Plant Syst. Evol. 234: 111–119 (2002)
DOI 10.1007/s00606-002-0187-z

Plant Systematics and Evolution
Printed in Austria

Do changes in floral odor cause speciation in sexually deceptive orchids?

F. P. Schiestl and **M. Ayasse**

Department of Evolutionary Biology, Institute of Zoology, University of Vienna, Vienna, Austria

Received November 22, 2001; accepted February 21, 2002
Published online: November 7, 2002

Abstract. We investigated differences in floral odor between two sympatric, closely related sexually deceptive orchid species, *Ophrys fusca* and *O. bilunulata*, which are specifically pollinated by *Andrena nigroaenea* and *A. flavipes*, respectively. We identified biologically active compounds by gas chromatography with electroantennographic detection using antennae of the pollinator bees. Alkanes, alkenes, aldehydes, and farnesyl hexanoate released electroantennographic reactions. The relative amounts of alkanes were mostly the same between the two orchid species, whereas the relative amounts of most alkenes were significantly different. On the grounds of these findings and behavioral experiments conducted in earlier studies, we suggest that the difference in relative amounts of alkenes is responsible for the selective attraction of pollinators in the two orchids. Speciation in this group of *Ophrys* orchids may be brought about by changes in pattern of alkenes, which lead to attraction of a different pollinator species and therefore reproductive isolation.

Key words: Pollination by sexual deception, floral odor, GC-EAD, pseudocopulation, *Ophrys fusca* Link, *Ophrys bilunulata* Risso, braune Ragwurz.

Floral odor is an important trait used by many plants to attract pollinators (Frisch 1919, Kugler 1970, Stebbins 1970, Vogel 1983). At the higher taxonomic level, different types of floral odor have been associated with adaption to different groups of pollinators (Stebbins 1970, Knudsen and Tollsten 1993, Dobson 1994). These observations of repeated adaptions of floral traits to pollinators have long been epitomized by the concept of the pollinator syndrome (Johnson and Steiner 2000). Additionally, odor differences between species have often been interpreted as a cue for attracting distinct pollinators (Dodson et al. 1969, Nilsson 1983, Gregg 1983, Ågren and Borg-Karlson 1984, Bergström 1987, Knudsen and Tollsten 1993, Raguso and Pichersky 1995). However, the role of floral odor in maintaining species integrity is questionable in cases where pollination is not specific, since generalized pollinators are necessarily attracted to several different odor bouquets. In a specialized system, however, where odor plays a major role in pollinator attraction and therefore in maintaining floral isolation, its alteration might influence the visitation of pollinators (Grant 1994). Although such specialized systems may be uncommon among angiosperms (Waser 1998, Johnson and Steiner 2000), sexual deception in the orchid genus *Ophrys* provides a rare example where odor communication is reasonably well understood (Pouyanne 1917, Kullenberg 1961; Borg-Karlson 1990, Schiestl et al. 1999).

The pollination mechanism of sexual deception is based on a mimicry of the sex pheromones released by the female of the pollinator species (Schiestl et al. 2000). Pollination is usually species-specific (Paulus and Gack 1990), which provides the mean of reproductive isolation between the intercrossable *Ophrys* species (Ehrendorfer 1980). The pollinator males perform pseudocopulations on the floral labella, transferring the pollinia in the process. The floral odor bouquet is primarily responsible for eliciting mating behavior in the pollinators. *Ophrys* orchids usually produce a wide array of floral odor compounds (Borg-Karlson 1990, Erdmann 1996). However, in *O. sphegodes*, Schiestl et al. (2000) demonstrated that only a subset of these, namely alkanes and alkenes, are detected by the pollinator and elicit mating behavior. It has been speculated that the highly specialized nature of pollination by sexual deception has led to adaptive radiation accompanied by changes in floral scent (Stebbins 1970, Paulus and Gack 1990, Grant 1994, Soliva et al. 2001). Empirical data support this hypothesis (Borg-Karlson et al. 1993), however, it has not yet been shown which compounds mediate the attraction of different pollinators, leading to reproductive isolation.

