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## Olfactory versus visual cues in a floral mimicry system

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**Abstract** We used arrays of artificial flowers with and without fragrance to determine the importance of olfactory and visual cues in attracting insects to a floral mimic. The mimic is a fungus, *Puccinia monoica* Arth., which causes its crucifer hosts (here, *Arabis drummondii* Gray) to form pseudoflowers that mimic co-occurring flowers such as the buttercup, *Ranunculus inamoenus* Greene. Although pseudoflowers are visually similar to buttercups, their sweet fragrance is distinct. To determine whether visitors to pseudoflowers were responding to fragrance we performed an experiment in which we removed the visual cues, but allowed fragrance to still be perceived. In this experiment we found that pseudoflower fragrance can attract visitors by itself. In other experiments we found that the relative importance of olfactory and visual cues depended on the species of visitor. Halictid bees (*Dialictus* sp.) had a somewhat greater visual than olfactory response, whereas flies (muscid and anthomyiids) were more dependent on olfactory cues. We also used bioassays to determine which of the many compounds present in the natural fragrance were responsible for attraction. We found that halictid bees were equally attracted to pseudoflowers and to a blend containing phenylacetaldehyde, 2-phenylethanol, benzaldehyde and methylbenzoate in the same relative concentrations as in pseudoflowers. Flies, on the other hand, only responded to pseudoflower scent, indicating that we have not yet identified the compound(s) present in pseudo-

flowers that are attracting them. The ability of insects to differentiate pseudoflowers from true flowers by their fragrance may be important in the evolution of the mimicry system. Different fragrances may facilitate proper transfer of both fungal spermatia and pollen, and thus make it possible for the visual mimicry to evolve.

**Key words** Halictid bees · Diptera · Floral fragrance · Pollination ecology · Rust fungi

### Introduction

In a simple world, one could imagine that floral mimicry should be favored whenever a species receives more visits when it co-occurs with another similar species. Over many generations, selection could favor mutations that increase floral similarity (Macior 1971; Proctor and Yeo 1972; Straw 1972; Bobisud and Neuhaus 1975; Little 1983; Rathcke 1983; Thomson 1983; Dafni 1984, 1986). However, there is a complication in pollination systems. More visits do not always translate into higher fitness due to improper pollen transfer. Pollen that is transferred between species is, at best, simply lost, or at worst, the wrong pollen on a stigma can lead to reduced seed set, either by clogging the stigmatic surface or by allelopathy (Sukada and Jayachandra 1980; Thomson et al. 1981; Kephart 1983; Little 1983; Rathcke 1983; Armbruster and Herzog 1984; Dafni 1984, 1986; Waser and Fugate 1986; Feinsinger 1987; Galen and Gregory 1989; Murphy and Aarssen 1989; Randall and Hilu 1990; Kunin 1993; Inouye et al. 1994). Thus, for a floral mimicry system to evolve, the gains in visitation must outweigh the fitness losses from improper pollen transfer.

Although floral mimicry is rare compared to animal mimicry (Williamson 1982), it does occur, so there must be special circumstances under which the losses from improper pollen transfer are reduced or are acceptable. One obvious such circumstance occurs when a species is sufficiently rare and sufficiently pollination-limited that any gain in visitation as a result of similarity to another

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species would increase fitness. Although this threshold effect is intuitive, and has been shown theoretically (Rathcke 1983; Feinsinger 1987), it has never been established experimentally. However, the circumstantial evidence that most documented cases of floral mimics are rare relative to their models (Dafni 1984) tends to support this hypothesis. A factor that would speed the evolution of floral mimicry is for the mimic to insure proper transfer of its own pollen. Kunin (1993) points out that most of the better documented cases of floral Batesian mimicry involve orchids which package their pollen in pollinia that can only be transferred when the pollinator lands on the proper species.

We propose that unique floral fragrances may be another way for flower mimics to increase the probability that their pollen gets to the proper species. Flowering species that are visually similar may form a common long-distance advertisement, but after the patch has been found, visitors may cue in on particular species by additional non-visual factors such as fragrance. Observational and experimental data on bee behavior in non-mimetic systems suggest that this mechanism is plausible: flowers are initially located by visual cues such as patch density or flower color, then scent is the short distance cue that induces the visitor to land and enter a flower (von Frisch 1923; von Aufsess 1960; Faegri and van der Pijl 1971; Williams 1983; Dobson 1987, 1993). The potential for fragrance to play an important role in separating mimic from model at close range is also suggested by the fact that fragrance has been shown to be an important isolating mechanism for closely related flower species that are similar in appearance (Dodson et al. 1969; Nilsson 1983; Pellmyr 1986).

To determine the plausibility of fragrance as a mechanism that may help maintain visitor constancy for floral mimics, we have been experimentally manipulating a floral mimicry system. The floral mimic is a fungus, *Puccinia monoica* Arth., which causes its crucifer hosts (here, *Arabis drummondii* Gray) to form pseudoflowers that mimic yellow-flowered angiosperms such as the buttercup *Ranunculus inamoenus* Greene (Roy 1993, 1994). The fungal flower mimics require insect visitation for sexual reproduction (Roy 1993), just as flowers do; insects carry fungal spermatia (fungal pollen equivalents) from one infected plant to another. Pollen can be transferred from flowers to the fungus, but it is not known whether this pollen decreases fungal reproduction (Roy 1996). Although spermatia cannot cause infection, the transfer of spermatia to flowers reduces seed set, probably due to an allelopathic or hypersensitive reaction on the part of the plant (Roy 1996).

Fungal pseudoflowers are rare. Even when the crucifer hosts are common, disease incidence in a population is rarely greater than 5–10%, and density of infected plants may be much less than 0.1 per m<sup>2</sup> (personal observation). Earlier work has shown that pseudoflowers receive more visits when they co-occur with flowering species that they resemble (Roy 1994). While the evolution towards mimicry by the fungus may have been favored

by a rarity threshold induced by an outcrossing mating system that requires insect visitation, the evolution of similarity would be facilitated by mechanisms that decrease improper pollen/spermatia transfer. For example, after insects have been attracted to a patch as a result of their combined densities, both pseudoflowers and flowers could increase the visitation fidelity of insects through distinctive fragrances. Although pseudoflowers on *Arabis drummondii* are visually similar to buttercups, the chemical profiles of pseudoflower fragrances, while flower-like, are chemically distinct from buttercups and the other flowers with which they co-occur. *Puccinia monoica* pseudoflowers emit at least nine volatiles, most of which are aromatic alcohols, aldehydes, or esters, while the floral scent profiles of co-flowering plants are dominated by terpenoid compounds (R.A. Raguso and B.A. Roy, in preparation). Thus, if insects are sensitive to the particular scent compounds involved, they should be able to distinguish pseudoflowers from true flowers. The incentive for learning to recognize pseudoflowers, even though they are rare, is that pseudoflowers produce more nectar than co-occurring species (70, 50 and 18 times more sucrose equivalent than one flower of co-occurring *Ranunculus inamoenus* Greene, *Claytonia lanceolata* Pursh, and *Mertensia fusiformis* Greene, respectively; see Roy 1993).

