

# An alternative hibernation strategy involving sun-exposed 'hotspots', dispersal by flight, and host plant finding by olfaction in an alpine leaf beetle

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*Key words:* insect-plant interaction, hibernation, sequestration, host plant finding, mark-release recapture, host plant odour recognition, wind tunnel, pyrrolizidine alkaloids (PAs), Coleoptera, Chrysomelidae, *Oreina cacaliae*, *Adenostyles alliariae*, *Petasites paradoxus*

## Abstract

*Oreina cacaliae* (Schrank) (Coleoptera: Chrysomelidae) has a 2-year life cycle that it has to complete within the short warm seasons of the harsh alpine environment. Three years of field observations and experiments revealed that not all beetles overwintered in the soil next to their principal host *Adenostyles alliariae* (Asteraceae), as was previously assumed, but that many *O. cacaliae* left their host in autumn and flew to overwintering sites that were extensively sun-exposed. In spring, these individuals became active 2 months earlier than their conspecifics that had remained in the soil close to the host plant. These early beetles flew from their hibernation sites against the direction of the prevailing wind. After a random landing in snow, they walked to the spring host *Petasites paradoxus* (Asteraceae) and fed on its floral stalks, the only plant parts present at that time. A few weeks later, they took flight again to locate newly emerging *A. alliariae* on which they would feed and deposit larvae as did individuals that had overwintered close to *A. alliariae*. Leaves of *A. alliariae* contain pyrrolizidine alkaloids (PAs), which the beetles sequester for their own defence. The dominating PA (seneciphylline) was also found to be present in the floral stalks of *P. paradoxus*. With additional behavioural assays in the field and laboratory, we demonstrated the importance of plant odours in the short-range host location process. This study reveals a unique hibernation behaviour in which part of the beetle population uses exceptionally warm locations from which they emerge in spring, long before all the snow has melted. This early, but risky emergence allows them to exploit a second, highly suitable host plant, which they locate first by wind-guided flight and then by odour-guided walking. The well-fed beetles then use odour again to move to their principal host plant, on which they reproduce.

## Introduction

The ability of many insects to fly enables them to exploit habitats they otherwise could not use and to increase their flexibility in response to environmental contingencies (Rankin & Burchsted, 1992). However, beetles of the genus *Oreina* Chevrolat (Coleoptera: Chrysomelidae), as well as many Chrysolinina, are usually recorded as flightless, which should hamper their ability to disperse and find new host plants over longer distances. Occasional flights of *Oreina* beetles have been reported only anecdotally (S. Dobler and

D. Conconi, pers. comm.), but they may suggest that certain *Oreina* species use flight in their foraging behaviour. The function of these occasional flights is as yet unknown.

*Oreina cacaliae* (Schrank) occurs at elevations higher than 1000 m in the Alpine regions, where winters are long and the vegetation is covered with snow between November and April. *Oreina cacaliae* beetles were thought to be sedentary within a patch, and to overwinter under the snow cover in the soil next to their principle host plant *Adenostyles alliariae* (Kerner) (Asteraceae), from where they emerge in May, when the snow cover has melted. The host plant forms patches, groups of plants with overlapping leaves, covering an area of between 10 and 600 m<sup>2</sup> and is chemically defended by pyrrolizidine alkaloids (PAs), mainly seneciphylline, present in the leaves (Pasteels et al.,

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1995). *Oreina cacaliae* is viviparous and deposits larvae directly on *A. alliariae* leaves. Larvae, as well as adult *O. cacaliae*, sequester seneciphylline when they feed on these leaves (Ehmke et al., 1991; Ehmke et al., 1999), which protects them from predation by birds (Rowell-Rahier et al., 1995). It is the only known *Oreina* species that has completely lost the capacity to synthesise cardenolides and relies exclusively on sequestering PAs from its host plant for chemical defence (Ehmke et al., 1991).

