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The sweet smell of success: floral scent affects pollinator attraction and seed fitness in *Hesperis matronalis*

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Summary

1. Patterns of floral scent are generally assumed to have been shaped by pollinator-mediated natural selection. However, while many studies document behavioural responses of pollinators to floral scent, few document the relationship between floral scent and fitness.

2. In this study, we explore the effect of variation in floral scent emission in colour polymorphic *Hesperis matronalis* on both pollinator visitation and seed fitness.

3. Using target inflorescences augmented with colour-specific floral scent extracts, we find that diurnal floral visitors significantly prefer night-scent extracts to non-augmented controls; inflorescences augmented with day-scent extracts receive an intermediate level of visits. Colour did not have a significant effect on visitation.

4. We characterized the relationship between natural variation in floral scent emission rate and seed production for plants under two settings: in small experimental arrays exposed to either day- or night-flying pollinators, and in wild populations exposed to all pollinators. In arrays, we found greater emission rate led to higher seed production, but only in plants exposed to day-flying pollinators. In contrast, we found a significant positive relationship between night-time floral emission rate and seed fitness in wild populations. In neither setting did floral anthocyanin concentration (colour) affect fitness.

5. This study reinforces the idea that scent-mediated pollinator visitation is an important component of plant fitness. Moreover, our results suggest that plants emitting more scent have higher fitness, although there is variation as to when this positive relationship occurs (i.e., at day or night). Research connecting floral scent and fitness is a necessary first step in understanding the evolution of floral scent.

Key-words: aromatics, natural selection, phenotypic variation, plant volatiles, pollinator attraction, syrphid flies, terpenoids, trait correlations

Introduction

Evolutionary studies of floral characteristics have traditionally focused on visual traits assumed to be attractive to pollinators, including floral display size, shape and colour (e.g., Stanton *et al.* 1986; Stanton 1987; Schemske & Bradshaw 1999; Caruso 2000; Worley & Barrett 2000; Ashman 2003; Irwin & Strauss 2005). Two key components of these studies are assessment of pollinator response to trait variation, and examination of the relationship between trait variation and plant fitness. A significant trait-fitness relationship is one of the three conditions necessary for evolution by natural selection (Endler 1986); finding this provides initial support for hypotheses involving pollinator-mediated natural selection. Similar processes are also invoked to explain the evolution of non-visual floral traits, with some recent studies describing and testing pollinator response to floral scent (e.g. Waelti *et al*. 2008). Such research continues to be rare, reflecting a disconnection between the fields of pollination ecology and floral chemistry (as reviewed in Raguso 2008). Consequently, little is known about the relationship between floral scent and realized plant fitness.

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Floral scent is highly variable and often assumed to be an important target of pollinator-mediated selection (Miyake & Yafuso 2003; Salzmann *et al*. 2007b). Observations of pollinator fauna on plants with known scent characteristics suggest that both day- (e.g., Galen & Kevan 1980; Pombal & Morellato 2000; Theis *et al.* 2007) and night-active floral visitors have distinct olfactory preferences (e.g., Knudsen & Tollsten 1993; Hoballah *et al*. 2005; Raguso & Willis 2005; Riffell *et al*. 2008), particularly for aromatic and terpenoid compounds (Dobson 2006). Likewise, behavioural choice assays show that bees, butterflies, and moths can discriminate in both qualitative (e.g., Heath *et al* 1992; Ômura *et al.* 1999; Cunningham *et al*. 2004, 2006; Theis 2006) and quantitative aspects of floral scent composition (e.g., Heath *et al*. 1992; Andersson 2003; Andersson & Dobson 2003; Wright *et al*. 2005). Floral visitors often prefer scented flowers over unscented ones, translating into increased pollinator approaches or landings (Knudsen *et al.* 1999; Kunze & Gumbert 2001; Raguso & Willis 2002; Schiestl 2004; Ashman *et al*. 2005). These findings provide strong evidence for the importance of floral scent across the specialization-generalization spectrum of pollination.

