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THE SCENT OF A MALE: THE ROLE OF FLORAL VOLATILES IN POLLINATION OF A GENDER DIMORPHIC PLANT

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Abstract. Most flowering plants rely on animal pollinators to transfer male gametes between individuals, and thus a significant problem for gender dimorphic plants is that pollinators often avoid female flowers. Here we show for the first time that one important reason pollinators shun female flowers is because they do not smell like males. We compared emission rates and floral scent composition in a gynodioecious wild strawberry (*Fragaria virginiana*) where females receive half as many visits by generalist pollinators as conspecific hermaphrodites. We used floral extracts to determine the source of sexually dimorphic odor and pollinator responses. Specifically, we used extracts of whole flowers and specific floral parts in choice tests to determine that pollinators preferred the scent of hermaphrodite flowers over those of females and that this discrimination was due primarily to the scent of hermaphrodite anthers. These data conclusively show that scent can be a major driver of pollinator behavior in gender dimorphic plants. Our results also indicate that scent is an important modulator of pollinator behavior even in a small flowered, weakly scented species visited by generalist pollinators, and not just peculiar to intensely scented, deceptive, or specialized pollination systems.

Key words: *dioecy; floral volatiles;* Fragaria virginiana; *fragrance; gynodioecy; pollen; pollen scent; 2-phenylethanol.*

INTRODUCTION

Inbreeding avoidance and resource reallocation favor the evolution of gender dimorphism (i.e., females and males or hermaphrodites) in flowering plants (reviewed in Charlesworth 1999), yet females, which produce no functional male organs, often suffer inadequate visitation and pollen limitation of seed set (e.g., Widen and Widen 1990, Ashman 2000, Williams et al. 2000, Ashman and Diefenderfer 2001). Consequently, pollinator preferences are believed to hinder the evolution of (Charlesworth 1993) or contribute to the extinction of (Vamosi and Otto 2002) plants with separate sexes (dioecy), and may be responsible for evolutionary transitions to wind pollination (reviewed in Culley et al. 2002). Understanding the basis of pollinator behavior on gender dimorphic plants is an important aspect of understanding the abundance, distribution, and ecological correlates of dioecy, and related sexual systems, such as gynodioecy (hermaphrodites and females).

Several authors have suggested that reductions in visual floral display (petal size and flower number) or rewards (pollen and nectar) are responsible for females' lower attractiveness to pollinators relative to male-fertile conspecifics (i.e., males or hermaphrodites; Bell 1985, Delph and Lively 1992, Ashman et al. 2000). However, the only study to evaluate the characters responsible for sex-differential visitation found that visual cues or reward status could not fully explain the male-bias in pollinator service (Ashman et al. 2000), leaving us with an incomplete understanding of the factors contributing to this visitation pattern.

One axis of floral attraction that has received little attention is the potential for olfactory cues to mediate sex-biased pollinator visitation patterns. This may be because dioecy is not generally associated with the types of flowers for which scent is thought to be a major modulator of pollinator activity (i.e., large, heavily scented flowers pollinated by specialists; Feinsinger 1983, Schatz 1990). However, plant volatile emissions mediate a variety of plant–animal interactions (Dobson 1989, Dobson and Bergstrom 2000), and the role of floral volatiles as pollinator attractants is known for several pollinator classes (reviewed in Dobson 1991, Raguso 2001). Given the differences in total floral investment (reviewed in Eckhart 1999) and in allocation to floral organs (e.g., Ashman 1994, Jones and Burd 2001), sex morphs of gender dimorphic plants are likely to differ in floral scent because emissions can be biomass dependent and the compounds emitted can vary among the floral organs (Dobson and Bergstrom 2000). Despite an often high degree of similarity in scent profile between sex morphs, differences are almost always found and they can sometimes be substantial (e.g.,Tollsten and Knudsen 1992, Knudsen and

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Tollsten 1993, Ervik et al. 1999, Knudsen et al. 1999, Grison-Pige et al. 2001). However, we do not yet know if this variation is functionally significant because only a rare study has determined the source of sex differences (see Dobson and Bergstrom 2000), and none have determined if they cause differential pollinator attraction.

