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ORIGINAL PAPER

Specific ant-pollination in an alpine orchid and the role of floral scent in attracting pollinating ants

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Abstract Several studies have recently shown that floral scent can deter ants from flowers. However, when ants serve as reliable pollen vectors, for example in harsh, windy habitats, where flying insects are less active, plants should have evolved floral signals to attract them to the flowers. We tested this hypothesis in the alpine orchid, *Chamorchis alpina*. *C. alpina* was found to be predominantly ant-pollinated, with some occasional pollination by ichneumonid wasps. In all three investigated populations, only two species of ants, *Formica lemni* and *Leptothorax acervorum* visited the flowers and removed pollinaria. These two pollinator ants were found to be among the most common ant species in all habitats, but other, non-pollinating ants were also frequently found, suggesting a factor that mediates specific pollination. Floral morphology was found to be compatible with at least one of the common non-pollinator ants. Floral scent consistently comprised five terpenoid compounds, β -phellandrene, 1,8-cineole, linalool, α -terpineol, and β -caryophyllene. A synthetic blend of these five compounds emitting from rubber septa, was found to be attractive to one pollinator ant-species, *F. lemni*, in the field. The floral scent of *C. alpina*, through attracting only specific ants, may thus play a role in filtering floral visitors.

Keywords Floral VOC · Floral evolution · Floral filter · Orchidaceae · Formicidae

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Introduction

Animal pollination is the most common mode of pollen transfer among angiosperms and represents a key mutualism for ecosystem functioning (Faegri and van der Pijl 1979). Plant adaptation to animal pollinators is seen as an important force for the astonishing diversification of angiosperms (Grant and Grant 1965; Stebbins 1970; Waser and Campbell 2004). Such diversification in relation to pollinators is thought to be driven through adaptations to a pollinator mosaic or “climate of pollinators” (Grant and Grant 1965), i.e. the community of efficient pollinators, which may vary along an ecogeographic transect. Besides adaptation to varying abundances of effective pollinators, specialization in pollination through adaptation to a subset of the available potential pollinator (guilds) plays a crucial role for pollinator-mediated plant evolution (Fenster et al. 2004; Kay and Sargent 2009; Schiestl and Schlüter 2009). Adaptations to pollinators leading to evolutionary specialization are mediated by floral traits, acting as filters by permitting the exploitation of rewards by only certain (groups of) pollinator animals (Johnson et al. 2006; Johnson and Steiner 2000). Such specialization can convey floral isolation, leading to assortative mating of plants sharing adaptive traits (Schiestl and Schlüter 2009). Despite the recent debate about the frequency of specialized pollination among plants, it is clear that highly specialized pollination systems can be found repeatedly across several plant families (Johnson and Steiner 2000). Orchids, however, provide perhaps the most illustrative examples, with sometimes only one or a few pollinator species involved (Schiestl and Schlüter 2009).

Specialized pollination can be mediated by floral morphology, rewards, and floral signals. Floral morphology can mediate functional specialization, for example by

concealing rewards in floral spurs (Nilsson 1988; Whittall and Hodges 2007). Floral nectar can act as filter by its chemical composition, conveying unpleasant taste to potential, unwanted visitors (Johnson et al. 2006). Because nectar does not act as a signal by itself, its taste is associated with floral signals by the visitors (Wright and Schiestl 2009). Among floral signals, color can mediate a switch between pollinator guilds, for example from bee- to hummingbird pollination, through learning and innate sensory preferences by pollinators (Melendez-Ackerman et al. 1997; Schemske and Bradshaw 1999). Only floral scent, however, has up to now been shown to mediate species-specific pollinator attraction, namely in sexually deceptive orchids (Schiestl 2005), a fungus “pollinated” by anthomyiid flies (Schiestl et al. 2006), and nursery pollination systems (Svensson et al. 2010). Floral scent is effectively a composite trait, because it normally consists of many different volatile organic compounds (VOCs) encoded by usually several different genes (Pichersky et al. 2006). Therefore, floral VOCs can have different functions, notably through attracting and/or deterring floral visitors (Junker and Bluthgen 2008; Schiestl 2010). Interestingly, several recent studies have highlighted the potential function of floral VOCs in deterring ants from flowers (Junker and Bluthgen 2008, 2010). Ants frequently interact in an antagonistic way with the reproductive functions of plants, by robbing nectar or repelling pollinators. In few cases, however, ants have been shown as effective pollinators of plants (Rostas and Tautz 2010).

