Phytochemistry 72 (2011) 74–81

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Geographic variation of floral scent in a highly specialized pollination mutualism

Catherine Soler [a,*], Martine Hossaert-McKey [a], Bruno Buatois [a], Jean-Marie Bessière [b], Bertrand Schatz [a], Magali Proffit [c]

[a] Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), UMR CNRS 5175, 1919 Route de Mende, 34293 Montpellier Cedex 5, France
[b] Laboratoire de Chimie Appliquée, Ecole Nationale Supérieure de Chimie de Montpellier, 8 rue de l'Ecole Normale, 34296 Montpellier Cedex 5, France
[c] Division of Plant Protection Biology, Chemical Ecology Group, Swedish University of Agricultural Sciences, 23053 Alnarp, Sweden

ARTICLE INFO

Article history:
Received 4 June 2010
Received in revised form 16 October 2010
Available online 23 November 2010

Keywords:
Ficus
Fig wasps
Interpopulation variation
Obligatory mutualism
Specific interaction
Volatile organic compounds
Headspace technique

ABSTRACT

Floral scents are important signals for communication between plants and pollinators. Several studies have focused on interspecific variation of these signals, but little is known about intraspecific variation in flower scent, particularly for species with wide geographic distributions. In the highly specific mutualism between *Ficus* species and their pollinating wasps, chemical mediation is crucial for partner encounter. Several studies show that scents, i.e. blends of volatiles, are species-specific, but no studies address interpopulation variation of scents in fig pollination mutualisms, which often have broad geographic distributions. In this study, using absorption/desorption headspace techniques, we analyzed variation in floral scent composition among three populations of each of two widely distributed Asian *Ficus* species. We identified more than 100 different volatile organic compounds, predominantly terpenes. In both species, significant differences were found between scent bouquets of East Asian and Indian populations. These differences are discussed in relation to geographical barriers that could disrupt gene exchange between these two areas, thereby isolating Indian populations from those of Eastern Asia.

1. Introduction

Volatile organic compounds (VOCs) emitted by plants often mediate interactions between plants and animals, as for example in many pollination systems (reviewed by Raguso (2008)) and other mutualistic interactions, such as animal-mediated seed dispersal (Borges et al., 2008; Hodgkison et al., 2007) and ant/plant protection mutualisms (Schatz et al., 2009). Among the different classes of compounds-fatty acid derivatives, benzenoids and terpenes-the last-named group is the largest, with the widest assortment of structural types (Degenhardt et al., 2009). Floral bouquets are usually mixtures of numerous components, varying among species in their composition, blend ratio, and overall concentrations (Raguso, 2008). In specific pollination mutualisms, such differences in floral scents, and in animal responses to them, contribute to reproductive isolation between closely related sympatric species (Levin et al., 2001).

Contrary to interspecific variation, variation among populations of a single species in production of floral scent has been studied only very recently and in a limited number of examples (Dötterl et al., 2005b; Füssel et al., 2007; Hossaert-McKey et al., 2010; Ibanez et al., 2010; Knudsen, 2002; Majetic et al., 2009; Schlumpberger and Raguso, 2008; Svensson et al., 2005). In a few cases, no interpopulation differences in floral scents were found (Dormont et al., 2010; Knudsen, 2002), or only quantitative differences could be observed (Svensson et al., 2005). However, in most studies, consistent interpopulation differences in floral scent have been reported (Azuma et al., 2001; Schlumpberger and Raguso, 2008). Why floral volatiles vary among different populations of the same plant species remains poorly understood. Intraspecific variation has been notably explained by hypotheses such as relaxed selective pressure, genetic drift, introgression of scent traits through hybridization, pleiotropic effects of plant defense on scent biosynthesis, or phenotypic plasticity resulting from edaphic or climatic differences (Raguso, 2008). Another explanation is that not all compounds contribute to the signal used by the pollinator, and only compounds without a signalling function are variable (Dötterl et al., 2006; Mant et al., 2005). In the literature, the two most frequently proposed explanations are (i) relaxed selection pressure on floral scents as visual cues assume an important role in pollinator attraction (Azuma et al., 2001; Ellis and Johnson, 2009), and (ii) the occurrence of different pollinators in different geographic areas, as in the case of species visited by a spatially variable set of generalist pollinators (Schlumpberger and Raguso, 2008). The extent to which selection on specificity of the plant-insect interaction leads to selection pressure on scent production is unclear. For example, different populations of *Echinopsis*