Thus, it is perhaps surprising that the direct relationship between variation in floral scent and plant fitness has rarely been explored. Many studies view the rate of pollinator visitation as a proxy for plant fitness (e.g., Ayasse *et al*. 2000; Diaz & Kite 2006). However, only a few studies specifically document fitness-scent relationships, with mixed results. In some cases, the presence of a less-preferred scent type or lower scent emission led to reduced fitness, through changes in pollinator activity (e.g., Galen 1985; Galen & Newport 1988; Miyake & Yafuso 2003). Other studies found no significant relationship between scent variation and fitness (e.g., Ackerman *et al.* 1997; Valdivia & Niemeyer 2006; Salzmann *et al*. 2007a,b). Recently, studies have found that pollinator responses to attractive and repellent components of floral scent interact in complex ways to optimize plant fitness (Kessler *et al*. 2008). Given the paucity of empirical evidence for scent-fitness relationships, and the mixed results of such research, we are not yet in a position to evaluate the role of pollinator-mediated selection in the evolution of floral scent.

Evaluating the relationship between floral scent and plant fitness may be further complicated by the complexity encountered in defining floral scent. Floral scent often consists of large numbers of volatile compounds that fall into different chemical categories, particularly terpenoids and aromatics (Knudsen & Gershenzon 2006), any or all of which can be attractive to pollinators (Dobson 2006). Moreover, some plant species emit scent in different rhythmic patterns. Pollinators use night vs. day scent cues differently, with scent often serving as a landing cue for day-flying pollinators and as an initial attractant for night-flying pollinators (i.e., Ômura *et al*., 1999; Raguso & Willis 2002; Andersson & Dobson 2003; Raguso & Willis 2005). Thus, it seems likely that through pollinator behaviour, certain elements of scent composition or timing of emission may greatly shape scent fitness relationships. Keeping such complexity in mind thus becomes vital for understanding which elements of floral scent are most biologically relevant for determining plant fitness.

Fig. 1. A purple inflorescence of *H. matronalis* being visited by a syrphid fly, one of the predominant pollinators of this plant species in western Pennsylvania.

To address this gap in our general knowledge about floral scent, we explored whether experimentally enhanced floral scent emission leads to increased pollinator visitation and whether natural variation in floral scent affects seed fitness in *Hesperis matronalis* (Brassicaceae; Fig. 1). This plant is well suited for such studies because there is wide variation in the amount of scent emitted by its flowers and its scent is rich in terpenoid and aromatic compounds (Nielsen *et al*. 1995; Majetic *et al*. 2007; Majetic, Raguso & Ashman 2008) known to be attractive to the insect taxa (Dobson 2006) that visit its flowers during day and night (Mitchell & Ankeny 2001; Majetic 2008). We conducted a three-part study focusing on the fitness effects of floral scent emission while controlling for flower colour: a scent augmentation experiment, an array experiment where we manipulated pollinator-access, and an observational experiment across several large wild populations. We asked three main questions: 1) How do diurnal floral visitors respond to scent augmentation? 2) Does scent emission influence seed fitness when plants are exposed to diurnal vs. nocturnal pollinators? If so, is the relationship due to the emission rates of specific subsets (aromatics or terpenoids) of the scent blend? 3) Can variation in seed fitness in wild populations be explained by floral scent emission during day vs. night? If so, is the relationship due to aromatic or terpenoid emission rate? We predicted that enhanced emission of floral scent (namely increased emission of terpenoids and/or aromatics) would lead to increased pollinator visitation and increased seed fitness. We expected stronger scent-fitness relationships for day-exposed plants, given greater abundance of day-flying pollinators observed in wild populations (Majetic 2008), as well as positive relationships between fitness and both aromatic and terpenoid volatile emission rates, given the demonstrated roles of both of these categories of compounds in pollinator attraction at day and night (rev. by Dobson 2006).

Materials and methods

STUDY SPECIES

Hesperis matronalis (Brassicaceae; Fig. 1) is an introduced, potentially invasive (Annen 2007), herbaceous biennial common to the northern tier of the US (Mitchell & Ankeny 2001). Plants over-winter as vegetative rosettes and flower in May. Inflorescences can have as many as 20 flowers open at a time (Mitchell & Ankeny 2001). *Hesperis matronalis* flowers are hermaphroditic and partially self-compatible in some populations (Majetic 2008, but see Mitchell & Ankeny 2001; Weeks & Frey 2007). Self-compatible plants set some seed autonomously, but those that receive pollinator visits have threefold higher seed set (Majetic 2008). During the day, flower visitors in Pennsylvania and Ohio are predominately bees (including *Bombus* spp. and *Apis mellifera*) and syrphid flies (a combined *c.* 80% of visitors), with less frequent visits by lepidopterans, including crepuscular moths (Mitchell & Ankeny 2001; Majetic 2008).