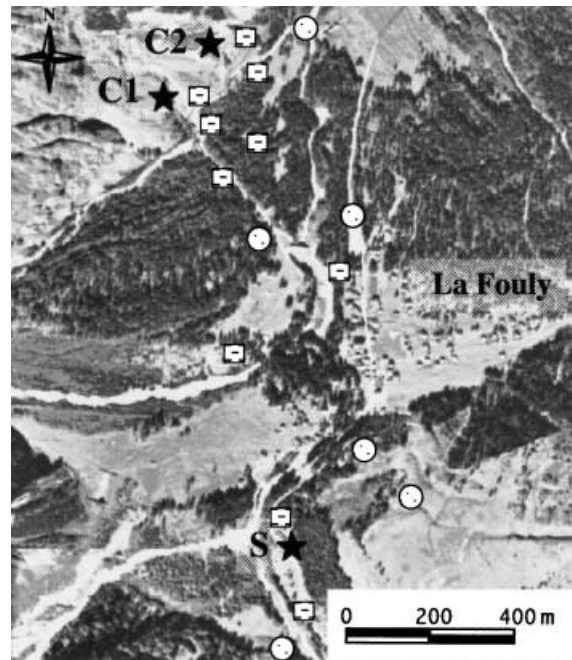
During preliminary observations, we noted however, that in early spring, when *A. alliariae* is not yet available, large numbers of beetles emerged from a few sun-exposed patches and took flight. Beetles were then seen feeding on flowers of *Petasites paradoxus* (Asteraceae), a herbaceous perennial plant that flowers very early in the season before it produces vegetative leaves. Later in the season, the beetles were observed to switch to *A. alliariae* (Kalberer & Rahier, 2003). Indeed, plants of the genus *Petasites*, as well as those of *Senecio*, have been reported as additional host-plants of *O. cacaliae* (Jolivet et al., 1986). All these plants are closely related and belong to the subtribe Senecioninae of the Asteraceae (Nordenstam, 1977). Adult Chrysomelidae commonly find their host plants by walking (Hsiao, 1988), and preliminary field observations with *O. cacaliae* also suggest that over short ranges the beetle only approaches host plants while walking. Nevertheless, the long distance between overwintering sites and patches with suitable host plants, as well as its observed flight behaviour suggest that, at an initial stage, the host plant finding process involves orientation by flight. We hypothesised that the use of sun-exposed overwintering sites is an alternative hibernation strategy, used by some individuals, while others use the less risky strategy of a much longer hibernation in the soil next to the principal host plant, *A. alliariae*.

Here we report on several field and one laboratory study that aimed to elucidate the intricacies of the host finding process of *O. cacaliae*. Field observations and mark-recapture studies were performed to study the role of flight behaviour and dispersal in host plant use by *O. cacaliae* emerging early in the season. Direct observations of field-released marked beetles suggest the importance of olfaction in its short-range host plant location behaviour in summer, when the beetles are flightless. Wind tunnel studies in the laboratory confirmed the odour-mediated host plant preferences of *O. cacaliae*.

## Materials and methods

### Field studies

*Study sites and direct observations.* All the fieldwork was carried out in the Swiss Alps near La Fouly in the Val Ferret (Valais) at 1500 m above sea level (45°56'10"N, 7°05'95"E),



**Figure 1** Map of the field site with patches of *Petasites paradoxus* represented by squares and patches of *Adenostyles alliariae* represented by circles. Hibernation sites (C1, C2, and S) were located at a distance of at least 200 m from the closest *A. alliariae* patches.

where the host plants *P. paradoxus* and *A. alliariae* both occur, but not *Senecio*. During the three following autumns we observed the behaviour of *O. cacaliae* on *A. alliariae* in order to determine their hibernation strategy. Three overwintering sites away from the plants were identified (Figure 1; see Results).

*Take-off directions in spring.* The following spring, individuals emerging from these sites were observed during weekly observation sessions of several hours between mid-March and mid-May. Whenever a spontaneous beetle take-off occurred, the take-off direction together with the prevailing wind direction and wind speed ( $\text{m s}^{-1}$ ) were recorded. Wind speed was measured using a portable anemometer (50518, Bioblock Scientific, Frenkendorf, Switzerland).

*Landing sites after spring flight.* To determine the beetles' flight destination near the hibernation sites, we searched for beetles in the area on foot along a 1.6 km transect, during an afternoon when we had observed beetle take-off around noon. At three spots, where the snow had already melted, flowering *P. paradoxus* had emerged, which was the only herbaceous plant present that day in the area. To test whether the beetles aggregated close to these plants, we

divided the 1.6 km long transect into sections of 30 m each, determined the number of beetles in each section, and ran a permutation test.

*Mark-recapture studies.* Beetles were marked over the whole season using a code of 2–7 dots of correction fluid (Optifluid, Carfa AG, Richterswil, Switzerland) on the elytra or pronotum in order to identify and differentiate beetles from different hibernation sites and patches of *P. paradoxus* and *A. alliariae*. After marking, the beetles were immediately released at their original positions. Once marked, a code could easily be read from a distance, while the insect was sitting on the upper leaf surface. Over 2 consecutive years, marked beetles were recorded weekly from March to September in order to follow the movement of individuals over time across the study site.