---

[*] Corresponding author. Tel.: +33 4 67 61 32 30; fax: +33 4 67 41 21 38.
E-mail address: catherine.soler@cefe.cnrs.fr (C. Soler).

0031-9422/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2010.10.012

ancistrophora (Cactaceae), pollinated by a broad spectrum of insects, were shown to emit different floral scents that attracted different pollinators (Schlumpberger and Raguso, 2008). But surprisingly, in the case of the interaction between *Yucca* and their pollinating moths (*Tegeticula yuccasella* and *Tegeticula cassandra*), in which specificity is high (but not complete), no difference of scent appeared among populations of *Yucca filamentosa* pollinated by different yucca moths (Svensson et al., 2005).

However, none of the cases mentioned above are entirely species-specific. Interpopulation variation of floral scents has never been investigated in obligatory, strictly species-specific pollination mutualisms (Hossaert-McKey et al., 2010). We can expect that in highly specific interactions, in which scents promote partner encounter, stabilizing selection should tightly constrain interpopulation variation of scent. Indeed, selection pressure could lead to strong conservatism of scents over a wide geographic scale, allowing the specific pollinator to recognize its host throughout its range, which can be quite large if the pollinator is a good disperser and often encounters host individuals a long distance from where it was born (Ahmed et al., 2009; Zavodna et al., 2005). The objective of the present study was to test whether intraspecific variation of scent exists in a highly specific plant-insect pollination interaction, the fig/fig wasp mutualism.

The genus *Ficus* (Moraceae) comprises about 800 species, most of them living in the intertropical region. The pollination of *Ficus* inflorescences (the fig or syconium) is carried out solely by agaonid wasps (Hymenoptera:Chalcidoidea:Agaonidae), which reproduce inside the figs. The relationship between *Ficus* and agaonid wasps is obligate for both partners and generally species-specific [one fig species is pollinated by one species of wasp [(Janzen, 1979; Wiebes, 1979); but see (Cook and Rasplus, 2003)]. In such a close-knit nursery pollination mutualism, the encounter between partners is a crucial step for the continuity of the life cycle of each partner. In tropical forests, where several *Ficus* species can live in sympatry, partner encounter is particularly problematic and requires specific chemical signals for each species pair. Several studies have shown that different species of *Ficus* emit clearly distinct chemical messages to attract only their specific pollinating wasp (Grison-Pigé et al., 2002b; Grison et al., 1999; Hossaert-McKey et al., 2010; Song et al., 2001). Behavioral evidence was also found for specific fig pollinator attraction to host volatiles (Chen et al., 2009; Grison-Pigé et al., 2002a; Hossaert-McKey et al., 1994; Proffit and Johnson, 2009; Song et al., 2001).

In general, variation in plant traits involved in pollinator attraction would be expected only when the most effective pollinators in each population exhibit divergent sensory preferences (Ellis and Johnson, 2009), even if some scent variation can be in part conditioned by phenotypic plasticity, in response to environmental variation on a large geographic scale, as shown in the family Brassicaceae (Majetic et al., 2009). In fig/fig wasp interactions, where (i) the plant is generally pollinated by only one pollinator species throughout its distribution, and (ii) scent is known to be responsible for the attraction of the obligate mutualistic partner, the interpopulation variation of the olfactory message might be limited (Hossaert-McKey et al., 2010). Indeed, a change in floral scents could disrupt partner recognition, leading to a drastic reduction in fitness of both partners. In this study, we examined the variation of floral scents emitted at receptivity in two *Ficus* species, one monoecious and one dioecious, and investigated variation among three Asian populations of each species. Our aim here is to test the hypothesis that, even on large geographical scales, the scent of both studied species is species-specific and invariant among populations. To our knowledge, this study is one of the rare ones (Svensson et al., 2005) to examine interpopulation variation in chemical signals in a pollination mutualism that is both obligatory and specific.