In this study we hoped to determine whether or not fragrance was important in attracting pollinators, and under what circumstances. Experiments to examine the role of fragrance in maintaining constancy necessarily have to wait upon the elucidation of the role of fragrance in visitation. Here, we used arrays of artificial flowers and fungal pseudoflowers to address the following basic questions about pollinator response to pseudoflower fragrance:

1. Does fragrance affect visitor behavior to pseudoflowers in the absence of other cues?
2. What are the relative roles of fragrance and color in attracting insects to pseudoflowers?
3. Does fragrance play a role in long distance attraction to pseudoflowers?
4. Which of the compounds found in pseudoflowers are attracting insects?

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## Methods and results

### Localities

All of the experiments took place between 12 and 18 June 1995. The primary locality was a south-facing slope at c. 3215 m, near the Copper Creek trail and approximately 1 km east of Judd falls, Gunnison County, Colorado. The long-distance attraction experiment was conducted about 3 km from the above site, in a meadow at the Rocky Mountain Biological Laboratory, Gunnison County, Colorado.

**Table 1** Effect of fragrance on pollinator behavior in the absence of other cues. Summary of number of visits and mean visit duration for all visitors summed together, and separately for halictid bees and ants (*inf. minus pseudo*=infected, pseudo flowers removed)

Treatment	Total no. of landings (% of group total)	Total time (s) (% of group total)	Duration/visit (no. seconds/no. landings)
<i>All visitors</i>			
Covered, uninfected plant	2 (3.3)	33 (1.8)	16.5±6.5
Covered, inf. minus pseudo.	9 (15.0)	45 (2.4)	5.0±0.8
Covered, pseudoflower	21 (35.0)	397 (21.5)	18.9±4.7
Exposed, pseudoflower	28 (46.7)	1368 (74.2)	48.9±8.2
<i>Halictids</i>			
Covered, uninfected plant	1 (3.3)	10 (0.73)	10
Covered, inf. minus pseudo.	0	0	0
Covered, pseudoflower	5 (16.7)	46 (3.4)	9.2±4.4
Exposed, pseudoflower	24 (80.0)	1309 (95.9)	54.5±9.1
<i>Ants</i>			
Covered, uninfected plant	1 (3.6)	23 (7.0)	11.5
Covered, inf. minus pseudo.	9 (32.0)	45 (13.8)	5±0.3
Covered, pseudoflower	16 (57.1)	232 (70.9)	14.5±5.9
Exposed, pseudoflower	2 (7.1)	27 (8.3)	13.5±6.0

### Dependent variables

We counted the number of insect landings, the number of approaches (defined as obvious orientation of the insect toward the "flower" but without actually landing), and measured the duration of each visit (s/visit) when a landing occurred. For analysis of variance (ANOVA) we converted the raw data into fractions of the total per replicate (for example, number of landings on a particular treatment in a replicate divided by the number of all landings in that replicate), then we used the arcsine transform to normalize the binomially distributed proportions. We used proportions rather than raw numbers because the number of visitors available in any given spot fluctuates for a variety of reasons not related to the experimental design, such as temperature and time of day. We were not interested in quantifying this variation in the numbers of visitors; instead, we wanted to know whether insects visited the treatments in the same relative proportions, regardless of their total abundance.

Q.1. Does fragrance affect visitor behavior to pseudoflowers in the absence of other cues?

#### *Experimental design for experiment 1*

To determine whether visitors to pseudoflowers were responding to fragrance we performed an experiment in which we removed the visual cues, but allowed fragrance to still be perceived. Visual cues were removed by covering both uninfected and infected plants with small cotton bags tied at the base of the plant. For this experiment we located two adjacent 1-m<sup>2</sup> patches (about 3 m apart). Each patch contained three infected plants and one uninfected plant. These plants were treated as follows: (1) bagged uninfected, unmanipulated *A. drummondii* in early bolting phase, (2) bagged pseudoflowers on infected *A. drummondii*, (3) bagged infected plants with the pseudoflowers removed, and (4) pseudoflowers exposed, but

the lower part of the stem covered with a bag. Each patch was observed for three 20-min observation periods (= 3 replicates per patch, for a total of 1 h of observations/patch). To reduce observer bias, we alternated plots after each 20 min observation period. We recorded visit number and visit duration to each treatment, but did not record the number of approaches (observations during this experiment convinced us to measure the approach variable in subsequent experiments). We used ANOVA to examine the effect of treatment on visitation. Means were compared to the controls (open pseudoflowers) by using Dunnett's tests.

#### *Results of experiment 1*

Most visits (30/60) were made by halictid bees (*Dialictus* sp.). The rest of the visits were made by three other species: 28 by ants (*Formica* sp.), 1 by a wasp, and 1 by a species of muscid fly.

There was a significant effect of treatment on overall visit number ( $F_3 = 6.16$ ,  $P = 0.0039$ ); uninfected plants received the least visits and exposed pseudoflowers the most (Table 1). There was not a significant difference in visit number between covered pseudoflowers and pseudoflowers ( $P \leq 0.05$ , Dunnett's test), suggesting that a majority of the attraction was due to fragrance. However, the two major groups of visitors, halictid bees and ants, behaved very differently with respect to the treatments (Table 1). Halictid bees clearly preferred the exposed pseudoflowers over covered ones (Table 1, 24 visits versus 5), suggesting a larger visual component to attracting halictids. There were very few visits to the infected plants from which the pseudoflower had been removed (Table 1) indicating that the pseudoflower is the source of the olfactory attractants. Ants on the other hand, preferred the covered pseudoflowers over exposed ones (16 versus 2 visits). We suspect, however, that the ant data is probably at least somewhat an artifact of treatment and proximity to an ant mound. Normally, ants visit pseudo-

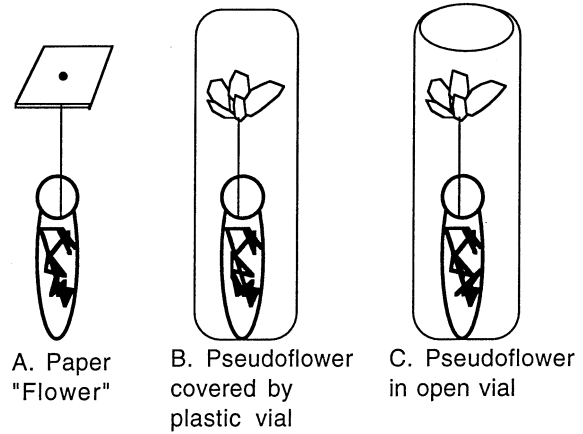
flowers rarely (0 to at most 7%, Roy 1994; 1996), however, in this particular experiment ants appeared to be quite interested in the bags used to cover the plants, perhaps because the covered plants tended to lie on the ground, whereas uncovered plants stood free on a thin stem. In addition, there was also a nearby ant mound, hence proximity to the nest is a likely explanation for some of the visits. This hypothesis is supported by data: the majority (13/16) of the visits by ants to the covered infected plants were during the three replicates where this treatment was near to the ant mound, while in the other three replicates the majority of ant visits (9/12) were also to the treatment adjacent to the mound (this time the treatment was: infected pseudoflowers minus infected parts).