*Short range host plant finding in the field in summer.* On four different dates in July, 50 marked beetles (25 of each sex) were released per date from a glass jar in the middle of a path, in order to study their host plant approach behaviour. On both sides of the point of release, *A. alliariae* were growing at a minimal distance of 3.2 m. The radius from 340° to 8° and 165° to 185° was free of *A. alliariae* plants. The area had been flooded the previous year and the soil was covered with small stones. All plants surrounding the release point at a distance of 20 m were searched for marked beetles seven times during the release days (15, 45, 60, 75, and 90 min, as well as 2 and 6 h after release). A portable wind vane constantly gave us the wind direction at a height of 0.3 m. Wind speed was measured with the portable anemometer. The position of each recaptured beetle was recorded and the distance to the point of release measured. We calculated the mean angle ( $\phi$ ), representing the mean direction of movement of all recaptured beetles, as well as the length of the mean vector  $R$ , representing the 'directedness' of the movements, ranging from 0 (random movement) to 1 (Rayleigh-test; Batschelet, 1981).

#### Laboratory studies

*Wind tunnel tests.* Plant material and beetles were collected simultaneously once a week. The beetles were transported to the laboratory in cooled containers, where they were maintained in glass jars in an incubator at 16 °C (day) and 12 °C (night) and under a L16:D8 light regime. They were sexed using sexual dimorphism of the tarsi (Lohse & Lucht, 1994), and used in experiments within 3 days of collection. Plants collected from the same field site were transplanted into 15 cm diameter ceramic pots and immediately transported to the laboratory, where they were placed near a window and kept at room temperature until use. The

spring host *P. paradoxus* produces flowers first; after about a month the flowers decay and the plant starts to produce leaves. The non-host plant *Tussilago farfara* was chosen for the wind tunnel studies because this plant emerges in a flowering state like *P. paradoxus* and because these two plants are the only perennial herbs present when *O. cacaliae* emerges, early in spring. All behavioural studies were dual-choice tests with walking beetles in a wind tunnel. The Plexiglas wind tunnel (0.6 × 0.6 × 1.8 m) was surrounded by a white tent to eliminate any visual distractions for the beetles. Air was pulled through the tunnel at 0.1 m s<sup>-1</sup> with the use of a tubular duct fan (RR 125 C, Melios Ventilatoren, Urdorf, Switzerland) and was exhausted outside the room. A charcoal-impregnated fabric cleaned the air at the tunnel entrance. Two potted plants were placed 0.3 m apart at the upwind end of the wind tunnel. A glass plate (0.5 × 1.5 m) was placed horizontally in the wind tunnel at a height adjusted such that the air from the volatile-emitting, upper plant parts would pass over it. Five beetles at a time were released from a glass Petri dish on to the glass plate at the downwind end of the wind tunnel. The distance between the point of beetle release on the glass plate and the plants was 1 m. Beetles that reached the end of the glass plate in front of a plant were considered to have made a choice for that plant. The glass plate was cleaned with 70% ethanol after testing 10 beetles. For each experimental plant combination, four replicates with 20 beetles each (10 females and 10 males) were made. Replication took place at intervals of at least 1 week with different plants and different beetles. The position of the plant in the wind tunnel was switched after testing 10 beetles, to avoid any directional bias. An individual beetle was used only once in each wind tunnel experiment. Glass jars with beetles were taken out of the incubator 30 min before an experiment and left near the wind tunnel to let the beetles acclimatize to the experimental conditions. Plant preferences were tested with a two-tailed binomial test, using the total number of beetles that made a choice for a particular plant and pooling the data from the four replicates.

*Analysis of pyrrolizidine alkaloids.* The leaves of *P. paradoxus* have been reported to be devoid of alkaloids (Rowell-Rahier et al., 1991), but *O. cacaliae* actually feed on the floral stalks of *P. paradoxus* in spring. We therefore tested the stalks for their alkaloid content, using the extraction method described by Pasteels et al. (1995). Pyrrolizidine alkaloids in the extracts were separated by capillary gas chromatography (GC) on fused silica capillary columns (15 m × 0.25 mm; SPB1, Supelco) using a Perkin-Elmer 8500 apparatus equipped with a flame ionisation detector. Conditions: injector, 250 °C; temperature program, 150–300 °C, 6 °C min<sup>-1</sup>; injection volume 1 µl; carrier gas, He at 0.75 bar. The PA

retrosine (Sigma R-0382) was used as an internal standard. Peaks were identified by comparing retention times with chromatograms of PAs detected in *A. alliariae* leaves.