Our study tests the hypothesis of differences in biologically active odor compounds within two sympatric, closely related species by investigating the composition of floral odor and its olfactory detection by the pollinators in *Ophrys fusca* Link and *O. bilunulata* Risso. Our findings indicate which changes in biologically active compounds are linked with speciation, which we suggest may have taken place in sympatry.

Materials and methods

Natural history. The *Ophrys fusca*-group comprises approximately 10 closely related species that are often hardly distinguishable by morphological traits (Paulus and Gack 1981, Delforge 1995). The two members of this group, *Ophrys fusca* and *O. bilunulata* are distributed throughout the Mediterranean basin and flower in early spring. At our study site in Majorca, the species occur sympatrically, with an overlap in blooming time of about two weeks. *O. fusca* is pollinated by males of the bee *Andrena nigroaenea* Kirby and *O. bilunulata* by males of *A. flavipes* Panzer (Paulus and Gack 1990). In both species, the pollinia get attached to the abdomen of the bees. *A. nigroaenea* also pollinates *O. sphegodes*; however, in this species the pollinia are placed on the head of the bee, which prevents hybridization with *O. fusca* (Kullenberg 1961, Paulus and Gack 1990). Both bee species are solitary, and the males patrol along shrubs in considerable numbers, searching for females. In Austria, the two species occur in the same locations and show a broad overlap in flight season, but *A. flavipes* tends to fly up to about 50 cm above ground, whereas *A. nigroaenea* usually patrols 1–2 m above ground (Schiestl unpublished).

Sample collection. Flowers of the two orchid species were collected in the field in Majorca, Spain, and individual labella were extracted in 400 µl pentane for 24 h. In the laboratory, samples were concentrated by evaporation of solvent to 50 µl and stored in a freezer. Male bees for the GC-EAD studies were collected in Oberweiden, Lower Austria.

Gas chromatography and electrophysiology (GC-EAD). GC-EAD analyses were conducted according to Schiestl et al. (2000) and Schiestl and Ayasse (2000). One µl of each odor sample was injected splitless at 50 °C (1 min) into a gas chromatograph (HP 6890), followed by opening the split valve and programming the temperature to increase to 310 °C at a rate of 10 °C/min. The GC was equipped with a DB-5 column (30 m × 0.32 mm); helium was used as carrier gas. A GC effluent splitter (press-fit-connection; split ratio 1:1) was used and the outlet was directed into a purified and humidified airstream, which was directed over a male bee's antenna. The antenna was prepared as follows. The tip of an excised antenna was cut off and the antenna mounted between two glass electrodes filled with insect ringer. The electrode holding the base of the antenna was connected to a grounded Ag-AgCl wire. The electrode at the distal end of the antenna was connected via an interface box to a signal acquisition interface board (IDAC; Syntech, Hilversum) for signal transfer to a PC. EAD signals and flame ionisation detector (FID) responses from the GC were simultaneously recorded.

For each orchid-pollinator species pair, approximately 10 GC-EAD runs were obtained. Reactions

from the antennae were distinguished from background noise by comparing the reproducibility of a response: peaks in the EAD recording were assumed to be responses from the antennal receptors if they occurred in at least half the recordings at exactly the same retention time. Active compounds were identified by coinjection with synthetic compounds, and EAD responses were verified in GC-EAD runs with synthetic compounds.

Quantitative analysis. Relative amounts of biologically active alkanes and alkenes were calculated separately for alkanes and alkenes. Mean

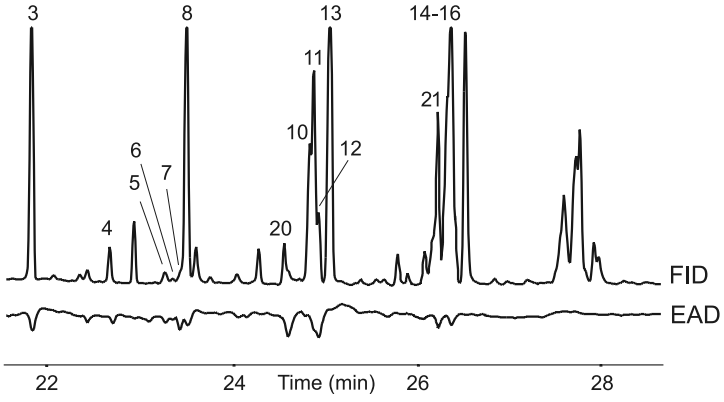

Fig. 1. Gas chromatographic analysis of an *Ophrys bilunulata* labellum extract with simultaneous recording of flame ionisation detector (FID) and electroantennographic detector (EAD) (GC-EAD) using an *Andrena flavipes* male antenna. Numbered FID peaks represent compounds that elicited electroantennographic responses in antennal receptors. For names of these active compounds see Table 1