There was a significant effect of treatment on the proportion of time insects spent on treatments ( $F_3 = 29.93$ ,  $P < 0.0000$ ). Insects spent a much larger proportion of their time on pseudoflowers than on any other treatment (Table 1), probably because the bags were not rewarding and pseudoflowers were. The actual visit duration per landing also varied (Table 1), with bees making the shortest visits to the bagged plants from which a pseudoflower had been removed, and the longest visits on exposed pseudoflowers.

Q.2. What are the relative roles of fragrance versus color in attracting insects to pseudoflowers?

#### Experimental design for experiment 2

To determine the relative roles of fragrance and color in attracting insects to pseudoflowers we performed experiments using artificial arrays composed of natural pseudoflowers placed in 2-ml microfuge tubes and artificial flowers constructed from a combination of microfuge tubes, colored paper, terry cloth wicks, and partially reconstituted pseudoflower fragrance (see Fig. 1A for the construction of artificial flowers). To reconstitute pseudoflower fragrance we collected and analyzed volatile scent compounds emitted from leaves of *Arabis holboellii* Hornemann infected with *Puccinia monoica* using dynamic head space collection, gas chromatography, and mass spectrometry (as described by Williams 1983, Patt et al. 1988; Raguso and Pichersky 1995). We enclosed 4.6 g of infected plant tissue within 0.5-l polyacetate bags (Reynolds, Inc.) and swept filtered, fragrance laden air across a glass cartridge (9 mm ID) packed with Tenax TA and activated charcoal (60–80 mesh) sorbents at a flow rate of 700 ml/h, for 7.5 h. Chromatogram peaks were identified by comparison with ambient and uninfected *Arabis holboellii* controls, and analyzed through comparison of mass spectra and retention times with those of standard compounds (Sigma, Inc.). The scent compounds emitted by *Puccinia* infected *Arabis drummondii*, the organisms used in all of the following experiments, do not differ appreciably from those found in *Arabis holboellii*; further details may be found in R.A.



**Fig. 1** Setup used in experiments. **A** Artificial flowers composed of microfuge tubes, a wick (2 cm by 5 cm strip of terry cloth, rolled), a square piece of white or yellow paper (1.25 cm, which is similar to the size of the pseudoflowers which averaged  $1.7 \pm 0.37$  cm) attached to an insect pin. Fragrance solution was added to the microfuge tube with a dropper until the wick was saturated. **B** Pseudoflower covered by a 2.5×6 cm plastic vial placed upside down. **C** Pseudoflower covered by a plastic vial that had both ends open. One end was forced into the ground, the other end was open to the air

Raguso and B.A. Roy (in preparation). We then chose the four most volatile constituents and prepared a 0.1% volumetric solution of these compounds in hexane, in the ratio in which they naturally occurred (see Table 2, C-1 blend). The resulting fragrance was indistinguishable, by our noses, from that of pseudoflowers. However, other compounds in trace amounts that were not included also may function as attractants.

We simultaneously observed two arrays composed of the following treatments: (1) color but no scent = artificial flowers without scent, one each of white and yellow, (2) color and scent = artificial flowers with scent, one each of white and yellow, (3) hexane controls = artificial flowers plus hexane, one each of white and yellow, and (4) one pseudoflower in an microfuge tube with a wick and water. The arrays were arranged as follows: two parallel rows of three, with the microfuge tubes 30 cm apart within the rows and 60 cm between the rows and the seventh tube was placed in the center between the two parallel rows. Treatments were randomly assigned to positions.

We observed insect behavior for 10-min periods, then we recharged the wicks (hexane evaporates rapidly) and exchanged plots. On the first day of observations we did not change the position of the treatments between observation periods (eight 10-min periods). On the second day of the experiment we moved the treatments to new random positions after each observation period (six 10-min periods) in a "double-blind" fashion: each observer set up the other person's plot and the tubes were identified only by location.

Each dependent variable was analyzed separately by ANOVA with treatment as a fixed effect, day as a fixed effect and the interaction of day and treatment as a ran-

**Table 2** A list of the fragrances used in experiments, including abbreviations and concentrations

1. *Bz*: Benzaldehyde (0.1% by volume in hexane)
2. *Blank*: Blank. Microfuge tube with wick but without fragrance.
3. *C-I Blend 1*: a solution of 0.1% total concentration by volume of the four most volatile compounds in the pseudoflower fragrance, in same relative ratio to each other in which they are found naturally: phenylacetaldehyde (88.7%), 2-phenylethanol (4.2%), benzaldehyde (4.9%), and methylbenzoate (2.1%), all diluted into hexane (100% HPLC grade).
4. *C-0I Blend 2*: As in 3, but in a 0.01% solution.
5. *C-i-1 Blend 3*: As in 3, but with the addition of indole in ethanol (see 9) which changed the ratios of the other compounds slightly: phenylacetaldehyde (86.9%), 2-phenylethanol (4.12%), benzaldehyde (4.8%), and methylbenzoate (2.06%), all diluted into hexane (100% HPLC grade), plus 2.06% indole (0.1% by mass in 95% ethanol).
6. *C-i-0I Blend 4*: As in 3 but in a 0.01% solution.
7. *Ethanol*: (95%)
8. *Hexane*: (100% HPLC grade)
9. *Indole*: (0.1% by mass in 95% ethanol)
10. *Methylbenzoate*: (0.1% by volume in hexane)
11. *P-A-A*: phenylacetaldehyde (0.1% by volume in hexane)
12. *2-P-E*: 2-phenylethanol (0.1% by volume in hexane)
13. *Pseudo*: pseudoflower caused by *Puccinia monoica* on *Arabis drummondii*
14. *Phx*: hexane extraction of pseudoflower
15. *Uninf*: uninfected plant

**Table 3** The relative effects of color and fragrance on pollinator behavior. Summary of number of visits and mean visit duration