Hesperis matronalis populations are polymorphic for floral colour, with purple or white morphs, although some populations contain a pink intermediate (Dvořák 1982; Mitchell & Ankeny 2001; Rothfels *et al.* 2002). Purple morphs have high levels of anthocyanins in their petals, whereas white morphs have little or no floral pigment. Floral colour morphs do not differ in size, shape, pollen or ovule production or vegetative characteristics, but differ in some aspects of scent composition (Majetic *et al*. 2007; Majetic 2008). *Hesperis matronalis* flowers emit a complex volatile blend (39 compounds) that varies diurnally (Nielsen *et al*. 1995; Majetic *et al*. 2007). Scent emission rates are twofold higher at dusk than at dawn (0·041 μg vs. 0·019 μg scent/flower/hour), and temporal variation in floral scent composition also occurs (i.e., more aromatics emitted at dusk than at day in some populations; Majetic *et al*. 2007). The focus of the current study is variation in flower scent emission; thus, in each component study we control for flower colour.

EXPERIMENTAL AUGMENTATION OF FLORAL SCENT AND RESPONSE BY POLLINATORS

Pentane extraction of whole flower scent and floral scent emitters

We used whole flower extracts to capture the complex floral scent of *H. matronalis* for augmentation of floral targets. We harvested purple and white *H. matronalis* inflorescences during morning ('day': between 7 am and 10 am) and evening ('night': between 6 pm and 9 pm) from wild populations near the University of Pittsburgh (Allegheny County, PA) and the Pymatuning Laboratory of Ecology (PLE; Crawford County, PA). We extracted volatiles for 20 min from these flowers using 2 mL pentane per gram fresh weight (Sigma-Aldrich, 99% purity, GC quality). Pentane is commonly used for extraction of scent from floral tissue (e.g., Ashman *et al.* 2005) and is an appropriate solvent for a broad range of plant volatiles (Prososki *et al.* 2007); indeed, pentane extracts are commonly used to test for pollinator behavioural responses to floral odours (Schiestl *et al.* 2004). All of the compounds we have found in the headspace of *H. matronalis* can be extracted with pentane (Kerrola *et al.* 1994; Antonelli *et al*. 1997; Gibernau *et al*. 1997; Gancel *et al*. 2003; Ashman *et al*. 2005; Jerković *et al.* 2006; Teixeira *et al*. 2007). We stored extracts at −20 °C until use. We prepared several separate extracts for each colour morph during each time period, using approximately 30 flowers for each extract.

We constructed floral scent emitters from 4 mL polypropylene culture tubes and wicks fashioned from coffee filters. We filled treatment emitters with 10 flower equivalents of scent-containing pentane extract (*c.* 1–2 mL of liquid) that was diluted to 3 mL with mineral oil, whereas we filled control tubes with 1 mL of pentane diluted to 3 mL with mineral oil. Thus, our treatment emitters represent the scent emission of an inflorescence, rather than individual flowers. We produced five treatments which were used in each trial and are hereafter referred to as 'control', 'purple day' (emitters containing scent extracts from day-collected purple flowers), 'purple night' (containing scent extracts of night-collected purple flowers), 'white day' (containing white day-collected flower extracts), and 'white night' (emitters with scent extracts of night-collected white flowers). By standardizing our methods for all colour morphs and time periods, we assume that we have effectively captured the documented differences in odour quality and quantity (as in Majetic *et al*. 2007) between plants.

Experimental arrays

We constructed hexagonal arrays at two sites in an open field at PLE, each consisting of six experimental units spaced with at least 1 m between nearest neighbour units. Each experimental unit was composed of a scent emitter plus a floral target; an array thus contained two control units (one of each floral colour) and one of each of the treatment units (i.e., purple day, purple night, white day, and white night). Floral targets were created by haphazardly selecting a white or purple inflorescence harvested from a wild *H. matronalis* population, trimming each to 10 open flowers and placing them into individual florist's pictures. On the morning of observation days, three purple and three white floral targets were assigned to alternating positions in the array. Each floral target was then randomly assigned to a control, day, or night emitter, within the constraint that target colour and colour of scent source matched. Targets were arranged in the hexagonal array such that flower colours alternated, but emitters were assigned positions within a colour at random. Emitters were given 5 min to equilibrate to ambient conditions before the onset of data collection.