Table 1. Floral odor compounds of *Ophrys fusca* and *O. bilunulata* that elicited electroantennographic reactions (i.e. biologically active compounds) in the antennae of their pollinator species *Andrena nigroaenea* and *A. flavipes*, respectively. Numbers correspond to peaks in Fig. 1

Peak No.	Compound	*Andrena nigroaenea*	*Andrena flavipes*
	A) Alkanes and alkenes		
1	Heneicosane*	X	–
2	Docosane*	X	–
3	Tricosane	X	X
4	Tetracosane	X	X
5	(*Z*)-9 + (*Z*)-10-Pentacosene	X	X
6	(*Z*)-7-Pentacosene	–	X
7	(*Z*)-5-Pentacosene	–	X
8	Pentacosane	X	X
9	Hexacosane*	X	X
10	(*Z*)-12 + (*Z*)-11-Heptacosene	X	X
11	(*Z*)-9-Heptacosene	X	X
12	(*Z*)-7-Heptacosene	–	X
13	Heptacosane	X	X
14	(*Z*)-12 + (*Z*)-11-Nonacosene	X	X
15	(*Z*)-9-Nonacosene	X	X
16	(*Z*)-7-Nonacosene	–	X
	B) Other compounds		
17	Octadecanal*	X	–
18	Nonadecanal*	X	–
19	Farnesyl hexanoate*	X	–
20	Tetracosanal	–	X
21	Unknown 1		X

* Not indicated in Fig. 1

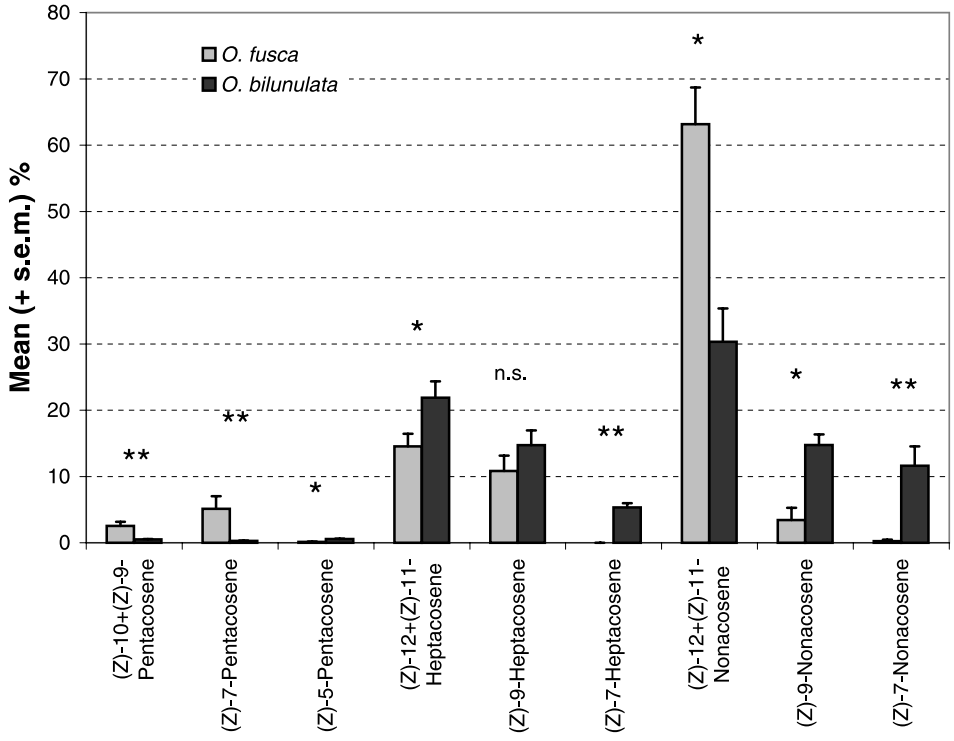

Fig. 2. Relative amounts of alkenes in the floral odor of *Ophrys fusca* (n = 10) and *O. bilunulata* (n = 8). *P < 0.05, **P < 0.001, Mann-Whitney U-Test

