Smells like aphids: orchid flowers mimic aphid alarm pheromones to attract hoverflies for pollination

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Most insects are dependent on chemical communication for activities such as mate finding or host location. Several plants, and especially orchids, mimic insect semiochemicals to attract insects for unrewarded pollination. Here, we present a new case of pheromone mimicry found in the terrestrial orchid *Epipactis veratrifolia*. Flowers are visited and pollinated by several species of aphidophagous hoverflies, the females of which also often lay eggs in the flowers. The oviposition behaviour of these hoverflies is mainly guided by aphid-derived kairomones. We show that the flowers produce α - and β -pinene, β -myrcene and β -phellandrene, and that these compounds attract and induce oviposition behaviour in female hoverflies. This floral odour profile is remarkably similar to the alarm pheromone released by several aphid species, such as *Megoura viciae*. We therefore suggest that *E. veratrifolia* mimics aphid alarm pheromones to attract hoverflies for pollination; this is the first time, to our knowledge, that such a case of mimicry has been demonstrated.

Keywords: deceptive pollination; chemical mimicry; pheromone

1. INTRODUCTION

The huge diversity of pollination systems in orchids has fascinated evolutionary biologists from the very beginning [1]. One main factor behind this fascination was the fact that one-third of the approximately 30 000 orchid species described have evolved a deceptive pollination mechanism [2,3]. Deceptive flowers do not offer a reward (e.g. nectar or pollen) to the pollinators but attract them by visual mimicry, olfactory mimicry or both. The deceptive mechanisms include food deception, brood-site mimicry, sexual deception or prey mimicry [4–6]. With approximately 11 per cent of orchid genera using brood-site mimicry, this is one of the most widespread deceptive pollination mechanisms in the Orchidaceae [7,8].

Hoverflies (Syrphidae) are important pollinators of flowering plants throughout the world [9] and, furthermore, some species are very efficient predators of aphids [10]. *Episyrphus balteatus* De Geer 1776 (Diptera, Syrphidae) is the most frequently encountered syrphid species at aphid-infested sites in temperate regions of the Northern Hemisphere [11]. While the larvae are aphidophagous (feed on aphids), adults feed on nectar and pollen from flowers [12]. Syrphid larvae are unable to disperse far [13], which makes female choice of oviposition site a crucial factor in offspring survival. Host search

and oviposition behaviour in *E. balteatus* have been intensively studied in the past decade (reviewed in Almohamad *et al.* [14]): the search for oviposition sites is mainly guided by plant- and aphid-derived semiochemicals (chemicals used for communication), used as kairomones (chemical signals that benefit the receiver) by the flies. Females of *E. balteatus* are able to locate plant and aphid species, discriminate between them and adjust their oviposition behaviour according to the performance of their larvae on different host species [15] and aphid colony size [16]. Furthermore, aphid honeydew and aphid alarm pheromone compounds (e.g. (*E*)- β -farnesene, α - and β -pinene) elicit oviposition in *E. balteatus* females [17,18]. Visual cues are important for the search for host plants and flowers, but do not trigger oviposition behaviour [14].

The genus *Epipactis* (Orchidaceae) contains 25–59 species with a predominantly Eurasian distribution [19,20]. *Epipactis veratrifolia* Don. (syn. *E. consimilis*) is found throughout the Middle East as well as eastwards to the Himalayas and southwards to Somalia and Ethiopia [21]. The plant consists of separate stems growing from a common rhizome. The stems reach a height of 1.5–2 m and produce 20–40 flowers. The pollination biology of *E. veratrifolia* has been studied by Ivri & Dafni [22]: *E. veratrifolia* is exclusively pollinated by five species of aphidophagous hoverflies (Syrphidae), namely *Sphaerophoria ruepelli* Wiedemann 1830, *Sphaerophoria scripta* (L. 1758), *Ischiodon aegyptus* Wiedemann 1830, *Eupeodes corollae* (F. 1794) and *E. balteatus*. Flies of the genus *Paragus* also visit the flowers, but are too small to carry the pollinia

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and effectively pollinate the flowers. The flowers produce small amounts of nectar that is presented freely on the labellum. Flowers are not autogamous, and the natural pollination rate is about 15 per cent [22].

Male hoverflies are often found in the vicinity of the orchids. Males occupy a territory comprising a few plants, in which they try to copulate with females approaching the flowers. Males occasionally visit the flowers in search of nectar and thereby also pollinate them.