Up to now, mechanisms of specific pollinator attraction have been analyzed in only few pollination systems, and never in ant-pollinated plants. In this study, we tested the role of floral scent in specifically attracting ant-pollinators in an alpine orchid to address the following questions: (1) which ant species pollinate the orchids across three disjunct populations? (2) What are the abundances of the pollinating and non-pollinating ants in the habitats of the orchid? (3) Which floral traits mediate the specific attraction of pollinators in this system?

Materials and methods

Plant populations, floral biology

Three populations of *Chamorchis alpina* (L.) L.C.M. Richard in the Swiss Alps were visited during a total of 6 days between 2008 and 2010: Cadagno (Ticino, N46°32'50.4" E008°41'30", 2,117 m) Munter (Graubünden, N46°37'43.1" E009°31'17.1", 2,179 m) and Albula (Graubünden, N46°34'54" E009°48'49.4", 2,220 m; for dates see Table 1). Flowering plants of *C. alpina* were observed for 3–4 h per day and all visitors were recorded and collected if possible. 12 randomly chosen plants were

investigated for removed pollinia and massulae deposited. To do so, pollinia and stigma were investigated using a hand lens with 10× magnification. Pollinia bending time was measured by taking out pollinia on a toothpick and measuring the time until bending was completed.

Ant species in the habitat

The composition of the ant fauna in *C. alpina* habitats was investigated by three different methods, nest counts, apple baits, and baited pit fall traps. Nest counts were done at all sites; ant colonies were counted by turning stones and exploring grass tussocks and other potential nest sites. Nest frequencies were estimated by the number of colonies sampled per hour (sample times and date: Munter: 70 min, 6 July 2010; Albula: 80 min, 7 July 2010; Cadagno, 75 min, 8 July 2009). From each colony, worker ants were collected and stored in 75% ethanol for determination. Counts on apple baits were also done at all sites; 13 apple pieces (ca. 3 cm diameter) were positioned with a distance of 2 m along a transect. Afterwards, ants sitting on the apple pieces were counted in intervals of 15 min. Single specimen which could not be determined in the field without doubt were collected and stored in 75% ethanol (observation times and date: Munter: 17:00–17:50 (CEST), 6 July 2010; Albula: 10:50–12:50, 7 July 2010; Cadagno, 9:40–12:40, 8 July 2009). Baited pit fall traps were only used in Cadagno; 25 plastic tubes (Saarstedt, length: 7.5 cm, diameter: 2 cm) were placed evenly with the ground surface with a distance of approximately 3 m, forming 4 parallel transects. Tubes were half filled with alpine herb liquor (“Gurktaler Almkräuter”, see Dietrich and Ölzant 1998) and exposed for 24 h between the 6 and 7 July 2010. After sampling, the ants were washed with water and stored in 75% ethanol.

Ant determination

Ants were determined using a Nikon SMZU (magnification 150× equipped with measuring eyepiece) following the keys in Seifert (2007) and Kutter (1977, males). The ant material was deposited into the collection of F. Glaser.