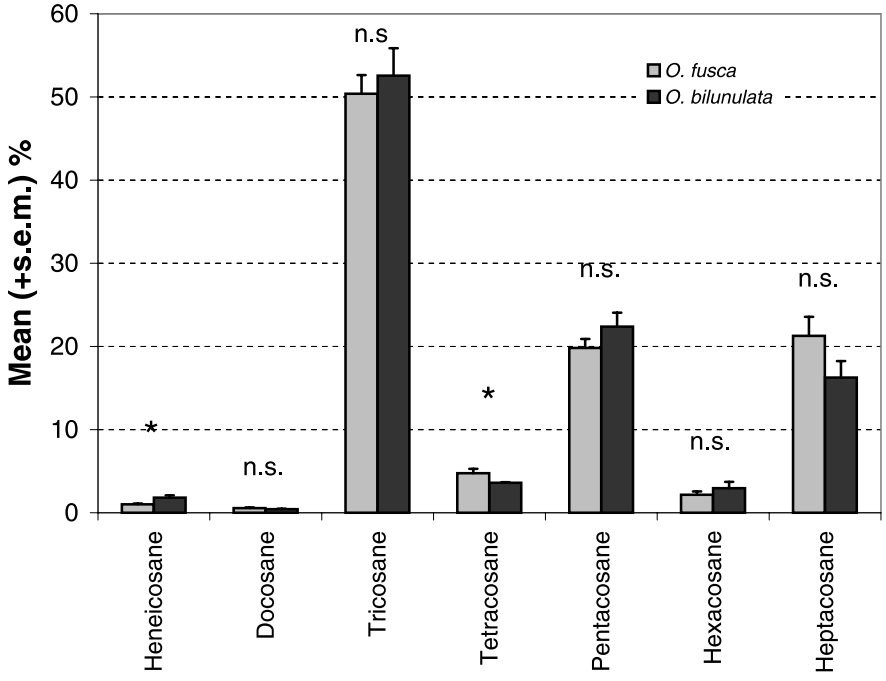

Fig. 3. Relative amounts of alkanes in the floral odor of *Ophrys fusca* (n = 10) and *O. bilunulata* (n = 8). *P < 0.05, Mann-Whitney U-Test

relative amounts were compared between the two orchid species using the Mann-Whitney U-test.

Results

Biologically active compounds

Our GC-EAD analyses detected compounds that trigger electroantennographic responses in olfactory receptors (Arn et al. 1975). These "biologically active" compounds in an odor bouquet are the ones that can be detected by the pollinating bee. In both *A. nigroaenea* and *A. flavipes*, 16 peaks comprising 19 compounds were found to be biologically active (Fig. 1, Table 1). Of these compounds, the (*Z*)-9 and (*Z*)-10 isomers of pentacosane, and the (*Z*)-11 and (*Z*)-12 isomers of both heptacosane and nonacosane could not be separated with the GC parameters used and hence were analyzed together.

There was a broad overlap in the orchid compounds detected by the males of both bee species. Among the alkanes and alkenes, 10 compounds proved to be active in *A. nigroaenea* and *A. flavipes*. The shorter chain alkanes heneicosane and docosane triggered a response only in *A. nigroaenea*, whereas the (*Z*)-5 isomers of pentacosene and the (*Z*)-7 isomers of pentacosene, heptacosene, and nonacosene were detected only by *A. flavipes* males. Within the aldehydes, *A. nigroaenea* responded to octadecanal and nonadecanal, whereas *A. flavipes* reacted to tetracosanal.