Hoverfly females approach the flowers in hovering flight, land on the labellum, may lick the exposed nectar droplets and lay an egg on the labellum or in other parts of the flower. During nectar feeding and egg laying, they pollinate the flowers [22].

The fact that females lay eggs on the flowers of the orchid is very interesting as aphidophagous hoverflies normally lay their eggs exclusively in places where aphids are present, because the larvae feed on aphids [23]. Based on this fact and on the aphid-like dark warts in *E. veratrifolia* flowers, Ivri & Dafni [22] suggested that the flower mimics the shape and colour of aphids to attract syrphid flies for pollination. However, as volatiles seem to play a key role in host location and oviposition behaviour of syrphid flies [14], we reasoned that the flowers would also have to mimic aphid volatiles if they were to achieve pollination by attracting female hoverflies searching for oviposition sites. Mimicry of aphid volatiles has so far not been demonstrated in any pollination system. To test this idea, we used a combination of behavioural experiments, electrophysiological investigations and chemical analyses.

2. MATERIAL AND METHODS

(a) Study site

Samples were collected in March 2009 in the Enot Tsukim Nature Reserve, Israel. The nature reserve is a small wetland on the Dead Sea coast, characterized by dense grass and reed vegetation. The population of *E. veratrifolia* comprises approximately 100–200 plants.

(b) Nectar collection

The amount of nectar produced by the flowers of *E. veratrifolia* was measured in the morning (09.00–10.00 h) and at noon (12.00–13.00 h) using 0.5 µl microcapillary tubes (Hirschmann, Germany).

(c) Volatile collections

For collection of headspace samples of *E. veratrifolia*, stems bearing several flowers or only buds were enclosed in a polyethylene terephthalate oven bag (Toppits, Germany) without cutting the stem. A headspace filter (50 mg Super-Q, Analytical Research Systems, USA) was put inside the bag and connected to a hand-held air-sampling pump (Casella, USA) using a silicone tube. Air was pumped out of the bag at a rate of 0.8 l min⁻¹ for 4 h. Filters were eluted with 500 µl pentane (Sigma–Aldrich, Germany) concentrated to 100 µl and samples were stored at –20°C until further analysis. To avoid contamination of plant volatiles, we carefully checked the plants to ensure that the stem, flowers and buds were free of aphids or syrphid larvae before headspace collection.

Volatiles of the aphid *Megoura viciae* Buckton 1876, a common species in the Middle East, were collected by extracting approximately 20 individuals of mixed stages for

120 s in 1 ml pentane. The extract was concentrated under a gentle stream of nitrogen and stored at –20°C until analysis.

(d) Chemical analysis

Headspace samples were analysed by gas chromatography–mass spectrometry (GC–MS) (Agilent 7890GC and 5975c MS). The gas chromatograph (GC) was equipped with a DB5-MS column (30 m long, 0.25 mm internal diameter, 25 µm film thickness; Agilent) with helium as a carrier gas (18.3 µl s⁻¹ constant flow). The inlet temperature was set to 250°C. The temperature of the GC oven was held at 40°C for 180 s and then increased by 5°C per 60 s to 280°C. The final temperature was maintained for 600 s. The MS transfer line was held at 300°C, the MS source at 230°C and the MS quad at 150°C. Mass spectra were taken in electron ionization mode (at 70 eV) in the range of 33–350 m z⁻¹ with a scanning rate of 4.42 scan s⁻¹. To evaluate the enantiomeric composition of α- and β-pinene, selected samples and authentic standards were also analysed on a chiral column (β-dex, 30 m long, 0.25 mm internal diameter, 25 µm film thickness; Agilent) under the same analytical conditions.

GC–MS data were processed with the MDS-CHEMSTATION software (Agilent). Compounds were provisionally identified with the NIST 2.0 mass spectra database using the NIST algorithm. Identification was confirmed by comparison with synthetic standards or in the case of β-phellandrene, by comparison of the Kovats retention index with the published data.

