FLORAL BIOLOGY OF NORTH AMERICAN *OENOTHERA* SECT. *LAVAUXIA* (ONAGRACEAE): ADVERTISEMENTS, REWARDS, AND EXTREME VARIATION IN FLORAL DEPTH[1,2]

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ABSTRACT

We studied the floral biology of five North American members of *Oenothera* L. sect. *Lavauxia* (Spach) Endl. (Onagraceae L.) in field and common greenhouse settings. *Oenothera* sect. *Lavauxia* floral morphology ranges from small, cleistogamous flowers (*O. flava* subsp. *flava* (A. Nels.) Garrett in Garrett) to some of the longest-tubed flowers in North America (*O. flava* subsp. *taraxacoides* (Wooton & Standl.) W. L. Wagner). Our goal was to compare qualitative and quantitative aspects of floral advertisement and reward among taxa in section *Lavauxia*. All taxa are night-blooming and self-compatible, have yellow petals with ultraviolet reflectance, and produce floral scents dominated by nitrogenous compounds and monoterpenes. Methyl nicotinate is present in the fragrances of all taxa of section *Lavauxia* regardless of flower size or putative mating system. Because this floral volatile is largely absent from other *Oenothera* species, we hypothesize that it is a synapomorphy for section *Lavauxia*. The rare *O. acutissima* W. L. Wagner, which is endemic to the Uintah Mountains, is polymorphic for odors dominated by linalool- or ocimene-derived compounds. Field observations in its type locality in northeastern Utah, U.S.A., revealed frequent floral visitation by crepuscular hawkmoths during the first 1.5 hours after anthesis, a pattern common to *O. flava* subsp. *taraxacoides* and other large-flowered *Oenothera* throughout western North America. Quantitative aspects of floral advertisement (flower size, scent emission) and reward (nectar volume) are dramatically reduced in putatively autogamous taxa (*O. flava* subsp. *flava*, *O. triloba* Nutt.), whereas qualitative aspects (flower color, scent, and nectar chemistry) remain comparable. All taxa could be distinguished through ordination of characters related to flower size, herkogamy, and scent chemistry. Extreme nectar tube length variation across the range of *O. flava* renders this an excellent model system for measuring the costs and mechanisms of shifts between outcrossing and autogamy.

Key words: biogeography, floral scent, fragrance, nectar, night-blooming, *Oenothera*, Onagraceae, pollination.

The genus *Oenothera* L., with about 120 species native to the Americas, has long served as a model system for the study of evolutionary pattern and process in flowering plants (Raven, 1979, 1988, and references therein). The basic floral ground plan is fairly conserved across the genus with tetramerous flowers, white, pink, or yellow-colored petals, and a long, tubular hypanthium. However, there have been numerous evolutionary shifts between outcrossing and autogamy, nocturnal and diurnal anthesis, annual and perennial habit, and xeric and mesic habitat specialization in this genus. Recent efforts to establish well-supported phylogenetic hypotheses for this genus and its closest relatives in the Onagraceae L. (Levin et al., 2003a, 2004) have been motivated in part by the goal of understanding the frequency of these shifts and

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their underlying processes. In parallel, we and our colleagues have initiated studies of evolutionary transitions in reproductive biology and life history strategies in several monophyletic sections of *Oenothera*, including section *Anogra* (Spach) Engl. (Evans et al., 2005), section *Calylophus* (Spach) Torr. & A. Gray, section *Pachylophus* (Spach) Endl. (R. A. Raguso, in prep.), and in *Gaura* L. (Clinebell et al., 2004).

Here we examine the floral biology of the North American species of *Oenothera* sect. *Lavauxia* (Spach) Endl. The recognition of *Lavauxia* as a distinctive entity dates from early in the study of Onagraceae, either as a genus (Spach, 1835: 367; Raimann, 1893) or a subgenus of *Oenothera* (Endlicher, 1840: 1190; Jepson, 1923–1925: 680). As monographed by Munz (1930) and amended by Wagner (1981, 1986), *Oenothera* sect. *Lavauxia* presently consists of three yellow-flowered North American species, *O. acutissima* W. L. Wagner, *O. flava* (A. Nels.) Garrett in Garrett (including *O. flava* subsp. *flava* and *O. flava* subsp. *taraxacoides* (Wooton & Standl.) W. L. Wagner), and *O. triloba* Nutt., and two white-flowered South American species, *O. acaulis* Cav. and *O. centauriifolia* (Spach) Steud. *Oenothera triloba* is a small-flowered, self-compatible spring annual or biennial herb native to south-central U.S.A. This species is broadly parapatric with the western members of section *Lavauxia*, which are perennial herbs occurring throughout montane western North America into central Mexico (Wagner, 1986). Typical *O. flava* is a small-flowered, self-compatible plant with a very broad distribution, whereas *O. flava* subsp. *taraxacoides* is a large-flowered plant known only from disjunct, high-elevation populations in southwestern U.S.A. and northern Mexico. Wagner (1986) combined these two taxa as subspecies of *O. flava* on the grounds that small-flowered, autogamous individuals and large-flowered, modally outcrossing individuals represent extreme phenotypes of a single species that intergrade extensively in zones of contact. In contrast, Wagner (1981) segregated large-flowered, self-compatible but presumably outcrossing plants endemic to the mountains of northwestern Colorado and northeastern Utah, U.S.A., as a new species, *O. acutissima* (Wagner, 1981), citing several vegetative and reproductive autapomorphies that distinguish it from *O. flava*, with which it co-occurs. Subsequently, *O. acutissima* was treated as a variety of *O. flava* by Welsh (1986), but the poor viability (and sterility) of artificial hybrids between *O. acutissima* and *O. flava* (Wagner, 1981) compels us to recognize *O. acutissima* sensu Wagner (1986). Regardless of nomenclatural treatment, the western members of *Lavauxia* are remarkably variable in floral characters, from the short-tubed (less than 6 cm), often cleistogamous *O. flava* subsp. *flava* to the extremely long-tubed (up to 18 cm) *O. flava* subsp. *taraxacoides*, which are among the deepest flowers in the North American flora (Gregory, 1964; Grant, 1983; see Fig. 1).

We studied floral traits (i.e., color, morphology, scent, nectar) associated with pollinator attraction and reward in only the North American species of section *Lavauxia*, because the South American species (*Oenothera acaulis* and *O. centauriifolia*) were not available to us at the time of study. Given the biogeographic, morphological, and taxonomic patterns described above, we sought to address several questions with such data.

(1) Are floral scent and nectar (or components thereof) reduced or absent in the small-flowered, putatively autogamous flowers of *O. triloba* and *O. flava* subsp. *flava*? The dramatic difference in visual display between these and outcrossing taxa (*O. acutissima*, *O. flava* subsp. *taraxacoides*) suggests reduced selective pressure for floral advertisement and reward when self-pollination is the predominant strategy.

(2) Regarding components of floral fragrance, are plants of the two subspecies of *O. flava* more similar to each other than to plants of *O. triloba* and *O. acutissima* or does fragrance chemistry mirror pollination strategy, rather than phylogenetic affinity?

(3) Do individuals from two isolated populations of *O. flava* subsp. *taraxacoides* share a uniform phenotype or do floral traits vary distinctively between populations? The mountaintop distribution of these plants suggests that their floral traits may have diverged, either through genetic drift or local adaptation (Slentz et al., 1999; Boyd, 2002).

(4) Are flowers of the narrow endemic *O. acutissima* visited by the same spectrum of medium- to long-tongued hawkmoths that pollinate other *Oenothera* throughout western North America (e.g., Wagner et al., 1985)? Pollinator data are especially needed for this geographically restricted entity, which could be vulnerable to extinction due to habitat loss or modification.

The results of this study will provide a baseline for quantitative investigations of the costs, benefits, and evolution of floral advertisement and reward in this fascinating lineage.

Methods

Plant care

Ten to 60 individuals of each taxon were cultivated in a greenhouse at the University of South Carolina, Columbia, South Carolina, where they were studied in

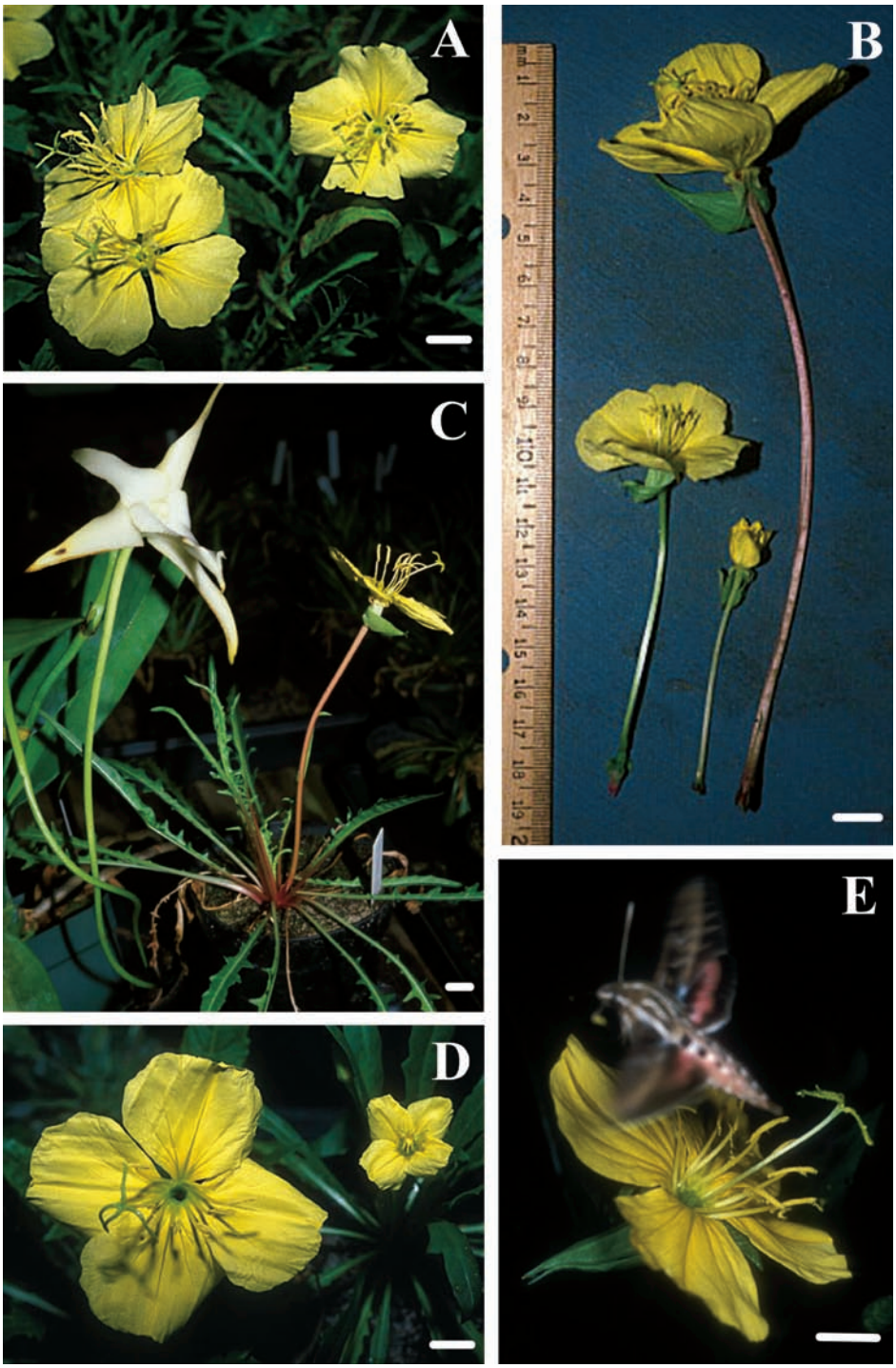

Figure 1. Floral variation in *Oenothera* sect. *Lavauxia*. —A. Newly opened flowers of *O. triloba*; note lack of strong herkogamy. —B. Nectar tube (hypanthium) length variation from putative autogamy (*O. triloba*, left, *O. flava* subsp. *flava*, center) to putative outcrossing (*O. flava* subsp. *taraxacoides*, right). —C. Side-by-side comparison of nectary depth between *O. flava* subsp. *taraxacoides* (right) and *Angraecum sesquipedale* Thouars (left), Darwin's Malagasy Star Orchid. —D. Differences in floral diameter between *O. flava* subsp. *taraxacoides* (left) and *O. flava* subsp. *flava* (right). —E. *Hyles lineata* leaving flower of *O. flava* subsp. *taraxacoides* in Sacramento Mtns., Otero Co., New Mexico. Photos in A–D by R. A. Raguso. Photo in E by M. S. Singer, with permission. All scale bars = 1 cm.