Qualitative and quantitative differences in odor compounds

The qualitative differences in biologically active compounds between the two orchid species were minor as most of the active compounds occurred in both species. (*Z*)-7-Heptacosene was only found in *O. bilunulata*, and (*Z*)-7-nonacosene occurred almost exclusively in the odor bouquet of *O. bilunulata*, whereas (*Z*)-7-pentacosene was predominantly found in *O. fusca* (Fig. 2). Quantitative analyses of relative amounts of alkanes and alkenes showed that most alkanes, except heneicosane and tetracosane, did not differ significantly between the two species (Fig. 3). However, among the alkenes, 8 of 9 biologically active compounds occurred in significantly different relative amounts in the two orchid species (Fig. 2).

Discussion

Biologically active compounds

In all *Ophrys*-pollinator systems so far investigated by GC-EAD, only a small subset of the long array of compounds produced by the flowers (Borg-Karlson 1990, Erdmann 1996) have been found to be detected by the pollinators (Schiestl et al. 2000). Our GC-EAD experiments here show that *A. nigroaenea* and *A. flavipes* males react to almost the same set of orchid odor compounds. *A. nigroaenea*, which pollinates *O. sphegodes* as well as *O. fusca* studied here, detects the same alkanes and alkenes present in the floral odors of the two orchid species (Schiestl et al. 1999). Behavioral tests in the field previously established that a synthetic mixture of the active compounds elicited copulation attempts by males of *A. nigroaenea,* and that, when tested separately, alkenes were more attractive than alkanes (Schiestl et al. 2000). Since *A. flavipes* reacts to most of the same compounds as *A. nigroaenea*, we assume the behavioral function of those compounds are the same as has been behaviorally demonstrated in *A. nigroaenea*.

Although knowledge about solitary-bees sex pheromones is scarce (Ayasse et al. 2001), the importance of alkenes has been documented in the leafcutter bee *Megachile rotunda,* where female-produced alkenes were found to stimulate copulatory activity in males and differences in the amounts of alkenes produced distinguished virgins from older females (Paulmier et al. 1999). Ayasse et al. (1999) also found alkanes and alkenes produced by female *Lasioglossum malachurum* bees to be attractive to males.

The functions of other orchid compounds found to be physiologically active in pollinators may be similar in *O. sphegodes*, *O. fusca* and *O. bilunulata*. Aldehydes, together with esters, in the floral odor of *O. sphegodes*, have been shown to influence the learning behavior of *A. nigroaenea* males (Ayasse et al. 2000). Farnesyl hexanoate has a repellent effect on *A. nigroaenea* males (Schiestl and Ayasse 2000) and influences the pollinators' choice between pollinated and unpollinated flowers of *O. sphegodes* (Schiestl and Ayasse 2001).

Specificity of pollinator attraction

Specificity in an odor signal may be reflected in the presence of different compounds and/or differences in the relative amounts of a given set of compounds (Linn and Roelofs 1995). In our study, we found only a few qualitative differences in the bees' physiological detection of specific compounds. Ten out of the total of 16 active alkanes and alkenes were detected by each of the bee species. However, heneicosane and docosane were detected by *A. nigroaenea* only, whereas the (*Z*)-5-isomer of pentacosene and the (*Z*)-7-isomers of three different alkenes are detected by *A. flavipes* males only. This receptor specificity is nevertheless not sufficient to explain the selective attraction, since all of these compounds are produced by both orchid species. In our study, the only compound that was found in only one orchid species, namely *O. bilunulata*, was (*Z*)-7-heptacosene, but Erdmann (1996) identified this compound in *O. fusca* as well. Therefore, in the case of *O. fusca* and *O. bilunulata,* it is likely that variation in the relative amounts of the same odor constituents leads to the pollinator specificity we observe in the field.

Sex pheromones are often mixtures of compounds, with the quantitative ratios of the compounds being important for species-specific behavioral activity (West-Eberhard 1984; Löfstedt 1990, 1993; Linn and Roelofs 1995). For example, the sex pheromone composition of small ermine moths species varies mainly in the relative amounts of (*Z*) and (*E*) isomers of 11-tetradecenyl actetate (Löfstedt et al. 1991). In *O. sphegodes,* Schiestl et al. (2000) showed that it was mostly the pattern of alkanes and alkenes that differed between flowers and leaves, where flowers are attractive to the pollinator *A. nigroaenea* and leaves bear no attractiveness. In this study, we found the relative amounts of alkanes to be mostly the same in both orchid species, whereas the relative amounts of most alkenes differed significantly between *O. fusca* and *O. bilunulata*. We therefore suggest that the specific attraction of each male bee pollinator to only one orchid, and therefore the reproductive isolation between the two orchid species, is mediated by the pattern of relative amounts of alkenes in the orchids' floral scent.

