Dosage-Dependent Impacts Of A Floral Volatile Compound On Pollinators, Larcenists, And The Potential For Floral Evolution In The Alpine Skypilot *Polemonium Viscosum*

By: Candace Galen, Rainee Kaczorowski, Sadie L. Todd, **Jennifer Geib**, and Robert A. Raguso

Abstract

All volatile organic compounds (VOCs) vary quantitatively, yet how such variation affects their ecological roles is unknown. Because floral VOCs are cues for both pollinators and floral antagonists, variation in emission may have major consequences for costs and benefits in plant-pollinator interactions. In Polemonium viscosum, the emission rate for the floral VOC 2-phenylethanol (2PE) spans more than two orders of magnitude. We investigated the ecological and evolutionary impacts of this immense phenotypic variation. The emission rate of 2PE varies independently of nectar rewards and thus is uninformative of profitability. Emission is elevated in flowers that are morphologically vulnerable to ant larcenists, suggesting that chemical deterrence may compensate for weak physical barriers. In nature, plants emitting more 2PE than their neighbors escape ant damage. Flower-damaging ants die when exposed to 2PE in the laboratory, and they avoid high 2PE emitters in the field. High 2PE also reduces bumblebee visitation and pollination, suggesting an ecological cost of defense in pollinator service. However, at more moderate emission rates, 2PE enhances the amount of nectar left in flowers, at no pollination cost. In conclusion, repellency of 2PE is highly sensitive to dosage, giving it a key role in shaping ecological interactions between skypilot plants and their floral visitors.

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Dosage-Dependent Impacts of a Floral Volatile Compound on Pollinators, Larcenists, and the Potential for Floral Evolution in the Alpine Skypilot *Polemonium viscosum*

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ABSTRACT: All volatile organic compounds (VOCs) vary quantitatively, yet how such variation affects their ecological roles is unknown. Because floral VOCs are cues for both pollinators and floral antagonists, variation in emission may have major consequences for costs and benefits in plant-pollinator interactions. In *Polemonium viscosum,* the emission rate for the floral VOC 2-phenylethanol (2PE) spans more than two orders of magnitude. We investigated the ecological and evolutionary impacts of this immense phenotypic variation. The emission rate of 2PE varies independently of nectar rewards and thus is uninformative of profitability. Emission is elevated in flowers that are morphologically vulnerable to ant larcenists, suggesting that chemical deterrence may compensate for weak physical barriers. In nature, plants emitting more 2PE than their neighbors escape ant damage. Flower-damaging ants die when exposed to 2PE in the laboratory, and they avoid high 2PE emitters in the field. High 2PE also reduces bumblebee visitation and pollination, suggesting an ecological cost of defense in pollinator service. However, at more moderate emission rates, 2PE enhances the amount of nectar left in flowers, at no pollination cost. In conclusion, repellency of 2PE is highly sensitive to dosage, giving it a key role in shaping ecological interactions between skypilot plants and their floral visitors.

Keywords: VOC, floral fragrance, conditional ecological trade-off, pollination, floral larceny, multimodal signaling.

Introduction

Volatile organic compounds (VOCs) affect the behavior of mutualists and antagonists with multiple, potentially conflicting consequences for plant fitness. Plants utilize volatile signals to attract predators as a mechanism for defending against antagonists, yet eavesdropping herbivores may recruit to the same cues (Karban 2007). Similarly, pollinators and floral larcenists track nectar resources via reception of floral volatile compounds with opposing effects on plant reproduction (Kessler et al. 2008). Several attributes of volatile compounds contribute to both their phenotypic complexity and their functional diversity. VOCs disperse readily, bringing new fragrance qualities to plant tissues that function as "downstream collectors" (e.g., floral and extrafloral nectar) and inducing new organ-specific functional responses in different parts of the plant (Heil and Karban 2010). Similarly, within complex organs such as flowers, the same VOC may provide directional cues for pollinator foraging when released from one structure (e.g., petals) yet act as a feeding deterrent elsewhere (e.g., in pollen; Bergström et al. 1995). Moreover, because volatile compounds dissipate as they move into the atmosphere, they can play discrete, dosage-specific roles over distance gradients in encounters with flower-visiting animals (Raguso 2008). VOCs have both positive and negative impacts on the visitation of flower foragers, consistent with such functional diversity (Kessler et al. 2008; Junker and Blüthgen 2010). Here, we explore the potential for VOCs to assume multiple ecological functions through dosage specificity, focusing on one of the most taxonomically widespread floral volatile compounds, 2-phenylethanol (2PE; Hanson 2007). In particular, we ask how emission rate of 2PE affects positive and negative interactions of the alpine skypilot *Polemonium viscosum* (Polemoniaceae) with flower-visiting insects.

Plant-pollinator relationships are shaped by ecological trade-offs between benefits and costs of exploiting pollinator services. Floral volatile compounds may moderate these trade-offs by signaling rewards or risks to potential visitors. Volatile compounds that act as honest advertisements of food profitability to legitimate pollinators should

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accumulate in the vicinity of flowers at levels that correlate positively with floral rewards (Goulson 2003; Schaefer et al. 2004; Raguso 2008). According to this idea, VOCs evolve under selection for pollinator service and mating success. Conversely, if they act to deter floral larcenists or flower-damaging herbivores, then signal strength should not correlate with reward. Instead, volatile compounds should act along with other deterrents to reduce cheating or collateral floral damage (Galen et al. 1987). For example, glandular hairs that prevent crawling insects from accessing floral reproductive organs often emit VOCs (Levin 1973; Raguso et al. 2006).

Floral visitors' behavioral responses to VOCs determine how information contained in these aerial signals impacts costs and benefits of ecological relationships between host plants and their animal pollinators (e.g., Adler and Irwin 2005). Documenting the behavior of multiple parties in response to floral volatile compounds can illuminate ecological costs that balance against advantages in pollination or defense. Past studies of floral volatile compounds have emphasized benefits for pollinator attraction and potential costs in apparency to antagonists (Raguso 2008). Only recently have defensive roles of floral VOCs and other chemical components of flowers come to light (e.g., bitter nectar; Johnson et al. 2006). Ecological costs in plant fitness may arise via either scenario. If cheaters intercept and exploit volatile signals for pollinators, costs may arise from competition with legitimate pollinators or corollary floral damage (Galen 1999; Maloof and Inouye 2000; Bronstein et al. 2006; Irwin et al. 2008). Although less studied, shared avoidance of VOCs by cheaters and pollinators may also generate ecological costs (Kessler and Halitschke 2009). For example, in some *P. viscosum* plants, a skunky scent emitted from calyces reduces floral larceny by ants at a cost to bumblebee visits (Galen 1983).

In addition to benefits and costs associated with attracting effective pollinators or deterring floral antagonists, VOCs may also affect reward consumption by pollinators, altering costs of resource investment in plant-pollinator interactions. Traits that negatively alter behavior or duration of interactions between individuals can act as ecological sanctions, reducing resource consumption and keeping benefits above costs in cooperative or mutualistic relationships (Kiers and Denison 2008). Whether ecological sanctions alter the balance of trade in relationships between host plants and nectar-foraging pollinators is not known, but such effects are important in plant-microbe mutualisms (Kiers and Denison 2008) and obligate ("nursery") pollination interactions (Pellmyr and Huth 1994; Jandér and Herre 2010). Distasteful nectar volatile compounds (e.g., nicotine) are thought to restrict nectar uptake and encourage long-distance foraging movement (Kessler et al. 2008). Nectar can be energetically costly and may incur an additional water cost in dry environments (Southwick 1984; Pyke 1991; Galen 2005; but see Harder and Barrett 1992). Addressing the idea of volatile sanctions requires knowledge of how volatile compounds affect pollinator foraging and the impact, in turn, on the distribution and presentation of floral rewards.

Floral volatile compounds, then, may have diverse roles as attractants, defenses, or sanctions. On the face of it, these functions seem mutually exclusive: how can a single trait simultaneously deter and attract foragers to a given plant resource? Yet emission of even a single volatile compound can encompass a continuous range of phenotypes, from weak to intense, just as flowers vary in size or shape. Here we consider how function changes over this gradient by experimentally manipulating emission rate and probing foraging responses of receivers and concomitant impacts on reward distribution and pollination success. We further ask whether, via effects on pollinator behavior, the release of floral VOCs constrains selection on display traits in multiple sensory modalities.