March–April 2002. *Oenothera acutissima* and *O. triloba* plants were transplanted from the field in July 2001 and March 2002, respectively. Plants from three populations of *O. flava* were grown from seed germinated in September 2001. Plants were grown in 60:40 potting soil:sand mix, bottom-watered daily, and fertilized with Miracle-Gro (15% N:30% P:15% K) once per month for the duration of the study. Voucher specimens were deposited at ARIZ, US, and USCH; collection numbers and source localities are provided in Appendix 1.

VISUAL REFLECTANCE

The spectral properties of newly opened flowers were measured using a Spectral Instruments (Tucson, Arizona) SI-440 CCD array UV-VIS Spectrophotometer, connected by a 400 μm fiber optic probe to a Labsphere (North Sutton, New Hampshire) 9 cm ID integration sphere. We used a 10 W tungsten light source to collect reflectance data from 350 (ultraviolet) to 700 nm (infrared) wavelengths from freshly excised flowers placed over a black felt cloth background. Spectral reflectance from upper (adaxial) petal surfaces was measured at both distal and proximal positions, as we anticipated a central "target pattern" of contrasting ultraviolet-absorbance and reflectance across the flower (Dement & Raven, 1974). Data were collected as percent reflectance relative to a white pigment (Duraflect) standard, with negligible (0.5%–1% of standard) signal contributed by the black cloth background.

NECTAR AND FLORAL MORPHOLOGY

Nectar volume was collected from at least 10 newly opened flowers of each species at dusk, using 5 or 10 μl glass capillary micropipettes. Because of the unusual length and narrowness of the floral tubes, nectar collection required floral dissection with a razor, but care was taken to avoid contaminating or diluting nectar with other plant fluids (Cruden et al., 1983). We measured sugar concentration in 5 μl aliquots from each sample with a hand-held refractometer (Leica, Brix50) designed to measure 0%–50% sucrose equivalents by weight, in units of 0.25%. Floral morphological measurements were taken on freshly excised flowers. We used a metric ruler to measure floral diameter (the greatest distance across the open corolla limbs, perpendicular to the nectar tube), tube flare (diameter across the mouth of the nectar tube, not including corolla limb), floral depth (length of nectar tube from mouth to ovary), stamen length and style length (distance from the ovary to the longest stamen and to the stigma, respectively), and herkogamy (difference in length between stigma and longest anther), to the nearest 0.5 mm. Fresh mass was recorded after nectar removal using a Mettler analytical balance, to the nearest 0.001 g. Flowers then were dried at 50°C for 24 hr., and dry mass was recorded on the same balance. Because plants of each species opened one to two flowers per night, most floral measures included flowers from different individuals.

FLORAL SCENT COLLECTION AND ANALYSIS

Floral odors were collected using two complementary methods. The first method, solid-phase microextraction (SPME) (Zhang & Pawliszyn, 1993), was combined with gas chromatography–mass spectrometry (GC-MS) to optimize the quality of mass spectra for scent compound identification. Headspace bags were prepared from Reynolds (nylon resin) oven bags using an impulse heat sealer, as described by Raguso et al. (2003a). Bags were placed over living, uncut flowers and cinched with plastic ties. Parallel collections were made from *Oenothera* leaves and greenhouse air in order to identify vegetative and ambient contaminants in floral samples. In other analyses, 5–10 μl samples of nectar were spotted onto filter paper wedges, sealed within headspace bags, and analyzed for nectar odors as described by Raguso (2004). All samples were equilibrated for 15 minutes, then allowed to adsorb onto a 100 μm polydimethylsiloxane (PDMS) SPME fiber for an additional 15 minutes, immediately followed by GC-MS analysis (see below). Results were unchanged when longer equilibration or exposure times were used.

The second method, dynamic headspace trapping (Raguso & Pellmyr, 1998), was used to quantify volatile compound emission rates during the first few hours after anthesis. Floral volatiles were concentrated within headspace bags (ca. 500 ml volume) and trapped on adsorbent cartridges using Supelco (Berwick, Pennsylvania, U.S.A.) personal air sampler vacuum pumps. Pasteur pipettes were packed with 100 mg of Super Q (80–100 mesh) adsorbent (Alltech Associates, Waukeegan, Illinois, U.S.A.) between plugs of quartz wool, and headspace air was pulled over the flowers and into the adsorbent trap at a flow rate of ca. 250 ml/min. Fragrance was collected for 6–8 hr. after anthesis (dusk in all taxa) in a protected area outside the greenhouse. Additional scent collection was performed for *Oenothera acutissima* plants growing at the type locality (see Appendix 1). Trapped fragrance was eluted with 3 ml of high-purity hexane and stored at –20°C in Teflon-capped borosilicate glass vials. Before GC-MS analysis, we used a flow of gaseous N_2 to concentrate samples to 75 μl, then

added 5 µl of 0.03% toluene (16 ng) as an internal standard. One µl aliquots of each sample were injected into a Shimadzu GC-17A equipped with a Shimadzu QP5000 quadrupole electron impact MS (Shimadzu Scientific Instruments, Columbia, Maryland, U.S.A.) as a detector. All analyses were done using splitless injections on a polar GC column (diameter 0.25 mm, length 30 m, film thickness 0.25 µm (Econo Cap's carbowax coating, known as EC WAX); Alltech Associates), as described by Raguso et al. (2003a). SPME fibers were directly injected into the GC injection port at 240°C and were analyzed using the GC-MS parameters described above. Compounds were tentatively identified using computerized mass spectral libraries (Wiley Registry of Mass Spectral Data, National Institute of Standards and Technology, and Robert Adams' libraries (> 120,000 mass spectra)). Chiral GC was not available to us, so compounds with chiral carbons (e.g., α-pinene) were assumed to represent racemic mixes. Whenever possible, GC peak identities were verified using co-retention with known standards on both EC WAX and EC-5 GC columns. Peak areas were integrated using Shimadzu's Class-5000 software and were quantified by comparison with the internal standard. Total scent emission rates (per hour) were calculated as sums of all peak areas, converted to nanograms using the internal standard, and expressed per flower, and per gram fresh and dry floral mass.

COMPARISON OF SCENT COMPOSITION

We compared variation in fragrance profiles within and among taxa using the relative amounts of floral volatiles. For each individual, we calculated the proportion of total scent contributed by each compound and standardized these data to *z* scores, which have means of zero and variances of one. These values were used to compute a matrix of pairwise dissimilarity values between all individuals in the study (Euclidean distance). Pairwise dissimilarity values among conspecific (and co-occuring) individuals were compared to those between heterospecific (or disjunct) individuals using the Wilcoxon rank sum test (SPSS 11.5). This constituted a two-tailed test of the null hypothesis that mean ranks of pairwise dissimilarity values between taxa or populations did not differ from mean ranks of pairwise dissimilarity values within groups (see Levin et al., 2001, 2003b).

FACTOR ANALYSIS OF FLORAL PHENOTYPE

Ordination was used to determine whether combinations of floral attributes were characteristic for different section *Lavauxia* taxa. Floral morphological measurements including floral diameter, tube flare, floral depth, herkogamy, dry mass, and total number of scent compounds were normally distributed and were not transformed. Scent emission rates (ng scent per flower per hour) were calculated for the sums of all (1) nitrogenous, (2) ocimene-derived, and (3) linalool-derived compounds; these rates were then natural log-transformed ($y = \ln(x+1)$) for analysis. These data were combined for principal component analysis using the varimax rotation option (SPSS 11.5), in which factors with eigenvalues greater than unity were retained. Discriminant function analysis (SPSS 11.5) was then used to determine whether factor loadings could be used to correctly assign individuals to their source population or taxon.

POLLINATOR OBSERVATIONS FOR *OENOTHERA ACUTISSIMA*

Floral visitation to a natural population of *Oenothera acutissima* was observed at the type locality in Daggett Co., Utah, U.S.A. (Appendix 1). Several hundred plants were found in bloom along with flowering individuals of *Achillea* L., *Geum* L., *Iris* L., *Opuntia* Mill., and *Potentilla* L. species in a moist meadow at the margin of a *Pinus ponderosa* Douglas forest. Individual flowers were watched on the evenings of 11 June 2001 and 25–28 June 2003, for a total of 19 observer hours. Insect visitors were photographed and collected for identification when necessary. Voucher specimens remain in the possession of the lead author. To relate flower visitation to ambient conditions, light levels and temperatures were measured at the study site at one- to three-minute intervals. Light levels were measured in cd/m^2 with a highly sensitive silicon detector attached to a radiometer (International Light, IL1700). The time for sunset was obtained from the NASA database (http://aa.usno.navy.mil/).

Results

FLOWER SIZE AND COLOR

Flower size varied dramatically among taxa. The small, short-tubed flowers of *Oenothera flava* subsp. *flava* did not open fully in the greenhouse or the field. When buds were forced open on the evening of floral maturity (determined by color and firmness), the mean floral diameter was only 25 mm (Table 1). Flowers of *O. triloba* were twice this large on average, whereas flowers of *O. flava* subsp. *taraxacoides* and *O. acutissima* measured 60–85 mm in diameter. The nectar tubes (hypanthia) of all taxa are long compared with other North American flowers and vary markedly among taxa and populations (Fig. 1). Flowers of *O. flava* subsp. *taraxacoides* from Arizona were 1.1–1.7-

Table 1. Flower morphological measurements and nectar characteristics; plants from localities in Appendix 1 were grown under common greenhouse conditions. AZ, Arizona; NM, New Mexico; OA, *Oenothera acutissima*; OFF, *Oenothera flava* subsp. *flava*; OFT, *Oenothera flava* subsp. *taraxacoides*; OT, *Oenothera triloba*; v/v, volume per volume.