## Results

### Field results

*Alternative hibernation strategy.* In autumn, several *O. cacaliae* were observed leaving their withering main host plant *A. alliariae* by flight, apparently to search for specific hibernation sites rather than to overwinter in the soil close to the host plant. Three such hibernation sites could be identified, all >200 m away from the nearest *A. alliariae* patch (Figure 1). Hibernation sites C1 and C2 were in the soil at the bottom of an 800 m cliff, and S was on a slope of open soil next to a new road. In late August and September, when *A. alliariae* as well as *P. paradoxus* were wilting, 43 beetles were found wandering around at these sites, whereas in mid-summer, no beetles were ever recorded there during our weekly observations. At all three hibernation sites, the snow melted as early as March 17, when the surrounding vegetation, especially the shady *A. alliariae* patches, were still hidden under a snow cover of about 0.5 m. Non-flyers emerged 2 months later, simultaneously with the main host *A. alliariae*. On sunny days, *O. cacaliae* were observed taking off from hibernation sites by flight, after wandering around exposed to the sun for several minutes. The beetles quickly gained altitude and were lost from sight soon after.

*Take-off directions in spring.* The flight direction of *O. cacaliae* was dependent on the prevailing wind direction at the time of take-off. The angle of the mean wind direction ( $\phi$ ) was significantly directed towards the SE ( $135^\circ$ ) with  $R = 0.64$  and  $P < 0.001$ . Beetle take-off directions were significantly directed towards the ESE (mean angle:  $116^\circ$ ,  $R = 0.67$  and  $P < 0.001$ ), observed for a total of 60 individuals at wind speeds between 0.5 and 3.6 km h<sup>-1</sup>. No flights were initiated at wind speeds of 6 km h<sup>-1</sup> with gusts up to 12 km h<sup>-1</sup>. The beetles were observed flying exclusively on sunny days, mostly between 12.30 hours and 14.30 hours, the warmest period of the day.

*Landing sites after spring flight.* When recording the beetles' flight destination after take-off from overwintering sites, 74 beetles were observed walking on the snow along an arbitrarily chosen transect (data not shown). None of them had landed directly on *P. paradoxus* flowers that had already emerged, and only eight beetles were found within a distance of 15 m from these early emerging plants. There was no aggregation of beetles close to *P. paradoxus* within 2–4 h after flight (permutation test,  $P = 0.70$ , with the null

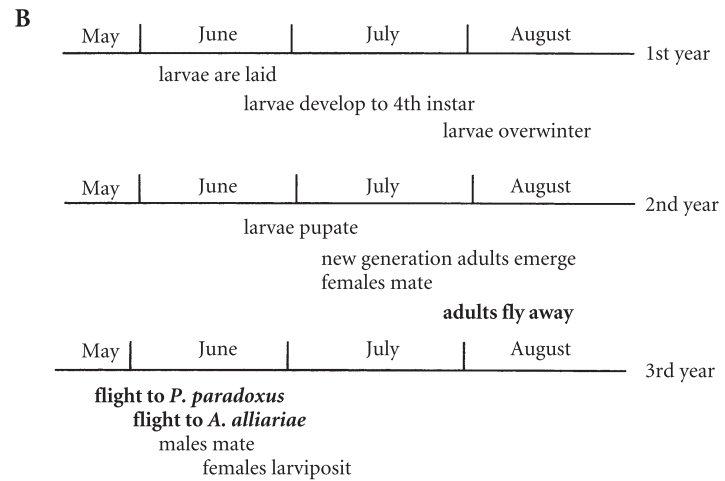
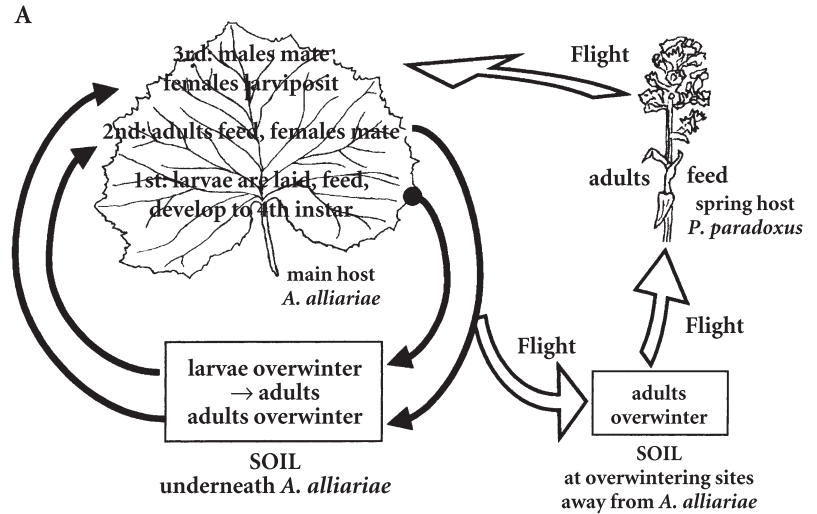
hypothesis that insects fall at random in 30 m sections). Two weeks later, at the beginning of May, hundreds of beetles were found on *P. paradoxus* flowers; presumably they had located the plants by walking. Hence, flowering *P. paradoxus* can be regarded as the spring host plant of those *O. cacaliae* that disperse by flight.