In this study we predicted that female flowers would have different scent profiles and/or emission rates than hermaphrodite flowers because they are smaller, allocate differently to floral organs, and do not produce pollen-bearing anthers. We tested these ideas in a gynodioecious wild strawberry (*Fragaria virginiana*) where females receive half as many visits by generalist pollinators as conspecific hermaphrodites (Ashman 2000). In particular, we sought to answer the following questions: (1) Do females emit lower amounts of floral (or foliage) volatiles than hermaphrodites? (2) Do the sex morphs differ in floral scent composition, and specifically, do females lack a ''male'' scent, due to the absence of functional anthers/pollen? (3) Do females receive fewer pollinator visits because they lack a male-specific volatile(s)?

Methods

Fragaria virginiana (Rosaceae), the Virginian wild strawberry, is a gynodioecious perennial herb that is native to eastern North America (sensu Staudt 1989). It flowers from late April until mid-June. Both sex morphs produce about 10 white flowers per inflorescence (Ashman and Hitchens 2000) and each flower supports a fleshy receptacle with numerous uni-ovulate carpels (Staudt 1989). The perfect flowers of hermaphrodites contain stamens with yellow anthers borne on long filaments, whereas the pistillate flowers of females contain staminodes with white anthers (devoid of pollen) borne on short vestigial filaments. Flowers of hermaphrodites have petals that are ~50% larger and produce 50% more nectar than those of females (Ashman 2000). *Fragaria virginiana* flowers are visited mostly (>80%) by small generalist bees (including several species of Apidae, Halictidae, Andrenidae, and Megachilidae), but also by flies (including species of Bombyliidae and Syrphidae) and ants (including species of Formicinae and Dolichoderinae; Ashman 2000, Ashman and King 2005). Bees are the most effective pollinators (T.-L. Ashman, *unpublished data*), and show a consistent preference for hermaphrodite flowers (Ashman 2000), but have been seen to move between female and hermaphrodite flowers when foraging for pollen and nectar.

Plant culture

Flowers and inflorescences used for both floral scent analysis and extract production were propagated in the greenhouse from plants originally collected from a northwestern Pennsylvania population (PR: see Ashman 1999). Plants were grown in 13-cm square pots and overwintered at the Pymatuning Laboratory of Ecology, Crawford County, Pennsylvania (PLE), or in the greenhouse at the University of South Carolina, Columbia, Richland County, South Carolina.

Floral scent collection

Complementary methods were used to collect flower fragrance. The first, very sensitive, method—static headspace—is ideal for qualitative identification of the chemical composition of odor blends, but is inappropriate for quantitative comparisons. It involves the equilibration of odors within a small volume of ''headspace'' air surrounding floral tissues. Nylon resin oven bags (Reynolds, Richmond, Virginia, USA) were used to concentrate floral headspace, within which solid phase micro-extraction (SPME) fibers were exposed to fragrant air. We used SPME fibers coated with polydimethylsiloxane (PDMS, 100-μm film thickness) or PDMS/divinylbenzene (DVB, 65 μm), which sensitively trap and identify a broad range of volatile plant compounds (Agelopoulos and Pickett 1998). Individual flowers, buds, or flower parts (0.09–0.25 g) were carefully excised and sealed within 5 × 5 cm bags to minimize headspace volume, using an impulse heat sealer (American International Electric, Whittier, California, USA). After 30 min equilibration, SPME fibers were exposed within headspace for 30 min before analysis; results did not improve with additional equilibration or exposure time (data not shown). In other experiments, intact inflorescences were enclosed within 12 × 9 cm bags cinched mid-bag with plastic ties for SPME scent trapping. In some cases, inflorescences were manipulated by selectively removing flowers or buds at least one hour before SPME exposure. Ambient contaminants and wounding artifacts were identified through control collections and these were omitted from all subsequent floral analyses.