Measurement, mean ± SEM (range, N)	OA	OT	OFF	OFT	
				NM	AZ
Floral diameter (mm)	86.3 ± 1.1 (80–98, 21)	42.4 ± 0.9 (32–53, 28)	24.4 ± 0.8 (19–32, 19)	65.0 ± 2.2 (49–78, 14)	82.9 ± 2.2 (65–101, 18)
Corolla flare (mm)	7.7 ± 0.2 (6.5–9.0, 21)	4.2 ± 0.1 (3.0–5.5, 28)	2.8 ± 0.1 (1.5–3.5, 19)	7.3 ± 0.3 (6.0–8.0, 8)	7.4 ± 0.2 (6–8.5, 18)
Floral depth (mm)	96.7 ± 4.0 (75–135, 21)	82.3 ± 1.7 (66–97, 28)	57.8 ± 1.7 (43–71, 19)	166.1 ± 3.8 (150–186.5, 14)	177.5 ± 3.6 (155–203, 18)
Stamen length (mm)	128.1 ± 4.6 (100–171, 21)	97.9 ± 2.1 (80–116, 28)	66.7 ± 1.9 (51–87, 19)	190.4 ± 4.3 (170–216, 14)	201.8 ± 4.1 (176–238, 18)
Style length (mm)	141.5 ± 4.7 (113–184, 21)	98.4 ± 2.1 (82–116, 28)	63.7 ± 1.9 (51–72, 19)	196.6 ± 4.0 (177–227, 14)	212.3 ± 3.9 (184–247, 18)
Herkogamy (mm)	13.4 ± 0.7 (10–21, 21)	0.6 ± 0.3 (−2–4, 28)	−2.9 ± 0.7 (−9–3, 19)	6.2 ± 1.1 (0–13, 14)	10.5 ± 1.0 (0–16, 18)
Nectar standing crop (μL)	9.7 ± 1.3 (3.5–15, 8)	11.5 ± 2.0 (4.3–22, 10)	2.3 ± 0.6 (0.8–5.0, 11)	11.2 ± 1.3 (6.2–15.9, 6)	40.9 ± 2.9 (26–56, 9)
Nectar concentration (% v/v)	25.4 ± 0.8 (19–30, 13)	27.4 ± 0.8 (22–31, 10)	25.9 ± 0.9 (23.5–32, 8)	26.6 ± 0.7 (23–30, 12)	25.2 ± 0.9 (16.5–31.5, 17)
Fresh mass (g)	1.26 ± 0.02 (1.21–1.29, 3)	0.32 ± 0.02 (0.19–0.44, 14)	0.17 ± 0.01 (0.14–0.25, 12)	1.55 ± 0.07 (1.22–1.81, 8)	1.92 ± 0.07 (1.63–2.38, 9)
Dry mass (g)	0.14 ± 0.01 (0.12–0.16, 3)	0.04 ± 0.003 (0.02–0.06, 14)	0.02 ± 0.001 (0.02–0.03, 12)	0.18 ± 0.006 (0.16–0.21, 8)	0.23 ± 0.01 (0.16–0.35, 9)

fold larger than *O. flava* subsp. *taraxacoides* from New Mexico in corolla diameter, nectar tube length, and anther-stigma separation. Stigmas of *O. acutissima* flowers were exserted the greatest mean distance beyond the anthers (positive herkogamy: 13.4 mm), whereas flowers of *O. flava* subsp. *flava* were consistently negatively herkogamous, with the open stigma lobes positioned 3 mm below the dehiscing anthers, on average. Flowers of *O. triloba* varied continuously from negative to positive herkogamy (Table 1).

All taxa produced flowers that were yellow to the human eye. Flowers of *Oenothera triloba* appeared to be the least saturated yellow (Fig. 1) but were not less reflective than other taxa in yellow wavelengths (Fig. 2). The inner petal surfaces of *O. acutissima* flowers were uniformly reflective above 500 nm but absorbed light of shorter wavelengths, including ultraviolet (UV) (Fig. 2). In all other taxa, the proximal portion of each petal (i.e., the central region of the corolla) absorbed UV, whereas the distal petal regions reflected strongly from 350 to 380 nm, creating a pattern of UV contrast at the flower's center (Fig. 2). Dissection of buds revealed this pattern to be present at least 24 hr. before anthesis (data not shown).

NECTAR VOLUME AND CONCENTRATION

Nectar volumes per flower varied considerably between taxa and populations, with means ranging from 2.3 μl in the cleistogamous *Oenothera flava* subsp. *flava* to more than 40 μl in *O. flava* subsp. *taraxacoides* from Arizona (Table 1). Surprisingly, the flowers of *O. triloba* produced nectar volumes comparable to those of the much larger flowers of *O. acutissima* and *O. flava* subsp. *taraxacoides* from New Mexico. On the other hand, flowers of *O. flava* subsp. *taraxacoides* from Arizona produced at least four-fold more nectar than those of any other taxon studied (Table 1), despite being grown on the same greenhouse bench. Nectar sugar concentrations and ranges were nearly identical (25%–27.5% volume per volume dissolved sucrose equivalents) among all taxa, including *O. flava* subsp. *flava*.

FLORAL SCENT EMISSION RATES AND CHEMICAL COMPOSITION

Odor emission rates varied dramatically, with large-flowered taxa producing up to 20-fold more scent per flower than small-flowered taxa (Fig. 3). Flowers of *Oenothera flava* subsp. *flava* and *O. triloba* had the lowest emission rates and were only half as strongly scented as large-flowered taxa even when standard-

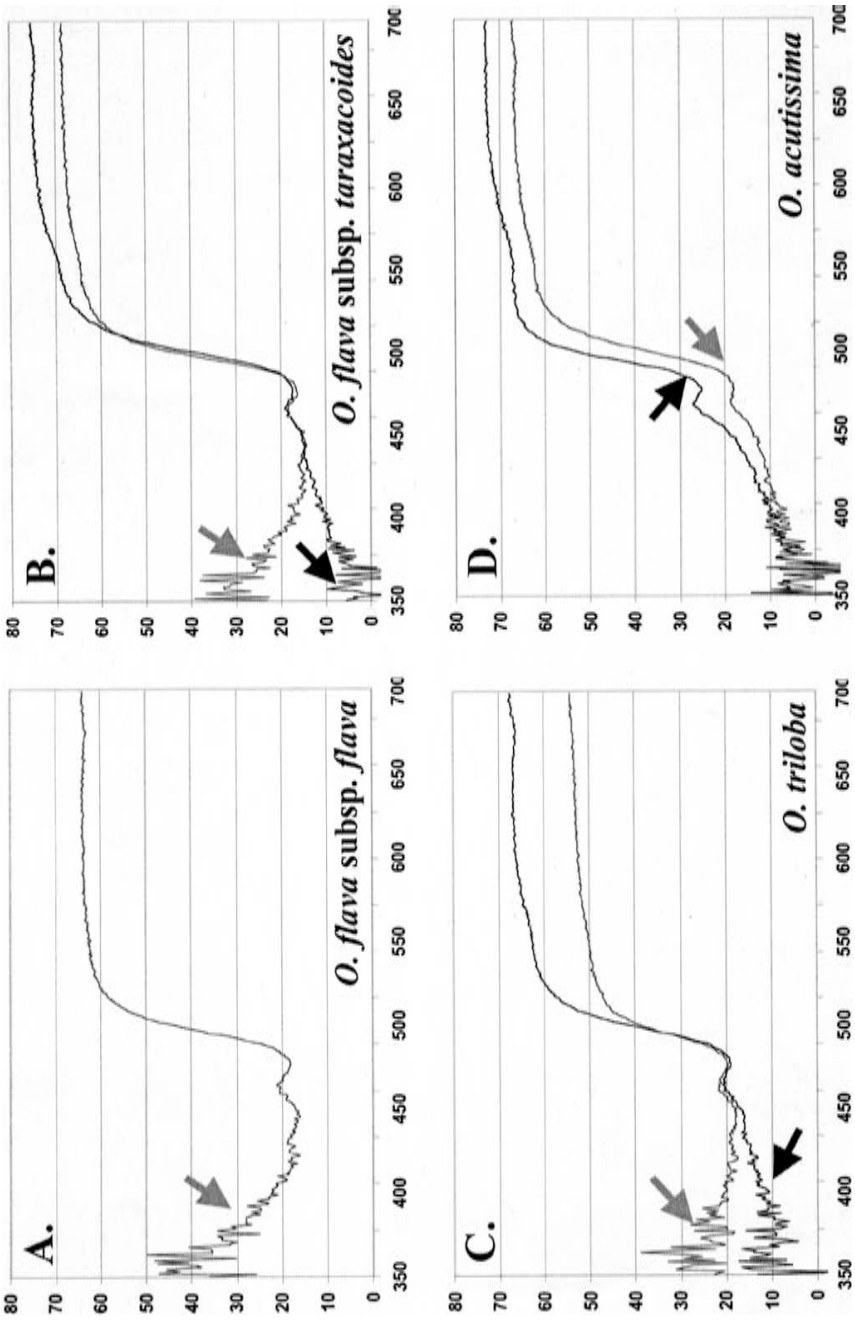

Figure 2. Spectral reflectance curves for upper (adaxial) petal surfaces, from 350–700 nm in wavelength. The y-axis represents percent reflectance, compared to white standard. In each panel, the curve marked by the grey arrow shows reflectance over a 1 cm diameter (0.78 cm^2 area) disc within the proximal (basal) region of the corolla lobe, and the curve marked by the black arrow shows reflectance over a similar area within the distal region of the petal. Petals of *Oenothera flava* subsp. *flava* (panel A) were too small to dissect in this way. *Oenothera flava* subsp. *taraxacoides*, *O. triloba*, and *O. acutissima* are shown in panels B, C, and D, respectively.

ized for fresh and dry floral mass. Although flowers of *O. flava* subsp. *taraxacoides* from Arizona were 20% larger than those from New Mexico, the latter were twice as strongly scented as the former, both per flower and per unit floral mass (Fig. 3). Emission rates of greenhouse-grown *O. acutissima* plants were comparable to those of *O. flava* subsp. *taraxacoides* from New Mexico but were less than half as strong as those measured from *O. acutissima* in the field (Fig. 3).

Fifty-four volatile organic compounds were detected in floral headspace, of which 32 were identified using known standard compounds or commercially available essential oil blends. Scent compounds represented several biosynthetic categories, including mono- and sesquiterpenoids, benzenoid (aromatic) compounds, fatty acid derivatives and, especially, nitrogenous compounds derived from amino acids (Appendix 2). The nitrogenous aldoximes, nitro- and nitrile compounds derived from valine, leucine, isoleucine, and phenylalanine (Fig. 4), were present in all taxa, whereas sesquiterpenoids were limited to *Oenothera acutissima*. As a class, the nitrogenous compounds accounted for 67%–96% of total scent emissions in all *O. flava* populations, but only 9.3% and 37% of emissions from *O. triloba* and *O. acutissima*, respectively. The scents of *O. triloba* and *O. acutissima* were dominated (62%–72% of emissions) by monoterpenoids (Fig. 5). Two additional nitrogenous compounds, nicotinic acid methyl ester and 1-pyrroline (Fig. 4), were present in all taxa surveyed. The monoterpene trans-β-ocimene constituted 30%–44% of total emissions in most taxa but was a minor scent component ($< 4\%$) in *O. flava* subsp. *flava* and *O. flava* subsp. *taraxacoides* from Arizona (Fig. 5). SPME analysis revealed that methyl benzoate and 1-pyrroline were present exclusively in the floral nectar of all taxa, whereas most other compounds were emitted by petals and other flower parts (data not shown).

Taxa with large, putatively outcrossing flowers produced the most complex fragrances, with 24–27 compounds in *Oenothera flava* subsp. *taraxacoides* and 34–37 compounds in *O. acutissima*, including the fruity-scented isoamyl alcohol and three of its esters (Appendix 2, Fig. 6). In contrast, the small, putatively autogamous flowers of *O. flava* subsp. *flava* and *O. triloba* produced the least chemically complex odors (13–21 compounds). This result is not an artifact of too little floral tissue being used for odor analysis, as some samples included up to five flowers. When the number of scent compounds of *O. triloba* was regressed against total floral mass per sample, odor complexity was not significantly correlated with sample floral mass (linear regression, $R^2 = 0.001$, $P = 0.94$).