All pollinator observations were conducted on eight warm sunny days in late-May and early June 2007. Two observers simultaneously recorded visitation on each half of an array (i.e., three experimental units per observer). Approaches and landings by all pollinator insect taxa by type (bees, syrphid flies and lepidopterans) to each of the six experimental units in the array were recorded during a 10-min period (hereafter, a replicate). Following the first replicate in a given array, we rotated each emitter to a new target, such that each target experienced each level of colour-specific scent (i.e., control, day and night); thus we conducted three replicates in a given array during a 30 min observation period. Observers also switched positions, such that they observed all possible target-emitter units during the observation period. New targets and emitters were used after 30 min to reset the array and begin a new observation period. We conducted a total of 50 observation periods for a total of 25 h of pollinator observation and recorded 1139 visits in all. Observation periods were conducted equally at both sites and no significant effect of site was found (data not shown), so sites were pooled in subsequent analyses.

Statistical analysis

We determined visitation rate as the sum of approaches and landings to each experimental unit. Most approaches led to landings, thus we pooled our data to concisely describe visitor behaviour. We performed an ANOVA (PROC GLM, SAS 2007) to determine the effect of target colour

(purple vs. white), colour-specific scent treatment (control vs. day scent vs. night scent), and their interaction on visitation rates, while controlling for observation period and 10-min replicate. Because initial analysis revealed that replicates within an observation period were not significantly different, we calculated the number of visits per emitter type across all replicates within a period as total number of visits/30 min/target of 10 flowers and reran our ANOVA without replicate.

FLORAL SCENT EMISSION RATE AND SEED FITNESS IN ARRAYS WITH SPECIFIC POLLINATOR ACCESS

Experimental design

In the early spring of 2006, we collected rosettes from four Pennsylvania populations, transplanted them into 1-gallon pots with Farfard™ #4 soil (Conrad Farfard, Agawam, MA) at PLE, and watered them daily. We constructed eight pollinator exclosures ($2 \times 2 \times 2$ m) from window screen and PVC pipe. We placed four exclosures at each of two locations at PLE. At each location two exclosures were designated as 'day-access' where only diurnal pollinators had access to the flowers, and two as 'night-access' where crepuscular and/or nocturnal pollinators had access to the flowers. Pollinator access was controlled by opening and closing the sides of exclosures at specific times of day (i.e., day – 7 : 00 am to 7 : 00 pm; night – 7 : 00 pm to 7 : 00 am). Upon flowering, a total of 98 potted plants were randomly assigned to one of the pollinator-access exclosures; thus, each array consisted of 12 or 13 plants.

Data collection

For each plant, we recorded inflorescence height at flowering as a measure of plant size (in cm), floral size (as petal length × petal width; mm^2) of a randomly chosen fully open flower, floral pigmentation and floral scent. Here, we characterized floral pigmentation quantitatively rather than qualitatively (i.e., purple or white). Specifically, we determined anthocyanin content using methanol extraction and spectrophotometry (modified from Harborne 1998). We read absorbance at 530 nm on a Spectronic 21D spectrophotometer (Model DV #332278, Milton Roy, Rochester, NY); a higher absorbance value reflects darker purple flowers. We sampled floral scent using dynamic headspace extraction and gas chromatography-mass spectroscopy as in Majetic *et al*. (2008) for day-access plants in the morning and night-access plants at night. We calculated floral emission rate (μg/flower/h) for each of the 39 identified compounds following Majetic (2008). Individual compounds were classified based on biosynthetic pathway (after Knudsen & Gershenzon 2006) to form two classes (aromatics and terpenoids). Emission rates for each group were summed and then were natural-log-transformed to improve normality and conform to the assumptions of parametric statistical analyses.

We observed pollinator visitation during several 15-min periods on clear days in late spring 2006. We observed plants in the day-access treatment between 7 : 00 am and 9 : 00 am and 12 : 00 pm and 3 : 30 pm, and plants in the night-access treatments between 7 : 00 pm and 9 : 00 pm. A total of 13–16 observation periods were made at each of the three times for a total of 11·25 h of observation. We observed a total of 56 visits. Most visits were made in the afternoon, 31 by bees, 21 by syrphid flies (58·5% and 39·6%, respectively), and 1 by a beetle. Two visits by bees were recorded in the morning, and only one evening visit (by a fly) was recorded at night. No inappropriate visitors were found in exclosures during our observations (i.e., no day-flying visitors appeared in night-exposed exclosures). We calculated visitation rate (visits/flower/h) for each observation period based on the number of available flowers during each period.

Once plants had been exposed to pollinator access treatments for one month, we counted the number of flowers produced during the experiment on each individual and fruits on these were allowed to mature. We recorded the number of seeds in each fruit. From these values, we estimated seed fitness as the number of seeds produced per plant during the experiment.