Treatment	Total no. landings (% of total)	Total time (s) (% of total)	Duration/visit (no.secs/no landings)
<b>A. All insects</b>			
White, unscented	1 (0.5)	4 (0.1)	4
White, hexane	4 (2.0)	26 (0.7)	6.5±3.0
White, scented	11 (5.4)	91 (2.3)	8.3±2.3
Yellow, unscented	39 (19.2)	417 (10.7)	10.7±2.3
Yellow, hexane	33 (16.2)	357 (9.2)	10.8±2.1
Yellow, scented	48 (23.6)	652 (16.7)	13.6±2.8
Pseudoflower	67 (33.0)	2341 (60.2)	34.9±5.2
<b>B. Halictids Only</b>			
White, unscented	1 (0.6)	4 (0.1)	4
White, hexane	4 (2.3)	26 (0.9)	6.5±3.0
White, scented	9 (5.2)	88 (3.0)	9.8±2.5
Yellow, unscented	34 (19.5)	386 (13.1)	11.4±2.6
Yellow, hexane	32 (18.3)	356 (12.0)	11.1±2.1
Yellow, scented	47 (27.0)	642 (21.7)	13.7±2.8
Pseudoflower	47 (27.0)	1444 (49.0)	30.7±6.0

dom effect. In this model, the main effect of day was considered to be fixed because it included the difference in methods (see above) as well as other differences there may have been between days (e.g., weather). When an analysis revealed a significant treatment effect, we then used four a priori contrasts to more closely examine differences among treatment means: (1) white versus yellow (= white, unscented+white, hexane+white, scented vs. the same three treatments in yellow), versus yellow, scented, (3) hexane control versus scented (= white, hexane+yellow, hexane vs. white, scented+yellow, scented), and (4) color, unscented versus color, scented (= yellow unscented+white unscented vs. white, scented+yellow, scented).

#### Results of experiment 2

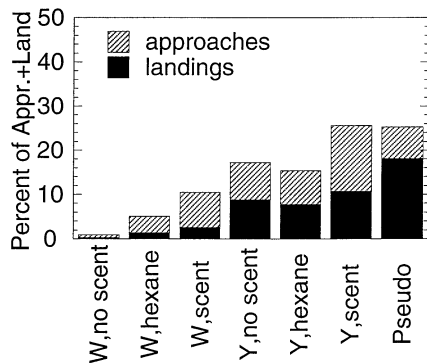
Most visits and approaches were made by halictid bees (*Dialictus* sp., 348/429). The rest of the visits were made

by five other insect species: 20 by *Andrena* sp. (andrenid bees), 33 by *Formica* sp.(ants), 6 by *Bombus* sp. (bumble bees), and 22 by a species of muscid fly.

Over all visitors there were significant differences in approaches, landings, and visit duration associated with the different treatments (Tables 3 and 4). When approaches and landings are combined, the yellow, scented artificial flowers received about the same amount of overall attention as the pseudoflowers (Fig. 2). However, more landings occurred on pseudoflowers, while most approaches were to the yellow, scented artificial flowers (Fig. 2). The overall pattern is clearly dominated by halictids since they made the most landings and approaches (Table 3). There was neither a significant date effect, nor an interaction between date and treatment in any of the analyses (Table 4), indicating that visitation patterns were similar for the two days and that the slight differences in methods between the days did not influence the results.

**Table 4** The relative roles of color and scent in attraction: results of ANOVA. In all models, treatment was a fixed effect whereas date and the treatment by date interaction were treated as random effects. All proportions were arcsine transformed

Source	SS	MS	df num	df denom	F ratio	P
<i>A. Dependent variable = proportion of landings plus approaches/treatment/replicate</i>						
Treatment	5.37	0.895	6	6	17.87	0.0014
Date	0.025	0.025	1	6	0.50	0.5080
Treatment×Date	0.301	0.050	6	181	0.42	0.2102
Contrast scent versus hexane control, $F_1=10.72$ , $P=0.0170$ ; more to scented						
Contrast scented versus unscented, $F_1=17.59$ , $P=0.0057$ ; more to scented						
Contrast yellow scented versus pseudoflower, $F_1=0.0109$ , $P=0.9204$						
Contrast all white versus all yellow treatments, $F_1=61.97$ , $P=0.0002$ ; more to yellow						
<i>B. Dependent variable=proportion of landings/treatment/replicate</i>						
Treatment	8.09	1.35	6	6	26.93	0.0004
Date	0.009	0.009	1	6	0.19	0.6800
Treatment×Date	0.301	0.050	6	181	0.64	0.6985
Contrast scent versus hexane control, $F_1=2.07$ , $P=0.2004$						
Contrast scented versus unscented, $F_1=4.91$ , $P=0.0686$						
Contrast yellow scented versus pseudoflower, $F_1=14.22$ , $P=0.0009$ ; more to pseudoflower						
Contrast all white versus all yellow treatments, $F_1=67.82$ , $P=0.0001$ ; more to yellow						
<i>C. Dependent variable=proportion of approaches/treatment/replicate</i>						
Treatment	3.59	0.598	6	6	9.27	0.0079
Date	0.06	0.060	1	6	0.93	0.3719
Treatment×Date	0.387	0.064	6	181	0.80	0.5678
Contrast scent versus hexane control, $F_1=2.07$ , $P=0.2004$						
Contrast scented versus unscented, $F_1=16.83$ , $P=0.0063$ ; more to scented						
Contrast yellow scented versus pseudoflower, $F_1=8.62$ , $P=0.0261$ ; more to yellow scented						
Contrast all white versus all yellow treatments, $F_1=25.16$ , $P=0.0024$ ; more to yellow						
<i>D. Dependent variable=proportion of time spent (duration) /treatment/replicate</i>						
Treatment	12.72	2.12	6	6	45.47	0.0001
Date	0.008	0.008	1	6	0.17	0.6944
Treatment×Date	0.28	0.047	6	181	0.55	0.7685
Contrast scent versus hexane control, $F_1=2.61$ , $P=0.1572$						
Contrast scented versus unscented, $F_1=4.29$ , $P=0.0836$						
Contrast yellow scented versus pseudoflower, $F_1=67.69$ , $P=0.0002$ ; longer on pseudoflower						
Contrast all white versus all yellow treatments, $F_1=50.96$ , $P=0.0004$ ; longer on yellow						

**Fig. 2** All visitor approaches and landings during the experiment designed to determine the relative roles of olfactory and visual cues in insect attraction in an area where pseudoflowers were present. The graph represents combined data from both days of the experiment (W white, Y yellow)

Insects clearly preferred yellow over white, and scented over unscented treatments (Fig. 2, Table 4, contrasts). Our results further suggest that scent was acting as an orientation cue, and yellow color was functioning as a

landing cue. Figure 2 shows that any yellow treatment receives more landings than any white, scented or not, and that white scented gets approaches, while white unscented does not.

For the number of landings and the duration of visits, there were no significant differences between the hexane controls and the scented artificial flowers (Table 4, contrasts). Thus, some of the attractiveness of the scent could also be a response to the hexane used as a solvent in the fragrance preparations. However, there were significantly more approaches to scented artificial flowers than to those with just hexane, and when landings and approaches were combined, scented artificial flowers received more attention overall than did the hexane controls (Fig. 2, Table 4). The proportion of time spent by insects on different treatments was even more strongly differentiated than visit number (Table 3); the most time was spent on pseudoflowers, the second most on yellow, scented artificial flowers and the least on white, unscented artificial flowers. Since the pseudoflowers were the only treatment that actually presented nectar, it is not surprising that insects spent longer on them.