*Mark-recapture studies.* The distances covered by *O. cacaliae* between the marking sites and the recapture sites were recorded over the season. In total, 2522 beetles found at the different sites in Val Ferret were marked over 2 years. These field observations allowed us to propose an extended life cycle for *O. cacaliae* as shown schematically in Figure 2, together with the more conventional cycle of exclusively feeding on *A. alliariae* and overwintering in the soil beneath it. Of the 611 beetles marked at the hibernation sites C1 and C2 (between 12 March and 1 April), 22 were found in *P. paradoxus* patches between 2 and 6 weeks after marking. Most of them had covered distances of between 70 and 110 m, but two individuals had travelled 190 and 230 m, and three between 370 and 540 m. Beetles marked at the same overwintering site were found in up to five different patches of *P. paradoxus*.

Of 1008 beetles marked on *P. paradoxus* (between April 13 and May 6), one had moved to another *P. paradoxus* patch at a distance of 75 m, two were recaptured on *A. alliariae* at a distance of 110 m, and three were recaptured at around 950 m on *A. alliariae*, where they fed, mated, and the females larviposited. Beetles marked at the same *P. paradoxus* patch were recaptured in up to three different *A. alliariae* patches. The beetle that travelled the farthest recorded a distance between *P. paradoxus* and *A. alliariae* of 1125 m, crossed two rivers and one street. Four beetles were found on *A. alliariae* after they had been marked 5–8 weeks earlier at overwintering sites at distances of 250 m or more.

A male beetle that was marked on *A. alliariae* in the summer was recaptured on *P. paradoxus* the next spring at a distance of 210 m, confirming that *O. cacaliae* leave *A. alliariae* patches in autumn and feed on *P. paradoxus* patches in the spring. No beetle was recaptured out of the 53 beetles marked in autumn (between 25 August and 23 September) at the hibernation sites. None of the 460 beetles marked on *A. alliariae* (between 20 May and 18 August) was recaptured in a different *A. alliariae* patch, during the same summer or in a following summer. Four beetles (three males and one female) marked on *A. alliariae*, were recaptured in the same patch the following year, indicating that not all beetles leave their patch.

*Short-range host plant finding in the field in summer.* Host plant finding was studied in summer, when beetles usually avoid flying. Groups of beetles were released on a path

