

RAPID COMMUNICATION

FLORAL ODOR VARIATION
IN TWO HETEROSTYLOUS SPECIES OF *Primula*

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Abstract—Floral traits such as odor, color, and morphology are important pollinator attractants. Variation in floral traits may influence floral constancy, the tendency of pollinating insects to visit flowers of only one type. We investigated for the first time variation in odor between floral morphs in heterostylous species. We analyzed inter- and intraspecific odor variation in the “pin” and “thrum” floral morphs of sympatric *Primula elatior* and *P. farinosa* (Primulaceae). Floral volatiles were sampled with headspace sorption. Quantitative analysis and chemical identification were performed by gas chromatography coupled to mass spectrometry. The species produced different floral bouquets. *P. elatior* emitted mostly limonene with small amounts of α -pinene, myrcene, and sabinene. *P. farinosa* produced benzaldehyde, 4-oxoisophorone (2,6,6-trimethyl-2-cyclohexene-1,4-dione), benzyl alcohol, and benzyl acetate. These interspecific differences may play a role in promoting floral constancy and maintaining species integrity. Conversely, no differences were detected between the scents of pin and thrum morphs within each species. Heterostyly relies on pollinators visiting both floral morphs. There may be stabilizing selection against divergences in traits that may cause pollinators to develop floral constancy to only one of the floral morphs.

Key Words—Floral odor, heterostyly, *Primula*, pollination, headspace sorption, gas chromatography-mass spectrometry.

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INTRODUCTION

Floral traits such as odor, color, and shape are important pollinator attractants and have evolved with the behavior, morphology, and physiology of pollinating animals (Chittka et al., 1999). An intriguing aspect of pollinator behavior is floral constancy, the tendency of an insect to visit flowers of only one type while ignoring other rewarding flowers. Taxa exhibiting floral constancy include honeybees (*Apis mellifera*), bumblebees (*Bombus* spp.) and solitary bees (*Trichocolletes* spp.), moths and butterflies (Lepidoptera), beetles (Coleoptera), and flies (Diptera; Chittka et al., 1999 and references therein). Pollinators use floral traits to discriminate among different flowers. Thus, there may be strong selection upon floral odors, colors, and shapes that encourage constancy if they increase pollen transfer efficiency and/or reproductive success.

Many plant species have variable floral traits and distinct morphs within a population. Heterostyly is a genetic polymorphism in which plant populations comprise two (distyly) or three (tristyly) mating types that differ in the reciprocally arranged female and male reproductive organs (Barrett, 2002). Distylous species have two floral morphs, 'pin' (long style and low anthers) and 'thrum' (short style and high anthers) (Barrett, 2002). Heterostyly occurs in 155 angiosperm genera, including *Primula* (Primulaceae). There are approximately 425 species of *Primula*, 91% of which are distylous (Richards, 2003).

In heterostylous species, pollen is efficiently transferred from the anthers of one plant to the stigma of another plant by its precise placement on the body of the pollinator (Barrett, 2002). Furthermore, self-fertilization is generally prevented by a self-incompatibility system that inhibits germination of pollen from the same floral morph. Successful pollen exchange requires a pollinator to visit both pin and thrum floral morphs during a foraging bout. Therefore, in heterostylous species, fertilization success relies on pollinators failing to distinguish between pin and thrum morphs of a species. Consequently, within a population, pin and thrum morphs are unlikely to differ in traits that may influence floral constancy. Between species, however, significant differences in floral traits are likely to be selected for to reduce pollen wastage and hybridization. Here, we test for low intraspecific and high interspecific variation in floral traits by considering variation in floral odor between floral morphs, and between species, in sympatric populations of heterostylous *Primula elatior* (L.) Hill and *P. farinosa* L. (Primulaceae).