(e) Electrophysiology

We used electroantennograms (EAGs) to test whether *E. balteatus* was able to perceive the floral compounds of *E. veratrifolia*. For an EAG, the head of a fly was cut off and pinned onto a glass electrode filled with insect saline. A second glass electrode was brought into contact with the tip of one of the antennae. The electrodes were connected by Ag–AgCl wires through a pre-amplifier to an amplifier (Syntech, Germany). Signals from the antenna were recorded on a PC running EAG recordings software (all Syntech, Germany). Test compounds were diluted in dichloromethane (DCM) with a concentration of 10 mg ml⁻¹. Discs of filter paper (100 mm²) were impregnated with 20 µl solution (containing 200 µg of compound) and put into Pasteur pipettes. The solvent was allowed to evaporate before the experiment. Pipettes were used to puff odour compounds into a filtered and humidified airstream (3.33 ml s⁻¹), which was directed over the antenna. Each compound was tested three times on each one of 18 female and 10 male flies. EAG reactions to single compounds were averaged for each individual. Differences in the EAG reactions to odour compounds and the control (DCM) were tested with a paired sample *t*-test and sequential Bonferroni corrections for multiple comparisons [24]. All tests were carried out using SIGMASTAT 3.5.

(f) Oviposition experiments

Bean plants (*Vicia faba*) were grown in the Botanical Garden of the University of Ulm. Hoverfly pupae (*E. balteatus*) were ordered from a commercial supplier (Katz Biotech, Germany), reared in a flight cage (0.6 × 0.6 × 0.6 m) in a climate-controlled room (13 L : 11 D cycle, 60–70% relative humidity, 24°C), and fed with sugar water, pollen and water ad libitum.

For the experiments, a membrane dispenser (Wilhelm Biological Plant Protection, Germany) was used to test the behavioural activity of the natural headspace samples from

Figure 1. GC–MS traces of (a) *Epipactis veratrifolia* flower headspace volatiles and (b) *Megoura viciae* aphid surface extract.

E. veratrifolia ($n = 10$), as well as the synthetic mixture of compounds identified in the headspace samples ($n = 13$). The dispenser guarantees a constant emission of the tested volatiles. We used the solvent pentane as a negative control. For each experiment, two *V. faba* plants (0.3–0.4 m tall), each with a dispenser containing either the odour sample or the solvent only, were placed in a flight cage ($0.6 \times 0.6 \times 0.6$ m) together with four gravid *Episyrphus* females. After 24 h, we counted the eggs laid on the plants. Experiments were conducted in a climate-controlled room at 24°C and 60–70% humidity. For the tests, we used *E. balteatus* females aged two to five weeks, mated and gravid. Two days before we tested the females they were allowed to lay eggs on a *V. faba* plant infested with *Aphis fabae*, as females with oviposition experience were more active in the behavioural test and laid a higher number of eggs. The synthetic mixture used consisted of $51 \text{ ng } \mu\text{l}^{-1}$ ($\pm$)- α -pinene; $41 \text{ ng } \mu\text{l}^{-1}$ ($\pm$)- β -pinene and $4 \text{ ng } \mu\text{l}^{-1}$ β -myrcene (all Sigma–Aldrich, Germany). The qualitative and quantitative composition of the synthetic mixture was the same as that found in the flowers as verified by GC analysis using the same parameters as for the chemical analysis of headspace volatiles.

3. RESULTS

(a) Nectar collection

We collected nectar from 13 flowers. In the morning (09.00–10.00 h), the flowers produced small amounts of nectar. Less than $1 \mu\text{l}$ was collected per flower. Because of high ambient temperatures at the field site, the nectar became too viscous at noon to be collected with microcapillary tubes.

(b) Volatile collections

In the headspace collections of *E. veratrifolia* flowers, we found four compounds: α -pinene ($51 \pm 1.0\%$, mean $\pm$ s.e.), β -pinene ($41.7 \pm 0.6\%$), β -myrcene ($3.7 \pm 0.4\%$) and β -phellandrene ($3.0 \pm 0.3\%$; figure 1a). By contrast, in the leaves and buds of *E. veratrifolia*, we found only trace amounts of α -pinene and β -pinene. *Epipactis*

veratrifolia produces both enantiomers of α - and β -pinene with a slight dominance of the (–)-forms in both compounds. The surface extracts of the aphid *M. viciae* (all stages) contained α -pinene (12%), β -pinene (80%), β -myrcene (3%) and one unidentified compound (4%; figure 1b).