Mechanism of speciation in Ophrys

Species-specific differences in floral odor may be a by-product of the speciation process, indicating selection for the attraction of a different pollinator after reproductive isolation has been established (Grant 1994). However, for *Ophrys*, where the flowers are pollinated by male bees or wasps through pseudocopulation, odor changes may be intricately associated with the evolution of reproductive isolation via pollinator-mediated selection. The mate signalling system of insects are usually expected to be species-specific, particularly when existing in sympatry. For sexually deceptive orchids utilizing such signals, the exploitation of single pollinator species among a pool of multiple sympatric pollinators is therefore likely to involve the production of a species-specific pheromone mimicry.

We also suggest that speciation may have taken place in sympatry, especially in present-day sympatric, closely related *Ophrys* species. It might be assumed that for *Ophrys* species to remain attractive to pollinators, the floral odor bouquet would be under strong stabilizing selection. However, negative frequency dependent selection in response to pollinator learning may favor the maintenance of some odor variability within orchid populations (Moya

and Ackerman 1993). In accordance with this hypothesis, Ayasse et al. (2000) confirmed that considerable odor variation exists within populations and even within individuals of *Ophrys sphegodes*.

The adaptive peak in floral scent for an *Ophrys* species may therefore encompass a degree of odor variability that remains within the range of the pollinators' response to its sex pheromone. If the odor of a mutant orchid differs too much for its original pollinator to respond, the odor might by chance more closely resemble the sex pheromone of another bee or wasp species, which consequently responds to the flower and becomes a new pollinator. These mutants would successfully reproduce, being reproductively isolated from their sympatric parent population, and the new odor would be established as the mutant becomes a new species.

A recent phylogenetic analysis of the genus *Ophrys* suggests that the group underwent a recent radiation (Soliva et al. 2001) and a comparative phylogeny of the sexually deceptive orchid genus *Chiloglottis* and its pollinators in Australia (Mant et al. 2002) indicates that these orchids are likely to have radiated onto an existing lineage of pollinators. Therefore, the process of speciation may have been dependent on the availability of pollinator species and the possibility of imitating their sex pheromones by genetic changes. In congruence with the phylogenetic results, we argue that speciation in *Ophrys* can be a rapid process, since acquiring a new pollinator species may not require the synthesis of new compounds, but only a change in pattern, which might involve only few mutations at certain loci (Haynes and Hunt 1990). In addition, the signal emitter – receiver system of orchids and their pollinators involves two different species, where a genetic change in the emitter may lead to a response of a new receiver; therefore, changes in emitter and receiver need not to happen in synchrony, making the process proceed at a potentially faster rate.

In conclusion, we suggest that in the two species of the *Ophrys fusca* group studied here, a change in the pattern of biologically active compounds in the floral scent may lead to the attraction of new pollinator species. Since pollination is highly specific in these orchids, a new pollinator species may lead to the reproductive isolation of mutant orchid plants in a population and therefore to speciation.

We thank Fernando Ibarra and Wittko Francke (Universität Hamburg) for their help with chemical identification of biologically active compounds, and Jim Mant and Rod Peakall (The Australian National University) for proof-reading of the manuscript. This study was financially supported by the FWF Austria (P12275-BIO) and the Austrian Academy of Sciences.

References

Ågren L., Borg-Karlson A. K. (1984) Responses of *Agogorytes* (Hymenoptera: Sphecidae) males to odour signals from *Ophrys insectifera* (Orchidaceae). Preliminary EAG and chemical investigation. Nov. Act. Reg. Soc. Sci. Ups. Ser. V: 111–117.

Arn H., Städler E., Rauscher S. (1975) The electroantennographic detector – a selective and sensitive tool in the gaschromatographic analysis of insect pheromones. Z. Naturforsch. 30c: 722–725.

