Appendix 1.

Plant material

Floral scent was collected from intact plants, in most cases growing in their natural habitat. Some plants were removed and potted for later scent sampling (CITES no. 023729), and were grown in a greenhouse at the Univ. of South Carolina (Columbia, SC, USA). If taxa could not be obtained in the wild, or additional samples were required, cultivated specimens from specialized nurseries (Uhlig Kakteen, Kernen, Germany; Kakteen Piltz, Düren, Germany; Mesa Garden, Belen, NM, USA) were used, preferably plants of documented origin with collection numbers. In *Echinopsis ancistrophora* ssp. *ancistrophora*, where population-specific flower morphs were compared, no nursery plants were studied. Voucher specimens were deposited in the herbarium of the Museo Botánico de Córdoba, Argentina (CORD).

Scent collection

Volatiles were usually collected for 4 to 5 h (depending on scent intensity) from the headspace of individual flowers enclosed within polyester oven bags (Melitta, Minden, Germany). Scent sampling took place during full anthesis, i.e. during the day in diurnal populations and during the night in primarily nocturnal ones (but see below, 'Scent emission day versus night'). In a closed loop system, floral scent was accumulated on 1.5 mg carbon traps (1.5 mg, CLSA-Filters, Daumazan sur Arize, France) by a constant air stream, provided by 12 V vacuum pumps (Fürgut, Tannheim, Germany), as described by Schlumpberger et al. (2004). The samples were eluted twice with 15 µl HPLC-grade acetone and stored in 1 ml glass autosampler vials with glass micro inserts at –20°C. Additional scent samples were taken from empty oven bags as ambient control to check for contaminants.

Scent analysis

Headspace samples were analyzed using a Shimadzu GC-17A gas chromatograph coupled with a Shimadzu QP5000 quadrupole electron impact mass spectrometer (GC–MS).

The samples were analyzed splitless by injecting 1 µl on a polar EC-wax column (length 30 m, diameter 0.25 mm, film thickness 0.25 µm), and a nonpolar EC-5 column (length 30 m, diameter 0.32 mm, film thickness 1 µm (Alltech Associates, Deerfield, IL)). Helium was used as carrier gas with a flow rate of 1 ml min^{-1} and a split ratio of 12. Injection port temperature was 240°C and detector temperature was 260°C. The oven temperature for the EC-5 column was held at 50°C for 3 min, then increased at 10°C min^{-1}, and held at 275°C for 3.5 min. For the EC-wax column, oven temperature was held at 40°C for 3 min, then in-

creased at 10°C min^{-1}, and held for 5 min at a final temperature of 260°C. Volatile compounds were identified by their mass spectra, using NIST and Wiley mass spectral libraries, by injecting known standards and essential oils with known components, and by comparing retention indices with data published by Adams (2001) and others. An internal standard (16 ng of toluene) was added to all scent samples in order to estimate emission rates of individual flowers as described by Svensson et al. (2005).

Hawkmoths

We obtained eggs from a laboratory colony of *Manduca sexta* from Dr. Lynn Riddiford (Univ. of Washington, USA), and raised larvae on an artificial diet developed by Bell and Joaquim (1976) as described by Raguso and Willis (2002). Larvae were reared in Precision, Inc. incubators with 16 h day length, day temperature set to 24°C, and night temperature set to 21°C, with a humidified atmosphere. Pupae were segregated by sex and emerged in flight cages (45 × 45 × 45 cm) (BioQuip, Rancho Dominguez, CA) stored within separate incubators, to avoid male exposure to female sex pheromones. Incubator photoperiods were shifted so that scotophase began at 16:00 h EST, and all behavioral assays took place within the first three hours of darkness.