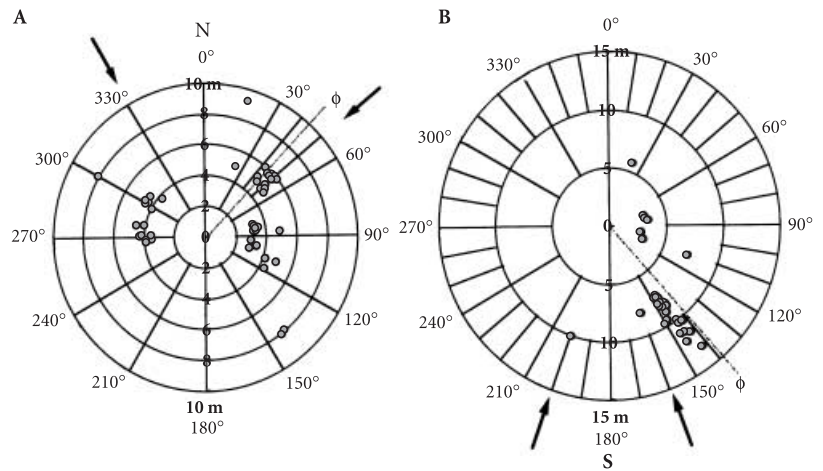


**Figure 2** (A) Life cycle of *Oreina cacaliae*, incorporating new elements of dispersal to hibernation sites away from *Adenostyles alliariae*, and feeding on *Petasites paradoxus* until the emergence of *A. alliariae* (open arrows). So far it was assumed that the life cycle was completed on *A. alliariae* (black arrows) starting with the first year (1st). (B) The temporal sequence for flyers; non-flyers stay within the *A. alliariae* patches to overwinter in the soil.

surrounded by *A. alliariae* plants (Figure 3). Of 150 beetles released under north wind conditions, a total of 45 (22 females and 23 males) found a host plant within 6 h (Figure 3A), at a mean distance of 4.9 m [mean ( $\pm$  SD) females:  $4.8 \pm 1.7$ ;

mean males:  $4.9 \pm 1.4$ ]. North wind direction during the 3 release days ranged from  $328^\circ$  to  $50^\circ$ , with calm winds and short gusts of up to  $10.2 \text{ m s}^{-1}$ . Of 45 beetles found on the plants, 43 were upwind from the release point. The fastest

**Figure 3** (A) Beetles (dots) recaptured on a host plant under north-wind conditions. The arrows indicate the wind direction, which was shifting between  $330^\circ$  and  $50^\circ$ . The mean angle of beetle movement ( $\phi$ ) was  $43^\circ$ . The data represent three releases of 50 beetles each, at different dates. (B) Beetles recaptured on a host plant under south-wind conditions. The arrows indicate the wind direction, which was shifting between  $160^\circ$  and  $200^\circ$ . The mean angle of beetle movement ( $\phi$ ) was  $139^\circ$ . Data represent one release of 50 beetles.



beetle (a female) reached a host plant leaf after a walk of 5.2 m in 33 min. Within 2 h, 22 beetles had reached a host (11 females and 11 males) at an average distance of 4.2 m from the point of release. Determination of the mean angle ( $\phi$ ) of beetle movement towards a host yielded a value of  $42.7^\circ$ , and the movement was significantly directed with  $R = 0.38$  and  $P = 0.002$ .

Of the 50 beetles released under rare south wind conditions, 34 beetles were recaptured on host plant leaves located upwind, within 6 h after release, whereas only one was recaptured downwind (Figure 3B). The mean distance walked from the point of release to a host plant was 8.2 m [mean ( $\pm$  SD) females:  $8.0 \pm 2.5$ ; mean males:  $8.3 \pm 2.5$ ], and the wind was blowing at an angle of between  $160^\circ$  and  $200^\circ$ , at a fairly constant speed around  $2.1 \text{ m s}^{-1}$ . The beetles' movement was significantly directed, with a mean angle of  $138.5^\circ$ , with  $R = 0.87$  and  $P = 0.001$ .