To achieve these goals, we experimentally manipulated fragrance profiles in *P. viscosum*, a model system for the study of balancing selection between pollinators and floral antagonists (e.g., Galen and Cuba 2001; Irwin et al. 2008). To our human perception, skypilot flowers have a strong, sweet floral odor (Galen and Kevan 1980), of which the dominant component is 2-phenylethanol (2PE), a compound common to flowers with generalized pollination systems (e.g., Ashman et al. 2005; Theis et al. 2007). The emission rates of 2PE vary over more than two orders of magnitude in *P. viscosum*, providing an excellent context for testing the hypothesis of dosage-dependent function (fig. 1; see Terry et al. 2007). Our study had four specific objectives: we tested whether quantitative variation in 2PE (1) informs receivers of resource profitability or correlates instead with physical defenses; (2) alters attractiveness to pollinators, vulnerability to floral larcenists, or potential costs of maintaining energetic rewards; (3) mediates costs and benefits of pollinator services; and (4) affects selection on other modalities of floral display in *P. viscosum*.

Methods

Study System

Field experiments and observations were conducted during the summers of 2006–2009 on tundra slopes of Pennsylvania Mountain (Park County, CO) at altitudes of 3,600–3,700 m. The field site is near the geographic center of the range for *Polemonium viscosum* and has been used for ecological and evolutionary studies of the species since 1978.

Polemonium viscosum is a self-incompatible alpine wild-

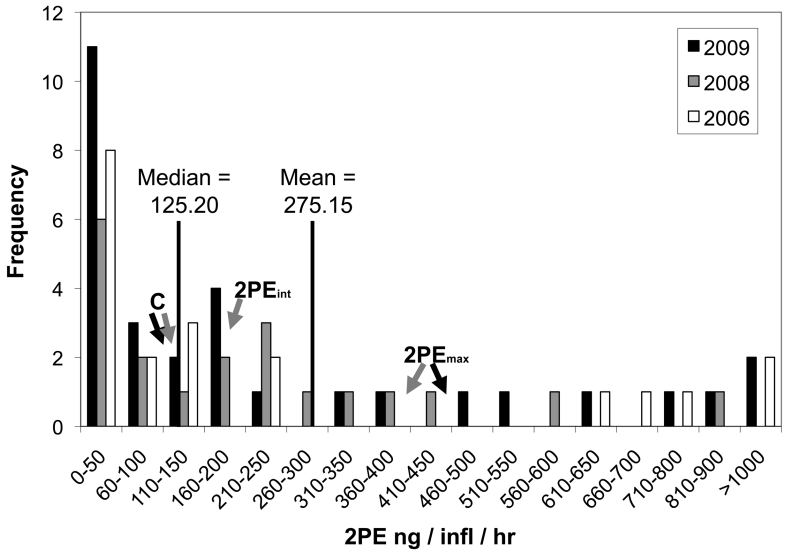

Figure 1: Frequency distribution of 2-phenylethanol (2PE) emission rates (ng inflorescence^{-1} h^{-1}) by flowers of *Polemonium viscosum*. Data shown represent randomly sampled, unmanipulated plants from the same population in 2006 ($n = 20$ plants), 2008 ($n = 20$), and 2009 ($n = 31$). Note the consistent Poisson distribution of emission rates over the period of study. Black arrows indicate mean emission rates for control (*C*) and 2PE$_{max}$ (synthetic 2PE at a concentration of 0.0015% by volume) treatments used in choice assays with free-flying bumblebees (see fig. 3). Gray arrows indicate mean emission rates for control, 2PE$_{max}$, and 50% 2PE$_{max}$ (a 50% dilution of synthetic 2PE at a concentration of 0.0015% by volume) fragrance manipulation treatments (see figs. 5, 6).

flower that depends entirely on insect visitors for pollination (Galen and Kevan 1980). Plants are long-lived perennials that bloom intermittently over their lifetimes, producing about six to 20 flowers per reproductive episode. Individuals vary in floral fragrance, with sweet and skunky floral scent morphs co-occurring in the Rocky Mountains (Galen et al. 1987). This study was conducted in a population primarily comprising sweet-flowered plants. We intentionally excluded rare skunky-flowered individuals, as their numbers were insufficient for a robust design. Sweet floral fragrance contains at least 16 volatile compounds. Of these, 2PE, emitted from the petals and absorbed into the nectar of sexually mature flowers, accounts for 50% or more of fragrance emission by volume (app. A in the online edition of the *American Naturalist*; table 1).

At our tundra study site and elsewhere in the Rocky Mountains, pollination by long-tongued nectar-foraging bumblebee queens of *Bombus balteatus* (formerly *Bombus kirbyellus*) accounts for most seed production (~80%–90%) in *P. viscosum* (Macior 1974; Galen 1996*a*; Galen and Geib 2007). Syrphid flies, anthomyid flies, and solitary bees also visit skypilots, but they contribute little to seed set in most years. Ants of *Formica neorufibarbis* remove nectar from skypilot flowers, but they provide no reciprocal pollination benefit. Theft of nectar rewards by ants has little impact on pollinator behavior in *P. viscosum* (Irwin et al. 2008). However, ants sever the style from its point of attachment to the ovary, disrupting seed set. Plants protected from ants set more seeds than do unprotected plants (Galen 1999). Ant visitation probably also decreases male fitness because exposure of anthers to ants reduces pollen germination (Galen and Butchart 2003).

Information Content of 2PE Emission

To address whether variation in 2PE signals floral profitability, we sampled 2PE emission rate, nectar standing crop (sugar content), flower size, and corolla shape for 16 randomly chosen individuals of *P. viscosum* in the field at an altitude of 3,640 m. Standing crop was sampled since it provides the most direct measure of reward profitability and is highly correlated with visual nectar advertisements in *P. viscosum* (flower size; Cresswell and Galen 1991). All plants were sampled in full flower on July 13–14, 2008 ($n = 8$ daily). To collect floral fragrances in the field, we used a dynamic headspace method (Raguso et al. 2006). Headspace chambers were created from Reynolds (nylon resin) oven bags that were cut and resealed to 8 cm × 12 cm in size using an impulse heat sealer (American International Electric). Odors were collected in bags placed over the inflorescences and were pumped to adsorbent cartridges using battery-operated PAS-500 pumps (Spectrex, Redwood City, CA). Clean air was pulled over the flowers

Table 1: Identified floral scent compounds of sky pilot, in ascending order of retention time (see fig. A1 in the online edition of the *American Naturalist*)

					Relative percentage of total scent produced	
Peak	Compound	Retention (min)	*N* (10)	CV	Mean	SEM
1	Benzaldehyde	12.86	9	1.80	10.22	4.25
2	Linalool	13.04	*3*	1.62	.43	.25
3	Methyl benzoate	14.13	7	1.22	1.91	.76
4	Phenylacetaldehyde	14.40	7	1.45	2.66	1.02
5	Methylphenylacetate	15.79	*3*	1.81	.09	.05
6	2-Phenylethylacetate	16.42	9	1.00	4.70	1.51
7	Benzyl alcohol	17.05	10	.93	11.90	3.78
8	2-Phenylethanol	17.43	10	1.35	50.43	7.69
9	(*Z*)-Methyl cinnamate	17.96	*3*	*2.48*	4.46	2.49
10	(*E*)-Methyl cinnamate	19.24	*3*	*2.46*	6.38	3.37
11	(*Z*)-Cinnamic alcohol	20.08	4	1.69	1.69	1.43
12	2-Aminobenzaldehyde	20.14	7	2.16	4.18	2.36
13	Methyl anthranilate	20.84	*1*	*3.20*	.01	.01
14	(*E*)-Cinnamic alcohol	21.16	*4*	*2.03*	.25	.14
15	Indole	22.69	4	1.68	.68	.32
16	Benzyl benzoate	24.27	*1*	*3.16*	.01	.01

Note: *N* (10) = frequency encountered out of 10 plants surveyed; CV = coefficient of variance. Values in italics are the most variable components of the floral headspace blend.

into the adsorbent traps (glass Pasteur pipettes packed with 10 mg of SuperQ adsorbent [80–100 mesh; W. R. Grace, Deerfield, IL] between plugs of quartz wool) at a flow rate of ~250 mL min^{-1} for 90 min. Traps were rinsed with 10 mL of acetone between uses. All collections were made under clear skies at midmorning during peak pollinator activity. Volatile compounds collected from ambient air and vegetative controls were subtracted from the gas chromatography (GC) chromatograms; only floral volatile compounds are discussed here.