(c) Electrophysiology

Synthetic copies of the volatiles found in the *E. veratrifolia* flower headspace (α -pinene, β -pinene and β -myrcene) released EAG responses in the antennae of *E. balteatus* males ($n = 10$) and females ($n = 18$; figure 2). Synthetic β -phellandrene could not be obtained and was therefore not tested. The antennae gave the highest responses to (+)- α -pinene (mean 2.4, s.e. 0.15 mV), (+)- β -pinene (mean 1.9, s.e. 0.14 mV) and β -myrcene (mean 2.1, s.e. 0.16 mV). The (–)-form of α -pinene released only a weak response (mean 0.9, s.e. 0.08 mV) as did (–)- β -pinene (mean 1.4, s.e. 0.12 mV). These values were, however, still significantly higher than the control (mean 0.6, s.e. 0.07 mV, $p < 0.001$ paired sample *t*-test, sequential Bonferroni correction for ties). There was no difference in the antennal reactions of males and females.

(d) Oviposition experiments

Episyrphus balteatus females laid significantly more eggs on plants combined with the headspace sample (mean 19.6, s.e. 2.1 eggs) than on plants with the solvent only (mean 7.9, s.e. 2.2; paired sample *t*-test, $n = 10$, $t = 6.8$, $p < 0.001$; figure 3a). The females also laid significantly more eggs on plants combined with the synthetic mixture (mean 14.6, s.e. 2.9 eggs) than on control plants (mean 3.3, s.e. 0.9 eggs; $n = 13$, $t = 4.8$, $p < 0.001$; figure 3b).

4. DISCUSSION

(a) Do orchid flowers mimic hoverfly alarm pheromone?

Our results provide evidence that *E. veratrifolia* flowers produce the same compounds as are found in the alarm

Figure 2. Antennal (EAG) responses of *E. balteatus* females and males to *E. veratrifolia* flower headspace compounds. Asterisks (*), significant difference from control (DCM) $p < 0.001$ (paired sample t -test, sequential Bonferroni correction for ties). Filled bars, females; white bars, males.

Figure 3. Effect of flower odour on oviposition of *E. balteatus* females (mean number of eggs $\pm$ s.e.). (a) Comparison of the headspace sample of the orchid *E. veratrifolia* and (b) the synthetic mixture against the solvent pentane (paired samples t -test, $n = 10/13$, $t = 6.8/4.8$, $*p < 0.001$).

pheromone of some aphid species, e.g. *M. viciae*, namely α - and β -pinene, and β -myrcene [25,26]. The flowers, therefore, appear to mimic the alarm pheromone of aphids, thus attracting hoverflies for pollination. This is supported by our behavioural experiments, in which we show that the flowers' scent, as well as a synthetic copy of it, induces oviposition behaviour in females of the hoverfly *E. balteatus*.

Aphids not only release alarm pheromone when under attack, but continuously release small amounts [27]. Predators can thus use aphid pheromones as faithful cues for locating aphid colonies. Although (*E*)- β -farnesene is the most commonly used aphid alarm pheromone, several other terpenoids are produced by aphids and also function as alarm pheromones [25,26]. The aphid *M. viciae* is a common species in the Middle East; its pheromone mainly consists of α - and β -pinene, and β -myrcene [25] and shows a striking similarity to the floral odour of *E. veratrifolia* (figure 1). Five more aphid species are known to produce α/β -pinene, three of them as the major pheromone component [26]. α/β -pinene and myrcene might, in fact, be more frequent components of aphid chemical communication than is currently recognized because, as yet, the alarm volatiles of only approximately 1 per cent of all aphid species have been analysed.

Our data suggest that *E. veratrifolia* does not mimic one aphid species specifically, as the orchid does not produce the same compounds in exactly the same proportions as a specific aphid species. The flower instead produces α - and β -pinene to mimic volatiles associated with a certain group of aphids, namely those using these compounds in their pheromone communication. This generalized mimicry makes sense as all five pollinating hoverfly species (i.e. their larvae) are not specialized in one certain aphid species, but can feed on a large number of hosts (*I. aegyptus*: 23 host species, *S. ruepellii*: 37, *S. scripta*: 79, *E. corollae*: 111, *E. balteatus*: 187; [28]). *Megoura viciae* is used as a host by three of these hoverflies (*S. scripta*, *E. corollae* and *E. balteatus*), and all but *I. aegyptus* use at least one additional pinene-producing aphid species as their host [28]. It is therefore plausible that most of the pollinating hoverflies do not react to the pheromone of only one aphid species, but use several aphid- and plant-derived volatiles for host location and as oviposition cues, as has been demonstrated for *E. balteatus* [18].