Floral scent blends generally were taxon-specific (Table 2). Fragrance composition between isolated populations of *Oenothera flava* subsp. *taraxacoides* was similar, except for the presence of trace amounts ($< 1\%$ of total emissions) of linalool and its furanoid oxides in plants from Arizona and substantial amounts (nearly 30%) of trans-β-ocimene in those from New Mexico. The exception to the pattern of taxon-specific odors was *O. acutissima*, in which the presence of two distinct scent phenotypes or "chemotypes" resulted in sufficient within-taxon variation such that the floral fragrance produced by conspecific individuals was not significantly more similar than that produced by flowers of different species (Table 2). The floral scent of individual plants was dominated either by linalool or by trans-β-ocimene, to the near exclusion of the other compound. Three of eight field-collected fragrance samples were linalool-dominated (mean ± SEM = 40.7 ± 4.2% of total emissions) with little trans-β-ocimene (6.0 ± 3.0%), whereas trans-β-ocimene was the dominant component of the remaining five plants (62.4 ± 2.6%), in which linalool was nearly absent (0.2 ± 0.06%). Similar patterns were observed in greenhouse-grown plants, none of which emitted large amounts of both compounds. Compositional differences between greenhouse- and field-grown *O. acutissima* were restricted to minor, derivative components. Field-grown plants produced unique linalool-derived compounds, whereas greenhouse-grown plants produced putative ocimene-derivatives, increased amounts of β-caryophyllene, and related sesquiterpenes not detected in the fragrance of field-grown plants.

FACTOR ANALYSIS OF FLORAL ADVERTISEMENTS AND REWARDS

Principal component analysis identified two factors with eigenvalues greater than unity, accounting for 85.8% and 12.5% of total sample variance, respectively. Floral dimensions above the nectar tube (floral diameter, corolla flare, and herkogamy) were highly correlated and loaded positively on factor 1, along with the total number of scent compounds and emission rates of linalool- and ocimene-derived scent compounds (Table 3, Fig. 7). This dimension clearly separated *Oenothera acutissima* from *O. flava* subsp. *flava* and *O. triloba* in floral phenotype space, and separated most individuals of *O. flava* subsp. *taraxacoides* from Arizona and New Mexico into their respective populations (Fig. 7). Floral depth, dry floral mass, and the emission of nitrogenous scent compounds had the strongest loading scores on factor 2, for which the only character with negative loadings was the emission of linalool and related scent compounds (Table 3). Floral diameter, herkogamy,

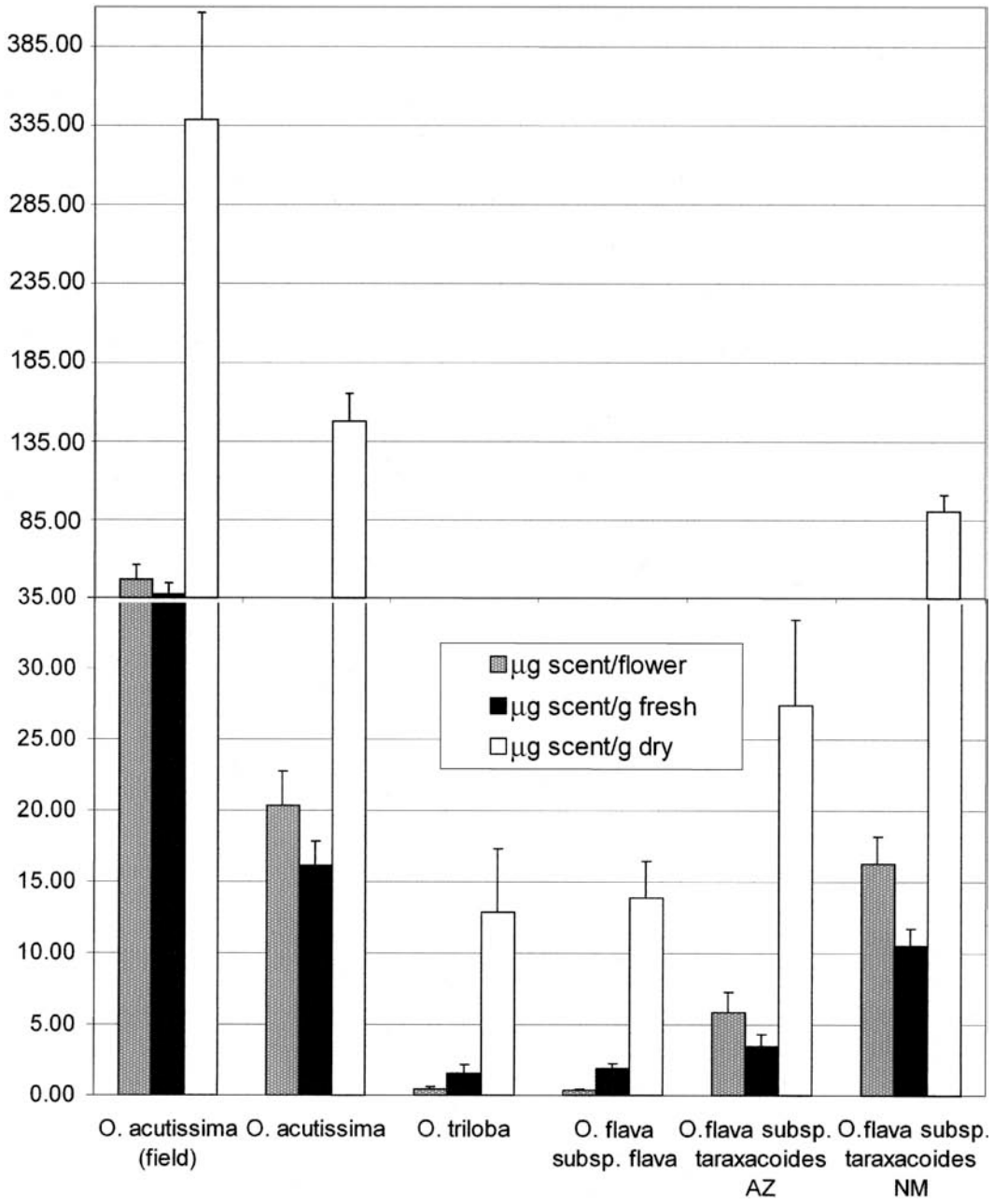

Figure 3. Hourly emission rates of floral scent, per flower (stippled bars), per gram fresh floral mass (dark), and per gram dry floral mass (light). In order to accommodate the substantial range of variation, the y-axis is discontinuous. Note the similarity in emission rates between the two putatively autogamous taxa (*Oenothera triloba* and *O. flava* subsp. *flava*) and between the outcrossing taxa (*O. acutissima* and *O. flava* subsp. *taraxacoides*). Also note the two-fold increase in emissions from *O. acutissima* collected in its native habitat. All other collections were made from plants grown in a common greenhouse setting.

and corolla flare loaded positively on both factors, with larger loadings on factor 1. Factor 2 clearly separated *O. flava* subsp. *taraxacoides* from all other taxa, and the combined factors separated the small-flowered taxa, *O. triloba* and *O. flava* subsp. *flava*, from each other (Fig. 7). Discriminant function analysis correctly assigned 95% of the 44 individuals sampled to the correct taxon and population except for

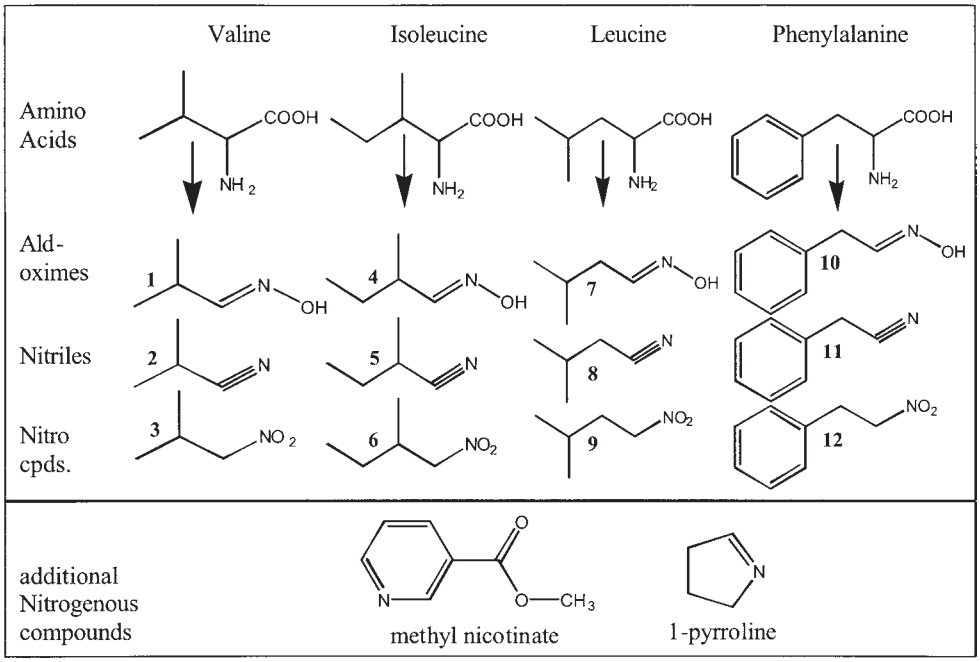

Figure 4. Nitrogenous volatile compounds prominent in the floral scent of *Lavauxia* species. The vertical series in the upper panel shows the derivation of aldoximes, nitriles, and nitro-compounds (in descending order) from their putative amino acid precursors, after Kaiser (1993). Numbered compounds are (1) 2-methylpropanaldoxime, (2) 2-methylpropylnitrile, (3) nitro-2-methylpropane, (4) 2-methylbutyraldoxime, (5) 2-methylbutyronitrile, (6) nitro-2-methyl butyrate, (7) 3-methylbutyraldoxime, (8) 3-methylbutyronitrile, (9) nitro-3-methyl butyrate, (10) phenylacetaldoxime, (11) phenylacetonitrile, and (12) nitro-2-phenylethane. Note that each aldoxime skeleton occurs in syn- and anti- isomers (not shown), and that compounds 2 and 12 were not detected in this study. Lower panel shows structures of methyl nicotinate, present in all *Oenothera* sect. *Lavauxia* but in few other *Oenothera* species, and 1-pyrroline, present in the nectar of all *Oenothera* studied to date.

O. flava subsp. *taraxacoides*, for which one plant in each population was incorrectly assigned to New Mexico or Arizona populations.