For the dynamic headspace analyses, scent traps were eluted immediately with 300 μL of gas chromatography–mass spectrometry (GC-MS)–grade hexane (Burdick and Jackson GC2) or dichloromethane (EMD Chemicals DX0831–6), and the eluate was stored at −20°C in Teflon-capped borosilicate glass vials. Before GC-MS analysis, we used a brief flow of gaseous N_2 to concentrate samples to 50 μL, and then we added 5 μL of 0.03% toluene (23 ng) as an internal standard. Aliquots of 1 μL of each sample were injected into a Shimadzu GC-17A equipped with a Shimadzu QP5000 quadrupole electron impact mass spectrometer as a detector. All GC-MS analyses were performed using splitless injections on a polar GC column (diameter, 0.25 mm; length, 30 m; film thickness, 0.25 μm [EC Wax]; W. R. Grace) or a nonpolar column (diameter, 0.35 mm; length, 30 m; film thickness, 0.25 μm [EC WAX]). The GC-MS operating conditions and temperature programs were as described by Raguso et al. (2003). Peak areas of total ion chromatograms (TICs) were integrated using Shimadzu's GC-MS Solutions software and were divided by the peak area of the internal standard (23 ng of toluene) to normalize slight variation in final sample volume. Emission rates were calculated for 2PE by fitting sample GC peak areas to regression equations derived from serial dilution curves of authentic 2PE (external-standard method; Raguso et al. 2006). Because the relationship between 2PE emission rate and the number of open flowers per inflorescence was not significant (see "Information Content of 2PE Emission" in "Results"), we express emission rate as nanograms of 2PE per inflorescence per hour.

All open flowers were counted on each plant. Corolla metrics are highly repeatable among flowers in plants of *P. viscosum* (Galen 1996*b*). Accordingly, and to minimize handling, we collected a single fully expanded flower for measurements of corolla length, flare, and nectar standing crop (sucrose equivalents). Corolla surface area and shape (ratio of corolla width to length, a measure of physical defense against ant access; Galen and Cuba 2001) were calculated nondestructively from these measurements. Flower measurements were taken at midday, 1 day before scent collection to reduce contamination by possible wound volatile compounds. Nectar was rinsed from the flower with distilled water from a syringe, frozen for transfer to the laboratory, and assayed for total sucrose equivalents by colorimetric analysis (anthrone test; Cresswell and Galen 1991).

We used Pearson's product-moment correlations (here and elsewhere, SAS ver. 9.2) to test for significant relationships of 2PE emission rate to flower number, nectar standing crop, and flower size and shape. Binned emission rate data were approximately Poisson distributed (fig. 1) and square root transformed for this and all further statistical analyses.

Behavioral Responses of Pollinators and Larcenists to 2PE

We allowed freely foraging queens of *B. balteatus* to choose between paired inflorescences having flowers supplemented with 30%-by-weight sucrose solution without 2PE (control) or with synthetic 2PE (Sigma-Aldrich, St. Louis, MO) added at a concentration of 0.0015% by volume. We refer to this addition treatment as $2PE_{max}$ because it yields an average emission rate bounded by those of the rare high-2PE-emitting individuals in our study population (fig. 1; see below). In control inflorescences, 2PE emission rate was ~25% of that in treated flowers, corresponding to the median value observed in our study population over three years (fig. 1). Inflorescence stems were collected in bud, placed immediately into water-filled florists' aqua-pics, and brought indoors until the flowers opened (following methods in Galen and Cuba 2001). Before presentation to bumblebees, stems were trimmed to six open flowers each to standardize display and were paired according to flower size dimensions. Corolla width and length of paired inflorescences fell within 1 SD on the basis of a preliminary survey of 30 randomly chosen plants from the population at large. Just before each choice trial, the two inflorescences in each pair were randomly assigned between treatments and placed 25 cm apart at opposite ends of a T-apparatus. Flowers were supplemented with artificial nectar using different pipettes for addition of sucrose solution (control) and $2PE_{max}$ (Pipetman precision microliter, Rainin Instruments, Oakland, CA). To four flowers on each stem, we added 1 μL of artificial nectar, doubling the average sugar reward per flower (Cresswell and Galen 1991). We left the remaining two flowers on each stem unsupplemented (nulls) so we could monitor bumblebee responses to 2PE at two levels: during approach flights to inflorescences and among flowers after alighting.

The actual 2PE emission rates for this experiment were estimated by applying either control sucrose or $2PE_{max}$ solution to flowers on randomly assigned, cut inflorescences of *P. viscosum* placed in florists' aqua-pics, as described above. We used dynamic headspace and GC-MS methods (see above) to analyze the trapped floral headspace from these manipulated inflorescences, resulting in $\bar{x} \pm$ SE (coefficient of variation [CV]) 2PE emissions of 476 ± 90 (0.57) ng inflorescence^{-1} h^{-1} for $2PE_{max}$ ($n = 9$), 124 ± 30 (0.68) ng inflorescence^{-1} h^{-1} for sucrose solution ($n = 8$), and 118 ± 28 (0.74) ng inflorescence^{-1} h^{-1} for unmanipulated control plants ($n = 10$).

These results show that our procedure for experimentally manipulating 2PE worked, as the mean emission rate of $2PE_{max}$-treated inflorescences fell squarely within the upper distribution of 2PE in natural populations (see fig. 1). Second, they show that the sucrose control treatment did not alter natural 2PE production. Mean 2PE emission from sucrose-supplemented inflorescences is within the range of emission for unsupplemented natural populations, despite addition of artificial nectar (fig. 2), and it does not differ significantly from that of unmanipulated control plants (Student's t-test, $t = 0.31$, df $= 16$, $P = .76$; fig. 2). Because 2PE is produced by petals and is absorbed secondarily into nectar or sucrose solution, we assume that 2PE from corolla tissues dissolves and equilibrates over time in artificial nectar much as it does in floral nectar (app. B in the online edition of the *American Naturalist*), in line with our results.

When a *B. balteatus* queen was observed on a wild *P. viscosum* individual, the T-apparatus was positioned using its stem as a handle so that the bee was equally distant from the $2PE_{max}$ and the control inflorescences. When the bee flew to an experimental inflorescence, we used a handheld voice recorder to monitor treatment choice ($2PE_{max}$ or control), flower choice (supplemented or null), total time spent probing supplemented and null flowers, and the number of visits made to flowers of each type. Between trials, both inflorescences were replaced with fresh, unvisited ones, and treatments were switched between arms of the T-maze. We monitored four bees in July 2007 and 17 more in June–July 2008. However, because bees were unmarked, we cannot completely dismiss the possibility of resampling.

To test for foraging bias during approach flights, we used a sign test with the null expectation of a 50/50 choice ratio. We used a second analysis to address whether, when alighted on an inflorescence, bees consume less reward from flowers supplemented with $2PE_{max}$ than from controls. For the latter test, we took advantage of a strong relationship between handling time (HT) and nectar consumption (NC) in *B. balteatus* (Cresswell and Galen 1991). For inflorescences where both supplemented and null flowers were visited (eight control and two $2PE_{max}$ individuals), we divided mean HT on visits to supplemented flowers by mean HT on visits to unsupplemented ones to obtain a standardized index of NC. This index has the advantage of eliminating effects of any unmeasured differences among inflorescences (e.g., in flower shape or size) on the difference in HT between treatments. We used a nonparametric Wilcoxon test to compare NC between bees foraging on $2PE_{max}$ and bees foraging on control flowers.

