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Hawk-moth pollination and elaborate petals in Cucurbitaceae: The case of the Caribbean endemic *Linnaeosicyos amara*

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ABSTRACT

Linnaeosicyos amara is a little known cucurbit endemic to the Caribbean island of Hispaniola. The species possesses conspicuously fringed petals which are known in just four other cucurbit genera and hypothesised to play a role in pollinator attraction. A population of flowering *L. amara* plants was observed for a total of 78 h, which revealed that Sphingid moths are the primary pollinators. Floral scent was dominated by the terpenoid (*E*)-β-ocimene. The elaborate petal fringes almost doubled the apparent diameter of *L. amara* flowers and the maximal extension of petal fringes was found to coincide with the peak visitation period of hawk-moths, with withering starting soon after. We conclude that petal fringes likely play a role in the visual attraction of hawk-moths by exploiting their preference for large flowers with deeply-divided petal margins, whilst limiting energy costs to the plant.

1. Introduction

Cucurbitaceae is a mostly tropical plant family with *c.* 980 species predominantly pollinated by generalist bees but also by birds, butterflies, bats, carrion flies and hawk-moths (Schaefer and Renner, 2011). Hawk-moth pollination is thought to have evolved in at least nine genera: *Ampelosicyos* A. Thouars, *Hodgsonia* Hook.f. & Thomson, *Ibervillea* Greene, *Lagenaria* Ser., *Linnaeosicyos* H. Schaef. & Kocyan, *Momordica* L., *Peponium* Engl., *Trichosanthes* L., and *Trochomeria* Hook.f. (De Boer et al., 2012; Morimoto et al., 2004; Schaefer and Renner, 2011). Direct observational data from the field is largely missing however and, for most species, hawk-moth pollination has been inferred based on floral morphology. Importantly, whilst distinguishing between flowers pollinated by moths and flowers pollinated by other animals is typically possible, it is often difficult to distinguish between hawk-moth (sphingophily) and settling-moth (phalaenophily) pollinated flowers (Haber and Frankie, 1989). Moreover, plants may be visited by both groups of moths with varying relative contributions (Dobson, 2006).

The genus *Linnaeosicyos* is monotypic and its only species, *Linnaeosicyos amara* H. Schaef. & Kocyan, is one of the rarest Cucurbitaceae worldwide, known only from a few collections made on the Caribbean island of Hispaniola. Like most cucurbits, the species is a tendril-climber; however, unlike the large yellow-orange flowers of the type genus *Cucurbita*, the genus *Linnaeosicyos* is characterised by white, conspicuously fringed petals and an elongated perianth tube. These traits make it superficially similar to some species of the Asian *Trichosanthes*, which led Linnaeus to describe it under the name *Trichosanthes amara* L. Molecular analysis, however, showed that it is a distinct lineage closely related to the New World Sicyoeae (Schaefer et al., 2008).

Records on the phenology, anthesis and pollinators of *L. amara* are absent from the literature; however, the pollination syndrome and similarity in floral morphology to *Trichosanthes*, for which pollination by hawk-moths has been observed, has led to sphingophilous pollination being inferred for *L. amara*. The pollination biology of *L. amara* is of particular interest as it represents the only fringed-petalled cucurbit species in the New World and, along with two or three species of *Ibervillea*, one of the few cucurbit species in the New World thought to be hawk-moth pollinated. As far as we are aware there has been no detailed investigation into the natural pollination system of any fringed-petalled cucurbit species. The only available studies so far are a study looking at fruit set of farmed *Telfairia occidentalis* Hook.f. (Akoroda, 1990) and a floral-scent analysis for *Trichosanthes kirilowii* Maxim (Miyake et al., 1998).

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Table 1
Mean percentage of scent compounds emitted by *Linnaeosicyos amara* flowers during anthesis. Compounds marked with an asterisk were identified based on the retention time and mass spectra of synthetic standard compounds. A full list of emitted compounds is provided in Appendix A.

			% Abundance		
#	Ret. Time	Compound	mean	min.	max.
1	14.277	Unknown 1 (*m/z* 91,119,134,77,105,92)	2.77	1.07	6.46
2	15.047	(*Z*)-β-Ocimene *	1.97	1.50	2.32
3	15.327	(*E*)-β-Ocimene *	73.02	50.73	87.53
4	17.003	Unknown 2 (*m/z* 82,43,81,39,54,53)	1.20	0.30	2.29
5	15.513	α-Ocimene *	1.02	0.83	1.33
6	17.59	*p*-1,3,8-Menthatriene	9.06	4.92	15.21
7	19.483	Unknown 3 (*m/z* 43,109,81,152,67,55)	2.11	0.00	4.17
8	24.56	Unknown 4 (*m/z* 105,91,107,135,79,41)	1.62	0.12	6.19
		Other compounds (mean; *n* = 30)	0.25	0.02	0.89

Elaborate petals are known in a number of lineages and their form and function is diverse (Endress and Matthews, 2006). De Boer et al. (2012) hypothesised that the fringed petals of *Trichosanthes* may act to attract hawk-moths visually either through the pattern created or by creating a waving motion, or by increasing the surface area for scent emission. Floral scent plays an important role in the long-distance attraction of hawk-moths; however, it is a character that is not preserved in herbarium specimens and frequently absent from pollination studies. The specific aim of this study was therefore to describe the pollination ecology of *L. amara*, including the components of floral scent.