Statistical analysis

Because our main interest was to determine how variation in floral scent emission affects seed fitness when different pollinators were allowed access, rather than to identify the sources of trait variation in our experiment, we conducted an ANOVA (PROC GLM, SAS 2007) to remove the effects of replicate, plant source population, and the interaction of replicate and population from all traits. We then regressed residual seed fitness on the residuals of plant traits for each pollinator access treatment separately (i.e., Model: residual total seed production = $\alpha + \beta_1$(residual ln-total day or night emission rate) + β_2(residual floral pigmentation) + β_3(residual floral size) + β_4(residual flower production) + β_5(residual inflorescence height) + ε_{ij}). When quadratic terms were included they were non-significant and therefore they were removed from the models. When our analysis indicated a significant relationship between total scent emission and seed fitness, we further investigated whether aromatic or terpenoid compounds had a tighter relationship with fitness. Because the emission rates of terpenoids and aromatics are highly correlated ($r = 0{\cdot}73$, $P = 0{\cdot}0001$), we did this by conducting the regression above, but substituting each class of volatiles for total scent separately.

SEED FITNESS AND FLORAL SCENT EMISSION RATE IN WILD POPULATIONS

To determine whether day- or night-produced scent affects seed fitness of *H. matronalis* in the wild, we located four populations that span the latitudinal range of this species in eastern North America: southern Ontario, Canada (44°01′ N, 79°31′ W), northwestern Pennsylvania, USA (41°36′ N, 80°25′ W; 41°36′ N, 80°27′W), and northern Virginia, USA (39°05′ N, 78°04′ W). In each population, 10 purple morphs and 10 white morphs were selected at random and marked for study. For each, we measured floral scent, inflorescence height, flower production, and seed production. We collected and analysed floral scent from *in situ* plants at day and night as for the plants in our pollinator-access exclosures and in Majetic *et al*. (2008) and we calculated emission rate for aromatics, terpenoids, and total scent as described above (see *Floral scent emission rate and seed fitness in arrays…*). When flowering was complete, we tallied the total number of flowers and fruits per plant and collected three fruits per plant. To estimate total seeds produced per plant, we multiplied total fruits per plant and mean seeds per fruit. Plant height at peak flowering was recorded as an estimate of plant size. As we were unable to locate some marked individuals, final sample size was 69 plants across four populations.

Statistical analysis

We used individual ANOVAS (PROC GLM, SAS 2007) to remove any effects of population, floral colour, and their interaction from all plant traits measured. Such an approach allows us to describe the

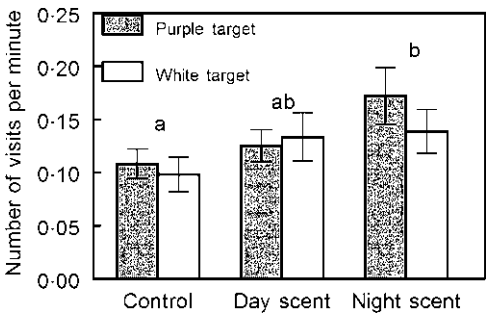

Fig. 2. Pollinator visits to colour-specific scent augmented floral targets (pentane control, day scent, and night scent). Letters above bars represent differences between overall treatment means as determined by Tukey's test.

scent emission-fitness relationship across all individuals in all populations while controlling for trait variation caused by location or colour specific effects. The resulting residuals were used to determine if there was a relationship between scent emission and seed fitness across populations (PROC REG, SAS 2007; Model: residual total seed production = $\alpha + \beta_1$ (residual ln-total day emission rate) + β_2 (residual ln-total night emission rate) + β_3 (residual total flower production) + β_4 (residual inflorescence height) + ε_{ij})). As above, when quadratic terms were included they were non-significant and therefore they were removed from the models. In addition, when we found a significant effect of total emission rate on seed fitness, we investigated whether aromatic or terpenoid components contributed to the relationship by replacing total emission rate terms in the regression model with aromatics or terpenoids individually.