### Q.3. Does fragrance play a role in long distance attraction to pseudoflowers?

#### Experimental design for experiment 3

For this attraction experiment we chose a location that had melted out of the snow only the day before, thus there were no flowers or pseudoflowers present within a radius of at least 100 m. We used eight treatments in this experiment:

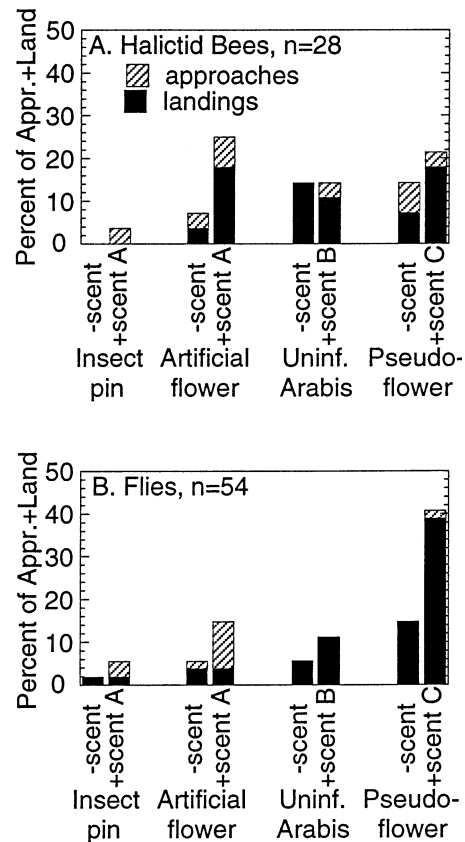
1. No scent, no color (= an insect pin stuck into a terry cloth wick in a microfuge tube)
2. Scent only (as in 1 plus the C-1 scent blend (Table 2))
3. Color, no scent (as in 1 plus yellow paper on the insect pin)
4. Color+scent (as in 3 but wick scented with C-1 blend)
5. Uninfected, open (= uninfected sprig of *Arabis drummondii* wrapped in a terry cloth wick and placed in a microfuge tube stuck in the ground, then an open 5 dram plastic vial was placed over the entire arrangement (see Fig. 1B))
6. Uninfected, covered (as in 5, but with an entire vial placed upside down over the plant, see Fig. 1C)
7. Pseudoflower, open (as in 5, but a pseudoflower)
8. Pseudoflower, covered (as in 6, but a pseudoflower)

Two complete arrays were set up 5 m apart and observed simultaneously. The treatments were arranged within each array in two parallel rows of four. The positions were assigned randomly and were changed and recharged at the end of each replicate. Twelve 10-min replicates (six per person) were observed between 1500 and 1630 hours under clear to partly cloudy skies. The dependent variables were the same as those used for earlier experiments. For analysis we used ANOVA with fragrance treatment as a fixed factor. If a model was significant we tested one a priori contrast: the average of all treatments where fragrance was present versus the average of all treatments where fragrance was absent.

#### Results of experiment 3

Most landings were made by flies (41/62): anthomyiids (23), muscid sp. A (3), muscid sp. B (3), small "gnat-like" fly (14), unidentified fly (1). The rest of the visits were made by two bee species: *Dialictus* (20), and *Andrena* (1).

Long distance attraction did occur and there were significant differences in response to the fragrances by insects. Since there were more landings than approaches, and the analysis of these two variables yielded similar results (Fig. 3), we present statistics only for the combination of approaches plus landings. When all visitors are considered, there was a significant effect of treatment ( $F_7 = 11.86$ ,  $P < 0.0001$ ). When only flies are considered, there was also a significant effect of treatment ( $F_7 = 2.14$ ,  $P = 0.0052$ ). There were too few visits by ha-



**Fig. 3** Pollinator approaches and landings during the experiment designed to determine the relative importance of visual and olfactory cues in long-distance attraction (*W* white, *Y* yellow) for **A** halictid bees, **B** flies

lictid bees to test them separately (28 visits and approaches spread over 12 replicates and 8 treatments). However, qualitatively, halictids showed a similar pattern to the flies, albeit with less extreme interest in the pseudoflowers (compare Fig. 3A with 3B).

Olfactory cues were more important than visual cues in long distance attraction (contrast between scented versus unscented treatments,  $F_1 = 6.90$ ,  $P = 0.0101$ ). When flies are separated out, the contrast is still significant ( $F_1 = 4.33$ ,  $P = 0.0407$ ). Halictid bees also tended to prefer scented to unscented treatments (Fig. 3A), but there were too few visits to test this result for significance.

The total proportion of time spent by insects on each treatment, and the mean duration per visit are given in Table 5. For all insects combined, there were significant differences in the proportion of time they spent on particular treatments ( $F_7 = 11.86$ ,  $P < 0.0001$ ), and the same was true for flies separately ( $F_7 = 8.93$ ,  $P < 0.0001$ ). Insects spent proportionately more time on scented versus unscented treatments ( $F_1 = 13.5$ ,  $P = 0.0004$ ).

**Table 5** Visitation data for the long distance attraction experiment

Type of treatment	Fragrance	No. visits (% of Visits)	Time (s) (% of time)	Duration/visit (s)±SE
<b>A. HALICTIDS</b>				
Insect pin	unscented	0	0	0
Insect pin	scented <sup>a</sup>	0	0	0
Artificial flower	unscented	1 (5.0)	75 (20.8)	75
Artificial flower	scented <sup>a</sup>	5 (25.0)	42 (11.6)	11.6±2.8
Uninf. <i>Arabis</i>	unscented	4 (20.0)	44 (12.2)	11.0±7.0
Uninf. <i>Arabis</i>	scented <sup>b</sup>	3 (15.0)	112 (31.0)	37.3±13.0
Pseudoflower	unscented	2 (10.0)	12 (3.3)	6.0±1.0
Pseudoflower	scented <sup>c</sup>	5 (25.0)	76 (21.1)	15.2±5.6
<b>B. FLIES</b>				
Insect pin	unscented	1 (2.3)	65 (2.0)	65
Insect pin	scented <sup>a</sup>	1 (2.3)	10 (0.3)	10
Artificial flower	unscented	2 (4.6)	11 (0.3)	5.5±4.5
Artificial flower	scented <sup>a</sup>	2 (4.6)	30 (1.0)	15.0±8.0
Uninf. <i>Arabis</i>	unscented	3 (6.8)	25 (0.8)	8.3±4.3
Uninf. <i>Arabis</i>	scented <sup>b</sup>	6 (13.6)	86 (2.7)	14.3±8.4
Pseudoflower	unscented	8 (18.2)	110 (3.5)	13.8±3
Pseudoflower	scented <sup>c</sup>	21 (47.7)	2839 (89.4)	135.2±41.0

<sup>a</sup> Scent was blend C-1 (see Table 2), placed on terry cloth wick in the microfuge tube; for the unscented treatments we did not apply scent to the wick

<sup>b</sup> Scent was the natural odor given off by leaves and the cut stem of an uninfected *Arabis* plant; for the unscented treatment we covered the plant with a plastic vial (see Fig. 1)