Morphological measurements

Head widths of five workers of each of the four dominant species *Leptothorax acervorum*, *Myrmica lobulicornis*, *Formica lemani* and *F. cf. paralugubris* were measured according to Seifert (2007) using a Nikon SMZU microscope with magnifications between 40× and 80×. The investigated specimens were collected at Cadagno in pitfall traps and were stored in 75% ethanol before measuring. Flowers of three individuals of *C. alpina* inflorescences were

Table 1 Visitors and pollinators of *C. alpina* in the study populations in Switzerland

Population	Munter		Albula		Cadagno	
	06.07.2009	08.07.2010	25.07.2008	08.07.2009	06.07.2010	07.07.2010
Date	06.07.2009	08.07.2010	25.07.2008	08.07.2009	06.07.2010	07.07.2010
<i>Formica lemni</i> ^a without pollinaria	11	3	6	2	1	14
<i>F. lemni</i> male without pollinaria	0	0	1	0	0	0
<i>F. lemni</i> ^a with pollinaria	3	0	0	0	0	1
Sum <i>F. lemni</i>	14	3	6	2	1	15
<i>Leptothorax acervorum</i> ^a without pollinaria	3	1	0	2		2
<i>L. acervorum</i> ^a with pollinaria	0	0	0	3	1	0
Sum <i>L. acervorum</i>	3	1	0	5	1	2
Ichneumonidae 1 with pollinaria	0	1 ^b	1	0		
Ichneumonidae 2 without pollinaria	0		0	1 ^b		
1 Fly (Brachycera) without pollinaria		1 ^b				

^a Worker ants ^bnot caught

collected and stored in 70% ethanol. The widths of the stigmatic cavity of these flowers were measured; this distance is meaningful for the fit of a potential pollinator, because nectar is produced just below the stigmatic cavity at the base of the labellum.

Floral scent collection

Floral scent was collected from 8 individual plants in the three populations (Munter and Albula: 3, Cadagno: 2). Scent collection was done in early afternoon. Plants of *C. alpina* were enclosed in nalophan oven bags; air was drawn out of these bags using battery-operated pumps (PAS-500 personal air sampler Spectrex, Redwood city, CA, USA) at a rate of 100 ml/min, through glass tubes filled with ca 20 mg of Tenax). Scent collection commenced for approximately 2 h. In each population, scent of the surrounding air was collected as a control using the same collection parameters. After scent collection, the tenax tubes were transported to the lab as soon as possible and stored at -30°C until gas chromatographic analysis.

Analysis of floral scent

For analysis of floral scent, gas chromatography with mass selective detection (GC-MSD) was used. Samples were injected into a GC (Agilent 6890 N) using a Gerstel thermodesorption system (TDS3, Gerstel Mühlheim, Germany) with cold injection system (KAS4; Gerstel). For thermodesorption, the TDS was heated from 30 to 240°C at a rate of $60^{\circ}\text{C}/\text{min}$ and held at the final temperature for 5 min. The KAS was set to -150°C during trapping of eluting compounds from the TDS. For injection, the KAS was heated to 250°C at a rate of $12^{\circ}\text{C}/\text{s}$, and the final temperature was held for 3 min. The GC was equipped with a DB-5 column (0.32 mm ID, 0.25 μm film thickness, 30 m length), helium

was used as carrier gas at a flow rate of 2 ml/min. Compound determination and quantification was done using a mass selective detector (Agilent MSD 5975). Compounds were identified by comparison of spectra obtained from the natural samples with those from a reference collection (NIST library). In addition, retention times and spectra of synthetic standard compounds were compared with those of natural samples. To quantify individual compounds, synthetic standards were analyzed in two different amounts (10 and 100 ng) on the GC-MSD system, and the resulting peak areas of the total ion chromatograms used to calculate a regression of amount and peak area. The linear regression coefficient was used to calculate the amounts in the natural samples from peak areas obtained in total ion chromatograms.

