Biochemical Systematics and Ecology 61 (2015) 44–53

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Pollination mode determines floral scent

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ARTICLE INFO

Article history:
Received 8 January 2015
Accepted 2 May 2015
Available online 1 June 2015

Keywords:
Entomophily
Anemophily
Floral emissions
Floral scent
VOC richness

ABSTRACT

The main objective of this study is to determine if the pollination vector influences the potential floral emissions of flowering plants. We hypothesized that flowers pollinated by insects would emit significantly higher amounts of volatile organic compounds (VOCs) and would present a higher diversity of these compounds than flowers pollinated by wind. The floral emissions of fifteen entomophilous species and eleven anemophilous species were captured by dynamic headspace sampling under field conditions and analyzed by gas chromatography–mass spectrometry. We searched for differences in the emission profiles between anemophilous and entomophilous flowers by considering the effects of phylogeny in our analysis. The floral emissions from the two groups were significantly different. Entomophilous species presented highly diverse emissions in both magnitude of emission rates and richness of compounds depending on the species, but overall, the flowers from entomophilous species had much higher VOC emission rates and VOC richness, both for terpenes and benzenoid compounds, than those from anemophilous species (two orders of magnitude higher emissions). The data thus confirm that the presence of intensely scented flowers with complex scents is strongly related to biotic pollination.

1. Introduction

Anemophilous plants entrust their pollen to the wind, which serves to deliver the pollen to the stigma and fertilize the ovules. Anemophily requires a large investment in the production of male flowers with abundant pollen to ensure the pollination of few female flowers (Friedman and Barrett, 2009). On the other hand, entomophilous plants rely on visiting insects to perform their pollination. These plants have lower investments in male flowers and pollen, but they generally have higher investments in the production of rewards and signals for attracting pollinators (Friedman and Barrett, 2009). The most common floral rewards are nectar and pollen (Simpson and Neff, 1981), but some species offer oils and other less common nutritive resources to the pollinators (Bittrich and Amaral, 1997; Capellari et al., 2012; Steiner et al., 2011). Floral signals from entomophilous flowers serve to attract the attention of pollinators. These signals can be visual, such as a perianth with brightly-colored pigmentation (Chwil and Weryszko-Chmielewska, 2009), or olfactory, such as the strong scents of flowers (Parachnowitsch et al., 2012). Pollinators can learn the floral odor of species that offer rewards and establish an association

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http://dx.doi.org/10.1016/j.bse.2015.05.007
0305-1978/

between the stimulus and the presence of these rewards (Riffell, 2011). Generalist pollinators use these specific blends of volatiles to find the flowers with the best rewards in the community, while specialist pollinators use them to find their host plants (Burger et al., 2010; Filella et al., 2011).

The reliance of plants on animal pollination has become a major driver of plant speciation and diversification of floral traits (Bronstein et al., 2006; Kay and Sargent, 2009; Whitney and Glover, 2007). Floral scents are considered to have evolved as attractants of pollinators and have diversified extensively with biotic pollination (Schiestl, 2010; Whitehead and Peakall, 2009). Floral bouquets of volatile compounds have been found to be under strong natural selection by pollinators (Parachnowitsch et al., 2012). The ability of plants to emit volatile organic compounds (VOCs) emerged early in the evolution of the plant kingdom (Chen et al., 2011; Fink et al., 2006; Kamenarska et al., 2002). Floral structures may have emitted VOCs before the need to attract pollinators appeared in angiosperms. Floral volatiles perform functions other than attraction, the most important of which is defense, which has the opposite effect on visitors to flowers (Galen et al., 2011; Junker and Blüthgen, 2010; Kessler et al., 2008; Schiestl, 2010). The attractive function of floral VOCs may have effectively appeared as a modification of pre-existent VOC emissions, such as defensive terpenes that deter detrimental organisms or emissions that protect plants against stressful environmental conditions (Pellmyr and Thien, 1986). The display of VOCs emitted by plants has coevolved with the sensory system of pollinators resulting in new species-specific floral bouquets directed to attract particular insect species (Farré-Armengol et al., 2013).