FLORAL BIOLOGY OF *OENOTHERA ACUTISSIMA*

Flowers of *Oenothera acutissima* opened in the evening, from 30 minutes before to 15 minutes after sunset. In 2001 and 2003, flowers were visited frequently at dusk by crepuscular hawkmoths, including *Hyles lineata* Fab., *Sphinx chersis* Hubner, *Sphinx vashti* Strecker, and *Manduca quinquemaculata* Haworth (Table 4, Fig. 8). On 25 June 2001, a mean of 0.9 visits per flower (N = 13 watched flowers) was observed during a 30-minute period, whereas on 27 June 2001, a mean of 1.3 visits per flower (N = 14 flowers) occurred during a 20-minute period (Table 4). *Hyles lineata* and the *Sphinx* species first arrived at flowers of *O. acutissima* 30 minutes after sunset (20:45 MST) and continued to forage sporadically until 22:00 hr., when observations ended due to darkness. *Manduca quinquemaculata* individuals were not seen until 40 minutes after sunset and continued visiting flowers until observations ceased. During hawkmoth visitation, ambient temperatures dropped from 15°C to 10°C, and light intensity decreased from 120 cd/m^2 at sunset to 1.25 cd/m^2 at first *H. lineata* visit, and 0.06 cd/m^2 at first *M. quinquemaculata* visit. *Hyles lineata* and *Sphinx* moths visibly removed large amounts of pollen on their head and legs (Fig. 8D), whereas *M. quinquemaculata* carried pollen on the legs and the extended proboscis (Fig. 8F). Female *H. lineata* moths oviposited on leaves of *O. acutissima* between flower visits, and several larvae of different developmental stages were observed eating flower buds in this population. Flowers remained open and bright yellow until 10:00 hr. on the following day before losing turgor and turning deep brick red. Small halictid bees, *Evyleus (Lasioglossum) aberrans* Crawford, collected pollen from individual anthers within an hour after sunrise but did not frequently touch the extended stigmas (Fig. 8B). Mule deer (*Odocoileus hemionus* Rafinesque) were abundant in 2003 and browsed

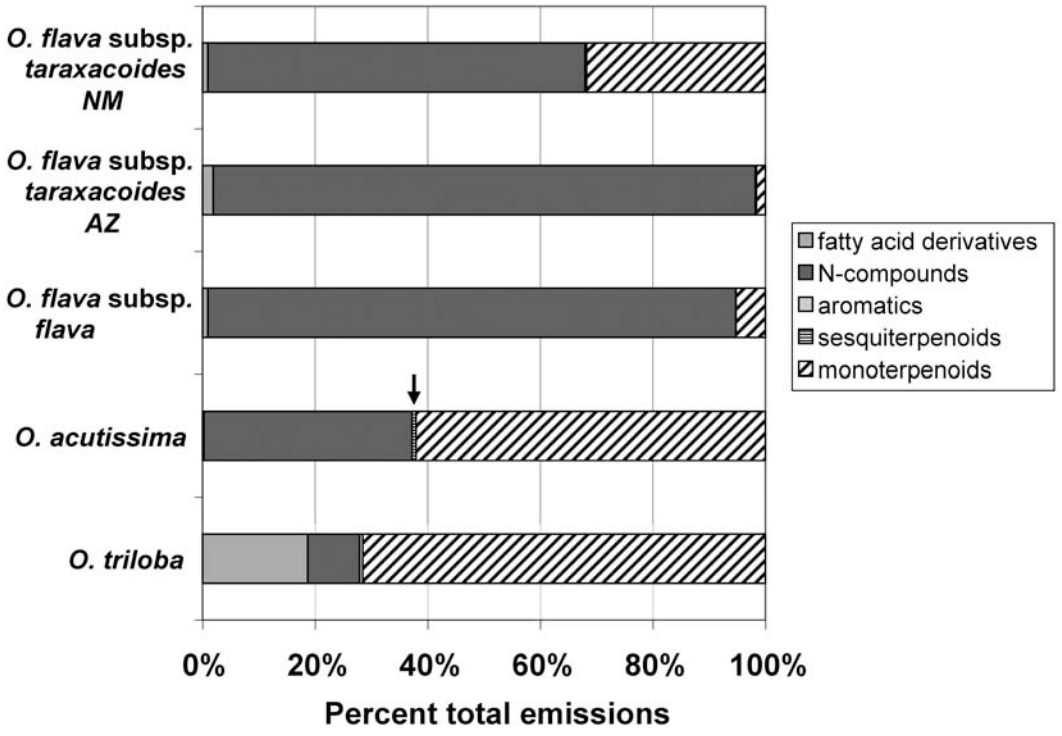

Figure 5. Contributions (relative %) of different compound classes to floral scent emissions in *Oenothera* sect. *Lavauxia* taxa. Nitrogenous compounds (dark solid bars) are dominant fragrance components in all *O. flava* populations, whereas monoterpenoids (striped bars), specifically trans-β-ocimene, dominate the fragrances of *O. acutissima* and *O. triloba*, and aromatic (white) and sesquiterpenoid (stippled, see arrow) compounds are present in trivial amounts (barely visible between nitrogenous and monoterpenoid bars). The seemingly large (ca. 20%) contribution of fatty acid derivatives (cis-3-hexenol-related compounds) to *O. triloba* emissions reflects this taxon's relatively weak floral scent.

heavily upon buds and newly opening flowers in the evening and upon flowers that remained open in early morning.

Discussion

Floral advertisements: visual and olfactory display

Floral visual cues and scent chemistry are relatively conserved among North American species of *Oenothera* section *Lavauxia*. All taxa studied have yellow flowers that, with the exception of *O. acutissima*, have contrasting patterns of distal UV-reflectance and proximal UV-absorbance on the upper petal surfaces (Fig. 2). This pattern creates a concentric "target" of UV contrast previously documented for other yellow-flowered *Oenothera* species (Dement & Raven, 1974). It is unclear whether this target is a phylogenetically conserved trait in the lineage that includes section *Lavauxia* or an adaptation for proboscis placement by hawkmoths, as suggested by Kawano et al. (1995) for *O. biennis* L. and *O. glazioviana* Micheli naturalized to Japan. Visual contrast (including UV wavelengths) is an important orientation or landing cue for several bee species (Jones & Buchmann, 1974; Chittka et al., 1994) and may indicate historical and/or present pollination by crepuscular or matinal bees, as documented for other yellow-UV–colored *Oenothera* species (Towner, 1977; Barthell & Knops, 1997). On the other hand, the diurnal hawkmoth *Macroglossum stellatarum* L. uses visual contrast to position itself before a flower and prefers flowers with centered marks of contrast over those with off-center or no marks (Kelber, 1997). If such preferences are shared by crepuscular and nocturnal hawkmoths, the UV patterns in section *Lavauxia* species would provide appropriate visual contrast.

Floral scent chemistry also is relatively constrained among North American *Oenothera* sect. *Lavauxia*, with most taxa producing nested subsets of the odor blend emitted by *O. acutissima* (Fig. 6). Four intriguing patterns emerge from our chemical data set. The first is the dominance of amino acid–derived nitrogenous odors, particularly the four aldoxime isomers derived from leucine and isoleucine (Figs. 4, 5). These compounds are produced by a number of

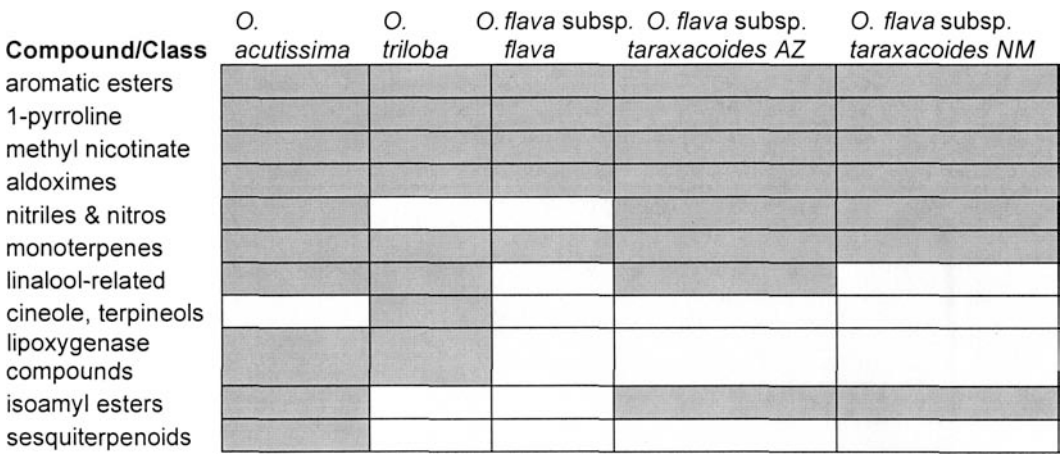

Figure 6. Nested representation of the presence/absence of specific compound classes in the floral scent of *Oenothera* sect. *Lavauxia* species. Sesquiterpenoids and oxygenated cyclohexane monoterpenoids (1,8-cineole, α-terpineol) are autapomorphic in *O. acutissima* and *O. triloba*, respectively.

night-blooming, obligately outcrossing species of *Oenothera*, including *O. primiveris* A. Gray (Raguso, 2004), *O. xylocarpa* Coville, *O. howardii* (A. Nelson) W. L. Wagner, and most species in section *Pachylophus* (R. A. Raguso, unpublished data). Aldoximes also are frequently produced by night-blooming, hawkmoth-pollinated flowers of diverse plant families, such as *Datura* L. and *Nicotiana* L. (Solanaceae), *Angraecum* Bory (Orchidaceae), and *Hedychium* J. Koenig (Zingiberaceae) species (Kaiser, 1993; Knudsen & Tollsten, 1993; Raguso et al., 2003a, b; Raguso, 2004). However, aldoximes are absent from two lineages of hawkmoth-pollinated Nyctaginaceae (*Acleisanthes* A. Gray and *Mirabilis* L.), in which the only nitrogenous floral volatiles are the tryptophan-derived indole and methyl anthranilate (Levin et al., 2001, 2003b). Behavioral experiments are needed to determine the function (if any) of aldoximes and their metabolites in the scents of night-blooming flowers, and whether other nitrogenous volatiles (e.g., indole) play similar roles in hawkmoth attraction to flowers.

The second notable result is the presence of methyl benzoate and 1-pyrroline in floral nectar throughout section *Lavauxia* and other sections of *Oenothera* (Raguso, 2004). Scented nectar occurs among disparate angiosperm families, but its role in plant reproductive ecology is poorly understood (Raguso, 2004). Methyl benzoate is widely distributed among floral scents (Knudsen et al., 1993) and elicits strong antennal responses from *Manduca sexta* L. and other

Table 2. Variation in scent profiles based on dissimilarity between pairs of individuals. Mean, minimum and maximum percent dissimilarity (Euclidean distances), and number of pairwise comparisons (N) within and between taxa or populations are shown. Mean ranks were compared using the Wilcoxon rank sum test for independent samples. Besides field data for *Oenothera acutissima*, all data were collected from a common greenhouse study. Note that between-group dissimilarity measures are significantly greater than within-group values overall, and for all taxa studied except *O. acutissima*. AZ, Arizona populations; NM, New Mexico populations.