The pollination mechanism of *E. veratrifolia* is thus similar to those found in other plant species mimicking oviposition sites. Many Araceae, for example, mimic dung or faeces, the oviposition sites for coprophilic flies.

These species also produce odours typical for such substrates, but do not mimic the odour profile of a specific kind of faeces perfectly [29,30].

The genus *Epipactis* shows a huge variety in pollination systems, with most species showing specialist pollination systems, e.g. wasp-pollinated or bumble-bee-pollinated [31]. Floral odours have been investigated only in wasp-pollinated *Epipactis* species; those produce a completely different floral odour than *E. veratrifolia* [32]. This strengthens our hypothesis that *E. veratrifolia* specifically mimics aphid volatiles.

Opinions diverge on whether such a generalized or non-model mimicry, which lacks a certain model (-species), should be referred to as mimicry *sensu* Bates [33] or perceptual exploitation [34]. As mimicry implies a very exact copy of the original, we would in the present case favour the generalized mimicry or perceptual exploitation terminology.

(b) Does *Epipactis veratrifolia* reward or harm its pollinator?

Epipactis veratrifolia does provide some nectar in its flowers (although very little) and might thus not be a truly deceptive species. On the other hand, the floral signal that attracts the pollinators advertises a different reward (aphids) than actually provided (nectar). Thus, *E. veratrifolia* has to be considered deceptive, at least in terms of pollinator attraction. A similar case has been found in the wasp-pollinated *Epipactis helleborine* and *Epipactis purpurata*, where the flowers mimic volatiles associated with the wasps' prey. But instead of the prey, the wasps get rewarded with nectar [32].

An important question that remains unanswered is the cost to the pollinators when they deposit eggs that cannot develop in the flowers. First-instar larvae of *E. balteatus* are limited in their dispersal [13]. If the larvae do not find a suitable host and consequently die, *E. veratrifolia* does harm its pollinators by reducing their fitness. Harming the pollinator is thought to be evolutionarily unstable and, therefore, very rare [35]. However, detailed data on the survival rate of hoverfly larvae on *E. veratrifolia* are needed to support this hypothesis.

(c) Deceptive pollination by syrphids

Hoverflies are frequent and well-known pollinators of rewarding flowers and, as such, have been thoroughly studied. By contrast, deceptive pollination systems involving hoverflies are rare and have so far received little attention. The Japanese *Epipactis thunbergii* may exhibit a similar pollination strategy to *E. veratrifolia*: it is also pollinated by several syrphids, which also deposit eggs in the flower [36]. The slipper orchids *Paphiopedilum dianthum* and *Paphiopedilum rothschildianum* are also exclusively pollinated by hoverflies, predominantly females, which also lay eggs in the flowers [37,38]. Unfortunately, no data on the floral chemistry are available for all these species. A third slipper orchid, *Paphiopedilum barbigerrum*, is also pollinated by hoverflies, but in this case no eggs are deposited by the flies and as no floral odour could be detected, it is assumed that the orchid rather exploits an innate colour preference of the flies [39].

(d) Evolution of floral odours—a case of pre-adaptation?

Pre-adaptation (the acquisition of a new function by the pre-existing traits) is thought to play a major role in the

evolution of specialized and deceptive pollination systems [40]. A prime example is the tropical genus *Dalechampia*. Here, resins produced as floral defence compounds appear to have been a pre-adaptation for the evolution of a resin-reward pollination system [41]. The use of aphid pheromones for herbivore defence is known from the wild potato *Solanum berthaultii* [42]. Its leaves produce the aphid alarm pheromone compound (*E*)- β -farnesene to deter aphids. α - and β -pinene are very common floral compounds (found in at least 350 species of Orchidaceae) [43,44]. We speculate that the initial function of pinene in flowers may have been to deter aphids away from the precious reproductive organs. Subsequently, α - and β -pinene may have gained importance in pollinator attraction, as the function switched from plant defence to pollinator attraction. It is of interest that the flowers of *E. veratrifolia* are mostly aphid free, even though the green parts of the flower are regularly infested [22]). However, one function does not exclude the other. Further studies are required to fully test the hypothesis that hoverfly pollination has evolved as a consequence of switching from aphid deterrence to pollinator attraction in this system.