Wind tunnel

Behavioral experiments were performed in a laminar flow wind tunnel with 3 × 1.5 × 1.5 m (length, width, height) dimensions, constructed from 1 cm thick plexiglas sheets and plywood sealed with epoxy. The distance between the moths' release point and the odor source was 2 m. Laboratory air was pulled through a flow-straightening honeycomb at 100 cm s^{-1} by a digitally controlled wall fan (Grainger, Inc.). Titanium chloride smoke was used to visualize air flow. Turbulent flow at this velocity was limited to air within 3 cm of the wind tunnel walls. The wind tunnel room was illuminated by two dimmable 25 W red tungsten lamps, directed toward the ceiling for indirect lighting. Further diffusion of light was accomplished by covering the top and far side of the wind tunnel with white cotton cloth, reducing reflectance that could have distracted the moths. Relative humidity was not measured daily but ambient temperature was held relatively constant by a dehumidifier-air conditioner at 24°C.

Presentation of stimuli

In order to present floral scent as a test stimulus, compressed air was passed through activated charcoal, then pumped through a nylon resin oven bag (Reynolds, Inc.) containing the test flower. Scent-enriched air was delivered to the wind tunnel using Teflon tubing (8 mm inner Ø) and was emitted from a point source 30

cm above the wind tunnel floor. Treatment-specific tubes were used to avoid odor contamination. Individual moths were transferred to small cylindrical wire mesh cages before the onset of scotophase, and were left undisturbed for 15–30 min. *Magnolia grandiflora* flowers were used as a positive control, because they were available throughout the period of study. Although these nectarless flowers are not visited by hawkmoths in nature (Thien 1974), previous studies showed their fragrance to be highly attractive to naïve *M. sexta* (Raguso et al. 2005). *Magnolia* flowers emitted about 28 times more scent (per fresh, individual flower) than the average flower of *E. ancistrophora* ssp. *ancistrophora*.

Behavioral assays

Individual moths were transferred to cylindrical screen cages before the onset of scotophase, and were left undisturbed for 15–30 min. Active moths were released in the down-wind end of the wind tunnel, at the same height and 2 m distance from the odor source, and flown 5 min each. Titanium chloride smoke showed this release point to be within the odor plume. A positive response was indicated by stereotypic upwind, zig-zag flight within the odor plume, which is distinguishable from random or escape flight, when moths explore the walls and ceiling of the tunnel (Raguso and Willis 2003). To get an additional measure of attractiveness, the length of the upwind flight was recorded (Plepys et al. 2002). A stronger or more attractive odor may enable the moths to detect the plume from a longer distance, and may prevent them from losing plume contact during upwind flight. The comparisons of responses to long tubed vs short-medium depth flowers were 1-tailed, due to our a priori expectations that the scent of long-tubed flowers should be more attractive than that of shorter tubed flowers, if such flowers are adapted to hawkmoths as pollinators. As another measure of attractiveness, we recorded whether or not moths showed the proboscis extension reflex (PER) when first exposed to the odor plume at the starting point in the wind tunnel (Dötterl et al. 2006). Proboscis extension is the unconditioned response shown by moths, bees and other nectar-feeding insects, which generally indicates appetitive motivation and becomes associated with floral odors through Pavlovian conditioning (Menzel and Bitterman 1983, Daly and Smith 2000).

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Appendix 2.

Table 3.

	ssp. *ancistrophora* n = 37			ssp. *arachnacantha* n = 4			ssp. *pojoensis* n = 3			ssp. *cardenasiana* n = 4		
	mean	SD	sem	mean	SD	sem	mean	SD	sem	mean	SD	sem
Monoterpenoides	5.15	7.53	1.24	18.44	32.42	16.521	17.16	11.55	6.67	6.94	6.32	3.16
Sesquiterpenoides	89.84	14.03	2.31	63.90	34.28	17.14	25.76	29.32	16.93	6.92	10.44	5.22
Fatty acid derivatives	3.92	9.21	1.51	14.85	25.68	12.84	24.97	20.77	11.99	85.25	10.39	5.19
Aromatics	0.77	2.18	0.36	2.60	2.89	1.45	32.11	39.54	22.83	0.04	0.07	0.03
Nitrogenous compounds	0.18	0.60	0.10	0.20	0.40	0.20	0.00	0.00	0.00	0.85	1.47	0.74
Irregular terpenoids	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Unknowns	0.14	0.34	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00