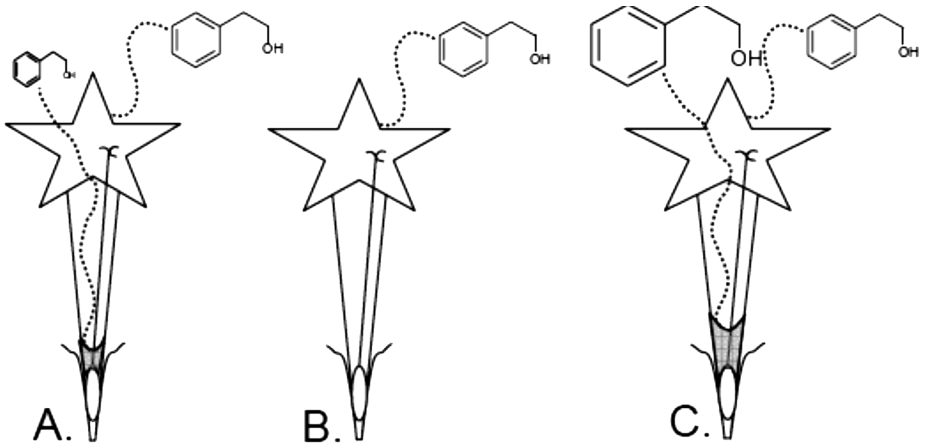

Figure 2: Schematic diagram of 2-phenylethanol (2PE) emissions from *Polemonium viscosum* flowers. 2PE is emitted from the corolla tissues (including the inner epidermal surface of the nectar tube) and also from the nectar (*shaded area at base of tube*), as indicated by dotted lines leading to 2PE molecules diffusing into the floral headspace (*A*). Thus, a flower recently depleted of nectar would still emit 2PE from corolla tissues (*B*), and the addition of sucrose to the nectary would only trivially and temporarily dilute 2PE in nectar because the sucrose solution itself serves as a 2PE sink in much the same way as nectar. The experimental augmentation of 2PE-scented sucrose solution increases 2PE emission from manipulated flowers (see text), as indicated by the larger schematic 2PE molecule emanating from the nectar tube in *C* (cf. *A*).

Gustatory Responses of Laboratory-Housed Bumblebees to 2PE. Because so few bumblebees foraged from 2PE-supplemented flowers in the field, we further explored gustatory responses to 2PE in the laboratory. Fourteen captive queens of *B. balteatus* were given a choice of artificial nectar solutions made without (control) or with 2PE added (as above). To avoid disrupting populations of bumblebees on Pennsylvania Mountain, queens were collected on tundra surrounding Kite Lake (~10 km north of the field site). Individuals were placed in vials and stored on ice for transportation to a large screen-house ~20 km away. Bees were kept cool in the refrigerator overnight and then were moved into the screen-house and placed in 15 × 17.5 × 10-cm nest boxes made of 1-cm-thick plywood. Nest boxes were connected on opposite sides to two smaller 13 × 9.5 × 6.5-cm wood feeding chambers assigned to $2PE_{max}$ and control treatments at random (adapted from Evans et al. 2007). The bottom of the entire apparatus was made of 3-mm wire mesh to improve airflow. We used clear Plexiglas to cover feeding chambers for observation, and we used plywood to cover the nest box. Each feeding chamber was provisioned via a 1.0 × 3.8-cm cotton wick (First Aid, New London, CT) immersed for 2 h before the experiment in either control or $2PE_{max}$ sucrose solution. Wicks held a total of ~3 g of solution. Saturated wicks were wrapped in 3.3-cm lengths of plastic cut from drinking straws to prevent evaporation and were inserted through the floor of the chamber into plastic cups (Solo Cup, Highland Park, IL) that held their tips just above the wire mesh. Bees rapidly discovered and drank from the wicks. Nest boxes were also provisioned with 0.33-cm-diameter pollen balls (Nectar Honey, Longmont, CO). Choice trials ran for 32 h on June 13–14, 2008. Wicks were weighed before choice trials and then reweighed afterward to measure NC, calculated as the difference between the final and initial weights. Individual bumblebees were observed for 15 min daily in a randomized order to ascertain time spent feeding at $2PE_{max}$ and control wicks.

Statistical analysis of feeding time per wick was performed on summed data from the two 15-min observation intervals. NC and feeding time data were not normally distributed, so nonparametric tests were used. We tested the relationship between feeding time and NC using Spearman's rank correlation. Differences in feeding time and NC between treatments were tested for significance by ranking each variable across replicates (bumblebees) and performing a Kruskal-Wallis test on the ranks.

Foraging Responses of Ants to Quantitative Variation in 2PE. In 2007, we tested whether ants forage nonrandomly in relation to 2PE emission rate, using inflorescences collected in bud (as above), trimmed to five flowers each, and assigned randomly among four nectar supplementation treatments: 30% sucrose control, $2PE_{max}$, $2PE_{min}$ (formulated as a 10-fold dilution of $2PE_{max}$), and null control (flowers probed with an empty pipette). Inflorescences were inserted in aqua-pics pushed into the soil to hold

the flowers at the height of natural *P. viscosum* inflorescences. Aqua-pics were placed 0.10 m away from nest rocks housing colonies of *F. neorufibarbis* ants. Ants were observed at a total of 36 nests on five days between July 6 and July 11. Observations were made during intervals of peak foraging activity on clear days from 0900 to 1400 hours Mountain Daylight Time (MDT). New inflorescences were installed each morning and replaced with fresh ones after 3 h. Treatments were randomly assigned among the four cardinal directions, with the nest rock at the center. Inflorescences were surveyed at 20-min intervals, and the number of ants on each was recorded.

We used mixed-model ANOVA (SAS Proc Mixed) with nest rock (random effect) nested within date to test for ant responses to quantitative variation in 2PE. Treatment, date, and their interaction were included as fixed effects in the model. Planned contrasts were used to compare the average number of ants observed per inflorescence (square root transformed to meet assumptions of ANOVA) among fragrance treatments.

Relationship of Ant Floral Larceny in the Field to Quantitative Variation in 2PE. We used style loss from flowers of *P. viscosum* as a sign of prior floral larceny by *F. neorufibarbis* ants, comparing 2PE emission of plants with styles severed from at least one 1 flower (mean = 2.3, range = 1–4) with that of neighboring plants with intact flowers on July 4, 2006. Ten pairs of neighboring ant-damaged and intact plants were sampled at 3,540 m on the tundra. To reduce possible variation in 2PE emission rate due to nectar removal by flower visitors, we excluded flying insects from inflorescences with pollination bags (Oxford Duraweld) and surrounded the inflorescence stems with Tanglefoot-coated drinking straws to protect flowers from ants for 24 h before sampling. Fragrance for each plant was sampled in the field using methods described above for dynamic fragrance collection. Logistic regression analysis (SAS PROC CATMOD) was used to test the hypothesis that variation among plants in 2PE emission rate predicts the likelihood of ant damage.

Ant Tolerance of 2PE in the Laboratory. In July 2008, we tested whether exposure to 2PE affects survival of *F. neorufibarbis* ants by placing groups of 10 ants in plastic petri dishes (16-mm depth × 60-mm diameter) with filter paper (20 mm × 20 mm) to which one of four concentrations of 2PE in 30% sucrose solution was applied: $2PE_{max}$, 50% $2PE_{max}$ (a 50% dilution of synthetic 2PE at a concentration of 0.0015% by volume), $2PE_{min}$, and no 2PE (sucrose control). Unique ant nests ($n = 15$) were used to populate each replicate (set of the four treatments). Ants were aspirated from the nest and stored briefly (< 30 min) in vials on ice to facilitate handling before transfer to dishes. Once in the dishes, ants regained activity almost immediately. The treated filter paper was then placed in the center of each dish, and the dish was covered. Ants could feed from the paper and were exposed to any volatile compounds evaporating from it. We counted total ants alive per dish at 15-min intervals over 2 h. Repeated-measures ANOVA was used to test for variation in the proportion of ants surviving per replicate (arcsine square root transformed) over time between treatments, with replicate as a random effect.