Bioassays with synthetic compounds

The following scent compounds were found to occur consistently in all *C. alpina* populations and were thus used for bioassays: β -phellandrene (Dragoco), eucalyptol (=1,8-cineole; Givaudan), linalool (racemic, Fluka) α -terpineol (Givaudan), β -caryophyllene (purum, Sigma). For bioassays with synthetic compounds, grey GC Septa (Supelco GR-2 septa, 11 mm diameter) were cut into four pieces of equal size. These septa were soaked for 2 h in a solution of synthetic scent compounds (β -phellandrene 3.7 μl ; eucalyptol 7.5 μl ; linalool 5 μl ; α -terpineol 2.5 μl ; β -caryophyllene 1.7 μl) in 900 μl dichloromethane. Then, septa were taken out of the solution, and left on aluminium foil for 1 h to ensure evaporation of the solvent. To control evaporation rates of synthetic compounds from the septa, compounds emitted from the septa were collected with headspace sorption using the same conditions as for collecting floral scent. The evaporation rate of compounds from septa treated in this way was about 10 times higher than from natural

inflorescences (Table 2), which was considered a good estimate of the actual amounts scent compounds in the habitat, because *C. alpina* plants often flower in dense clones of 5–15 ramets. The proportion of most compounds emitting from the septa was within the range of concentrations found in *C. alpina* populations (Table 2). Odorless control septa for bioassays were soaked in solvent only but otherwise treated in the same way. For the bioassays in the field, septa were positioned on the ground, in places with little vegetation, in the *C. alpina* habitat, but approximately 50 m away from flowering plants. Behavior of ants was classified as (a) approach 1 cm towards the septum and (b) touch of the septum with either antennae or legs, or walking over the septa. Bioassays were run for 10 min; each septum was used for only one assay, and after each assay, the test location was moved for approximately 10 m.

Statistical analysis

Mann–Whitney *U* tests (2 species) and Kruskal–Wallis tests (more than 2 species) were used to compare numbers of ants recorded with apple and pitfall traps. To statistically compare numbers of individual species within populations and trap-type (if more than two species were found), Mann–Whitney *U* tests were used for a posteriori multiple comparison, with the level of significance set to $P = 0.02$ (3 species) or $P = 0.01$ (5 species). Mann–Whitney *U* tests were also used to compare behavioral responses of ants towards scentless and scented septa. All statistical analysis was done in SPSS 16 (SPSS Inc.).

Results

Floral biology, breeding system

Flowers of *C. alpina* produce openly accessible nectar in the stigmatic cavity. In all three populations, only two species of ants were found to visit the flowers for nectar and thereby to remove pollinaria (Table 1). During 6 observational days, spread over three flowering seasons, 38 individuals of *Formica lemni* were found visiting flowers, of which 4 carried

pollinaria. In addition, nine individuals of the considerably smaller *Leptothorax acervorum* were found visiting flowers, of which also four carried pollinaria. We noted several times that this ant species is difficult to see on the flowers, because of its small size, slow movements, and inconspicuous color. The lower representation of this species may therefore be an artifact of its cryptic appearance to some extent. All pollinaria on ants were situated in the central or lateral region of the head (frons), always between the frontal carinae (see Fig. 1 showing a worker of *Leptothorax acervorum* carrying pollinia). We did not find pollinaria in other parts of the ant body. It is therefore likely that the removed pollinia also fitted into the stigma thus enabling pollination. In addition, two ichneumonid wasps were observed visiting flowers, of which one took pollinaria; one fly was visiting a flower but did not remove pollinaria.

The available data suggest *C. alpina* is dependent on animal pollinators for seed set. Pollinator exclusion experiments, however, were not yet done in this species. Several old flowers, however, were seen with pollinaria unaltered in their anther sacs, suggesting a lack of spontaneous autogamy. Of the 12 plants with a total of 57 open flowers (mean \pm SD open flower per plant: 4.75 ± 2.18) investigated for deposited massulae, a mean of $45 \pm 33.3\%$ had received at least a single massula. Individual inflorescences had a mean of 6.42 ± 2.68 pollinaria removed. One pollinium contained a mean of 46.8 ± 5.37 massulae. Thus, the mean number of massulae removed from an inflorescence was 300.3 ± 125.35 . Inflorescences received a mean of 14 ± 14.66 massulae. The pollen transfer efficiency, calculated from this data, was 4.66%. Pollinarium bending time was 3.67 ± 0.29 min.