Results

POLLINATOR RESPONSE TO SCENT AUGMENTATION

Approximately 95% of floral visitors were syrphid flies, with the remaining 5% of visits by small bees and lepidopterans. Minority visitors showed qualitatively similar patterns of behaviour to majority visitors but were too infrequent to be analysed separately. Total flower visitors did not discriminate between purple and white target inflorescences ($F_{1,240} = 0{\cdot}72$, $P = 0{\cdot}40$) but did discriminate between scent augmentation treatments ($F_{2,240} = 4{\cdot}74$, $P = 0{\cdot}01$; Fig. 2); this occurred regardless of observation period in our experiment ($F_{48,240} = 3{\cdot}10$, $P < 0{\cdot}0001$). Floral visitors visited targets augmented with night-scent extracts significantly more often (59%) than the control targets (Fig. 2). In contrast, targets augmented with day-scent extracts received slightly but not significantly more (16%) visits than control targets (Fig. 2) and this was similar across colour morphs (scent-by-colour interaction: $F_{2,240} = 0{\cdot}75$, $P = 0{\cdot}48$).

FLORAL SCENT EMISSION RATE AND SEED FITNESS IN ARRAYS WITH SPECIFIC POLLINATOR ACCESS

Plants exposed only to day-flying pollinators experienced little visitation during the morning (0·006 ± 0·006 visits/flower h/h), but greater visitation during the afternoon (0·312 ± 0·167 visits/flower/h). In this access treatment, seed fitness increased significantly with natural log day floral emission rate ($\beta = 12{\cdot}526$; $P = 0{\cdot}009$; Fig. 3a) and flower production ($\beta = 3{\cdot}9$; Fig. 3 legend). In contrast, none of the other plant traits (floral size, floral pigmentation and inflorescence height) significantly affected seed fitness (Fig. 3 legend). Natural log day emission rate of terpenoids had a similar positive relationship with seed fitness ($\beta = 12{\cdot}868$, $P = 0{\cdot}01$). The relationship between fitness and natural log day aromatic emission rate was also positive, but only marginally significant ($\beta = 8{\cdot}044$, $P = 0{\cdot}06$). To verify our results, we also ran this analysis removing two potential outliers (one high, one low; see Fig. 3a). Removal of these data points did not change our results – total and terpenoid scent remained significant, whereas aromatics were marginally significant. At night we observed a very low level of flower visitation (a single visit during 13 observation periods) and the treatment was

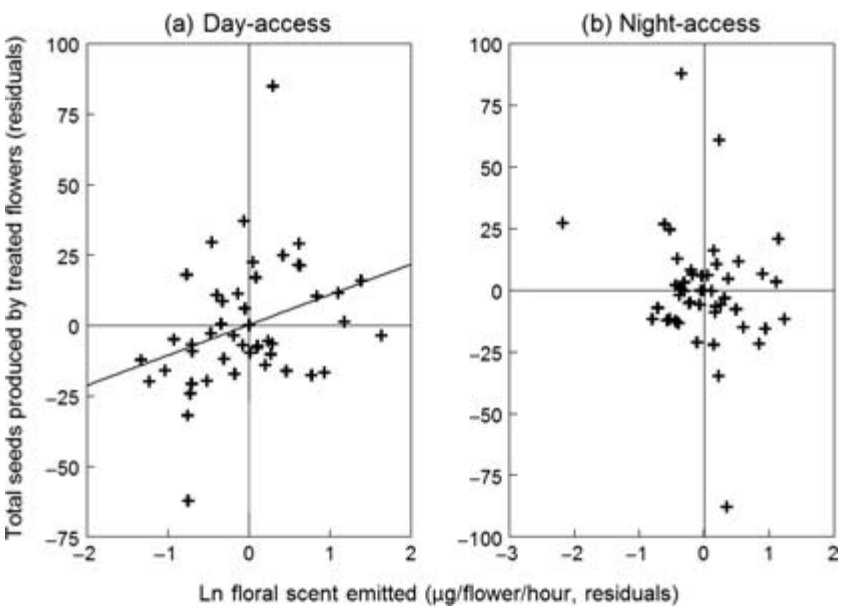

Fig. 3. Relationship between *H. matronalis* seed fitness and floral scent emission for plants in exclosures that allowed pollinators access only during the day (a) or night (b). Complete models are as follows: Day-access ($R^2 = 0{\cdot}58$; $P < 0{\cdot}0001$): residual total seed production = **12·526** (residual ln-total day emission rate) + 4·084 (residual floral pigmentation) + 0·12 (residual floral size) + **3·9** (residual flower production) + 0·1 (residual inflorescence height) + 0·07; Night-access ($R^2 = 0{\cdot}14$; $P = 0{\cdot}24$): residual total seed production = – 9·750 (residual ln-total night emission rate) – 24·455 (residual floral pigmentation) – 0·11 (residual floral size) + 0·1 (residual flower production) + 0·7 (residual inflorescence height) – 0·01. Parameters in bold are significant at $P \leq 0{\cdot}02$.