Impact of 2PE Emission Rate on Costs and Benefits of Pollination and Opportunity for Pollinator Selection

In July 2008, we manipulated fragrance experimentally for inflorescences of intact plants in the field to test how quantitative variation in 2PE emission affects nectar standing crop, pollen receipt, seed set, and opportunities for pollinator selection on floral display traits. Thirty spatially blocked replicates of three fragrance treatments were established along five parallel transects at altitudes from 3,560 to 3,640 m on the tundra slopes. Adjacent replicates were separated by a minimum of 10 m along each transect, with transects spaced 20 m apart down the slope.

Five fully expanded flowers on each plant were supplemented with artificial nectar (1 μL of 30% sucrose solution per flower). Control flowers received sucrose solution only, high-2PE flowers received $2PE_{max}$, and intermediate-2PE flowers received 50% $2PE_{max}$. The emission of 2PE averaged ($\bar{x} \pm$ SEM [CV]) 120 ± 46 (0.93) ng inflorescence^{-1} h^{-1} in controls, 183 ± 89 (1.09) ng inflorescence^{-1} h^{-1} in the 50% $2PE_{max}$ addition treatment, and 397 ± 204 (1.15) ng inflorescence^{-1} h^{-1} in the $2PE_{max}$ addition treatment ($n = 5$–6 for all measurements; fig. 1). The CVs for these treatments indicate that high variability among individuals in 2PE emission rates was spread relatively evenly across each treatment in this experiment. Artificial nectar was added daily before 0800 hours (MDT) over the flowering period of each plant (July 8–17, 2008), using a separate Eppendorf 5-μL-capacity repipette for each solution. Tips were discarded after nectar addition to each inflorescence to prevent accidental cross-pollination.

Enamel paints were used to mark calyces on three unsupplemented flowers. Pistils were removed from one marked untreated and one supplemented flower after the corollas wilted, placed in 3 : 1 (ethanol : acetic acid) fixative, and transferred to the laboratory for staining and visualization of germinating outcross pollen (methods follow Galen and Cuba 2001). We sampled pollen receipt in an untreated flower as well as a treated one because of the concern that some grains might be dislodged during pipetting. Because pollen loss (the difference in pollen receipt between treated and unsupplemented flowers on the

same plant) did not differ among treatments ($F_{3,31} = 1.57$, $P > .216$), counts of germinating grains per pistil were averaged for the two kinds of flowers on each plant. For another marked unsupplemented flower, nectar standing crop was sampled in the afternoon (typically between 1400 and 1600 hours MDT unless afternoon thundershowers began earlier; methods were as described above: see "Information Content of 2PE Emission" in "Methods"). Standing crop was sampled as a way to elucidate the impact of 2PE emission rate on NC. Accordingly, measurements were taken as late as possible in the afternoon to maximize the opportunity for prior pollinator visitation. Cages were placed over plants after flowering to prevent elk herbivory. Fruits were collected and seeds were counted in late September.

Among treatments, we analyzed variation in nectar standing crop, outcross pollen receipt, and relative fitness based on average seed set per flower, using separate mixed-model ANOVAs (Proc Mixed) with treatment (fixed) and replicate (random) as main effects. Many nectar samples were lost when lids opened accidentally in transit, reducing the total sample size to 64 individuals. Nectar standing crop and pollen receipt per flower were square root transformed before analysis to meet assumptions of ANOVA. Planned contrasts were used to compare means among treatment groups.

To determine whether 2PE emission rate influences the relationship of fitness to pollen delivery (selection gradient on pollen delivery), we conducted an ANCOVA in relative fitness with outcross pollen receipt as the covariate, treatment as a categorical fixed effect, and replicate as a random effect. Here, significance of the interaction between pollen receipt and fragrance treatment tests whether pollinator-mediated selection on floral display traits varies with 2PE emission rate.

Results

Information Content of 2PE Emission

We found no evidence at the flower or the inflorescence level that 2PE is a signal of energetic rewards. Relationships of 2PE emission rate to flower number, flower size (a correlate of nectar rewards; Cresswell and Galen 1991), and nectar standing crop were negligible ($P > .57$ for all; fig. 3*A*, 3*B*, and 3*C*, respectively). Conversely, 2PE emission varied with flower shape in *Polemonium viscosum*. Emission of 2PE increased in inflorescences containing shorter, more broadly flared flowers ($r = 0.59$, $P < .0165$; fig. 3*D*). Removal of the outlier point at the upper extreme of the data reduces the significance of this relationship, but it does not eliminate the trend ($r = 0.43$, $P < .10$).

Behavioral Responses of Pollinators and Larcenists to 2PE

Foraging Responses of Bumblebees to Quantitative Variation in 2PE. When offered a choice of $2PE_{max}$-treated inflorescences or sucrose controls, queen bumblebees of *Bombus balteatus* visited controls in 86% of foraging trials (sign test, $P < .005$; fig. 4), showing strong olfactory discrimination. Within inflorescences, bumblebees probed longer and likely consumed more nectar from flowers supplemented with plain sucrose than from flowers supplemented with $2PE_{max}$. Standardized NC rate was significantly greater for bees on sucrose-supplemented flowers than it was for bees on $2PE_{max}$-supplemented ones (medians for NC = 1.35 and 0.37, respectively; Wilcoxon test $\chi^2 = 4.586$, $P < .032$). Although visits to 2PE-augmented inflorescences were few, this comparison indicates that $2PE_{max}$ deters bumblebees from consuming nectar (gustation).

Gustatory Responses of Laboratory-Housed Bumblebees to 2PE. For nesting bumblebee queens, NC was correlated with time spent foraging from wicks (Spearman's $r = 0.56$, $n = 28$, $P < .0017$). Similar to field results, bumblebees in the laboratory consumed less artificial nectar when it was enriched with $2PE_{max}$. Median NC by individual *B. balteatus* was reduced by 14% when nectar was augmented with $2PE_{max}$ (Kruskal-Wallis $\chi^2 = 19.837$, df = 1, $P < .0001$). Although lengthy foraging bouts were not observed in either treatment, bumblebees fed longer (median = 7 s) on nectar containing only sucrose than they did on nectar with $2PE_{max}$ (median = 0 s, Kruskal-Wallis $\chi^2 = 8.68$, df = 1, $P < .0032$).

Foraging Responses of Ants to Quantitative Variation in 2PE. Fragrance manipulation significantly affected ant visitation to experimental inflorescences of *P. viscosum* ($F_{3,93} = 5.92$, $P < .0011$). Ants foraged most frequently on flowers enriched with plain sucrose, and they avoided unsupplemented inflorescences (fig. 5*A*). Enrichment with nectar containing $2PE_{max}$ reduced visitation by 60% ($P < .05$). Conversely, dilute 2PE had little impact on ant visitation relative to plain sucrose ($P > .05$) and did not eliminate ant preference for high sucrose rewards (fig. 5*A*). Results suggest that defensive attributes of 2PE against ant floral larceny depend on emission rate.

Relationship of Ant Floral Larceny in the Field to Quantitative Variation in 2PE. Plants with ant-damaged flowers had much lower 2PE emission rates than did neighbors escaping damage (10 ± 4 vs. 72 ± 32 ng inflorescence^{-1} h^{-1}, respectively). Logistic regression analysis showed that low 2PE emission rate increased the odds of floral damage by *Formica neorufibarbis* (Wald $\chi^2 = 3.9618$, df = 9,