<sup>c</sup> Scent was the natural odor given off by pseudoflowers on infected *Arabis* plants; for the unscented treatment we covered the pseudoflower with a plastic vial

**Table 6** Visitation data for each treatment for both days combined of the bioassay experiment: 16 June was cold and windy, and fragrances were recharged every 10 min; 18 June was warmer and

calmer, and fragrances were recharged every 20 min. See Table 2 for explanation of chemical treatments

Type of treatment	Treatment	16 June 1995				18 June 1995			
		No. appr.	No. visits (%)	No. s (%)	S/visit. ±SE	No. appr.	No. visits (%)	No. s (%)	S/visit. ±SE
<b>A. Halictids</b>									
Controls	Uninf.	1	0	0		1	0	0	0
	Blank	1	5 (9.1)	87 (4.9)	17.4±8.2	3	3 (2.0)	28 (0.9)	9.3±1.8
	Hexane	1	2 (3.6)	174 (9.9)	87.0±74.0	11	9 (6.0)	186 (5.8)	20.7±5.8
	Ethanol	3	4 (7.3)	60 (3.4)	15.0±6.7	1	4 (2.7)	69 (2.2)	17.3±9.4
Single Compounds	Benzald.	0	4 (7.3)	25 (1.4)	6.3±1.5	4	4 (2.7)	35 (1.1)	8.8±4.2
	Indole	5	3 (5.5)	27 (1.5)	9.0±3.5	2	14 (9.3)	264 (8.3)	18.9±5.3
	Methylb.	5	3 (5.5)	147 (8.3)	49.0±38.5	5	15 (10.0)	361 (11.3)	24.1±7.5
	2-P-E	6	6 (10.9)	31 (1.8)	5.2±1.0	1	10 (6.7)	154 (4.8)	15.4±5.6
	P-A-A	3	5 (9.1)	174 (9.9)	34.8±10.2	4	22 (14.7)	460 (14.4)	25.6±5.6
Blends	Phx	6	1 (1.8)	4 (0.2)	4	1	11 (7.3)	287 (9.0)	26.1±7.9
	C-i-01	1	8 (14.5)	285 (16.2)	35.6±11.4	4	9 (6.0)	305 (9.6)	33.9±14.6
	C-i-1	4	2 (3.6)	205 (11.6)	102.5±87.5	8	18 (12.0)	460 (14.4)	25.6±5.6
	C-01	4	3 (5.5)	11 (0.6)	3.7±2.2	9	8 (14.5)	106 (3.3)	13.3±4.6
	C-1	4	4 (7.3)	136 (7.7)	34.0±28.1	4	15 (10.0)	380 (11.9)	25.3±8.8
Pseudo.	Pseudo.	3	5 (9.1)	396 (22.5)	79.2±32.9	6	8 (5.3)	89 (2.8)	11.1±2.9
<b>B. FLIES</b>									
Controls	Uninf.	0	0	0	0	0	0	0	0
	Blank	0	0	0	0	1	1 (1.9)	5 (0.2)	5
	Hexane	0	0	0	0	0	1 (1.9)	16 (0.6)	16
	Ethanol	0	0	0	0	0	5 (9.4)	194 (7.8)	38.8±17.5
	Single Compounds	Benzald	0	0	0	0	1	0	0
Indole	0	0	0	0	0	1 (1.9)	8 (0.3)	8	
Methylb.	1	0	0	0	1	2 (3.8)	8 (0.3)	4	
2-P-E	0	0	0	0	0	0	0	0	
P-A-A	0	0	0	0	1	0	0	0	
Blends	Phx	2	0	0	0	0	2	17 (0.7)	8.5±1.5
	C-i-01	0	0	0	0	1	5 (9.4)	32 (1.3)	6.4±2.4
	C-i-1	1	0	0	0	3	3 (5.7)	20 (0.8)	6.7±1.2
	C-01	1	0	0	0	0	2 (3.8)	44 (1.8)	22.0±3.0
	C-1	0	0	0	0	1	3 (5.7)	14 (0.6)	4.7±2.7
Pseudo.	Pseudo.	2	0	0	0	2	28 (52.8)	2144 (85.7)	76.6±13.6



#### Q.4. Which of the compounds found in pseudoflowers are attracting insects?

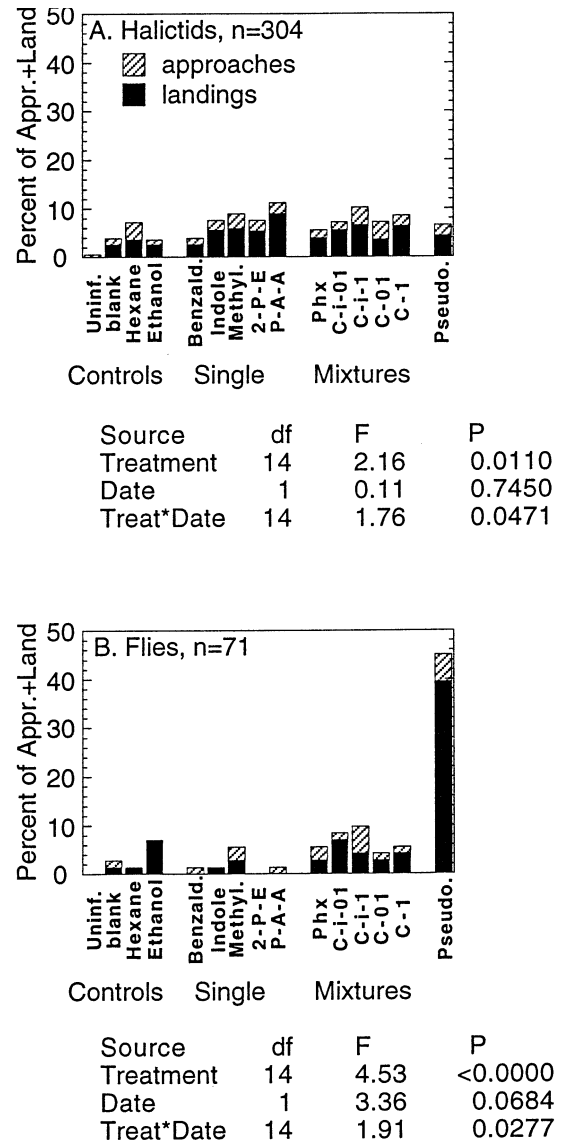
##### Experimental design for experiment 4

We presented insects with arrays containing 15 different scent treatments for them to choose among. The compounds, the concentrations used, and our abbreviations are listed in Table 2. The fragrances were proffered in the same kind of artificial flowers as the previous experiment (Fig. 1), except that the color of all the artificial flowers was yellow. Within an array, the 15 scent treatments were randomly arranged in three rows of five, with each artificial flower 20 cm from the next. During each observation period we each observed one array; the two arrays were placed 5 m apart. Observations were double-blind: after each observation period we re-randomized the treatments of the other person's array so that treatment identity was not known to the observer. This basic experiment was performed on two different days, but with different amounts of time between recharging the fragrances. On 16 June 1995 we observed twelve 10-min replicates and recharged the fragrances after each replicate because it was windy and evaporation was rapid. On 18 June, we recharged the fragrances every 20 min, but switched observers after 10 min. By switching observers every 10 min on both days observations were separable into 10-min intervals for a total of sixteen 10-min replicates. We analyzed these experiments together in an ANOVA by including a date effect in the model. Date was considered to be a fixed effect because it not only represented different dates, but also a difference in methods. The dependent variables were the fraction of landings + approaches, and visit duration on each treatment per replicate, arcsine transformed to achieve normality of residuals. Differences among treatment means were assessed with five a priori contrasts: (1) controls (uninfected, hexane, ethanol, blank) versus all the other treatments, (2) blends containing indole versus pseudoflowers, (3) blends not containing indole versus pseudoflowers, (4) blends versus single compounds, and (5) blends + indole versus blends without indole.