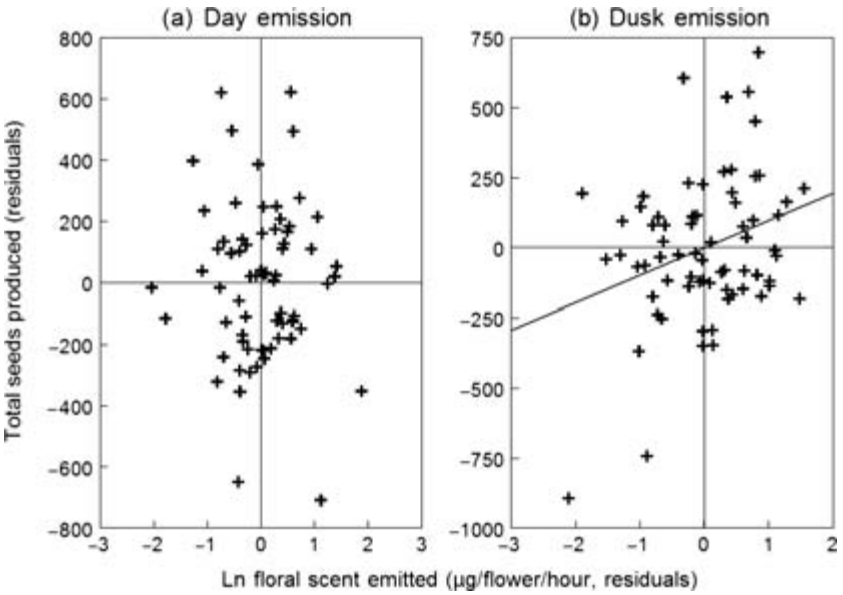

Fig. 4. Relationship between seed fitness and (a) day floral scent emission rate and (b) night floral scent emission rate across four wild populations of *H. matronalis*. Complete regression model ($R^2 = 0{\cdot}77$; $P < 0{\cdot}0001$): residual total seed production = −25·297 (residual ln-total day emission rate) + **100·554** (residual ln-total night emission rate) + **14·9** (residual total flower production) + **5·9** (residual inflorescence height) − 1·37. Parameters in bold are significant at $P \leq 0{\cdot}05$.

effective at removing day-flying pollinators, but average seed set was similar to that of day-exposed plants (data not shown). When plants were exposed to visitors only at night, the regression model explained little variation ($R^2 = 0{\cdot}14$) and was not statistically significant (Fig. 3b and legend).

SEED FITNESS AND FLORAL SCENT EMISSION IN WILD POPULATIONS

Across wild populations of *H. matronalis*, we found no significant effects of day emission rate on seed fitness (β = –25·297, $P = 0{\cdot}58$; Fig. 4a), but a significant positive relationship between natural log night emission rate and seed fitness ($\beta = 100{\cdot}554$, $P = 0{\cdot}01$; Fig. 4b). In addition, total flower production and plant height both had a significant positive effect on seed fitness ($\beta = 14{\cdot}9$, $P < 0{\cdot}0001$; $\beta = 5{\cdot}9$, $P = 0{\cdot}04$; Fig. 4 legend). Further analysis found a significant effect of night terpenoid emission rate on seed fitness ($\beta = 94{\cdot}672$, $P = 0{\cdot}01$), but no significant effect of night-time aromatic emission on seed fitness ($\beta = 51{\cdot}893$, $P = 0{\cdot}16$), which suggests that night-time terpenoid emissions drive the overall relationship between scent emission and seed fitness.

Discussion

Our results suggest that floral scent in *H. matronalis* can have significant impacts on both pollinator visitation and seed fitness, with additions of scent extracts and increases in scent production leading to higher pollinator visitation and seed production, respectively. Such patterns may have profound implications for our understanding of pollinator-mediated floral scent evolution, which we explore in more detail below.