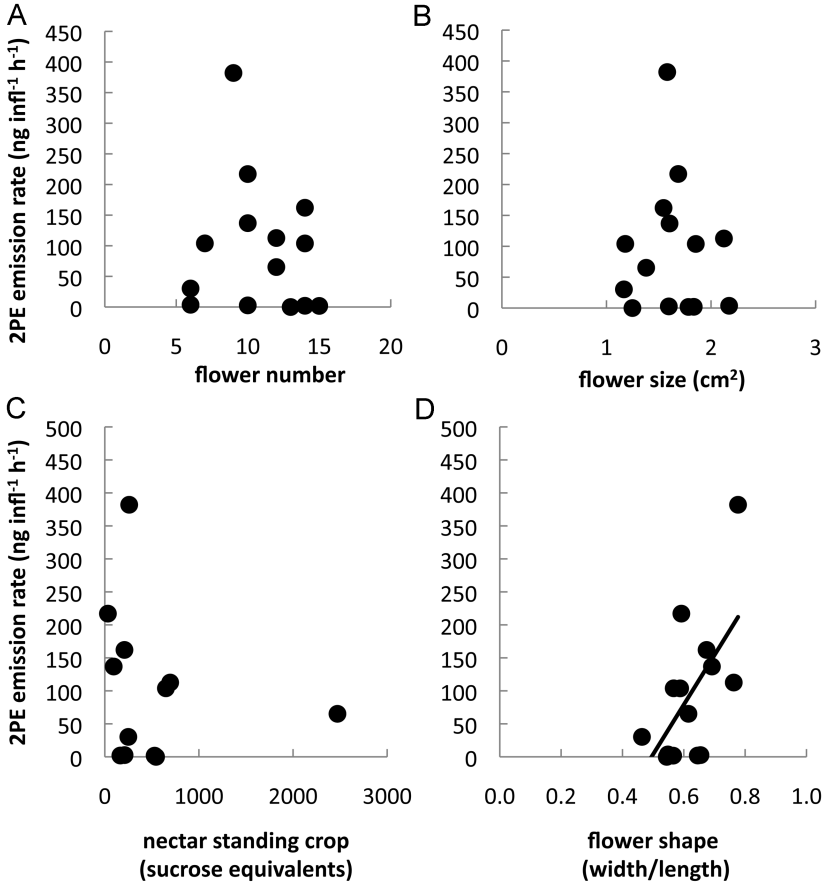

Figure 3: Relationship of 2-phenylethanol (2PE) emission to flowers per inflorescence (*A*), flower size (corolla surface area; *B*), nectar standing crop (*C*), and flower shape (corolla width/length ratio; *D*) in *Polemonium viscosum*. Only flower shape correlates significantly with 2PE emission ($r = 0.59$, $P < .0165$).

$P < .0465$). Although plants without ant damage had a broad range of 2PE emission, all plants with damaged flowers had extremely low emission rates (fig. 5*B*). Results concur with behavioral assays in supporting the view that low 2PE emission increases susceptibility to ants.

Ant Tolerance of 2PE in the Laboratory. Toxicity of 2PE to *F. neorufibarbis* ants was dosage dependent (for the time × treatment interaction, $F_{12,168} = 83.27$, $P < .0001$; table 2). About one-half of the ants exposed to the $2PE_{max}$ treatment died after 60 min, and all were dead after 2 h. When data from this treatment are removed from the analysis, treatment ($P > .44$) and the time × treatment interaction ($P < .08$) effects are no longer significant, indicating that ants tolerate 2PE over a wide range of lower emission rates found in nature (fig. 1).

Impact of 2PE Emission on Costs and Benefits of Pollination and the Opportunity for Pollinator Selection

Manipulation of floral VOCs significantly affected nectar standing crop ($F_{2,33} = 3.94$, $P < .0292$), with more sucrose per flower remaining late in the day in inflorescences provisioned with $2PE_{max}$ at full strength or at 50% dilution than inflorescences of controls (planned contrasts, $P < .05$ for both; fig. 6*A*). Pollen receipt also tended to vary among fragrance treatments ($F_{2,56} = 3.00$, $P < .0578$). Flowers on inflorescences supplemented with $2PE_{max}$ received less pollen than did flowers on sucrose controls (fig. 6*B*; $P < .0212$). In contrast, supplementation with 50% $2PE_{max}$ had no negative impact on pollination (fig. 6*B*). Surprisingly, given the impact of fragrance on pollination success, 2PE supplementation had little effect on average fitness ($F_{2,47} = 1.29$, $P > .28$). However, the relationship between relative fitness based on seed set per flower and

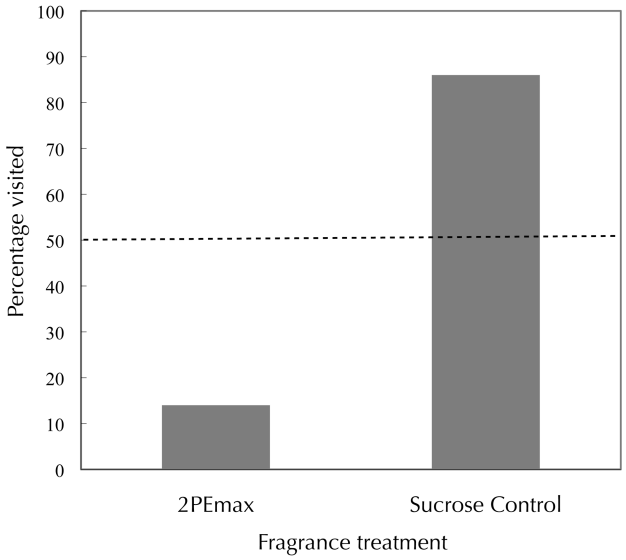

Figure 4: Discrimination against 2-phenylethanol (2PE) by freely foraging bumblebees. Percentage visitation to *Polemonium viscosum* inflorescences augmented with $2PE_{max}$ (synthetic 2PE at a concentration of 0.0015% by volume) and controls augmented with plain sucrose solution. $2PE_{max}$ significantly reduced visitation rate ($P < .005$) relative to expectations with random foraging (*dashed line*).

pollen receipt varied among fragrance treatments (interaction $F_{2,42} = 4.24$, $P < .021$; fig. 7), increasing in strength (slope) as emission of 2PE rose from its low level in controls ($\beta_1 = 0.0044 \pm 0.0038$, $P > .25$; fig. 7*A*) to a moderate level in 50% $2PE_{max}$-supplemented inflorescences and a high level in $2PE_{max}$-supplemented inflorescences ($\beta_1 = 0.0098 \pm 0.0032$, $P < .0053$, and $\beta_1 = 0.0247 \pm 0.0084$, $P < .0074$, respectively; fig. 7*B* and 7*C*, respectively).

Discussion

Emission rate of the floral volatile compound 2PE is not indicative of energetic rewards at flower or inflorescence scales. Nor is variation in 2PE emission correlated with flower size, a positive visual indicator of nectar rewards. Instead, 2PE emission varies with flower shape, a trait associated with the physical defense of *Polemonium viscosum* flowers against ants (Galen and Cuba 2001). Plants with more vulnerable, broadly flared flowers emit 2PE at higher rates. Our behavioral assays show that at the highest levels found in nature, 2PE defends against floral larcenists but incurs an ecological opportunity cost in lost pollination, creating a conditional ecological trade-off. At moderate levels (intermediate strength), 2PE discourages NC by pollinators (fig. 6*A*) without significantly interfering in pollinator service (fig. 6*B*). In other words, as the emission of 2PE drops, its costs in pollinator deterrence shift to a potential resource benefit from reduced consumption of nectar rewards per unit pollen delivery. We were unable to eliminate 2PE emission experimentally in the field. However, lack of ant deterrence for $2PE_{min}$ inflorescences coupled with the low 2PE emission rate of plants showing a history of ant damage suggest that very low 2PE phenotypes incur an ecological cost in susceptibility to floral larceny. Thus, ecological costs and benefits of 2PE emission for *P. viscosum* vary with signal strength in currency and magnitude.