Ant species in the habitat

Overall, seven ant species were recorded in the habitat of *C. alpina* (Table 3). The two pollinator species, *F. lemni* and *L. acervorum*, and the non-pollinator *Myrmica lobulicornis* were recorded at all sites and showed the highest nest frequencies, too (Table 3).

Nest counts Nests of three ant species were recorded in the *Chamorchis* habitats (Table 3). In addition, two nest

Table 2 Composition and variability of floral scent in *Chamorchis alpina*, and emission of synthetic compounds from septa used in the bioassays

Compounds	Relative amounts in floral emission (mean \pm SD, %)	Relative amounts in floral emission (min–max, %)	Emission from septa (%)
β -Phellandrene	6.80 \pm 1.42	5–9	17.19
Eucalyptol	28.65 \pm 8.07	19–45	52.80
Linalool	30.13 \pm 12.86	10–48	18.97
α -Terpineol	25.05 \pm 7.09	14–38	9.49
β -Caryophyllene	9.36 \pm 9.00	0–24	1.54
Sum total amount (ng/liter)	19.65 \pm 3.35		190.54

Fig. 1 Flowering plants of *Chamorchis alpina* and one of its pollinator ant-species, *Leptothorax acervorum*, carrying a pollinarium of the orchid

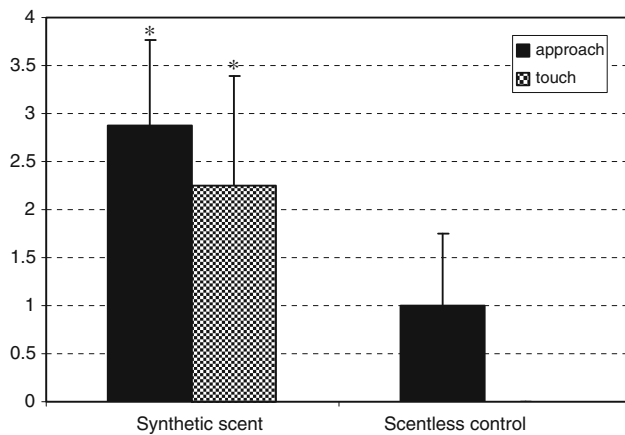


Fig. 2 Responses of *Formica lemani* workers in bioassays with synthetic scent compounds. The ants showed significantly more approaches and touches to septa soaked with synthetic scent compounds compared to scentless control septa

mounds of *Formica exsecta* and one of *F. paralugubris* were found approximately 50 m away from flowering *Chamorchis* plants at the Cadagno population. *F. lemani* and *L. acervorum* showed the highest nest frequencies per hour, whereas *M. lobulicornis* was rarer (Table 3). Populations, however, differed in abundances of nests; at Munter *L. acervorum* nests were the most abundant ones, whereas no colonies of the other species were found. At Albula, nests of *L. acervorum* also dominated, but we also found several nests of *F. lemani* and one nest of *M. lobulicornis*. At Cadagno *F. lemani* nests were the ones most frequently found, whereas nest numbers of the other two species were similar to Albula. **Apple baits** No significant differences were detected in the occurrence of *F. lemani* and *M. lobulicornis* on apple baits at Munter and Albula. At Cadagno,