2. Methods

2.1. Locality

Search for the species was based on information from herbarium labels, with most recent collections coming from Sierra Martín García, a mountain range in the south–west of the Dominican Republic. The area, totalling *c.* 320 km^2 and with a maximum elevation of 1359 metres above sea level, was designated a national park in 1996. A single population of *c.* 100 *Linnaeosicyos amara* plants was found close to sea level in January–February 2014; however, all plants were either dry or fruiting. Whilst within the national park and appearing largely well protected, the area is affected by free-ranging cattle and firewood collection from a nearby salt works. The vegetation is xeric shrubby-forest with seasonal rains occurring between May and November. This location was revisited in December 2014 when three patches of plants were found to be in flower; two smaller patches of <10 individuals (10–15 m^2) and one larger patch with *c.* 30 individuals (25–30 m^2). *Linnaeosicyos amara* was predominantly found climbing up through the shrubby forest to flower on the canopy (*c.* 5 meters high) of *Prosopis juliflora* DC. (Fabaceae). To facilitate floral measurements, scent collection and observations, a temporary observation platform was built in the canopy. Plant habit and size, and flower orientation and frequency were recorded at all three patches. Sunrise during the observation period was *c.* 07:05 and sunset *c.* 18:05.

2.2. Floral characteristics

Petal, fringe, and perianth tube length were measured on flowers during peak anthesis. Nectar characteristics were assessed as standing crop from unbagged flowers. Nectar volume was measured using graduated 5 μL micropipettes (Brand GmbH, Wertheim, Germany) and nectar concentration (total sugar) was measured using a manual handheld refractometer (0–90% Brix) (Müller Optronic, Erfurt, Germany). Nectar characteristics were taken throughout the anthesis period.

2.3. Floral scent

Floral scent was collected from the flowers of five individuals (one flower per individual) growing in the largest patch during the peak time for floral visitors, using a method modified from Dötterl et al. (2014). A control sample was taken from a young flower bud. To collect scent, open flowers were enclosed for 5 min in polyester oven bags (Toppits, Minden, Germany) and the emitted volatiles were trapped for 2.5 min in an adsorbent tube by using a membrane pump (G12/01 EB ASF Rietschle-Thomas Inc., Puchheim, Germany) adjusted to a flow rate of 200 mL min^{-1}. ChromatoProbe quartz microvials (length 15 mm, inner diameter 2 mm; Varian Inc., California, USA) were used as adsorbent tubes after cutting the closed end, and filling them with a mixture (1:1) of 1.5 mg Tenax-TA (mesh 60–80; Supelco, Pennsylvania, USA) and 1.5 mg Carbotrap (mesh 20–40; Supelco, Pennsylvania, USA). The adsorbents were held in the tubes with glass wool. The trapped volatiles were analysed by GC/MS using an automatic thermal desorption (TD) system (TD-20, Shimadzu, Kyoto, Japan) coupled to a Shimadzu GCMS-QP2010 Ultra equipped with a ZB-5 fused silica column (5% phenyl polysiloxane; 60 m, i.d. 0.25 mm, film thickness 0.25 μm, Phenomenex). The samples were run with a split ratio of 1:1 and a constant helium carrier gas flow of 1.5 mL/min. The GC oven temperature started at 40 °C, then increased by 6 °C/min to 250 °C and held for 1 min. The MS interface worked at 250 °C. Mass spectra were taken at 70 eV (EI mode) from *m/z* 30 to 350. GC/MS data were processed using the GCMSolution package, Version 2.72 (Shimadzu, Kyoto, Japan). Identification of the compounds was carried out using the NIST 11, Wiley 9, FFNSC 2, and Adams (Adams, 2007) databases as well as the database available in MassFinder 3. Some of the compounds were confirmed by comparing mass spectra and retention times with those of synthetic reference compounds (Table 1).

2.4. Floral visitors

Flowering plants were monitored between the 13th and 20th December 2014 via direct observation of a patch of flowers and observation of a small cluster of flowers using a video camera (DCR-SR35, Sony, Tokyo, Japan). A variety of observation regimes were implemented to cover the entire 24 h period, these were specifically: daytime (08:00–17:00), morning (05:00–12:00), evening (12:00–22:00) and night (17:00–08:00). During hours of darkness direct observations were facilitated using head-torches with a red LED light-bulb (Petzl, Crolles, France) and the video camera was switched into NightShot (infrared) mode.

3. Results

Linnaeosicyos amara is a vigorous climber with stems up to 10 m long and 30 mm in diameter. It appears to be dioecious although