The second method—dynamic headspace—is suitable for measuring odor emission rates, as headspace air is sampled with replacement. Scent is collected from intact, bagged inflorescences as it is emitted, by sweeping headspace air through an adsorbent polymer trap over a given time (Raguso and Pellmyr 1998). To measure emissions from live flowers, plants were moved from a greenhouse to a growth chamber (818 incubator, Precision Scientific, Chicago, Illinois, USA) at 24°C for 10:00 hours and watered. One hour later, inflorescences (or leaves) were placed within 9 × 12 cm bags and cinched mid-bag. Two slits were cut into the bags, one to admit ambient air, the other to hold scent traps constructed from cut Pasteur pipettes packed with 10 mg of SuperQ adsorbent (80/100 mesh size, DVB/ethylvinylbenzene polymer; Alltech Associates, Deerfield, Illinois, USA) between two plugs of silanized quartz wool. Scented air was drawn through scent traps using Personal Air Sampler-500 vacuum pumps (Supelco, Bellefonte, Pennsylvania, USA) at 200 mL air/min, for 4 h. Trapped scent compounds were

eluted with 300 μL of hexane, concentrated to 75 μL with gaseous N_2, and supplemented with 16 ng of toluene as an internal standard. Eluted samples were stored in Teflon-capped glass vials at −20°C until they could be analyzed by gas chromatography–mass spectrometry (GC–MS).

To evaluate the absolute and relative similarity of emission rates from experimental ''emitters'' used in behavioral assays (see *Methods: Pentane extractions* and *Methods: Pollinator behavioral assays*) to emission rates from live flowers, bundles of five emitters (each loaded with 0.5 mL of extract in mineral oil) were mounted on florists' Styrofoam blocks and enclosed within headspace bags (100 mL volume). Bags were suspended above the emitters using aluminum wire halos, to prevent wicking of extracts onto the bags' surfaces. Headspace odors were collected, eluted, and concentrated as with volatiles from live tissue.

Pentane extractions

We prepared extracts of whole flowers and floral organs using pentane. For each extract fresh flowers from hermaphrodite and female plants were collected and used whole or separated into petals and anthers. Anthers from hermaphrodites were allowed to dehisce at room temperature for 1–2 h prior to extraction. ''Anthers'' of female staminodes are vestigial and do not produce pollen. Pentane was added to floral tissues at ~2.5 mL pentane/1.0 g biomass of floral organs (1.5 mL/g for whole flowers) and floral compounds were extracted at room temperature for ~3 h. Extracts were used in either quantitative or behavioral assays. For quantitative analysis extracts were filtered with quartz wool to remove waxy substances and concentrated to 100 μL volumes for GC–MS analysis. For behavioral assays, extracts were suspended and diluted in mineral oil to achieve 500-μL aliquots equal to the extract from 10 flowers (hereafter, ''flower equivalents'') and these were used in emitters.

GC–MS Analysis

Either SPME fibers or 1-μL aliquots of solvent-trapped odor samples were injected into a Shimadzu GC-17A gas chromatograph with a Shimadzu QP5000 quadrupole-electron impact mass spectrometer (MS; Shimadzu Scientific Instruments Columbia, Maryland, USA) as a detector. Scent components were separated on polar (EC wax) and nonpolar (EC5; Alltech Associates, Deerfield, Illinois, USA) GC columns as described by Raguso et al. (2003). Putative compound identification via comparison with mass spectral libraries (Wiley and NIST libraries [>120 000 mass spectra]) was verified through co-injection of known standards. GC peak areas were integrated using Shimadzu's Class-5000 software and expressed as a relative percentage of all summed peak areas. For solvent-eluted samples, peak areas were quantified by comparison with the internal standard and expressed as nanograms scent per flower per hour and dry biomass per hour. For pentane extractions and emitter calibration, key compounds (e.g., 2-phenylethanol) were quantified by fitting peak areas to dose-response curves of external standards analyzed using GC–MS. Response factors were log-linear throughout the range of concentrations observed in this study.

Scent data analysis

To address whether female flowers or leaves are less strongly scented than those of hermaphrodites, we calculated total volatile emission rates using dynamic headspace data collected over 4–4.5 h from living inflorescences and foliage ($N = 5$ per gender) and expressed them as nanograms scent per flower (or per leaf) per hour and dry biomass per hour to control for the smaller size of female flowers (Ashman 2000). Mean emission rate data were $\log(x + 1)$-transformed and analyzed using unpaired *t* tests, in a one-tailed test of the null hypothesis that female plants were less scented than hermaphrodites.