2. Results and discussion

A total of 114 different VOCs, including six unidentified compounds, were found in the bouquets emitted by receptive figs (i.e. those at the developmental stage ready to be pollinated) of these two species. The 108 identified VOCs belong to four different classes of compounds, including 25 monoterpenes, 59 sesquiterpenes, 10 benzenoids and 14 fatty acid derivatives. In the bouquets of *Ficus racemosa* individuals, we found 57 different VOCs, and in those of *Ficus hispida* 94 compounds (37 VOCs were common to both species; see major compounds for both species in Table 1). Most of these volatile compounds are quite common in floral scents (Knudsen et al., 2006). Only 51 VOCs had a mean relative proportion in the bouquet higher than 1% (respectively 10 VOCs for *F. racemosa* and 45 for *F. hispida*).

2.1. Interspecific variation in floral scents

To test if each species had a particular blend of VOCs allowing reproductive isolation of sympatric species in our highly specific system, we performed multivariate analyses on our dataset of relative abundance of each compound for each individual of the two species. The PCA (Principal Component Analysis) showed a separation of the scents emitted by receptive figs of the two species (Fig. 1a). This difference between the two species was confirmed by the MANOVA performed on the coordinates (two first components) of the PCA ($F_{(15;91)} = 11.42$; $p < 0.0001$. Furthermore, the species effect was highly significant in the MANOVA performed on the relative abundance dataset (Wilks's Lambda ["species effect"]: $F_{(19;17)} = 7.95$; $p < 0.0001$). Eleven different VOCs are responsible for this interspecific variation of scents (see Table 1). These results confirm previous studies showing that the chemical signals emitted by different *Ficus* species are sufficiently distinct to allow specific attraction of their own pollinating wasps (Chen et al., 2009; Grison-Pigé et al., 2002b; Grison et al., 1999; Proffit and Johnson, 2009). Indeed, in the case of sympatric species, the differences between species bouquets may be reinforced by selection pressure reducing the frequency of mistakes by the pollinating wasps and scents may act as a barrier to host shifts (Chen et al., 2009; Proffit et al., 2009). Moreover, our results are in agreement with previous studies on odour extracts of receptive figs of *F. hispida* (Proffit et al., 2008, 2009) where similar VOCs have been identified. However, our results differ from those presented in Song et al. (2001). That study used solvent extraction, a technique that recovers not only volatile compounds emitted by figs, but in addition chemicals present in the superficial cell layers of the plant. Our study, as well as those of Proffit et al. (2008, 2009) based on dynamic headspace extraction, reported only volatile compounds, which are potentially detectable by pollinators and other animals at distance.

2.2. Intraspecific variation in floral scents

In this very specialized pollination system, we examined whether the blends of VOCs emitted by receptive figs of these two *Ficus* species vary over a large geographic scale. We first performed MANOVAs on our global dataset on relative abundance of each compound in different populations of each species (VOCs > 1% for each species), testing for a population effect. There was significant variation among populations of a single species in scents emitted by receptive figs (Wilks' Lambda ["population within species effect"]: $F_{(76;69)} = 5.62$; $p < 0.0001$). Most of the compounds contributing to interpopulation differentiation are monoterpenes and sesquiterpenes (Tables 2 and 3). In fact, previous studies on fig/fig wasp interactions showed that volatiles from these two