##### Results of experiment 4

Most of the landings were made by halictid bees (205/274), the rest were made by: muscid flies (41), ants (12), anthomyiid flies (9), andrenid bees (3), sarcophagid flies (2), syrphid fly (1), and a grasshopper (1). On the first day of observations, it was cold and partly cloudy; during 120 min there were 59 landings and 47 approaches for a combined rate of 53/h. On the second day of observations, it was warm and sunny and there were twice as many visitors: during 160 min we observed 215 landings and 78 approaches for a combined rate of 110/h.

The ANOVA revealed significant treatment differences in visit number and duration, and flies and halictid bees reacted differently to the fragrance treatments (Fig. 4). Halictids approached and landed on scented



**Fig. 4** Pollinator approaches and landings during the bioassay experiment for **A** halictid bees, **B** flies. Combined results of 2 days of observations

treatments significantly more often than on controls ( $F_1 = 13.45$ ,  $P = 0.0003$ ), but did not distinguish very well among the scented treatments in terms of visitation (Fig. 4A). However, for halictids, the amount of time spent investigating an artificial flower depended on the specific scent (Table 6). Bees were obviously attracted by the fragrance of the hexane controls, which may explain why there was little difference between the hexane control and fragrance blends containing hexane (treatments 1–6, 8, 10–12, 14 in Table 2). Like the halictid bees, flies visited the controls less often than the treatments ( $F_1 = 5.73$ ,  $P = 0.0177$ ), however, flies showed a strong preference for the pseudoflowers over all other treatments (Table 6, Fig. 4B). After pseudoflowers, flies spent the most time on the ethanol control, indicating some interest, but the number of visits to ethanol was not very high (Fig. 4B). Contrary to our expectations, flies

responded less strongly to the nitrogenous compound indole than did bees (Fig. 4B, Table 6).

The significant interaction between date and treatment in the ANOVAs (Fig. 4) indicates that at least some treatments received different proportions of visitation on the different days of the experiment. Table 6 clarifies what these differences are: (1) there were no visits by flies on 16 June, and the presence of flies on 18 June doubled visits to ethanol, and more than tripled the number of visits to pseudoflowers, and (2) mean visit duration was usually longer on 16 June, probably reflecting the cooler, cloudier weather on that day (insects fly less frequently when they are cold). Alternatively, the insects could have stayed longer on 16 June due to more concentrated fragrances (the recharge time was twice as frequent on that day). There was not a significant effect of date in this experiment so even though visitation varied among the treatments on different days, the net changes in visitation balanced out: some treatments received more and others received less (Table 6).

## Discussion

The relative role of visual and olfactory attractants

### Bees

There are both visual and olfactory components in attraction to *Puccinia monoica* induced pseudoflowers, but the relative importance of these cues depends on whether the visitors are flies or halictid bees. For the halictids, several lines of evidence suggest that visual cues are more important initially than olfactory cues, although both play a role. When we removed visual cues by covering pseudoflowers with muslin bags, visits by halictids dropped 80% when compared to visits to exposed pseudoflowers (Table 1). When we presented pollinators with either white or yellow artificial flowers, with and without scent, the halictids showed a strong ability to differentiate among visual cues, since they preferentially visited yellow artificial flowers. Both white, scented and yellow, unscented artificial flowers elicited approaches and landings, but the combination of yellow color and scent was required to reconstitute the observed levels of visits to pseudoflowers (Table 3). The long-distance attraction experiment illustrates the interaction between yellow color and odor very clearly: no halictids landed on the colorless treatment whether it was scented or not, but in the first replicate, when insects were assumed to be naive, 10/12 of the observed visits were to scented, yellow treatments. Finally, in the bioassay experiment, all of the artificial flowers were yellow and differed only by the scents they proffered. Each treatment received about the same number of visits, suggesting that landings may have been occurring due to color alone, or that the bees could not differentiate among the fragrances, or that the individual components were equally attractive.

How do these results compare to those of other studies? Outside of our work, little is known about the behavior of halictid bees in response to fragrance, but there is some literature on other bees. Most work on honey and bumble bees suggests that they locate flowers visually, then use floral fragrance for orientation in landing (Butler 1951; Manning 1957; von Frisch 1971; Galen and Kevan 1980; Dobson 1991). However, some recent work with *Marjorana syriaca* suggests that whole-plant volatiles can also be important in long- and close-range attraction (Beker et al. 1989). The orientation to fragrance by honey bees is most likely a conditioned response (reviewed in Waller et al. 1973; Pham-Deleque et al. 1986), and there is little evidence that honey bees have innate preferences for particular floral odors (Ribbands 1955; Faegri and van der Pijl 1971; Waller et al. 1973; Pham-Deleque et al. 1986; Henning et al. 1992).

Oligolectic (pollen-specific) bees, such as *Colletes spp.*, use floral and pollen fragrances to locate their specific host plants (Dobson 1987). Recently, Borg-Karolson et al. (1996) found that male *Colletes cunicularis* bees approached but rarely landed on false flowers of black velvet squares attracted to insect pins when the velvet was charged with floral extract of *Daphne mezereum* (Thymeliaceae) in hexane, or linalool in hexane, or hexane controls. Interestingly, bees also approached the hexane controls, and, similar to our findings, the absence of the appropriate color stimulus (purple) may explain the lack of landings in the bioassays.

A famous scent-driven pollination system involves males of several genera of bees including *Andrena*, *Anthophora*, *Argogorytes*, *Eucera*, and *Campsocolia* which are attracted by *Ophrys* orchids, which they pollinate accidentally when they attempt to copulate with the labellum of the orchid (= pseudocopulation, reviewed in Bergström 1978; Dobson 1993). The flowers attract the bees through a combination of visual (shape, size, color), tactile (hairiness), and olfactory cues (they emit volatiles that mimic sex pheromones). In *Ophrys* pollination, fragrance is important both at long distances (when the flower is not and cannot be in sight) and for close orientation to the flower (Bergström 1978). Our experiments were designed to determine whether there was a response to odor by wild insects, and thus could not directly differentiate between learned and innate responses. However, in the long distance attraction experiment, the bees were almost certainly naive to pseudoflowers due to the isolation of the experimental plot by distance, yet more landings occurred when floral odors were present than when they were absent (Fig. 3).