To address whether floral scent composition differs between female and hermaphroditic plants, we first compared overall variation in the chemical composition of fragrances within vs. between five replicates of each gender. For each replicate, floral scent was collected from large inflorescences (21.9 ± 1.1 flowers, 8.8 ± 1.3 buds, mean ± SE) cut from ramets of the same genotype and placed into florists' water pics, using SPME as described previously to maximize odor signal. For each sample, we calculated the relative amount of each compound as a proportion of total scent, standardized each to a mean of 0 and variance of 1 (i.e., *Z* scores) and calculated a dissimilarity matrix based on Euclidean distances (SPSS ''Correlate-Dissimilarity'' command; SPSS 2003). The Wilcoxon rank sum test (SPSS 2003) was used to perform a one-tailed test of the null hypothesis that variance between genders is not greater than variance within genders (see Levin et al. [2001] for details of and justification for this approach). Rejection of this hypothesis would provide evidence for sexual dimorphism in overall scent composition.

Second, to determine if females lack a ''male'' scent due to the absence of anthers with pollen, we verified exploratory analyses, which indicated that two aromatic compounds, benzyl alcohol and 2-phenylethanol, were consistently more abundant in hermaphrodite flowers, the latter compound specifically associated with stamens. To verify this, we collected odor from 120 flower equivalents of anthers ($N = 6$) placed within 4-mL glass vials as headspace chambers using SPME as described previously. We then compared the amount of each compound (nanogram scent per flower) present in pentane extracts of hermaphrodite vs. female flowers ($N = 5, 5$), petals ($N = 2, 3$), and anthers from stamens (staminodes, $N = 4, 3$), using paired *t* tests of log-

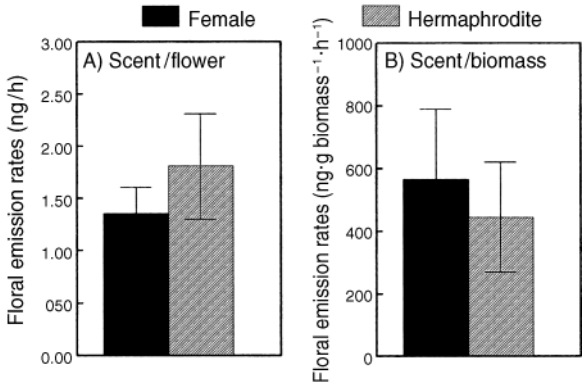

FIG. 1. Floral emission rates (mean ± SE) from hermaphrodite and female *Fragaria virginiana* flowers on the basis of (A) flowers or (B) grams dry floral biomass. Emission rate is significantly lower in females than in hermaphrodites on a per-flower basis ($P < 0.05$), but equivalent on a per-biomass basis ($P > 0.3$). Note the difference in *Y*-axis scale between panels. $N = 5$ flowers per gender.

transformed data, quantified through the use of external standards.

Pollinator behavioral assays

To assess pollinator response to floral odor we used female flowers as visual targets and enhanced their odor with floral extracts. Two types of arrays were used and each consisted of three treatments (a ''triplet''): (1) whole flower extracts from females or hermaphrodites plus a pentane control, and (2) extracts from hermaphrodite petals or anthers plus a pentane control. Flower targets of each triplet were positioned linearly ~20 cm apart. Flower targets consisted of a florist's ''aqua pic'' with a female inflorescence trimmed to two open flowers, and each had a volatile emitter positioned behind it. Emitters were fashioned from 500-μL green microcentrifuge tubes, embroidery thread (used as wicks), and green florist sticks. Each triplet was observed for up to 90 min. During an observation period the three flower targets were rotated among the three emitter treatments, and the emitters were rotated among the three positions such that every target–emitter–location combination was observed for 10 min/replicate. Fresh flowers and solvents were used for each replicate.

Pollinator observations were conducted on sunny days between 27 May and 2 June 2002 at PLE. A total of 33 hours of observations were conducted, and the vast majority (>95%) of pollinators visiting the arrays were small native bees (Ashman 2000). We recorded pollinator approaches to and landings on the target flowers. Bees approached emitters and occasionally landed, and we included these data in the analyses. Thus, visitation was analyzed as visits per target per 10 min of observation, but for presentation purposes we converted the data to the more conventional measure of ''visits per flower per minute.'' Visitation data were analyzed by two-way ANOVA with treatment (scent enhancement) and replicate (block) as fixed factors. When the treatment-by-replicate interaction was not significant, it was removed from the ANOVA.

RESULTS

Floral scent analysis: do females emit lower amounts of floral or foliage volatiles than hermaphrodites?