Covariance of 2PE with Other Floral Traits

If volatile compounds function as honest signals of energetic reward, then selection should favor correlation of emission rate with profitability. For example, experimental

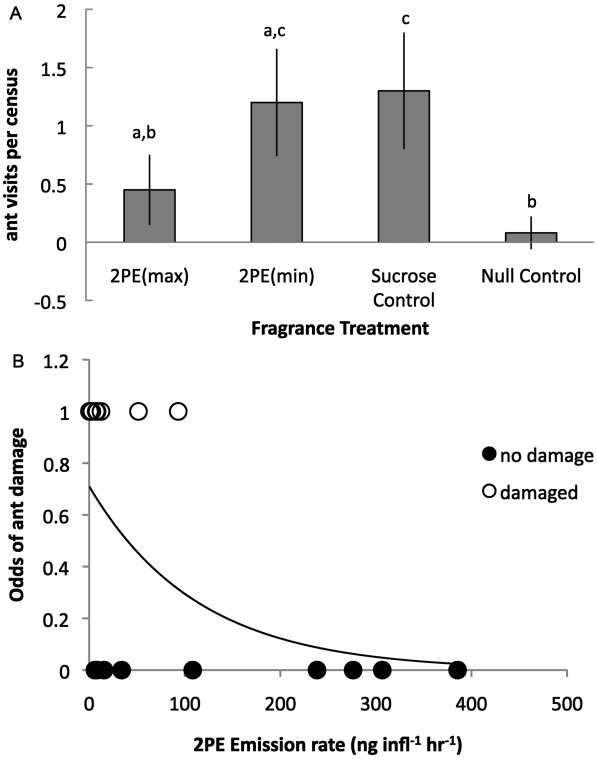

Figure 5: Impact of 2-phenylethanol (2PE) emission from flowers of *Polemonium viscosum* on (*A*) foraging frequency of ants and (*B*) ant damage to plants in the surrounding population. In *A*, bars show mean frequency of ants per inflorescence and error bars denote standard errors after augmentation with plain sucrose (control), no reward (probed with an empty pipette), $2PE_{max}$ (synthetic 2PE at a concentration of 0.0015% by volume) at full strength, or $2PE_{min}$ (a tenfold dilution of $2PE_{max}$). Treatments sharing superscripts do not differ at $P < .05$. In *B*, the curve shows the best-fit line from logistic regression. Open circles indicate plants with ant-damaged flowers and filled circles indicate neighboring plants without damage.

Table 2: Repeated-measures analysis of variance for mortality rate of *Formica neorufibarbis* under exposure to a range of concentrations for the nectar volatile compound 2-phenylethanol

Effect	*F*	df	*P*
Exposure time	116.34	4	<.0001
2-Phenylethanol concentration	57.19	3	<.0001
Ant nest	2.71	14	<.0063
Time × concentration	83.27	12	<.0001
Time × ant nest	.85	56	NS

Note: Each of 15 ant nests (random effect) was used to populate an entire replicate of the four treatments.

evidence suggests that bees can exploit nectar volatile compounds as remote (prelanding) cues for assessment of reward level (Heinrich and Raven 1978; Howell and Alarcon 2007). Our results fail to support this view for *P. viscosum*: although 2PE is present in floral nectar, its emission into inflorescence headspace does not correlate with reward availability at the flower or inflorescence scales (fig. 3). Furthermore, because the entire corolla emits 2PE independently of its presence in nectar, bumblebees could not predictably use the odor of 2PE to assess nectar rewards. Instead, bees avoid *P. viscosum* flowers with artificially enhanced 2PE emission (fig. 4).

Correlations of VOCs with other floral traits could evolve if, by signaling poor reward quality to antagonists, volatile compounds alter the ecological arena in which pollinator selection occurs. For example, if volatile compounds deter larcenists, then display traits in other signal modalities might evolve with less ecological cost (e.g., Galen 1999; Irwin et al. 2004). In skypilots, broad, short flowers achieve an advantage in bumblebee pollination that is partly offset by vulnerability to ant damage (Galen and Cuba 2001). The association between 2PE emission and flower shape could tip the balance against cheating, providing enemy-free space for the evolution of broader flowers under bumblebee selection. Ideally, we would have explored this idea by comparing selection on flower shape by ants under different levels of 2PE emission. However, in 2008, ants were very scarce on Pennsylvania Mountain, so our manipulation of VOCs could not adequately address their impact. Of course, covariation among traits may reflect mechanical or genetic constraints rather than adaptive evolution. For example, in *Medicago* (alfalfa), flower color alters microclimate, affecting volatile emission rates (Pecetti and Tava 2000). For skypilots, corolla flare may allow inner petal surfaces to experience turbid airflow in exposed alpine environments, promoting greater evaporation of volatile compounds. The relationship between floral form and fragrance volatility in the absence of specialized scent glands is poorly understood (see Effmert et al. 2006), but it may involve biophysical factors that are similar to those mediating pollen movement by wind (reviewed by Niklas 1985). Alternatively, pleiotropy (e.g., through regulatory elements; Lloyd et al. 1992) may integrate allelic variation in biosynthetic aspects of scent production with genes controlling floral form.

Impacts of 2PE Emission on Flower Visitors are Interaction Specific

Findings suggest that at high levels, 2PE is toxic to ants and repellent to both ants and bumblebees (figs. 4, 5). At intermediate doses (100–300 ng inflorescence^{-1} h^{-1}), 2PE, although not toxic in the laboratory, defends against ants in nature (fig. 5) and restricts NC by *Bombus balteatus* pollinators. Willmer et al. (2009) concur that VOCs may play a key role in deterring ants from flowers of temperate plant species. Nonetheless, it is difficult to predict how different flower visitors will respond to the same VOC. Roy and Raguso (1997) found that *Dialictus* bees were attracted to artificial flowers augmented with 0.1% 2PE in hexane. Similarly, Ashman et al. (2005) showed that preference of solitary bees for hermaphroditic flowers over female ones of *Fragaria virginiana* is due to the emission of 2 ng of 2PE flower^{-1} h^{-1} from pollen. In skypilot flowers, the olfactory repellence of $2PE_{max}$ (>300 ng inflorescence^{-1} h^{-1}) to ants and bumblebees appears to be a dosage-specific effect. Additional experiments are needed to test whether bumblebees prefer or simply tolerate very weak 2PE in *P. viscosum* flowers.

In specialized interactions, the same trait can deter cheaters and encourage more beneficial behavior from pollinators (e.g., Dunn et al. 2008; Junker and Blüthgen 2010), raising the question of what partner-specific coadaptations give rise to these different responses. For flower visitors of *P. viscosum*, body size and tongue length, two traits underlying differences in pollination effectiveness, may also determine exposure to 2PE. Because *Formica neorufibarbis* ants are much (10-fold) smaller than queens of *B. balteatus*, they must crawl completely into the corolla tube and spend more time navigating it to access nectar at its base. Both behaviors could exacerbate exposure to 2PE vapor. Larger bumblebees simply extend their proboscises into the corolla tube, restricting olfactory exposure to volatile compounds. Such coordination of plant defensive filters and pollination efficacy is predicted when visitors differ in costs as well as benefits to plant reproduction (Thomson 2003).

In skypilots, defense against cheaters reduces the ecological cost in seed loss for the pollination mutualism, while discouragement of nectar uptake by pollinators reduces potential water and carbohydrate costs. Water in