The absence of a need for communication with the pollinator in anemophilous plants, leads to our hypothesis: attractive VOCs are less diversified and less abundantly emitted in anemophilous than in entomophilous flowers.

Magalhães et al. (2005) and Wragg and Johnson (2011), measuring and comparing the emission of volatiles from flowers of diverse species from the same genus or family, found that the presence and abundance of floral volatiles depended mainly on the mode of pollination, i.e. by insects (entomophily) or by wind (anemophily). Raguso et al. (2007) found that emissions from flowers of different species of *Oenothera* were higher when flowers are pollinated by insects than by self-pollination. Doubleday et al. (2013) demonstrated that floral fragrance is dramatically lower in selfing compared to outcrossing populations of the species *Abronia umbellata*. Here we largely increase the range of species studied and analyze the floral emissions of several entomophilous and anemophilous Mediterranean species from diverse families to test our hypothesis while considering the effect of the phylogeny and thus to determine if the two modes of pollination have significant quantitative and qualitative differences in floral emissions. We thus aim to determine the importance of the mode of pollination on the amount and display of volatiles emitted by flowers.

2. Materials and methods

2.1. Scent sampling

Scent samples were captured by dynamic headspace sampling (Stashenko and Martínez, 2008) under field conditions. Flowers or inflorescences were enclosed in an oven bag (Nalophan, 20 cm × 30 cm), without separation from the plant. Air filtered through activated carbon was pumped into the bag via a Teflon entrance tube. Another Teflon tube collected the air exiting the bag, with one side of a T-tube connected to an adsorbent tube that collected the VOCs, followed by a flowmeter and a pump. The flux of air into the bag was always higher than the flux through the adsorbent tube to ensure that all the air from which we sampled the VOCs came from the bag. The influx was between 800 and 2000 mL min^{-1}, and the flow through the adsorbent tube was between 400 and 800 mL min^{-1}. The other side of the T-tube was open to release the excess air that did not pass through the adsorbent tube. Adsorbent tubes were filled with 114.6 mg of Tenax and 236.8 mg of Carbotrap adsorbents. Floral VOC samples were collected for five minutes. Blank samples with empty bags were collected to confirm the presence or absence of contaminating VOCs in the surrounding air and the sampling system. The filter of activated carbon used to clean the air introduced into the sampling bags did not generate air completely free of VOCs, so we collected additional controls to differentiate the environmental VOCs from those emitted by the samples. We also analyzed the air from clean unused tubes to identify possible contaminating compounds from the decomposition of tube adsorbents during thermal desorption or other contaminants from the system (Vercammen et al., 2000). When the sampling of floral scents was completed, flowers of each sample were cut and dried to obtain the dry weights of the emission sources and to calculate the emission rates relative to dry weight.

The samples were collected during 2012 from different locations in central Catalonia. The criteria used to choose the species were basically two. First, we decided to select species that belong to different plant families to have a more diverse and representative sample for each pollination mode. Second, we chose species that flowered successively along the year. Five samples and one or more blank controls were collected for each species. Replicates of each species were taken on different individual plants on the same day and location. Sampling was conducted under field conditions on sunny days. Eleven anemophilous species were sampled: *Acer negundo* L., *Alnus glutinosa* L., *Coriaria myrtifolia* L., *Corylus avellana* L., *Fraxinus angustifolia* Vahl, *Olea europaea* L., *Pistacia lentiscus* L., *Populus nigra* L., *Quercus pubescens* Willd., *Ulmus minor* Mill., and *Vitis vinifera* L. Fifteen entomophilous species were sampled: *Calendula arvensis* L., *Diplotaxis erucoides* DC., *Euphorbia characias* L., *Helichrysum stoechas* L., *Lepidium draba* L., *Ligustrum japonicum* Thunb *Prunus dulcis* Mill., *Rhamnus alaternus* L., *Salvia verbenaca* L., *Sambucus nigra* L., *Syringa vulgaris* L., *Thymus vulgaris* L., *Tilia platyphyllos* Scop., *Viburnum lantana* L., and *Viburnum tinus* L.