Ayasse M., Engels W., Lübke G., Francke W. (1999) Mating expenditures reduced via female sex pheromone modulation in the primitively eusocial halictine bee, *Lasioglossum (Evylaeus) malachurum* (Hymenoptera: Halictidae) . Behav. Ecol. Sociobiol. 45: 95–106.

Ayasse M., Paxton R. J., Tengo J. (2001) Mating behavior and chemical communication in the order hymenoptera. Annual Rev. Entomol. 46: 31–78.

Ayasse M., Schiestl F. P., Paulus H. F., Löfstedt C., Hansson B., Ibarra F., Francke W. (2000) Evolution of reproductive strategies in the sexually deceptive orchid *Ophrys sphegodes*: How does flower-specific variation of odor signals influence reproductive success? Evolution 54: 1995–2006.

Bergström G. (1987) On the role of volatile chemical signals in the evolution and speciation of plants and insects: why do flowers smell and

why do they smell differently? In: Labeyrie V., Fabres G., Lachaise D. (eds.) Insects-Plants. Netherlands, Dr. W. Junk Publishers, Dordrecht, pp. 321–327.

Borg-Karlson A. K. (1990) Chemical and ethological studies on pollination in the genus *Ophrys* (Orchidaceae). Phytochemistry 29: 1359–1387.

Borg-Karlson A. K., Groth I., Ågren L., Kullenberg B. (1993) Form-specific fragrances from *Ophrys insectifera* L. (Orchidaceae) attract species of different pollinator genera. Evidence of sympatric speciation? Chemoecology 4: 39–45.

Delforge P. (1995) Orchids of Britain and Europe. Harpercollins London.

Dobson H. E. M. (1994) Floral volatiles in insect biology. In: Bernays E. A. (ed.) Insect–Plant Interactions. CRC Press, pp. 47–81.

Dodson C. H., Dressler R. L., Hills H. G., Adams R. M., Williams N. H. (1969) Biologically active compounds in orchid fragrances. Science 164: 1243–1249.

Ehrendorfer F. (1980) Hybridisierung, Polyploidie und Evolution bei europäisch-mediterranen Orchideen. Die Orchidee Sonderheft: 15–34.

Erdmann D. H. (1996) Identifizierung und Synthese flüchtiger Signalstoffe aus Insekten und ihren Wirtspflanzen. Dissertation Universität Hamburg.

Frisch K. V. (1919) Über den Sitz des Geruchssinns bei Insekten. Zool. Jb. Abt. Allg. Zool. u. Physiol. 38: 449–516.

Grant V. (1994) Modes and origins of mechanical and ethological isolation in angiosperms. Proc. Natl. Acad. Sci. USA 91: 3–10.

Gregg K. B. (1983) Variation in floral fragrances and morphology: incipient speciation in *Cycnoches*? Bot. Gaz. 144: 566–576.

Haynes K. F., Hunt R. E. (1990) A mutation in pheromonal communication system of the cabbage looper moth, *Trichoplusia ni*. J. Chem. Ecol. 16: 1249–1257.

Johnson S. D., Steiner K. E. (2000) Generalization versus specialization in plant pollination systems. Tree 15: 140–143.

Knudsen J. T., Tollsten L. (1993) Trends in floral scent chemistry in pollination syndromes. Floral scent composition in moth-pollinated taxa. Bot. J. Linn. Soc. 113: 263–284.

Kugler H. (1970) Blütenökologie. Gustav Fischer, Stuttgart.

Kullenberg B. (1961) Studies in *Ophrys* pollination. Zool. Bidrag. (Uppsala) 34: 1–340.

Linn E. C., Roelofs L. W. (1995) Pheromone communication in moths and its role in the speciation process. In: Lambert M. D., Spencer G. Hamish (eds.) Speciation and the recognition concept. The John Hopkins University Press, Baltimore and London, pp. 263–300.

Löfstedt C. (1990) Evolution of Moth Pheromones. Proc. Conf. Insect Chem. Ecol. 57–73.

Löfstedt C., Herrebout M. W., Menken B. J. S. (1991) Sex pheromones and their potential role in the evolution of reproductive isolation in small ermine moths (Yponomeutidae). Chemoecology 2: 20–28.