METHODS AND MATERIALS

Floral odor samples were collected from 30 plants of *P. elatior* (15 pin, 15 thrum), 39 plants of *P. farinosa* (23 pin, 16 thrum), and 10 from ambient air

at Klausenpass (1950 m), Switzerland, during July 2004. The odors were collected by headspace sorption by isolating the inflorescence in a polyethylene bag. Air was drawn from the bag and through a filter attached to a pump at ~100 ml/min, for 4 hr. Filters consisted of 5 mg of Porapak Q absorbent material sealed in glass capillaries. Before each use, filters were cleaned with 200 μ l of dichloromethane. Filters were eluted with 50 μ l of a 9:1 solution of hexane and acetone.

Gas Chromatography. Before analysis, 100 ng of octadecane (Fluka, purity 99.8%) were added as an internal standard to all samples. A 1.5- μ l portion of each odor sample was injected splitless into a gas chromatograph (GC) (Agilent 6890N) at 40°C (3 min), followed by opening the split valve and programming to 150°C at a rate of 2.5°C/min, and then 250°C at a rate of 10°C/min. The GC was equipped with an HP-Innowax column [30 m \times 0.32 mm (diam) \times 0.25 μ m (film thickness)] and a flame ionization detector (FID); helium was the carrier gas. Compounds were identified by analyzing samples on a GC coupled to mass spectrometry (MS) and comparing spectra and retention times with reference compounds.

Statistical Analyses. Statistics were calculated by using relative amounts of floral odor compounds. As data were not normally distributed and resisted transformation, nonparametric Mann–Whitney *U*-tests were used to compare the relative amounts of each compound between pin and thrum plants. There was no difference in the compounds produced by pin and thrum plants within each species (Mann–Whitney *U*-tests, all *P* values > 0.5), so the pin and thrum data were pooled for each species. Between-species comparisons were then conducted, also with Mann–Whitney *U*-tests.

RESULTS AND DISCUSSION

In our study populations, *P. elatior* and *P. farinosa* had relatively simple bouquets composed of common floral odors (Table 1, cf. Gregg, 1983; Knudsen et al., 1993; Knudsen, 2002). The two species produced qualitatively different bouquets. *P. elatior* emitted primarily limonene with small amounts of three other monoterpenes: myrcene, α -pinene, and sabinene (Table 1). *P. farinosa* produced benzaldehyde, 4-oxoisophorone (2,6,6-trimethyl-2-cyclohexene-1,4-dione), benzyl alcohol, and benzyl acetate (Table 1).

Some plant individuals overlapped in scent between the species: five of the 30 *P. elatior* samples contained some benzaldehyde, and two of the 23 *P. farinosa* samples contained a small amount of limonene (Table 1). This overlap may be a part of the natural variation in each species rather than hybridization, since *P. elatior* and *P. farinosa* are classified in different sections

TABLE 1. MEAN (\pm SEM) RELATIVE AMOUNTS OF VOLATILE COMPOUNDS IDENTIFIED FROM 'PIN' AND 'THRUM' FLORAL MORPHS OF *Primula elatior* AND *Primula farinosa* (PRIMULACEAE)

| Volatile compounds | <i>Primula elatior</i> | | <i>Primula farinosa</i> | | <i>U</i> (<i>P</i>) |
|--------------------|-------------------------|---------------------------|-------------------------|---------------------------|-----------------------|
| | Pin (<i>n</i> = 15) | Thrum (<i>n</i> = 15) | Pin (<i>n</i> = 16) | Thrum (<i>n</i> = 23) | |
| α -Pinene | 0.75 \pm 0.22 | 0.74 \pm 0.22 | 0 | 0 | 320 (<0.001) |
| Sabinene | 2.71 \pm 0.44 | 2.05 \pm 0.45 | 0 | 0 | 180 (<0.001) |
| Myrcene | 2.01 \pm 0.50 | 1.99 \pm 0.50 | 0 | 0 | 280 (<0.001) |
| Limonene | 93.9 \pm 1.13 | 94.1 \pm 1.34 | 0.10 \pm 0.10 | 0.31 \pm 0.30 | 0.0 (<0.001) |
| Benzaldehyde | 0.63 \pm 0.44 | 1.10 \pm 0.72 | 34.5 \pm 2.46 | 36.4 \pm 2.75 | 0.0 (<0.001) |
| 4-Oxoisophorone | 0 | 0 | 27.5 \pm 2.28 | 26.6 \pm 1.77 | 0.0 (<0.001) |
| Benzyl alcohol | 0 | 0 | 29.5 \pm 2.66 | 28.4 \pm 2.31 | 15 (<0.001) |
| Benzyl acetate | 0 | 0 | 8.41 \pm 1.08 | 8.33 \pm 1.00 | 60 (<0.001) |