	Raw mean % dissimilarity (range) [N]		Mean rank			
	Within-group	Between-group	Within-group	Between-group	*z* Score	*P*
All species	3.54 (0.03–9.83) [167]	7.35 (1.10–10.71) [653]	25.4	88.5	−10.7	<0.001
O. acutissima (field)	5.33 (0.63–9.78) [28]	6.68 (0.39–10.40) [328]	8.0	22.0	−1.9	0.059
O. acutissima	5.14 (0.57–9.83) [10]	6.34 (1.10–10.63) [180]	2.7	6.7	−2.0	0.047
O. triloba	3.92 (0.03–7.55) [55]	7.49 (1.10–10.71) [400]	21.2	29.5	−4.7	<0.001
O. flava subsp. *flava*	1.68 (0.63–2.79) [21]	7.46 (1.14–10.64) [294]	0.0	11.0	−4.0	<0.001
O. flava subsp. *taraxacoides* (NM)	3.48 (0.78–9.03) [36]	6.03 (1.14–10.61) [330]	9.9	21.0	−4.0	<0.001
O. flava subsp. *taraxacoides* (AZ)	3.65 (0.77–7.05) [45]	8.23 (2.45–10.71) [390]	0.0	23.0	−5.8	<0.001

hawkmoths (Raguso et al., 1996; Fraser et al., 2003). Its potential as a gustatory stimulant or, possibly, an anti-microbial nectar component deserves further study. Wagner (1981: 155) described the fragrance of *O. acutissima* flowers as a "light, semen-like odor." This is undoubtedly due to 1-pyrroline, which bears a sharp, semen-like odor (Amoore et al., 1975), is a known derivative of the amino acid proline, and is produced by rearrangement of the polyamine putrescine (Robacker, 2001). The narrowness and extreme depth of the hypanthium in most *Lavauxia* taxa, combined with the mode of pollen dehiscence in viscin threads, argues against proline contamination (via pollen) as a source of this nectar component. The biological function of 1-pyrroline in *Oenothera* nectar is unknown, but it is present in the nectars of other long-tubed night-blooming flowers (e.g., *Datura wrightii* Regel, *Ipomoea alba* L.; R. A. Raguso, unpublished data) and is emitted by the inflorescences of many plant species that produce numerous,

Table 3. Principal component analysis of floral metrics and scent emissions. Loadings are given for two factors accounting for 98.3% of total sample variance. Loadings greater than ± 0.5 are shown in boldface. Values for scent were natural log (ln) transformed for the analysis.

	Loadings	
Floral Character	Factor 1 (85.8%)	Factor 2 (12.5%)
Floral depth	0.164	**0.987**
Floral diameter	**0.881**	0.468
Corolla flare	**0.731**	**0.598**
Herkogamy	**0.788**	0.454
Dry floral mass	0.421	**0.836**
Number of scent compounds	**0.709**	0.376
ln [Nitrogenous scent]	0.059	**0.661**
ln [Ocimene-derived scent]	**0.521**	0.122
ln [Linalool-derived scent]	**0.756**	−0.229

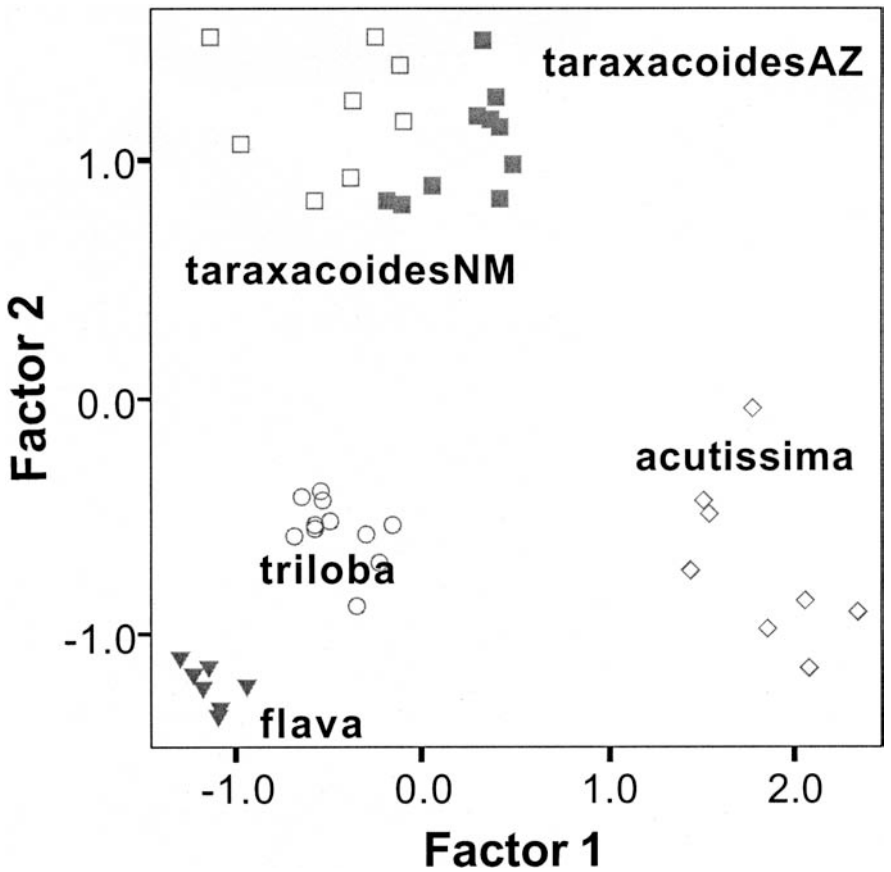

Figure 7. Scatterplot of loadings on factors 1 and 2 from different *Oenothera* sect. *Lavauxia* taxa. These variables clearly separate all taxa, including the two populations of *O. flava* subsp. *taraxacoides*, as 98% of individuals are correctly assigned to their population of origin using discriminant function analysis (see text).

Table 4. Number of observed visits to flowers of *Oenothera acutissima* at type locality, Uinta Mountains, Utah. Total flowers were not counted on days indicated by*.

Insect	11 June 2001	25 June 2003	27 June 2003	28 June 2003	Totals
Lepidoptera, Sphingidae					
Hyles lineata	4	8	1	0	13
Manduca quinquemaculata	0	3	3	2	8
Sphinx vashti and *S. chersis*	15	2	11	4	32
Hymenoptera, Halictidae					
Evyleus aberrans	5	0	0	0	5
Totals	24	13	15	6	58
Mean visits per flower	*	0.9	1.3	*	
Cumulative observer hours	3	10	5	1	19

small, white flowers in spikes or umbels (Raguso, 2006).

The third notable finding was the presence of the nitrogenous ester, methyl nicotinate, in all taxa studied, regardless of flower size or pollination system. We have identified this compound in other night-blooming plants (e.g., *Selinocarpus chenopodioides* A. Gray (Nyctaginaceae), Levin et al., 2001; *Nicotiana suaveolens* Lehm. (Solanaceae), Raguso et al., 2003a), but its biological function, if any, has not been examined in the context of pollination. Methyl nicotinate may represent a biosynthetic artifact if nicotinic acid is unusually common in the petals of *Lavauxia* flowers and is converted to a volatile ester by a biosynthetic enzyme that normally uses another compound as a substrate (see D'Auria et al., 2002). Given the absence of methyl nicotinate from most other hawkmoth-pollinated *Oenothera* studied to date (R. A. Raguso, unpublished data) and its presence in all North American *Lavauxia*, we consider this compound to be a phylogenetically informative character.

The fourth noteworthy result was the identification of two "chemotypes" dominated by either trans-β-ocimene or linalool among individuals of *Oenothera acutissima*. Clearly delineated linalool- and ocimene-dominated "chemotypes" were not observed in other taxa. No *O. acutissima* plants of intermediate phenotype were found, but field collections may have been too few to detect such phenotypes if they are rare. Both scent morphs have compounds that are associated with sphingophily in other plant groups (Kaiser, 1993; Knudsen & Tollsten, 1993). Similarly distinct chemotypes have been observed for hawkmoth-pollinated *Platanthera* Rich. orchids in Scandinavia, in this case dominated by trans-β-ocimene, linalool, or geraniol (Tollsten & Bergström, 1993). Distinct chemotypes likewise are present in *O. caespitosa* Nutt. in the Great Basin Desert of North America (R. A. Raguso, unpublished data). The adaptive importance of intraspecific variation in floral scent is poorly understood in general (Azuma et al., 2001; Knudsen, 2002; Dötterl et al., 2005), and determination of the biological significance, if any, of discrete scent chemotypes in *Oenothera* will require manipulative behavioral and ecological experiments.

COVARIATION BETWEEN FLORAL PHENOTYPE AND PUTATIVE MATING SYSTEM

North American members of *Oenothera* section *Lavauxia* exhibit remarkable variation in quantitative aspects of floral phenotype: flower size, hypanthium length, nectar volume, and fragrance intensity. These traits varied consistently in plants grown under common garden/greenhouse conditions, along a continuum from small, weakly scented, putatively autogamous flowers (*O. flava* subsp. *flava*, *O. triloba*) to large, strongly scented, putatively outcrossing flowers (*O. acutissima*, *O. flava* subsp. *taraxacoides*). Specific combinations of floral characters clearly distinguish most of these entities from each other using ordination techniques (Fig. 7). The least clearly separated populations (*O. flava* subsp. *taraxacoides* from Otero Co., New Mexico, and Apache Co., Arizona) differ measurably in additional floral characters (Table 2, Fig. 6).

Olfactory and visual floral displays are dramatically reduced in taxa for which pollinator attraction is unnecessary. Floral scent in putatively autogamous taxa is two orders of magnitude less intense than in outcrossing taxa, even when floral size differences are standardized (Fig. 3). Thus, reduction of odor in *Oenothera flava* subsp. *flava* and *O. triloba* is not merely a consequence of scaling differences in flower size and probably involves regulatory elements of fragrance biosynthetic pathways (e.g., Raguso & Pichersky, 1999). However, chemical composition remains relatively similar among taxa (Figs. 5, 6). The absence of minor scent compounds (nitriles, nitrocompounds, and isoamyl esters) in small-flowered species is correlated with greatly reduced absolute