Dynamic headspace analyses revealed that emission rates of total floral scent were significantly lower in females than hermaphrodites on a per-flower basis ($t_{1,6} = 3.03$, $P < 0.05$; Fig. 1A). When gender-based differences in flower size were standardized, floral emissions per gram dry biomass were comparable between genders ($t_{1,6} = 0.95$, $P > 0.3$; Fig. 1B). In addition, the foliage of female plants was not significantly less scented than that of hermaphrodites at either per leaf (H vs. F: 3.82 ± 1.39 vs. 0.89 ± 0.48 ng scent·leaf^{-1}·h^{-1}; $t_{1,6} = 2.07$, $P_{1\text{-tail}} = 0.06$) or per gram dry biomass (H vs. F: 57.74 ± 21.86 vs. 10.78 + 4.08 ng scent·g dry mass^{-1}·h^{-1}; $t_{1,6} = 2.11$, $P_{1\text{-tail}} = 0.06$).

Floral scent analysis: do the sex morphs differ in floral scent composition, and specifically, do females lack a ''male'' scent?

We identified 38 scent compounds through 73 SPME analyses of female and hermaphrodite flowers, buds, and flower parts (Appendix A). Floral scent was composed of 16 monoterpene hydrocarbons and alcohols,

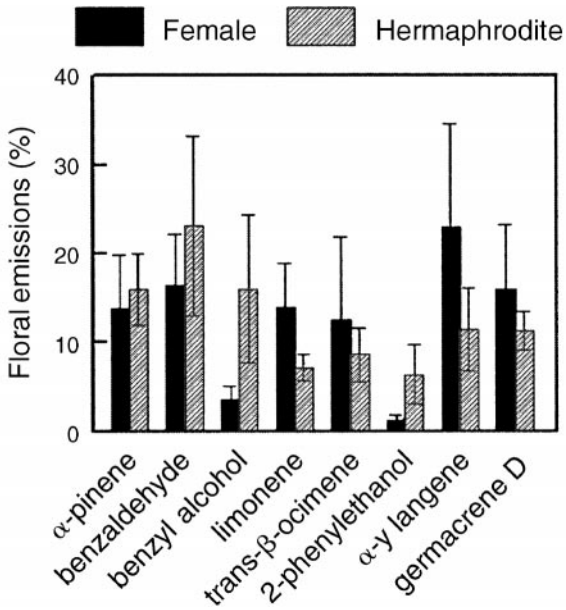

FIG. 2. Scent composition of *Fragaria virginiana* flowers from dynamic head space analyses. Percentage composition (mean ± SE) for the eight most abundant floral compounds detectable using this method is shown. There is no overall difference between the sex morphs (see *Results: Floral scent analysis: do the sex morphs differ*. . .). $N = 5$ flowers per gender.

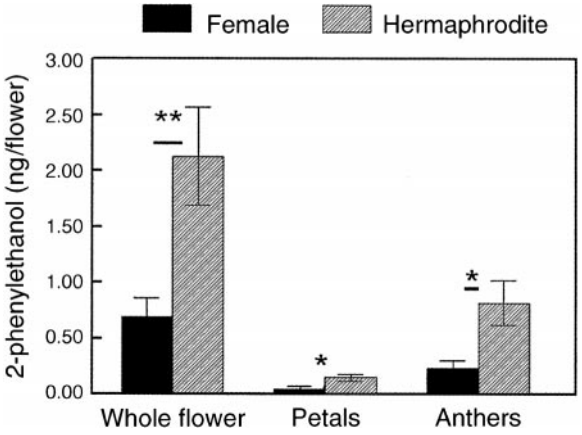

FIG. 3. Amount of 2-phenylethanol (mean ± SE) extracted from whole flowers, petals, or anthers (from stamens [H] or staminodes [F]) of female and hermaphrodite *Fragaria virginiana*. Significance as determined by one-tailed *t* tests is as follows: * $P < 0.05$; ** $P < 0.01$. Tests significant after Bonferroni correction are indicated by a bar under the asterisk(s). $N_{(F,H)} = 5, 5$ for flowers; 3, 2 for petals; 3, 4 for anthers.