5. CONCLUSIONS

We provide new evidence that *E. veratrifolia* attracts aphidophagous hoverflies by generalized mimicry of alarm pheromone, adding a new pollination strategy to the long list of diverse and intriguing pollination strategies that have evolved in the Orchidaceae. There is an ongoing discussion among evolutionary biologists as to whether species richness in Orchidaceae is a cause or a consequence of specialized (and deceptive) pollination systems [45–48]. Compared with the high number of species, orchids are still under-represented in scientific investigations of pollination biology and, more generally, of reproductive isolation and speciation mechanisms [49]. The present results add another piece to the puzzle of the complex evolution of orchids.

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REFERENCES

- 1 Darwin, C. R. 1862 *The various contrivances by which orchids are fertilised by insects*. London, UK: John Murray.
- 2 Van der Pijl, L. & Dodson, D. H. 1966 *Orchid flowers: their pollination and evolution*. Coral Gables, FL: University of Miami Press.
- 3 Nilsson, L. A. 1992 Orchid pollination biology. *Trends Ecol. Evol.* **7**, 255–259. (doi:10.1016/0169-5347(92)90170-G)
- 4 Dafni, A. 1984 Mimicry and deception in pollination. *Annu. Rev. Ecol. Syst.* **15**, 259–278. (doi:10.1146/annurev.es.15.110184.001355)
- 5 Jersakova, J., Johnson, S. D. & Kindlmann, P. 2006 Mechanisms and evolution of deceptive pollination in