significant differences between the three species found on apple baits were found ($\text{Chi}^2 = 13.77$, $P = 0.01$). *F. lemani* was significantly more common than *M. lobulicornis* ($U = 17$, $P < 0.001$); *F. paralugubris* was not significantly different from the other two species. There was a temporal difference in the detection of the apple baits by the ant species. *F. lemani* and *M. lobulicornis* detected the baits at all three sites after 15–20 min. *M. lobulicornis* exploited the baits exclusively within the first hour, whereas *F. lemani* could be observed during the whole exposition time. *F. paralugubris* appeared at the baits after one hour. **Baited pitfall traps** were used only at the Cadagno site. With this method, six ant species could be recorded, in significantly different capture rates ($\text{Chi}^2 = 55.46$, $P < 0.001$). *F. lemani* was significantly more common in the traps than all the other ant species (Table 3; *F. lemani* compared to: *F. paralugubris*: $U = 80.5$, $P < 0.001$; *L. acervorum*: $U = 26$, $P < 0.001$; *M. lobulicornis*: $U = 55.5$, $P < 0.001$; *M. lonae* $U = 22$, $P < 0.001$; *M. sulcinodis*: $U = 34$, $P < 0.001$); *F. paralugubris* and *M. lobulicornis* were significantly more common than *M. lonae* ($U = 134.5$, $P = 0.007$; $U = 144$, $P = 0.007$). Numbers of the other ant species were not significantly different from each other. *L. acervorum*, however, was underrepresented in the traps compared with the data of nest counts (Table 3). Overall, *M. lonae* und *M. sulcinodis* were only rarely found in traps.

Morphological measurements

Head widths of ant species varied between 0.75 ± 0.07 mm (*L. acervorum*) and 1.89 ± 0.15 mm (*F. paralugubris*; Fig. 3). Mean width of stigmatic cavity of *C. alpina* was 1.32 ± 0.03 mm. It seems likely that all species with

Table 3 Ant species recorded in the three *Chamorchis alpina* populations Munter, Albula, and Cadagno in 2009 and 2010 using different methods

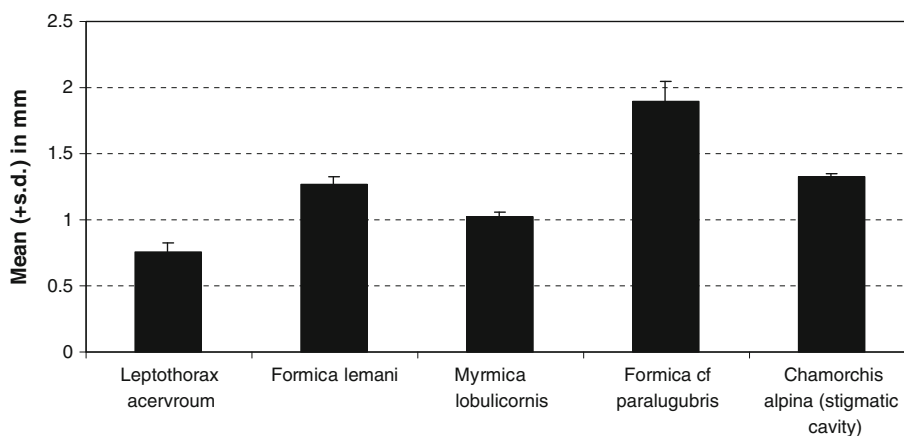
	Populations							
	Munter		Albula		Cadagno			
	Type of recording							
	NC	B	NC	B	NC	B	P	
<i>Formica exsecta</i> Nylander, 1846	0	0	0	0	2	0	0	
<i>Formica lemani</i> ^a Bondroit, 1917	0	0.23 ± 0.36 ^A	2.3	0.15 ± 0.31^A	7.2	0.89 ± 0.79^A	9.7 ± 8.4^A	
<i>Formica paralugubris</i> Seifert, 1996	0	0	0	0	1	0.42 ± 0.83 ^{AB}	2.9 ± 6.5 ^B	
<i>Leptothorax acervorum</i> ^a (Fabricius, 1793)	9.4	0	4.6	0	4.8	0	0.2 ± 0.4 ^{BC}	
<i>Myrmica lobulicornis</i> Nylander, 1857	0	0.48 ± 1.19^A	0.8	0.03 ± 0.06 ^A	0.8	0.02 ± 0.05 ^B	1.3 ± 2.2 ^B	
<i>Myrmica lonae</i> Finzi, 1926	0	0	0	0	0	0	0.1 ± 0.2 ^C	
<i>Myrmica sulcinodis</i> Nylander, 1846	0	0	0	0	0	0	0.5 ± 2 ^{BC}	