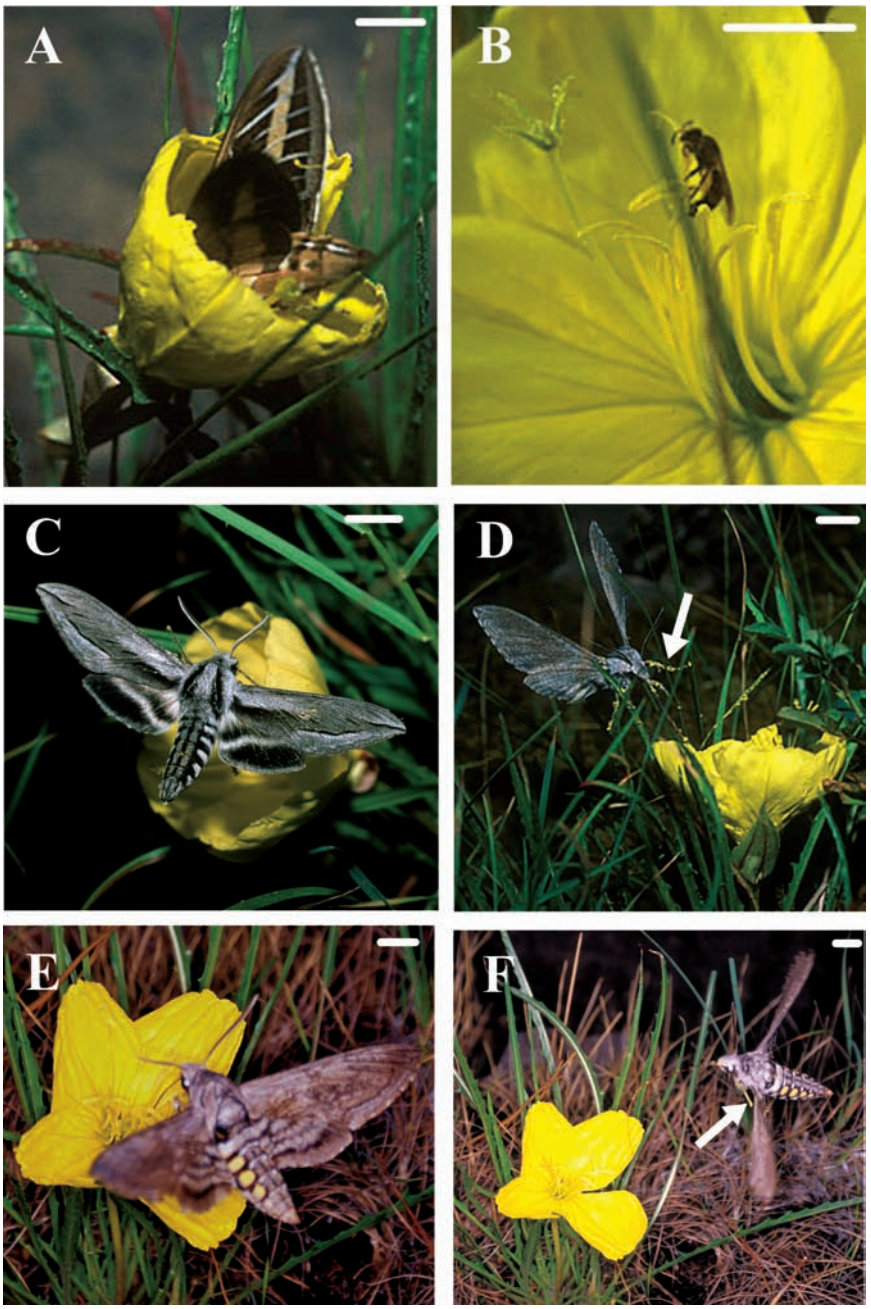

Figure 8. Floral visitors to *Oenothera acutissima* at its type locality in the Uinta Mountains, Daggett Co., Utah. Hawkmoths, including *Hyles lineata* (A), *Sphinx vashti* (C, D), and *Manduca quinquemaculata* (E, F), visit newly opened flowers at dusk. Note the yellow pollen adhering to the moth's legs in D and F (arrows). In panel B, a small *Evyleus* (*Lasioglossum*) *aberrans* bee strips pollen from an anther. Photos in A–D by R. A. Raguso, photos in E, F by M. Pfaff. All scale bars = 1 cm.

amounts of structurally similar aldoximes (Fig. 6), from which they may be derived. Thus, downregulation of biosynthetic enzymes and/or substrate flux (e.g., amino acids), rather than null mutations in specific pathway steps, are the most likely proximate mechanisms for scent reduction in small-flowered section *Lavauxia* taxa (see Dudareva et al., 2000). Further physiological and ecological experiments will

be required to determine whether resource allocation costs or herbivore-mediated selection (e.g., Mothershead & Marquis, 2000) are the ultimate selective forces driving reduced floral display in *O. flava* subsp. *flava* and *O. triloba*.

In contrast, the qualitative aspects of floral phenotype—color, nectar, and overall scent chemistry—were quite similar among taxa, regardless of flower size or mating system. All taxa produced floral nectar with mean sugar concentrations within the range of diverse hawkmoth-pollinated flowers (Cruden et al., 1983; Haber & Frankie, 1989; Hodges, 1995; Raguso et al., 2003b); only the cleistogamous flowers of *Oenothera flava* subsp. *flava* produced nectar volumes that were likely too small to be rewarding (Table 2). Indeed, the relatively high nectar volumes in small-flowered *O. triloba* suggest the potential for a mixed mating system, rather than strict autogamy, and indicate the need for detailed field studies of this species across its range. These patterns suggest that evolutionary reversals from small autogamous flowers to large, outcrossing flowers may be easily accomplished in section *Lavauxia*, because the fundamental components of floral advertisement and reward remain intact in small-flowered taxa (see Armbruster, 1993).

EXTREME FLORAL VARIATION WITHIN *OENOTHERA FLAVA*

Our study populations of *Oenothera flava* represent the extreme large and small floral phenotypes known for that species. However, Wagner (1986) described continuous floral variation among populations of *O. flava* subsp. *flava* and *O. flava* subsp. *taraxacoides* across western North America, encompassing a range of variation similar to that presented here for the entire section *Lavauxia*. Flowers with intermediate morphology might represent mixed mating systems in which both self-pollination and hawkmoth-mediated outcrossing occur. Ashman and Schoen (1994) found that *O. flava* with intermediate-sized flowers in western Colorado accrued 82% and 97% of male and female function, respectively, within hours of anthesis through hawkmoth visitation and, presumably, self-pollination. Variation in the degree of self-pollination must be quite high in *O. flava*, as has been documented for a related species with a similar degree of variation in floral characters, *Calylophus serrulata* (Nutt.) P. H. Raven (Cruden & Lyon, 1989). Given the broad geographic range of *O. flava* and the distances between isolated, mountaintop populations of *O. flava* subsp. *taraxacoides* in Arizona and New Mexico, U.S.A., and Chihuahua, Mexico, it is unclear whether populations of *O. flava* subsp. *taraxacoides* have evolved independently as high-elevation, outcrossing ecotypes of *O. flava* subsp. *flava* or represent a distinct monophyletic entity that has dispersed among high-elevation habitats or has been isolated by changing climate (see Strand et al., 1996). The morphometric and chemical characters used in ordination provided only a modest degree of separation between individuals of *O. flava* subsp. *taraxacoides* from Arizona and New Mexico in phenotype space (Fig. 7). However, these populations also differed significantly in mean nectar volume ($t = 9.13$, $df = 11$, $P_{2\text{-tail}} < 0.001$) and total scent emission per dry mass ($t = 5.22$, $df = 11$, $P_{2\text{-tail}} < 0.001$, Fig. 3). High-elevation populations of *O. flava* subsp. *taraxacoides* clearly are differentiated to some degree, but it is unclear whether this reflects separate origins, genetic drift, or local pollinator adaptation (see Slentz et al., 1999; Boyd, 2002). Phylogeographic and population genetic analyses of these and other isolated populations will be required to better understand the systematic placement of *O. flava* subsp. *taraxacoides* with respect to other populations of *O. flava* and *O. acutissima*. However, flowers of *O. acutissima* were clearly differentiated from those of all taxa studied (Fig. 7) and produced the most complex odor blends (Appendix 2, Fig. 6). These differences in floral biology, combined with edaphic specialization, autapomorphic vegetative characters, and the poor viability (and sterility) of artificial hybrids between *O. acutissima* and *O. flava* (Wagner, 1981), cast doubt on Welsh's (1986) contention that *O. acutissima* is a form of *O. flava*.

HAWKMOTHS, DEEP FLOWERS, AND MONTANE HABITATS

We present the first pollinator observations for the rare, endemic species, *Oenothera acutissima*, taken at its type locality in the Uinta Mountains of Utah, U.S.A. The observed pattern of visitation by short- and long-tongued hawkmoths at dusk, followed by occasional visits of halictid bees the following morning, is consistent with observations of night-blooming *Oenothera* species in western North America over the past half century (Linsley et al., 1963a, 1963b, 1964; Gregory, 1964; Towner, 1977; Wagner et al., 1985). Although visitation rates generally were high (Table 4) and moths carried copious pollen on their appendages (Fig. 8), fruit set across the population was low. Capsules in section *Lavauxia* usually are indehiscent and accumulate among leaf scars from the previous year's growth (Wagner, 1986). However, in June 2001, Raguso found only three mature capsules on more than 100 plants. The densely crowded growth of *O. acutissima* in moist depressions, combined with low observed fruit set, suggests that plants may propagate clonally, or that capsules may abscise in this species. Further experiments are needed to determine the extent to which neighboring plants are

pollen compatible. Another possibility is that, despite pollinator abundance, the observed florivory by hawkmoth larvae and mule deer sharply reduced the reproductive success of *O. acutissima*. Plants were extremely cryptic when not in bloom, growing low among grasses, and flowers faded rapidly the next morning. Perhaps these aspects of plant form and flower duration have been shaped by selective pressure from herbivores, including browsing deer (see Herrera, 1993; Mothershead & Marquis, 2000).

Populations of *Oenothera flava* subsp. *taraxacoides* were collected from similarly mesic, montane habitats several hundred kilometers to the south. The nectar tubes of these plants, which sometimes exceed 20 cm in length, are among the deepest in the North American flora (Grant, 1983). A line of reasoning developed by Darwin (1862) and tested by Nilsson (1988) and Alexandersson and Johnson (2001) is that long nectar tubes or spurs result from extreme directional selection mediated by long-tongued hawkmoths. Gregory (1964) and Grant and Grant (1983a, b) predicted that a guild of widespread North American hawkmoths (*Agrius cingulata* Fab., *Manduca quinquemaculata*, *M. rustica* Fab., *M. sexta*) with proboscis lengths ranging from 8 to 12 cm are most likely to pollinate long-tubed, night-blooming flowers of the southwestern North American deserts, such as *Acleisanthes longiflora* A. Gray, *Mirabilis longiflora* L. (Nyctaginaceae), and *Datura wrightii* (Solanaceae) (Spellenberg & Delson, 1977; Raguso et al., 2003b). Unfortunately, few observations have been made on the floral visitors of *O. flava* subsp. *taraxacoides* at any one population. Gregory (1964) studied *O. flava* subsp. *taraxacoides* at its type locality in the Sacramento Mountains, New Mexico, and found that short-tongued (3–4 cm) *Hyles lineata* moths were abundant visitors and effective pollinators just after flowers opened (Fig. 1E), whereas fewer individuals of *Manduca quinquemaculata* visited these flowers later in the evening and no crepuscular bees were observed. Gregory (1964), who pioneered the use of paired control and emasculated flowers to determine the rate of outcrossed pollen deposition, found that most stigmas of *O. flava* subsp. *taraxacoides* received non-self pollen within the first 45 minutes after sunset. Similarly, studies of three different populations of *O. macrocarpa*, a related species with flowers whose floral tubes range from 9.2–12.3 cm in length, indicate that this species is pollinated effectively by *H. lineata*, *Dolba hyloeus* Drury, and other short-tongued (3–5 cm) hawkmoths (Nonnenmacher, 1999; Mothershead & Marquis, 2000; Moody-Weis & Heywood, 2001).

If flowers of *Oenothera flava* subsp. *taraxacoides* are effectively pollinated by short-tongued hawkmoths, why are their nectar tubes so spectacularly long? One hypothesis concerns the plants' acaulescent, rosette growth form combined with the moist, grassy meadows in which *O. flava* and its relatives often are found (Munz, 1930; Wagner, 1981). Hawkmoths generally prefer individual flowers that are highest above the ground (Herrera, 1993; Mothershead & Marquis, 2000), perhaps reflecting a predator avoidance strategy (Wasserthal, 1993; Dukas, 2001). In the absence of a bolting stem or inflorescence, directional selection for hypanthium length in *O. flava* subsp. *taraxacoides* may result in the projection of the floral visual display and/or landing platform higher above the substrate, as was suggested for *Nierembergia* Ruiz & Pav. by Cocucci (1991). A similar function is thought to be served by the sterile stipe of flowers in a related lineage, *Camissonia* Link sect. *Tetrapteron* (Munz) P. H. Raven, which also grow in wet, grassy habitats (Raven, 1969). An alternative hypothesis is that increased floral tube length of high-elevation *O. flava* ecotypes is a consequence of tight genetic correlation between this trait and some other character (e.g., leaf length), as was suggested for *O. caespitosa* subsp. *marginata* Munz by Wagner et al. (1985). Furthermore, it is possible that extreme tube length somehow enhances the interplay between short- and long-tongued hawkmoth visitors in ways that optimize the plants' male and/or female fitness. Direct manipulative experiments will be required to test these hypotheses, and additional research is needed to better understand the relative importance of autogamy and outcrossing in the North American species of *Oenothera* section *Lavauxia*.