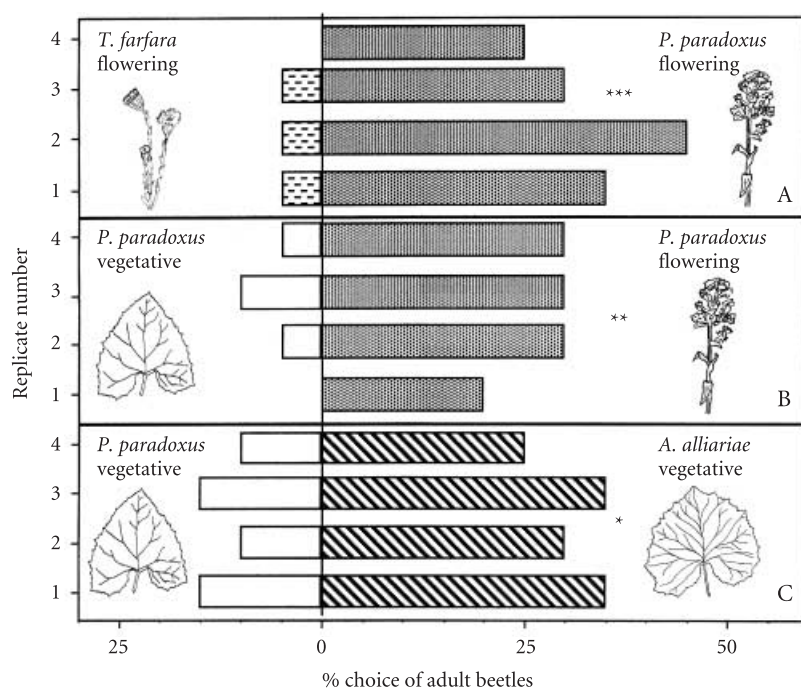
### Laboratory results

**Wind tunnel assays.** In the wind tunnel, *O. cacaliae* was more attracted by flowering *P. paradoxus* than by flowering *T. farfara* ( $P < 0.001$ ; Figure 4A). Flowering *P. paradoxus* was more attractive than its vegetative state ( $P < 0.01$ ; Figure 4B). It should be noted that *P. paradoxus* flowers in the spring and only produces leaves in summer. Comparing vegetative stages, the main summer host *A. alliariae* was significantly more attractive than the spring host *P. paradoxus* ( $P < 0.05$ ; Figure 4C).

**Analysis of pyrrolizidine alkaloids.** Gas chromatography analysis of extracts from *P. paradoxus* revealed the presence of seneciophylline in the calyx, the petals, and the flowerhead stems (data not shown), which represent the plant parts fed on by *O. cacaliae*. This plant defence compound is also the dominating PA in leaves of *A. alliariae* and is readily sequestered by the beetles for their own protection against predators (Ehmke et al., 1991, 1999; Pasteels et al., 1995; Rowell-Rahier et al., 1995).

### Discussion

This study has revealed a unusual polymorphism in the overwintering behaviour of the alpine leaf beetle *O. cacaliae*, as summarized in Figure 2. At the end of the summer season, part of the population migrates to specific hibernation sites, whereas the remaining individuals overwinter in the soil next to the primary host plant, *A. alliariae*. The following spring, the migrating individuals emerge much earlier than their conspecifics due to the higher temperatures at the sun-exposed hibernation sites. The adults are thus able to exploit a second host plant, *P. paradoxus*, which flowers very early in the season and from which they can sequester the defence compound seneciophylline. Later in the season, at about the same time that their conspecifics emerge from under *A. alliariae*, the migratory morph also moves from *P. paradoxus* onto *A. alliariae*. The travelling beetles disperse by flight, but appear to orient towards the plants only by walking, using olfactory cues to locate their host plants.



**Figure 4** Response of *Oreina cacaliae* towards odours emitted by potted host (*Petasites paradoxus* and *Adenostyles alliariae*) and non-host plants (*Tussilago farfara*) in a wind tunnel. Beetles were presented with a choice between (A) *P. paradoxus* and *T. farfara*, (B) the flowering state of the spring host *P. paradoxus* and its vegetative state, and (C) *A. alliariae* and *P. paradoxus*, both in a vegetative state. Similar shadings represent the same plant species. Each experiment was repeated four times with 20 beetles. Significance levels are indicated as \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  (binomial test).

The beetles that left the host plant patches in autumn flew distances of up to 1 km to the hibernation sites. How they located these sites remains unknown. The relatively high temperature at the sun-exposed overwintering sites must be the key factor explaining why flying *O. cacaliae* emerged earlier than non-flyers that overwintered in shady *A. alliariae* patches. The fact that the snow melted early at the hibernation sites indicated that the temperature at the microclimatic scale was increased, compared to the surroundings. Temperature has also been found to be the most important extrinsic factor controlling diapause termination in the Colorado potato beetle (de Kort, 1990). The observed early emergence could be an adaptation to a short season of activity in an alpine environment.

In spring, *O. cacaliae* appeared to be relatively weak flyers, and take-off direction was against the prevailing wind. Beetles were observed crash landing on the snow from where subsequent take-off attempts were unsuccessful, presumably because of the cold. The distribution of beetles after landing on the snow in the surroundings of a hibernation site was found to be random rather than aggregated, and often far removed from the three spots where the first herbaceous host plant, *P. paradoxus*, had already emerged. Since the beetles reached *P. paradoxus* by walking, we consider *O. cacaliae* incapable of precisely approaching a host plant by flight. However, orientation by walking was efficient, as a few weeks later *P. paradoxus* plants, in the area surrounding the hibernation sites, were crowded with *O. cacaliae*.