n = Number of plants sampled. Statistics in final column compare the two species using data pooled from pin and thrum plants. Test statistic *U* and *P* values are derived from Mann–Whitney *U*-tests.

(*Primula* and *Aleuritia*, respectively), and no hybrids between these two species have been reported (Richards, 2003). The quantitative differences in the odor bouquets between the species were also highly significant for all compounds (Table 1).

The different odors emitted by *P. elatior* and *P. farinosa* may attract different pollinators. Limonene and the other monoterpenes emitted by *P. elatior* are produced by many plants with diverse pollinating taxa including bees, Lepidoptera, beetles, and fly species (e.g., Knudsen et al., 1993; Knudsen, 2002 and references therein). The few published observations of visitors to *P. elatior* suggest pollinators include species of bumblebees, Lepidoptera, beetles, and thrips (Schou, 1983). The compounds emitted by *P. farinosa* are often found in Lepidoptera-pollinated flowers (Andersson et al., 2002). Butterflies and day-flying moths reportedly visit *P. farinosa*, but Diptera (Syrphidae, Bombyliidae), bees (*Osmia* spp.), and bumblebees have also been observed (Hamblen and Dixon, 2003). Some insect taxa may be attracted to both *Primula* species.

The insects pollinating *Primula* spp. at our study site have not yet been identified, nor has the specific role of floral odor in pollinator attraction been investigated in any *Primula* species. However, odor is a principal pollinator attractant in a wide range of flowering plants and is a major factor promoting floral constancy (Knudsen et al., 1993; Chittka et al., 1999 and references therein). Both *P. elatior* and *P. farinosa* are largely self-sterile and rely heavily on pollinators for fertilization (Richards, 2003).

Even if some pollinator species are shared, cross-pollination between the two *Primula* species seems improbable. Individual insects are unlikely to visit

both *Primula* species during a foraging bout, since the striking quantitative and qualitative differences in the floral bouquets should promote floral constancy to only one of the species. Even minor qualitative differences in floral scent can affect pollinator behavior. For example, trained moths can distinguish between flowers differing by a single volatile compound (Cunningham et al., 2004). Thus, scent differences could contribute to the maintenance of species integrity, and/or provide a selective advantage, e.g., by reducing pollen wastage or clogging of the stigmas with heterospecific pollen.

Intriguingly, we found no intraspecific variation in scent between the floral morphs in either *Primula* species. Even the relative amounts of each compound produced by the pin and thrum morphs were remarkably similar (Mann–Whitney *U*-tests: *P. elatior* all *P* values > 0.74, *P. farinosa* all *P* values > 0.58). Similarly, low intraspecific scent variation was detected in two other species: *Cycnoches densiflorum* (Orchidaceae; Gregg, 1983) and *Geonoma macrostachys* (Arecaceae; Gregg, 1983; Knudsen, 2002). As in heterostylous primulas, both *C. densiflorum* and *G. macrostachys* require pollinators to visit two floral morphs for successful fertilization. Thus, in these four species, stabilizing selection might operate against any divergence in scent that would cause pollinators to develop floral constancy to only one of the morphs.

Chittka et al. (1999) argue that floral constancy is rarely strong enough to isolate floral morphs or maintain species integrity. If this is so, some variation in traits between morphs or species may have little impact on pollinator behavior. However, if there are additional factors that limit which individual plants can exchange pollen for successful reproduction (for example, self-incompatibility in heterostylous primulas), then low scent variability that promotes constancy to both floral morphs may provide a significant reproductive advantage.

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