### Flies

It was difficult to assess the relative importance of visual versus olfactory cues for flies, because they were nearly or completely absent on 2 out of 5 days of experimentation, and when flies were present, they were less numerous than halictid bees. It was unfortunate that flies were

so uncommon in this particular year as flies are normally the most abundant visitors to pseudoflowers and the flowers with which they co-occur (Roy 1993, 1994, Roy, 1996). The year of the fragrance experiments was the wettest in many years, with 180% more snowfall than normal (Art Mears, personal communication). These conditions apparently favored the bees, perhaps because the late snowmelt meant that the experiments were conducted a month later in the season and under warmer temperatures. Nevertheless, some general patterns were evident in the data. First, flies reacted even more positively to the presence of scent than did bees (Fig. 3). Second, none of the blends or single compounds we used in the bioassay experiment received nearly as many visits as pseudoflowers and thus none of the compounds or compound blends we chose was as attractive to flies as the aroma of pseudoflowers (Fig. 4). Since we tested only the most volatile compounds found in pseudoflowers, there are several other potential attractants that remain to be tested. Third, following attraction to pseudoflowers, ethanol controls were the next most attractive to flies. Ethanol is not a natural constituent of pseudoflowers but was used to dissolve indole in the treatments containing indole (C-i-1 and C-i-01 blends, as well as indole alone), so we included an ethanol control in the arrays. The attraction to ethanol may have resulted from its being a novelty. Insects often examine novel stimuli in their environment; it is assumed that these visits are a form of reconnaissance, a way to assess the foraging options within a resource patch (Krebs 1978). Alternatively, these flies may have an innate attraction to ethanol. Fourth, although the literature on fly behavior is replete with references to flies being attracted to amines and nitrogen-containing compounds such as indole (Smith and Meeuse 1968; Meeuse 1978; Williams 1983; Beehler et al. 1993; Kaiser 1993; Knudsen 1993; Wall and Warnes 1994), there was no evidence of strong attraction to indole in this study (Fig. 4). Indole is present in pseudoflowers only in trace amounts, but we included indole in the bioassay because it has been reported from another floral-scented rust fungus (Connick and French 1991).

When considering the relationship between flies, pollination, and odor, biologists typically connote fly attraction with foul odors, such as those emitted by carrion-mimicking flowers like *Rafflesia* (Beaman et al. 1988) and *Stapelia* (Meve and Liede 1994). However, flies from diverse families are attracted to, and pollinate, flowers that show a broad spectrum of different colors, scents, and morphological classes. For example, flowers of *Leontopodium alpinum* (Asteraceae), which are pollinated by muscid flies in the Swiss Alps, produce some of the same scent compounds that we have identified in *Puccinia* induced pseudoflowers (benzaldehyde and 2-phenylethanol; Erhardt 1993). In eastern North America, a chloropid fly, *Elachiptera formosa* Loew, is the obligate pollinator of an aroid, *Peltandra virginica* Kunth, whose sweet-musty floral scent is devoid of amines (Patt et al. 1995). The fragrances of normal (not carrion mimicking) flowers that are visited by flies range from sweet or un-

scented (according to our noses) (Vogel 1954; Faegri and van der Pijl 1971; Dobson 1993; Johnson and Steiner 1995) to "skunky" (Galen and Newport 1987a, b). Anthomyiid and muscid flies are important pollinators, particularly in Arctic and alpine habitats, and places where bees are rare or absent, such as Australia and New Zealand (reviewed in Kearns 1992). Fly pollination is also important in tropical habitats. For example, flies in the families Cecidomyiidae and Ceratopognidae have been shown to be important pollinators of the source of cocoa, *Theobroma* (Young 1989).

Given the diversity of flies that visit flowers, it should not come as a surprise that the relative roles of visual and olfactory cues vary among species. For flies that normally feed or oviposit on carrion or excrement (sapromyophily), odor is sometimes a more important attractant than visual cues, but neither cue is very successful alone. For example, Beaman et al. (1988) found that when both olfactory and visual cues were present, a fly was found on *Rafflesia* flowers 95% of the time. When just color was removed, flies (*Lucilia papuensis*, Calliphoridae) were present only 35% of the time, and when just odor was removed, flies were present 7–47% of the time (the degree of visitation loss depended on how effective the treatment was for the removal of odor; the more effective, the less visits there were). Kugler (1951) found a different pattern exhibited by a different species of *Lucilia*; these flies approached from a distance based on visual cues, but fragrance determined whether or not the flies landed. The few experimental studies of flies that visit more conventional flowers suggest that these flies, like honey bees, rely mostly on visual cues to initially locate flowers (reviewed in Dobson 1993).

Finally, our observation that anthomyiid and muscid flies were not preferentially attracted to yellow color alone (Figs. 3, 4) is interesting in that many species of anthomyiid and tephritid flies show strong, possibly innate preferences for yellow colored objects in behavioral assays (Agee et al. 1982; Vernon and Borden 1983; Katsoyannos 1986; Robacker et al. 1990; Vargas et al. 1991). In addition, some sapromyophilic muscid flies are attracted to objects with high scattered visible (white) and ultraviolet reflectance (Agee and Patterson 1983; Pickens 1983). Unfortunately, flies were not abundant during experiment 2, when yellow and white artificial flowers, with and without scent were presented in an attempt to decouple scent and color cues as visitation stimuli. The great disparity in attraction of flies to *Puccinia* pseudoflowers over artificial flowers in our experiments suggests that the quality or quantity of visual and olfactory cues presented by our artificial flowers were insufficient to attract these flies. Potential explanations for this observation include: (1) absence of a critical compound from the scent blend, (2) subtle differences in reflectance between pseudoflowers and paper lures, (3) negative behavioral responses to hexane, and (4) the possibility that the fructose-rich nectar of the pseudoflowers had its own odor and this was important in attracting flies.

## Implications for floral mimicry

Pseudoflowers and buttercups receive more visits when they are mixed together than when they are separated, and both species are rewarding to visitors (Roy 1994). Here we hypothesized that the unique fragrance of pseudoflowers may be important for the evolution of this mimicry system by reducing the probability of improper transfer of gametes between the fungus and flowers. Under this scenario, visual mimicry could occur if long-distance attraction was primarily by color, and short-distance differentiation among species could be accomplished through differential odor. In this study, we have shown that olfactory cues are important attractants: visitors prefer scented to unscented artificial flowers, and they are attracted to the scent of pseudoflowers even when visual cues are not available. However, our results also suggested that odor may be an even more important long-distance attractant than color, particularly for flies. In the future we plan to explore further the roles of odor and color in long-distance attraction, and the role of fragrance in enhancing flower constancy. In addition, we are exploring the evolution of the fragrance chemistry and floral morphology by completing a phylogenetic analysis of both the hosts and their pathogens.

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