Several beetles were found to move from one *P. paradoxus* patch to another, indicating that they may fly or walk to another patch when they exhaust a food source. The time of emergence of the main host *A. alliariae* coincided with the wilting of flowers and the formation of leaves on *P. paradoxus* plants. It was at that time of the season that *O. cacaliae* left *P. paradoxus* by flight and switched to *A. alliariae*, where they fed and larviposited. The beetles travelled as much as 1 km before arriving at an *A. alliariae* patch. Mark-recapture studies with two other chrysomelids reported travel distances around 500 m (Herzig, 1995; Follett et al., 1996). Beetles marked at hibernation sites were recaptured on *P. paradoxus* 2–6 weeks after marking and about 3 weeks after that on *A. alliariae*.

The results obtained from these mark-recapture surveys reveal a much more complicated life cycle of *O. cacaliae* than was previously thought (Figure 2). *Petasites paradoxus* can be considered the spring host on which adult *O. cacaliae* feed exclusively until the emergence of *A. alliariae*. One advantage of aggregating at hibernation sites may be the opportunity to mate prior to dispersal, which allows a female arriving in a suitable habitat to immediately produce offspring with no need to locate a mate (Caldwell

& Rankin, 1974). Females of *O. cacaliae* that mate before hibernation, and do not re-mate in spring, larviposit as successfully as females that do re-mate in spring (Dobler & Rowell-Rahier, 1996). Thus, single females are able to disperse and find new colonies on *A. alliariae* patches so far devoid of beetles. The rate of inbreeding can be high for *O. cacaliae* in established *A. alliariae* patches (Knoll et al., 1996). Mating at the hibernation sites with unrelated partners and the spread of *O. cacaliae* into a new host plant patch would therefore be adaptive. Moreover, if winter mortality is significant, females may increase their fitness by re-mating after dormancy with males that were able to survive the winter (Stevens & McCauley, 1989). Indeed, *O. cacaliae* were observed mating after a successful hibernation. *Petasites paradoxus* plants fed on by conspecifics are more attractive to *O. cacaliae* than undamaged plants (Kalberer et al., 2001), which should further facilitate aggregation and mating after hibernation.

The observed autumn migration may also simply be the result of leaving overexploited habitats to try to find more suitable plant patches the next season. Flyers (males as well as females) collected from *P. paradoxus* have been found to be smaller in size than non-flyers (Kalberer & Rahier, 2003). Within non-flyer groups, small females deposit significantly fewer larvae than large females (Kalberer & Rahier, 2003). Sub-optimal nutrition in a bad host plant patch is likely to result in smaller beetles, thus it may be adaptive for adults to fly away from poor habitats to locate plant patches that offer ample nutrition for their offspring (Kalberer & Rahier, 2003).

The importance of olfaction in the host finding process is implied by the short-range field experiment. Beetles of both sexes walked upwind either under north- or south-wind conditions and located host plants within hours. Considering that the beetles were released under identical visual surroundings, and located host plants in different directions under different wind conditions, we conclude that *O. cacaliae* relies primarily on olfactory rather than on visual cues to locate their host plants.

The use of olfaction in host plant selection by *O. cacaliae* had previously been shown in wind tunnel experiments, whereby plant odours were piped into the tunnel, thus omitting visual cues (Kalberer et al., 2001). The results from the wind tunnel experiments presented here confirm the host plant preferences observed in the field. The flowering spring host *P. paradoxus* was clearly more attractive for walking *O. cacaliae* than flowering *T. farfara*, a related non-host. After their flowers had wilted, *P. paradoxus* were no longer attractive, and the beetles showed a strong attraction to *A. alliariae* leaves. The PA seneciphylline was present in all plant tissues of flowering *P. paradoxus* that were eaten by *O. cacaliae*, such as the calyx, the petals, and

the stems of the flower heads. Seneciphylline also represents up to 95% of the total amount of PAs detected in leaves of *A. alliariae* (Hägele & Rowell-Rahier, 1999). The unattractive vegetative tissues of *P. paradoxus* are devoid of PAs, as was previously shown by Rowell-Rahier et al. (1991). By feeding on the flowering rather than the vegetative parts of *P. paradoxus*, in addition to vegetative *A. alliariae*, the beetles ensure the continuous presence of PAs in their diet, which they sequester for their chemical defence (Rowell-Rahier et al., 1991).

In conclusion, our field observations over 2 consecutive years revealed a sophisticated migration process by part of the *O. cacaliae* population, which allows it to prolong its season of activity and to utilise an additional spring host, *P. paradoxus*. The beetles travel by flight to and from hibernation 'hotspots', but precise orientation towards plant patches is done by walking with the use of olfactory cues.

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