits of the robbers, e.g., damaging ovaries.

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