NC nest counts (nests per hour), B apple baits (mean ± SD worker ants recorded on apple pieces), P baited pit fall traps (mean ± SD worker per trap)

The most common ant species for each type of recording is given in bold. Different letters indicate significant differences among species (tested separately for each population and trapping method)

^a Pollinator species

Fig. 3 Head widths of different ant species recorded in the habitat of *Chamorchis alpina*, as well as width of the stigmatic cavity of *C. alpina* flowers



head widths smaller than the stigmatic cavity will be able to reach the nectar; according to this criterion, all ants except *F. paralugubris* could in principle serve as pollinator (Fig. 3).

Floral scent

The floral scent bouquet of *C. alpina* consisted of five terpenoids compounds (Table 2), the monoterpenes β -phellandrene, eucalyptol, linalool, α -terpineol, and the sesquiterpene β -caryophyllene (Table 2). These compounds were found to be emitted consistently in all three populations, however, β -caryophyllene was not detected in one individual from Cadagno. None of the compounds was

found in the collections from surrounding air used as controls. These five compounds were thus used for bioassays.

Bioassays with synthetic scent compounds

The results of the bioassays showed that *Formica lemani* was attracted to the blend of scent compounds used. Significantly more ants approached the scented septa than the scentless septa ($U = 13$, $P = 0.04$); in addition, several ants touched the scented septa with their antenna or legs, whereas this behavior was never observed with scentless septa ($U = 16$, $P = 0.027$). In some experimental runs, the scented septa appeared highly attractive to the ants as several of them aggregated around it or walked over it. No other ant

species or other insects were seen approaching the septa during the experiments.

Discussion

In this study, we show that the alpine orchid *Chamorchis alpina* is pollinated by two species of ants, and occasionally by ichneumonid wasps. The two pollinator-ant species were generally the most frequent ant species in all populations, but other potential pollinator species were also present. A key trait mediating this specialization may be the floral scent that we show here to selectively attract one of the pollinator ants, *Formica lemani*.

Ant pollination is generally considered rare among angiosperms (Beattie 2007; Peakall et al. 1991; Rostas and Tautz 2010), but is more frequently found in harsh environments, such as arid or alpine environments (Beattie 2007; Gomez and Zamora 1999). Ant pollination may provide a rather reliable mechanism for pollen transfer in alpine plants, as ants visit flowers even at windy and cold weather conditions (own personal observations). A disadvantage of ant pollination may be its rather low efficiency, suggested by our estimate of pollen transfer efficiency of under 5%, and potential high figures of inbreeding (Peakall and Beattie 1991). Many ants also produce antimicrobial secretions in metapleural glands that reduce pollen viability (Beattie et al. 1984). While such toxic surface chemistry of ant cuticles may reduce their effectiveness as pollinators for most plants, many orchids, however, have their pollen transported in stalked pollinia, which provide protection against toxic cuticular secretions (Peakall et al. 1990). Nevertheless, ant pollination is also rare among the orchids (Beattie 2007). Up to now, only two orchid species are known to be exclusively pollinated by ants, namely the sexually deceptive *Leporella fimbriata* (Peakall 1989), relying on pseudocopulating male ants, and the nectar rewarding *Microtis parviflora*, pollinated by foraging worker ants (Peakall and Beattie 1989). The nectar rewarding *Chamorchis alpina*, is a new example of specialized ant pollination, recorded the first time by Baumann and Baumann (2010). In our study, ants were the dominant pollinators, however, we also found few ichneumonid wasps as flower visitors capable of taking pollinaria, whereas flies as occasional visitors never removed pollinaria from the flowers (Table 1). Pollination by ichneumonid wasp was suggested for *C. alpina* by Müller (1881) based on its inconspicuous flowers resembling *Listera ovata*. The latter orchid is indeed pollinated mainly by ichneumonids, and ants act as occasional side-pollinators (Nilsson 1981). Interestingly, the two plants share their main floral scent compound, linalool, which may play a role in attracting ichneumonid wasps or ants to the flowers (Nilsson 1981).