Löfstedt C. (1993) Moth pheromone genetics and evolution. Philosophical Transactions of the Royal Society of London B 340: 167–177.

Mant J., Schiestl F. P., Peakall R., Weston P. (2002) A phylogenetic study of pollinator conservatism among sexually deceptive orchids. Evolution 56: 888–898.

Moya S., Ackerman J. D. (1993) Variation in the floral fragrance of *Epidendrum ciliare* (Orchidaceae). Nord. J. Bot. 13: 41–47.

Nilsson L. A. (1983) Processes of isolation and introgressive interplay between *Platanthera bifolia* (L.) Rich. and *P. chlorantha* (Custer) Reichb. (Orchidaceae). Biol. J. Linn. Soc. 87: 325–350.

Paulmier I., Anne-Geneviève B., Afonso C. M. M., Dusticier G., Rivière G., Clement J. L. (1999) Alkenes as a sexual pheromone in the alfalfa leaf cutter bee *Megachile rotundata*. J. Chem. Ecol. 25: 471–490.

Paulus H. F., Gack C. (1981) Neue Beobachtungen zur Bestäubung von *Ophrys* (Orchidaceae) in Südspanien, mit besonderer Berücksichtigung des Formenkreises *Ophrys fusca* agg. Plant Syst. Evol. 137: 241–258.

Paulus H. F., Gack C. (1990) Pollinators as prepollinating isolation factors: Evolution and speciation in *Ophrys* (Orchidaceae). Israel J. Bot. 39: 43–97.

Pouyanne A. (1917) Le fecondation des *Ophrys* par les insectes. Bull. Soc. Hist. Nat. Afr. Nord 8: 6–7.

Raguso R. A., Pichersky E. (1995) Floral volatiles from *Clarkia breweri* and *C. concinna* (Onagraceae): recent evolution of floral scent and moth pollination. Plant Syst. Evol. 194: 55–67.

Schiestl F. P., Ayasse M., Paulus H. F., Löfstedt C., Hansson B. S., Ibarra F., Francke W. (1999) Orchid pollination by sexual swindle. Nature 399: 421–422.

Schiestl F. P., Ayasse M., Paulus H. F., Löfstedt C., Hansson B. S., Ibarra F., Francke W. (2000) Sex pheromone mimicry in the Early Spider Orchid (*Ophrys sphegodes*): patterns of hydrocarbons as the key mechanism for pollination by sexual deception. J. Comp. Physiol. A 186: 567–574.

Schiestl F. P., Ayasse M. (2000) Post mating odor in females of the solitary bee, *Andrena nigroaenea* (Apoidea, Andrenidae) inhibits male mating behavior. Behav. Ecol. Sociobiol. 48: 303–307.

Schiestl F. P., Ayasse M. (2001) Post-pollination production of a repellent compound in a sexually deceptive orchid: a new mechanism for maximising reproductive success? Oecologia 126: 531–534.

Soliva M., Kocyan A., Widmer A. (2001) Molecular phylogenetics of the sexually deceptive orchid genus *Ophrys* (Orchidaceae) based on nuclear and chloroplast DNA sequences. Molec. Phylog. Evol. 20: 78–88

Stebbins G. L. (1970) Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. Annual Rev. Ecol. Syst. 1: 307–326.

Vogel S. (1983) Ecophysiology of zoophilic pollination. In: Lange O. L., Nobel P. S., Osmond C. B., Ziegler H. (eds.) Encyclopedia of Plant Physiology, Physiological plant ecology, new series vol. 12c. Springer, Berlin, pp. 560–611.

Waser N. M. (1998) Pollination, angiosperm speciation, and the nature of species boundaries. Oikos 81: 198–201.

West-Eberhard M. J. (1984) Sexual selection, competitive communication and species specific signals in insects. In: Lewis T. (ed.) Insect communication. Academic, London, pp. 283–323.

Addresses of the authors: Florian P. Schiestl* and Manfred Ayasse, Department of Evolutionary Biology, Institute of Zoology, University of Vienna, Althanstrasse 14, A-1090 Vienna. *Present address: Geobotanical Institute ETH, Zollikerstrasse 107, CH-8008 Zürich. (e-mail: schiestl@geobot.umnw.ethz.ch)