- orchids. *Biol. Rev.* **81**, 219–235. (doi:10.1017/S1464793105006986)
- 6 Brodmann, J., Twele, R., Francke, W., Yi-bo, L., Xi-qiang, S. & Ayasse, M. 2009 Orchid mimics honey bee alarm pheromone in order to attract hornets for pollination. *Curr. Biol.* **19**, 1368–1372. (doi:10.1016/j.cub.2009.06.067)
 - 7 Vogel, S. 1983 Ecophysiology of zoophilic pollination. In *Encyclopedia of plant physiology, physiological plant ecology* (eds O. L. Lange, P. S. Nobel, C. B. Osmond & H. Ziegler), pp. 560–611. Berlin, Germany: Springer.
 - 8 Van der Cingel, N. A. 1995 *An atlas of orchid pollination: European orchids*. Rotterdam, The Netherlands: Balkema.
 - 9 Larson, B. M. H., Kevan, P. G. & Inouye, D. W. 2001 Flies and flowers: taxonomic diversity of anthophiles and pollinators. *Can. Entomol.* **133**, 439–465. (doi:10.4039/Ent133439-4)
 - 10 Ankersmit, G. W., Dijkman, H., Keuning, N. J., Mertens, H., Sins, A. & Tacoma, H. M. 1986 *Episyrphus balteatus* as a predator of the aphid *Sitobion avenae* on winter wheat. *Entomol. Exp. Appl.* **42**, 271–277. (doi:10.1007/BF00629314)
 - 11 Schneider, F. 1969 Bionomics and physiology of aphidophagous Syrphidae. *Annu. Rev. Entomol.* **14**, 103–124. (doi:10.1146/annurev.en.14.010169.000535)
 - 12 Irvin, N. A., Wratten, S. S., Frampton, C. M., Bowie, M. H., Evans, A. M. & Moar, N. T. 1999 The phenology and pollen feeding of three hover fly (Diptera: Syrphidae) species in Canterbury, New Zealand. *NZ J. Zool.* **26**, 105–115.
 - 13 Chandler, A. E. F. 1969 Locomotory behaviour of first instar larvae of aphidophagous syrphidae (Diptera) after contact with aphids. *Anim. Behav.* **17**, 673–678. (doi:10.1016/S0003-3472(69)80011-4)
 - 14 Almohamad, R., Verheggen, F. J. & Haubruge, E. 2009 Searching and oviposition behavior of aphidophagous hoverflies (Diptera: Syrphidae): a review. *Biotechnol. Agron. Soc. Environ.* **13**, 467–481.
 - 15 Almohamad, R., Verheggen, F. J., Francis, F. & Haubruge, E. 2007 Predatory hoverflies select their oviposition site according to aphid host plant and aphid species. *Entomol. Exp. Appl.* **125**, 13–21. (doi:10.1111/j.1570-7458.2007.00596.x)
 - 16 Scholz, D. & Poehling, H. M. 2000 Oviposition site selection of *Episyrphus balteatus*. *Entomol. Exp. Appl.* **94**, 149–158. (doi:10.1023/A:1003904423390)
 - 17 Budenberg, W. J. & Powell, W. 1992 The role of honeydew as an ovipositional stimulant for two species of syrphids. *Entomol. Exp. Appl.* **64**, 57–61. (doi:10.1007/BF00688994)
 - 18 Verheggen, F., Arnaud, L., Bartram, S., Gohy, M. & Haubruge, E. 2008 Aphid and plant volatiles induce oviposition in an aphidophagous hoverfly. *J. Chem. Ecol.* **34**, 301–307. (doi:10.1007/s10886-008-9434-2)
 - 19 Richards, A. J. 1982 The influence of minor structural changes in the flower on breeding systems and speciation in *Epipactis* Zinn (Orchidaceae). In *Pollination and evolution* (eds J. A. Armstrong, J. M. Powell & A. J. Richards), pp. 47–53. Sydney, Australia: Royal Botanic Gardens.
 - 20 Delforge, P. 2006 *Orchids of Europe, North Africa and the Middle East*. London, UK: A&C Black Publishers Ltd.
 - 21 Cribb, P. J. 1979 The orchids of Arabia. *Kew Bull.* **33**, 651–678. See <http://www.jstor.org/stable/4109808>.
 - 22 Ivri, Y. & Dafni, A. 1977 The pollination ecology of *Epipactis consimilis* Don (Orchidaceae) in Israel. *New Phytol.* **79**, 173–177. See www.jstor.org/stable/2433729.
 - 23 Rotheray, G. & Gilbert, F. 1999 Phylogeny of Palaearctic Syrphidae (Diptera): evidence from larval stages. *Zool. J. Linn. Soc.* **127**, 1–112. (doi:10.1111/j.1096-3642.1999.tb01305.x)
 - 24 Benjamini, Y. & Hochberg, Y. 1995 Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* **57**, 289–300. See www.jstor.org/stable/234610.
 - 25 Pickett, J. A. & Griffiths, D. C. 1980 Composition of aphid alarm pheromones. *J. Chem. Ecol.* **6**, 349–360. (doi:10.1007/BF01402913)
 - 26 Francis, F., Vandermoten, S., Verheggen, F., Lognay, G. & Haubruge, E. 2005 Is the (E)- β -farnesene only volatile terpenoid in aphids? *J. Appl. Entomol.* **129**, 6–11. (doi:10.1111/j.1439-0418.2005.00925.x)
 - 27 Almohamad, R., Verheggen, F. J., Francis, F., Lognay, G. & Haubruge, E. E. 2008 Emission of alarm pheromone by non-preyed aphid colonies. *J. Appl. Entomol.* **132**, 601–604. (doi:10.1111/j.1439-0418.2008.01309.x)
 - 28 Rojo, S., Gilbert, F., Marcos-Garcia, A., Nieto, J. M. & Mier, M. P. 2003 *A world review of predatory hoverflies (Diptera, Syrphidae: Syrphinae) and their prey*. Alicante, Spain: Centro Iberoamericano de la Biodiversidad (CIBIO).
 - 29 Kite, G. C. 1995 The floral odour of *Arum maculatum*. *Biochem. Syst. Ecol.* **23**, 343–354. (doi:10.1016/0305-1978(95)00026-Q)
 - 30 Kite, G. C., Hettterscheid, W. L. A., Lewis, M. J., Boyce, P. C., Ollerton, J., Cocklin, E., Diaz, A. & Simmonds, M. S. J. 1998 Inflorescence odours and pollinators of *Arum* and *Amorphophallus* (Araceae). In *Reproductive biology* (eds S. J. Owens & P. J. Rudall), pp. 295–315. Kew, UK: Royal Botanic Gardens.
 - 31 Burns-Balogh, P., Szlachetko, D. L. & Dafni, A. 1987 Evolution, pollination, and systematics of the tribe Neottieae (Orchidaceae). *Plant Syst. Evol.* **156**, 91–115. (doi:10.1007/BF00937204)
 - 32 Brodmann, J., Twele, R., Francke, W., Hölzler, G., Zhang, Q.-H. & Ayasse, M. 2008 Orchids mimic green-leaf volatiles to attract prey-hunting wasps for pollination. *Curr. Biol.* **18**, 740–744. (doi:10.1016/j.cub.2008.04.040)
 - 33 Bates, H. W. 1862 Contribution to an insect fauna of the Amazon Valley. Lepidoptera: Heliconidae. *Trans. Linn. Soc. Lond.* **23**, 495–566.
 - 34 Schaefer, H. M. & Ruxton, G. D. 2009 Deception in plants: mimicry or perceptual exploitation? *Trends Ecol. Evol.* **24**, 676–685. (doi:10.1016/j.tree.2009.06.006)
 - 35 Vereecken, N. J. 2009 Deceptive behavior in plants. I. Pollination by sexual deception in orchids: a host-parasite perspective. In *Plant environment interactions* (ed. F. Baluska), pp. 203–222. Berlin, Germany: Springer.
 - 36 Sugiura, N. 1996 Pollination of the orchid *Epipactis thunbergii* by syrphid flies (Diptera: Syrphidae). *Ecol. Res.* **11**, 249–255. (doi:10.1007/BF02347782)
 - 37 Atwood, J. T. 1985 Pollination of *Paphiopedilum rothschildianum*: brood site deception. *Natl Geogr. Res.* **1**, 247–254.
 - 38 Shi, J., Cheng, J., Luo, D., Shangguan, F.-Z. & Lou, B. 2007 Pollination syndromes predict brood-site deceptive pollination by female hoverflies in *Paphiopedilum dianthum* (Orchidaceae). *Acta Phytotaxon. Sin.* **45**, 551–560. (doi:10.1360/aps07025)
 - 39 Shi, J., Luo, Y. B., Bernhardt, P., Ran, J. C., Liu, Z. J. & Zhou, Q. 2009 Pollination by deceit in *Paphiopedilum barbigerrum* (Orchidaceae): a staminode exploits the innate colour preferences of hoverflies (Syrphidae). *Plant Biol.* **11**, 17–28. (doi:10.1111/j.1438-8677.2008.00120.x)
 - 40 Schiestl, F. P. & Cozzolino, S. 2008 Evolution of sexual mimicry in the orchid subtribe orchidinae: the role of preadaptations in the attraction of male bees as pollinators. *BMC Evol. Biol.* **8**, 27. (doi:10.1186/1471-2148-8-27)