eight sesquiterpene hydrocarbons, one 11-carbon sesquiterpenoid derivative (trans-4,8-dimethyl-nona-1,3,7-triene), and six benzenoid compounds. The sex morphs did not differ in overall scent composition as determined by SPME: mean rank differences between genders (26.06) were not significantly greater than those within genders (27.20; $U_{0.05} = 306$, df = 20,32, $P_{1\text{-tail}} = 0.40$). However, one compound (2-phenylethanol; Appendix A) was detected only in trace amounts (<0.01%) in female flowers, and further SPME analyses of dissected anthers confirmed this compound to be emitted from hermaphrodite anthers (Appendix A). Estimates of Euclidean pairwise distances from dynamic headspace data (Fig. 2) also confirmed these patterns for the eight most-abundant volatiles, i.e., there were no significant overall between-sex differences (between-gender mean rank = 18.85, within-gender = 18.06; $W_{0.05} = 289$, df = 20,36, $P_{1\text{-tail}} = 0.42$), but 2-phenylethanol was significantly more abundant in hermaphrodites than in female flowers ($t_{1,4} = 2.792$, $P_{1\text{-tail}} = 0.016$).

To further evaluate the production/emission of the putative anther-specific compound, 2-phenylethanol, we quantified the amount of 2-phenylethanol in the pentane extracts of whole flowers, petals, and anthers of both genders (Appendix B). More 2-phenylethanol was extracted from hermaphrodite flowers, petals, and anthers than from homologous structures of females (Fig. 3), and these gender differences for whole flowers and anthers remained significant even after Bonferroni correction for multiple tests. The only other compound to potentially show differences between the sexes was benzyl alcohol (Fig. 2); however, it did not differ significantly in the extracts of the anthers of the genders ($P_{1\text{-tail}} = 0.15$; Appendix B) and was not detected in flower part-specific SPME assays.

Pollinator behavioral assays: do females receive fewer pollinator visits because they lack a male specific volatile?

To determine if pollinators respond to floral scent and if they respond differently to the scent of female or hermaphrodite flowers, we compared pollinator approaches to and landings on female flowers enhanced with whole flower extracts of females, hermaphrodites, and pentane controls. We found that pollinators approached female flowers enhanced with extract of hermaphrodite whole flowers 50% more often than those scented with female whole-flower extract or with pentane only ($F_{2,224} = 7.7$, $P < 0.001$; Fig. 4A). These data suggest that pollinators respond to flower scent and prefer the scent of hermaphroditic flowers. This could result from hermaphrodites' greater scent production per flower or be due to the production of unique compounds. To evaluate these possibilities, we first compared emission from emitters with female extract to those with hermaphrodite extract. In the laboratory, we found that emission rates from emitters were nearly fourfold higher than those from live flowers (live flower data in Fig. 1A), but that the relative difference in emission between the sex morphs from extracts (H, 7.1 ± 2.4; F, 4.6 ± 1.7 ng·flower^{-1}·h^{-1}; $t_{1,4} = 2.30$, $P_{1\text{-tail}} = 0.074$) was comparable to that between live flowers (F/H: 0.76 vs. 0.64, respectively). Second, we separated hermaphrodite flowers into the two primary scent producing organs, petals and anthers, and enhanced female flowers with extracts from these. We found hermaphrodites' anther odor, but not their petal odor, significantly enhanced approaches to female flowers ($F_{2,239}$

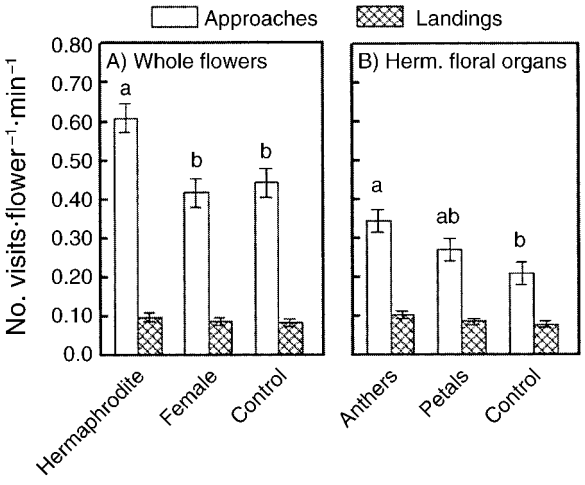

FIG. 4. The effect of scent augmentation on bee visitation to female flowers of *Fragaria virginiana*. Female flowers were augmented with (A) whole-flower extracts or (B) extracts from hermaphrodite floral organs, and visitation was compared to controls (pentane in mineral oil). $N = 234$ for floral extracts; $N = 252$ for floral organs.