Our comparison of *C. alpina* populations in regard to use of pollinators highlighted two main findings. First, the plants use the same ant pollinators across populations, and second, the plants use only a subset of the available ants for pollination. Relatively few pollination studies have incorporated pollinator observations in multiple populations, however, those that did frequently found variation in plant-pollinator systems across geographic ranges (summarized in Herrera et al. 2006; Gomez and Zamora 1999; Cosacov et al. 2008; Perez-Barrales et al. 2007). A lack of variation in pollinating ants among populations can be a consequence of highly specific, non-variable adaptations to given pollinators, or a lack of variation in pollinator abundances across habitats. We detected evidence for the latter reason in *C. alpina*, as the two pollinating ants were among the most common ant species in all three habitats. In addition to the geographic consistency in pollinator assemblage in *C. alpina*, we showed that this plant only uses a subset of the potentially available ants as pollinators. Earlier reports of ant pollination in *C. alpina* confirm our finding of *F. lemani* as the dominant pollinator in several populations, but did not record *L. acervorum* (Baumann and Baumann 2010). In our study, *F. lemani* and *L. acervorum* were among the most commonly occurring ants in all habitats, but other, non-pollinator species were also frequently found. *Myrmica lobulicornis* was present in all three habitats and found in similar abundances to the pollinator species on apple baits at Munter and Albula. This suggests that the species, which is of similar size as *F. lemani* (Fig. 2), occurs in similar frequency and has at least partly overlapping food preferences. *Formica paralugubris* was abundantly found in pitfall traps in Cadagno, and also foraged on apple pieces, however, this species is considerably larger than the two pollinator species and may hence not be able to exploit nectar produced in *C. alpina* flowers. The apparent specialization in pollinator use by *C. alpina* suggest that either (a) the non-pollinating ant species, because of their ecology, are not interested in floral nectar and therefore never visit these or even any flowers, or (b) floral traits act as filters for visitors (Raguso 2008).

Among traits that can act as specific attractants or repellents against ants and thus filter potential flower visitors (Beattie 2007), floral scent is a likely candidate in *C. alpina*. Whereas the cryptic green coloration of the flowers may make it difficult for bees and flies to find the flowers, visual cues are generally of lower importance in ants (Hölldobler and Wilson 1990), which may use specific floral VOCs to find the flowers. Floral scent is generally believed to be the most important trait for mediating highly specific pollinator attraction (Schiestl and Schlüter 2009). Chemical signals play diverse roles in the ecology of ants (Hölldobler and Wilson 1990), and several studies focused on the repellence effect of floral scent (Junker and Bluthgen 2008; Willmer et al. 2009). A potential function of floral VOCs in attracting

ants as pollinators has up to now mostly been ignored (Rostas and Tautz 2010). In our study, bioassays with synthetic blends mimicking the floral bouquet, clearly showed the attractiveness of these VOCs to one of the pollinator ants. The other pollinator ant-species, as well as all other ant species in the habitat were never seen close to scented septa. This suggests that the scent bouquet used did not attract these ants, or even repelled them. Alternatively, some of the ant species may simply avoid open spots bare of vegetation, or be less likely to walk on open ground. Repellency is difficult if not impossible to test in behavioral experiments in the natural habitat, therefore, bioassays in the lab would be necessary to test repellency of scent compounds. One of the compounds in the here tested blend, linalool, showed a significant repellent effect on other ants species in an earlier study using a four arm olfactometer assay (Junker and Bluthgen 2008). Thus, this compound could act as effective filter, by attracting specific ants but repelling others. Future experiments will shed light on the role of individual scent compounds emitted by *C. alpina* in filtering floral visitors and the maintenance of this specialized, unusual pollination system.

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