Conclusions

Our comparative study provides conclusive answers to the four questions posed in the introduction. First, flower size, fragrance intensity and complexity, and nectar volume are reduced to some extent in the small-flowered, putatively autogamous taxa of *Oenothera* section *Lavauxia*, whereas flower color, scent, and nectar composition are comparable among all taxa. Thus, reduced floral advertisement and reward in autogamous taxa is a quantitative, rather than qualitative phenomenon. Second, the floral scent of *O. flava* subsp. *flava* is more similar to that of large-flowered *O. flava* subsp. *taraxacoides* than to the other putatively autogamous taxon, *O. triloba*, suggesting that phylogenetic affinity is more responsible than mating system for chemical composition in small-flowered section *Lavauxia* taxa. Third, the flowers of *O. acutissima* are distinct from those of *O. flava* subsp. *taraxacoides* in all measured characteristics, whereas the two isolated populations of *O. flava* subsp.

taraxacoides produce flowers that are similar in floral dimensions and scent chemistry but differ in nectar volume, floral mass, and odor intensity. These results suggest the intriguing hypothesis of independent derivation of large-flowered, high-elevation forms of *O. flava*. The remarkable variation in floral phenotypes within *O. flava* provides an unusual opportunity to study the genetic architecture and physiological costs of resource allocation to pollinator attraction through scent production and visual display. Finally, flowers of the narrow endemic *O. acutissima* were visited abundantly by the same spectrum of medium- to long-tongued hawkmoths that pollinate other night-blooming *Oenothera* throughout western North America. Additional field studies will be required to determine the relative contributions of self-pollination and outcrossing to the reproductive ecology of all section *Lavauxia* species.

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Appendix 1. Vouchers for *Oenothera* sect. *Lavauxia* examined in this study. All vouchers were deposited at ARIZ, US, and USCH. *RAR[R. A. RagusoJ01-65* and *RAR99-46* were studied at the type localities for their respective taxa.

Oenothera acutissima W. L. Wagner. U.S.A. **Utah:** Daggett Co., Flaming Gorge Natl. Rec. Area, Greendale Campgr. & Hwy. 260, 1880 m, *RAR01-65* (ARIZ, US, USCH).

Oenothera flava subsp. *flava* (A. Nels.) Garrett. U.S.A. **Wyoming:** Teton Co., Teton National Forest, Hwy. 89, 1924 m, *RAR01-63* (ARIZ, US, USCH).

Oenothera flava subsp. *taraxacoides* (Woot. & Standl.) W. L. Wagner. U.S.A. **Arizona:** Navajo Co., mile marker 357 on Rte. 260, near Pine Top, 2350 m, *RAR98-146* (ARIZ, US, USCH).

Oenothera flava subsp. *taraxacoides* (Woot. & Standl.) W. L. Wagner. U.S.A. **New Mexico:** Otero Co., Rte. 82-Rte. 244 junction, 1.4 miles SE of Cloudcroft, 2800 m, *RAR99-46* (ARIZ, US, USCH).

Oenothera triloba Nutt. U.S.A. **Texas:** Bexar Co., Trinity University campus, San Antonio, 246 m, *RAR02-01* (collected by Donald Miller III) (ARIZ, US, USCH).

See Appendix 2, next page.

Appendix 2. Volatile compounds emitted by *Oenothera* section *Lavauxia* taxa. Numbers represent mean percentage (out of 100%) ± SE of total scent emitted. The data in the first column were collected in the field from the type locality of *O. acutissima*; all other data were collected in a common greenhouse environment. Compounds marked with * were identified by co-chromatography with known standards, those with ** using essential oils or natural products for which published GC-MS data are available. For remaining compounds, putative names are provided when MS showed > 90% identity with entries from mass spectral libraries. MS of unidentified compounds are given in descending order of mass per unit charge (m/z), with % abundance relative to the base peak (100) in parentheses. AZ, Arizona populations of *O. flava* subsp. *taraxacoides*; NM, New Mexico populations of *O. flava* subsp. *taraxacoides*; nr., suggests that the compound shares a structural affinity to cis-cinerone but is not verifiably that compound; RT, retention time on carbowax GC column, in minutes; SPME, solid-phase microextraction. M + ? indicates a potential molecular ion for the unknown mass spectrum.

Scent Compound	RT	*O. acutissima* field	*O. acutissima*	*O. triloba*	*O. flava* subsp. *flava*	*O. flava* subsp. *taraxacoides* AZ	*O. flava* subsp. *taraxacoides* NM
Monoterpenes (7)							
α-pinene*	2.76			0.26 ± 0.16			0.12 ± 0.02
β-pinene*	4.08			0.33 ± 0.18			
sabinene*	4.31			0.04 ± 0.04	0.50 ± 0.46		0.04 ± 0.01
β-myrcene*	5.07	0.25 ± 0.07	0.38 ± 0.11	0.38 ± 0.08			0.18 ± 0.02
Z-β-ocimene*	6.36	0.49 ± 0.13	0.85 ± 0.25	0.94 ± 0.20	1.37 ± 1.18	1.44 ± 0.85	0.47 ± 0.06
E-β-ocimene*	6.67	41.24 ± 10.48	48.96 ± 13.13	66.40 ± 8.50	3.38 ± 2.01	0.07 ± 0.03	31.14 ± 3.36
1,3,8 p-mentha-triene	9.93		0.01 ± 0.01				
Oxygenated Monoterpenoids (9)							
1,8-cineole*	5.69			0.70 ± 0.70			
furanoid linalool oxide*	9.82	0.005 ± 0.003	0.01 ± 0.01			0.01 ± 0.01	
furanoid linalool oxide*	10.22	0.003 ± 0.002	0.02 ± 0.01			0.004 ± 0.004	
linalool*	11.19	15.39 ± 7.54	11.51 ± 8.76	2.43 ± 2.43		0.10 ± 0.04	
linalool epoxide	11.52	0.17 ± 0.10					
δ-terpineol	12.81			0.29 ± 0.29			
α-terpineol*	13.11			0.10 ± 0.10			
2,6-dimethyl-3,7-octadien-2,6-diol	15.90	0.01 ± 0.005					
2,6-dimethyl-1,7-octadien-3,6-diol	17.70	0.004 ± 0.002					
Unidentified terpene-like compounds (5)							
137(M + ?), 109(5), 95(8), 83(38), 69(24), 67(65), 55(33), 53(18), 43(92), 41(100)	8.59		0.03 ± 0.01				
137(M + ?), 123, 109(2), 96(6), 95(11), 83(38), 82(22), 69(30), 67(67), 55(34), 53(20), 43(31), 41(100)	9.17		0.20 ± 0.12				
nr. *Z*-cinerone	11.45		0.02 ± 0.01				
nr. *E*-cinerone	12.02		0.03 ± 0.02				
nr. isophytol	19.11	0.01 ± 0.01	0.04 ± 0.04	6.77 ± 2.62	0.23 ± 0.19	0.58 + 0.15	0.08 ± 0.02
Sesquiterpenoids(4)							
E-β-caryophyllene*	12.04	0.03 ± 0.02	0.51 ± 0.34				

Appendix 2. Continued.

Scent Compound	RT	*O. acutissima* field	*O. acutissima*	*O. triloba*	*O. flava* subsp. *flava*	*O. flava* subsp. *taraxacoides* AZ	*O. flava* subsp. *taraxacoides* NM
α-humulene*	12.93		0.06 ± 0.04				
caryophyllene oxide*	16.43		0.10 ± 0.10				
E,E-farnesol	19.75		0.01 ± 0.01				
Aromatic Esters (2)							
methyl benzoate*	12.27	0.01 ± 0.007	0.02 ± 0.008	0.53 ± 0.32	In SPME only	0.18 ± 0.16	In SPME only
amyl benzoate*	15.61	0.01 ± 0.002	0.01 ± 0.004			0.02 ± 0.008	0.02 ± 0.004
Aliphatic compounds (9)							
isoamyl alcohol	6.12	0.12 ± 0.03	0.02 ± 0.01		0.72 ± 0.15	0.87 ± 0.15	0.41 ± 0.06
isoamyl butanoate	7.19	0.003 ± 0.002				0.17 ± 0.05	0.04 ± 0.008
isoamyl pentanoate	7.66	0.04 ± 0.01	0.06 ± 0.03			0.13 ± 0.04	0.24 ± 0.07
Z-3-hexenyl acetate*	7.91	0.08 ± 0.05	0.02 ± 0.02	7.58 ± 3.99			
Z-3-hexen-1-ol*	9.07	0.02 ± 0.01	0.01 ± 0.01				
isoamyl hexanoate	10.07					0.02 ± 0.01	<0.005
111(43), 97(40), 84(49), 83(57), 82(39), 69(92), 57(36), 55(92), 43(75), 41(100)	13.00	0.006 ± 0.005					
1-hexadecene	19.62			2.12 ± 1.79		0.05+0.04	0.19
octadecene	21.34			1.72 ± 1.24			
Nitrogenous Compounds (16)							
1-pyrroline	2.43	In SPME only	In SPME only	In SPME only	In SPME only	In SPME only	In SPME only
2-methylbutylnitrile	4.08	0.02 ± 0.01	0.20 ± 0.12			1.82 ± 0.51	0.52 ± 0.12
3-methylbutylnitrile	4.86	1.02 ± 0.12	2.01 ± 0.66		1.82 ± 0.72	5.10 ± 0.83	5.87 ± 0.84
nitro-2-methylpropane	6.52	0.005 ± 0.002	0.01 ± 0.007			0.06 ± 0.03	
4-methylpentylnitrile	6.73	0.05 ± 0.02	0.05 ± 0.03			0.03 ± 0.01	
nitro-2-methylbutane	8.07	0.03 ± 0.01	0.12 ± 0.05			0.95 ± 0.27	0.09 ± 0.02
nitro-3-methylbutane**	8.24	0.59 ± 0.06	0.74 ± 0.04		2.96 ± 1.28	0.94 ± 0.23	0.67 ± 0.08
2-methylpropanaldoxime	9.07	0.19 ± 0.08	0.08 ± 0.004			0.15 ± 0.04	0.14 ± 0.04
2-methylpropanaldoxime	9.31	0.09 ± 0.04	0.03 ± 0.01			0.03 ± 0.01	0.05 ± 0.01
2-methylbutyloxime*	10.48	6.10 ± 1.28	5.15 ± 1.62	1.48 ± 1.55	5.55 ± 1.24	36.16 ± 4.81	12.37 ± 1.51
3-methylbutyloxime*	10.56	17.81 ± 1.35	15.88 ± 4.65	3.14 ± 2.35	47.56 ± 2.27	27.14 ± 3.39	25.43 ± 1.93
2-methylbutyloxime*	10.74	2.16 ± 0.28	2.03 ± 0.51	0.71 ± 0.71	3.39 ± 0.65	8.23 ± 0.85	4.46 ± 0.54
3-methylbutyloxime*	11.03	13.11 ± 1.20	10.35 ± 2.93	2.97 ± 2.36	32.25 ± 1.49	14.06 ± 2.39	17.42 ± 2.13
methyl nicotinate*	14.09	0.92 ± 0.14	0.44 ± 0.13	1.02 ± 1.02	<0.005	1.65 ± 0.25	0.29 ± 0.06
phenylacetonitrile*	15.78	0.02 ± 0.01	0.03 ± 0.01		0.28 ± 0.12	0.06 ± 0.02	0.04 ± 0.01
nitro-2-phenylethane	17.80	0.003 ± 0.002					