$= 7.6$, $P < 0.005$; Fig. 4B). Taken together, these data suggest that the discrimination between female and hermaphrodite whole flower scent (Fig. 4A) may be due more to the lack of pollen-bearing anthers in female flowers rather than to differences in petal (or total) scent production.

While data on approaches suggest pollinators are attracted at short distances to the scent enhanced female targets, they do not reflect a commitment to forage. In fact, landings by pollinators were rare and not significantly affected by odor enhancement in any of the trials (all $P \geq 0.20$; Fig. 4A, B), suggesting that at very close range pollinators were not receiving the visual cues (the presence of pollen, perhaps) they needed to land. However, by including an additional five hours of pollinator observations and concentrating on the comparison between anther extract and pentane control, we found that landings are significantly enhanced by anther extract (0.122 ± 0.0078 vs. 0.088 ± 0.0077 landings·flower^{-1}·min^{-1}, mean $\pm$ SE; $F_{1, 315} = 11.3$; $P < 0.001$), indicating that anther scent alone can be a strong enough cue to get a portion of the pollinators to land.

Discussion

We demonstrate for the first time that female flowers are deficient in a significant pollinator attractant emitted solely from pollen-bearing anthers and that this contributes to pollinator discriminatory behavior between the sexual morphs. Thus, this work identifies a previously unrecognized mechanism for pollinator discrimination in gender dimorphic plants and thereby informs significantly on a main factor believed to hinder the evolution of separate sexes.

Our data also support the hypothesis that anther odors provide a critical signal not available in petals (Dobson et al. 1990, 1999); but rather than confirming species specificity, our data suggest that the anther signal may be general, as the pollinator fauna studied here was composed largely of generalist bees (Ashman 2000) and 2-phenylethanol is one of the most attractive and salient odors known for bees and a variety of other flower-visiting insects (Raguso 2004). Specifically, 2-phenylethanol has been found in flowers from multiple families including Rosaceae (*Rosa rugosa*; although produced by the petals and sepals rather than the anthers; Dobson et al. 1990), and those in the basal angiosperm group Trimeniaceae (*Trimenia moorei*; Bernhardt et al. 2003), and has been shown to elicit behavioral responses in several classes of insects (reviewed in Bernhardt et al. 2003). In addition, a pilot study indicated that the bee fauna observed in the present study do respond positively to 2-phenylethanol (T.-L. Ashman, D. Cole, and M. Bradburn, *unpublished data*). Additional work, however, is needed to verify that 2-phenylethanol is the major attractive component of anther scent. This would require pollinator assays of 2-phenylethanol, alone and in combination with other floral compounds (e.g., benzyl alcohol), and appropriate controls. Regardless, by showing that male organs are a production site of important volatile attractants, we provide an additional adaptive explanation for the retention of staminodes in females (Mayer and Charlesworth 1991). Furthermore, given that female anthers continue to produce 2-phenylethanol, but at extremely low rates, there may be the potential for natural selection to enhance the production of this attractant in females, perhaps even in the absence of increasing staminode size (i.e., by increasing enzymatic rate). Focused enzymatic and physiological studies would be needed to determine the site and kinetics of 2-phenylethanol, and hence the constraints on evolution to increase its emission.

Last, our results also indicate that scent is an important modulator of pollinator behavior even in a small flowered, weakly scented, diurnal species visited by generalist pollinators, and is not just peculiar to intensely scented or specialized pollination systems, such as pheromone-mimicking orchids (Schiestl et al. 1999) or those pollinated by fragrance-collecting euglossine bees (Gerlach and Schill 1991). We suspect that floral scent, whether alone or in concert with visual cues, probably impacts reproductive success to some extent in all animal-mediated pollination systems.

Acknowledgments

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APPENDIX A

A table showing a summary of floral volatiles detected by static head space analysis (SPME) from *Fragaria virginiana* is available in ESA'S Electronic Data Archive: *Ecological Archives* E086-111-A1.

APPENDIX B

A table showing a summary of floral volatiles detected by dynamic head space analysis from floral extracts of *Fragaria virginiana* is available in ESA's Electronic Data Archive: *Ecological Archives* E086-111-A2.