POLLINATOR RESPONSE TO FLORAL SCENT

Augmentation of target inflorescences with night-scent extracts led to a significant increase in visitation by pollinators, primarily syrphid flies, whereas augmentation with day-scent extracts did not, and colour did not affect visitation under any scent level (Fig. 2). Our study joins other studies showing increased visitation by a variety of insects in response to augmentation with floral extracts (i.e., Knudsen *et al* 1999; Ashman *et al*. 2005; Theis & Raguso 2005). However, our results also contribute to this body of knowledge by providing some of the first data on the scent preferences of syrphid flies, especially in the context of generalized pollination systems (e.g., Pombal & Morellato 2000; Dobson 2006). Although we have not yet determined the biologically active aspects of the night extracts in this experiment, previous research on harvested *H. matronalis* inflorescences indicated that flowers emit more scent at dusk, and this can contain proportionately more aromatics in some populations than scent emitted during the day (Majetic *et al*. 2007). The fact that day extracts elicited less of a response than night extracts suggests that our extracts successfully captured some of these scent differences. However, further study will be required to determine whether differences in composition, amount or both were responsible for differences in visitation.

SEED FITNESS AND FLORAL SCENT EMISSION

We found positive relationships between floral scent emission rate and total seed production in both small arrays with specific pollinator access and wild populations (Figs 3a and 4b). This suggests that non-visual characters like floral scent can have a strong impact on overall plant fitness in a variety of environments. Moreover, the results of both experiments point to terpenoid emissions as a major contributor to the scent-seed production relationship both during the day (in the small arrays) and at night (in wild populations). While aromatics are well-known as attractive to a wide array of pollinators in a number of systems (Raguso 2008), terpenoids are also known to attract a range of both day- and night-flying pollinators, particularly linalool and β-ocimenes (Dobson

2006). These compounds are major components of the floral scent bouquet for *H. matronalis* scent at both day and night, further illustrating the potential for this species to attract a variety of pollinators at different times of day. Moreover, our previous work on floral scent variation suggests that both overall and terpenoid scent emission rates vary among populations in North America (Majetic *et al*. 2008). Future studies designed to compare the fitness of plants with known high or low terpenoid emission would provide a test of the importance of floral terpenoids for plant reproductive success.

Interestingly, we see differences in the timing of scent emission and increased fitness in our different experiments: in our pollinator access experiment, we find a positive relationship between day scent emission rate and seed production in our day-pollinator access treatment, whereas in the wild populations we see a significant relationship between night scent and fitness. These results suggest that in our access experiment, pollinators positively selected on day-emitted floral scent, whereas in wild populations floral scent emitted at night had the stronger effect on fitness. Such a result may seem puzzling on the surface, especially given similarity in night visitation rates between wild populations and our pollinator exclosures (C. Majetic, pers. obs.). However, it is not uncommon to find temporal variation or context-dependent trait-fitness relationships (e.g., Schemske & Horvitz 1989; Nagy 1997; Caruso *et al.* 2003). Differences in context may account for the differences seen here, given that plant density differed between our small, isolated arrays (12–13 plants in a 2×2 m area isolated from other *H. matronalis* populations) and large wild populations (where densities ranged from 21 to 51 flowering stems per m^2; Majetic 2008), but we should also note that pollinator access was different between the experiments (limited to day or night in the arrays vs. open access in the wild), and this necessitated different approaches to the analyses. For the pollinator-access arrays, scent measurements were restricted to the time of pollinator exposure, and thus analyses only included scent emission at a single time period. In contrast, for the wild populations scent was quantified both during the day and night and we were able to account for both emission rates in our analyses. Despite these differences, it is noteworthy that both experiments demonstrated floral scent impacted seed fitness of *H. matronalis*.

Conclusion

Our research finds that floral scent has a significant positive effect on both pollinator visitation and seed fitness for *H. matronalis*. This latter result is particularly important as only a few previous studies have attempted to explicitly examine the relationship between floral scent and any measure of plant fitness beyond pollinator attraction, and many of these have met with equivocal results (Galen 1985; Galen & Newport 1988; Ackerman *et al*. 1997; Miyake & Yafuso 2003; Valdivia & Niemeyer 2006; Salzmann *et al.* 2007a; Salzmann *et al* 2007b). Moreover, our study suggests that this relationship may be driven in large part by terpenoid emission rate, compounds known to attract a variety of pollinators. Whereas the exact nature of the scent-fitness relationship varies among experiments, these results provide further support that non-visual plant characters play an important role in determining plant fitness. Our study is particularly valuable because we simultaneously controlled for aspects of visual display (specifically flower size and colour) and found them not to contribute significantly to differential pollinator visitation or seed fitness. Instead, floral scent and, to an extent, flower production and plant height, were largely responsible for the observed differences in female fitness through pollinator activity. Understanding the effects of floral scent on both pollinator activity and plant fitness is necessary if we are to truly understand the evolutionary trajectory of floral scent and its importance relative to other floral traits.

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