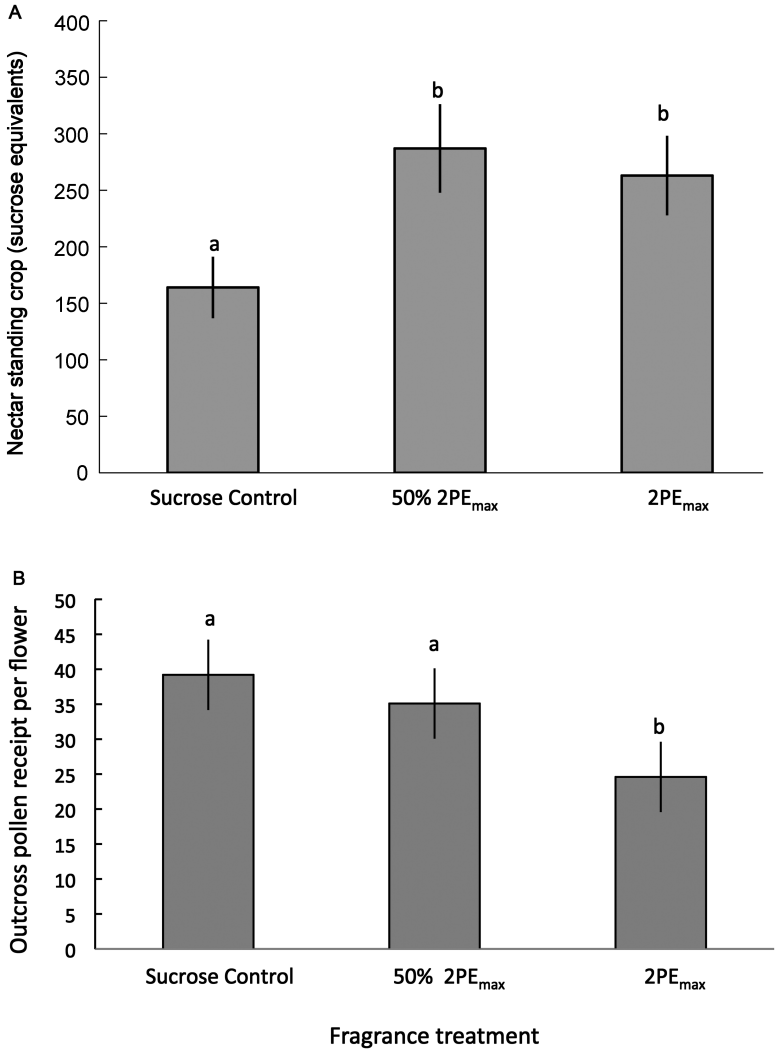

Figure 6: Standing crop of nectar (in sucrose equivalents) per flower (*A*) and outcross pollen receipt per flower (*B*) in *Polemonium viscosum* subjected to floral fragrance manipulation. Groups sharing superscripts do not differ at $P < .05$. Presence of 2-phenylethanol (2PE) in nectar significantly increased standing crop ($P < .05$) of both $2PE_{max}$ (synthetic 2PE at a concentration of 0.0015% by volume)-supplemented flowers and 50% $2PE_{max}$ (a 50% dilution of synthetic 2PE at a concentration of 0.0015% by volume)–supplemented flowers (*A*), but it significantly decreased pollen receipt for $2PE_{max}$-supplemented flowers only ($P < .05$; *B*).

particular is limiting for *P. viscosum*, with drought generating a trade-off between floral display traits and seed production (Galen et al. 1999). When traits alter the behavior of a pollinator without eliminating visitation, their roles as sanctions seem plausible. Chemical sanctions may be widespread in plant-pollinator mutualisms, but thus far they have been viewed mainly as defenses (Adler 2000). Honeybees foraging on almond nectar consume sucrose solutions with dilute concentrations of amygdalin more rapidly than they consume concentrated amygdalin solutions (London-Shafir et al. 2003). Nicotine reduces NC while encouraging pollinator movement between plants of *Nicotiana attenuata* (Kessler et al. 2008). The efficacy of sanctions may vary with reward level: Gegear et al. (2007) found that bumblebee tolerance of the nectar alkaloid gelsamine increased with sucrose reward. Benefits of sanctions also likely depend on pollinator efficiency. If pollination saturates at low NC, behavior modification through sanctions may optimize pollinator service per unit investment in partner attraction (Kessler and Baldwin 2006). This seems likely for skypilots, because *B. balteatus* are highly efficient pollinators (Galen and Stanton 1989). Nonetheless, our study focuses solely on female components of fitness, and it assumes that moderate emission of

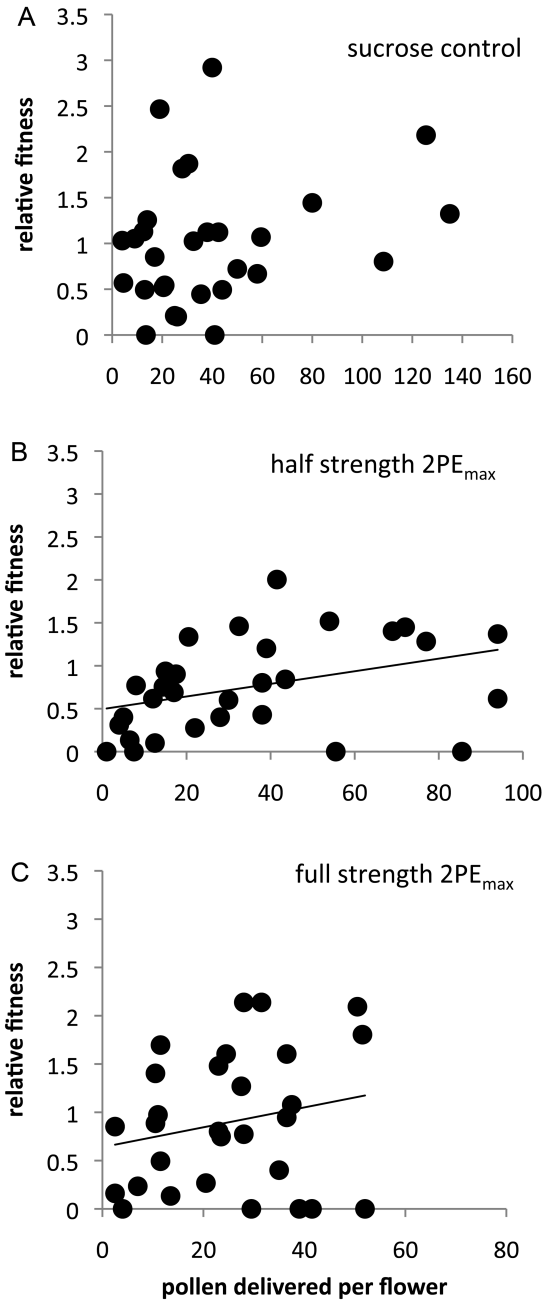

Figure 7: Variation among fragrance treatments in the relationship between outcross pollen receipt and fitness: sucrose control (*A*); augmentation with 50% 2PE$_{max}$ (a 50% dilution of synthetic 2-phenylethanol at a concentration of 0.0015% by volume; *B*), and augmentation with 2PE$_{max}$ (synthetic 2-phenylethanol at a concentration of 0.0015% by volume; *C*). In *A*, the slope of the best-fit line is not significantly different from 0 ($P > .20$), indicating negligible selection on traits associated with pollen delivery; however, slopes for best-fit lines in *B* and *C* are 0.0089 ± 0.002 ($P < .0047$) and 0.0203 ± 0.007 ($P < .0084$), respectively, which is consistent with the idea that pollinator deterrence by 2PE causes increasing selection on such traits.

2PE would not reduce male function. Further work is needed to test this idea.

2PE as a Modulator of Pollinator-Mediated Selection in other Sensory Modalities

As we increased 2PE emission, pollen delivery decreased, generating an opportunity cost of defense in lost pollination for 2PE$_{max}$-supplemented flowers that was negligible in 50% 2PE$_{max}$-supplemented flowers (fig. 6*B*). Additionally, the relationship between pollen delivery and seed set became increasingly tight, promoting pollinator selection on other floral cues. Results indicate that selection pressures on floral traits may be driven by emission rate of volatile defenses, promoting correlated evolution of defenses and floral attractants (Herrera et al. 2002; Kessler and Halitschke 2009). Interestingly, the observed trade-off between costs and benefits of the pollination mutualism at a high 2PE emission rate in skypilot flowers is opposite that envisioned by Raguso (2004*a*) and Theis et al. (2007). They suggested that stronger fragrances would attract pollinators at a cost in apparency to antagonists (see Baldwin et al. 1997). Our results indicate otherwise, suggesting that pollinator attraction should not be a default assumption in studies of highly fragrant flowers (see Ômura et al. 2000).

Conclusions

Plant volatile compounds have multiple sources of variation in function: in addition to quantitative variation, identities of source organs and their developmental phase may alter ecological impacts of VOCs. Our results suggest that for *Polemonium viscosum*, high 2PE emission provides an olfactory deterrent when emitted into inflorescence headspace and a gustatory deterrent when dissolved in nectar, restricting approach and consumption behaviors of ants and bumblebees alike. Similarity in olfactory and gustatory responses to VOCs may reflect the architecture of sensory pathways in plant-feeding insects rather than selection for coordinated defense. For example, aromatic compounds in pine resin attract and stimulate feeding in specialized insect herbivores to the detriment of the host (Harborne 1993). In contrast, changes in emission rate appear to fine-tune VOC function. In *P. viscosum* flowers, results range from a potentially lower resource cost for a given level of pollinator service to higher ecological cost with more effective defense against larceny. Perhaps the sensitivity of 2PE function to dosage explains why it is so widespread in morphologically generalized flowers. Such sensitivity provides a powerful mechanism for modifying ecological interactions using a simple biochemical cue.

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