- 41 Armbruster, W. S., Howard, J. J., Clausen, T. P., Debevec, E. M., Loquvam, J. C., Matsuki, M., Cerendolo, B. & Andel, F. 1997 Do biochemical exaptations link evolution of plant defense and pollination systems? Historical hypotheses and experimental tests with *Dalechampia* vines. *Am. Nat.* **149**, 461–484. (doi:10.1086/286000)
- 42 Gibson, R. W. & Pickett, J. A. 1983 Wild potato repels aphids by release of aphid alarm pheromone. *Nature* **302**, 608–609. (doi:10.1038/302608a0)
- 43 Knudsen, J. T., Eriksson, R., Gershenzon, J. & Stahl, B. 2006 Diversity and distribution of floral scent. *Bot. Rev.* **72**, 1–120. (doi:10.1663/0006-8101(2006)72[1:DAD OFS]2.0.CO;2)
- 44 El-Sayed, A. M. 2009 The Pherobase: database of insect pheromones and semiochemicals. See www.pherobase.com.
- 45 Cozzolino, S. & Widmer, A. 2005 Orchid diversity: an evolutionary consequence of deception? *Trends Ecol. Evol.* **20**, 487–494. (doi:10.1016/j.tree.2005.06.004)
- 46 Otero, J. T. & Flanagan, N. S. 2006 Orchid diversity: beyond deception. *Trends Ecol. Evol.* **21**, 64–65. (doi:10.1016/j.tree.2005.11.016)
- 47 Armbruster, W. S. & Muchhala, N. 2009 Associations between floral specialization and species diversity: cause, effect, or correlation? *Evol. Ecol.* **23**, 159–179. (doi:10.1007/s10682-008-9259-z)
- 48 Scopece, G., Cozzolino, S., Johnson, S. & Schiestl, F. P. 2010 Pollination efficiency and the evolution of specialized deceptive pollination systems. *Am. Nat.* **175**, 98–105. (doi:10.1086/648555)
- 49 Peakall, R. 2007 Speciation in the Orchidaceae: confronting the challenges. *Mol. Ecol.* **16**, 2834–2837. (doi:10.1111/j.1365-294X.2007.03311.x)