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Hunger-driven response by a nectar-eating jumping spider to specific phytochemicals

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Abstract The jumping spider *Evarcha culicivora* (Salticidae) has unusual links to *Lantana camara*, a plant species to which it is attracted. Three phytochemicals from the headspace of *L. camara* (1,8 cineole and especially β-caryophyllene and α-humulene) attract adult *E. culicivora*. These spiders, especially early-instar juveniles, feed on nectar, but adults may use *L. camara* as mating sites. The hypothesis we consider here is that, for *E. culicivora* juveniles, although not for adults, responding to plant odor is relevant in the specific context of acquiring nectar meals. We show that juveniles resemble adults by responding to β-caryophyllene and α-humulene, but we found no significant attraction of juveniles to 1,8 cineole. We also show that, compared to sated *E. culicivora* juveniles, juveniles subjected to a 5-day pre-trial fast responded more strongly to living *L. camara* plants and to β-caryophyllene and α-humulene, but we found no significant effect of hunger level on response by adults to living plants. These results suggest that attraction to *L. camara* may have different functions for *E. culicivora* depending on the stage of its life history.

Keywords Plant–arthropod interactions · Sensory ecology · Plant volatiles · Sesquiterpene · Salticidae · *Evarcha culicivora*

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Introduction

Many examples are known of specific volatile compounds, or blends of compounds, attracting insects to plants that serve as feeding or oviposition sites (e.g., Bruce et al. 2005; Kessler and Morrell 2010). Although less is known about spider–plant relationships, visiting plants can reward spiders with opportunities to feed on the insects that visit the same plants (Morse 2007; Romero et al. 2008) and many spiders also feed on nectar and other plant products (Pollard et al. 1995; Taylor and Pfannenstiel 2008; Chen et al. 2010).

In the tropics, jumping spiders (Salticidae) are common on plants (e.g., Nahas et al. 2012), and there appear to be especially many examples of nectar feeding by tropical salticids (e.g., Jackson et al. 2001). Here, we investigate the salticid *Evarcha culicivora*, a tropical East African species known for its distinctive preference for blood-fed mosquitoes as prey (Jackson et al. 2005). *E. culicivora* is also unusual because it associates with the plant *Lantana camara*, the odor of which is attractive to adults (Cross and Jackson 2009). Furthermore, the courtship sequences of *E. culicivora* become more intricate and both sexes become more receptive to mating when encounters take place on *L. camara* (Cross and Jackson 2009). In addition, both juveniles and adults of *E. culicivora* feed on the nectar of *L. camara*, although nectar feeding may be up to an order of magnitude more common in early-instar juveniles than in adults (Kuja et al. 2012), suggesting that, for adults, being attracted to *L. camara* odor may function primarily as a mechanism for finding mating sites.

Recently, 11 volatile compounds from the headspace of *L. camara* in Kenya were used in olfactometer experiments with naïve adults (Nelson et al. 2012),

and of these, adults were attracted to the odor of a monoterpene, 1,8-cineole, and particularly to the sesquiterpenes β-caryophyllene and α-humulene. In an effort to clarify the role of *L. camara* in *E. culicivora*'s life history, here we carried out comparable olfactometer experiments to determine the specific compounds to which early-instar *E. culicivora* juveniles respond, while also varying hunger level. As nectar feeding may be considerably more common among young *E. culicivora* than among adults (Kuja et al. 2012), which may be attracted to *L. camara* to find mates (Cross and Jackson 2009), we considered the prediction that pre-trial fasts would make early-instar *E. culicivora* juveniles, but not adults, more responsive to the odor of *L. camara*.

Methods

Research was carried out using salticids from laboratory cultures (F2 and F3 generation; no prior experience with plants or the compounds used in experiments; no individual was tested more than once). Spiders were fed non-biting midges (Chironomidae and Chaoboridae) that were collected locally. Testing was carried out between 0800 hours and 1400 hours (laboratory photoperiod 12L–12D, lights on at 0700 hours). Rearing methods, as well as the basic procedures used in olfactometer experiments, were as in earlier studies (see Jackson et al. 2005; Nelson et al. 2012) and only essential information is provided here.

As a way of assessing repellence or attraction to the odor, olfactometers were designed to test the spider's latency to leave the vicinity of a given odor ('retention testing'). These tests followed a paired design: each spider was tested twice over successive days, one day with an odor source and the other day with a no-odor control (sequence random). For odor sources, we used living plants (*L. camara*) and preparations made from the same 11 compounds with which *E. culicivora* adults were tested previously (Nelson et al. 2012).

The test spiders were juveniles that had recently emerged from their egg sacs (body length 1.5–2.0 mm) and unmated adult females (body length 5 mm; matured 2–3 weeks before testing or pre-trial fasting began). Juveniles (see Table 1 for sample sizes) were tested with living plants and with individual compounds, but adults ($N = 25$) were tested only with living plants.

The juveniles were fed to satiation 2 days after emerging from the egg sac. 'Sated' juveniles were fed to satiation again on the fifth day after the initial meal and then tested on the following 2 days. 'Hungry' juveniles were kept without prey for 5 days and then tested on the following 2 days. Adults were fed to satiation and then tested the following day (sated) or, because the nutritional stress of a 5-day fast in adults is not especially high (Nelson and Jackson 2012), after a fast of either 10 or 20 days.

During testing, air was pushed by a pump (1,500 mL/min) in succession through three glass segments of the olfactometer, the odor chamber, the holding chamber and then the exit chamber (see Nelson et al. 2012). Other than using a shorter holding chamber (45 mm) when testing juveniles, olfactometer design and procedure were as in the earlier study (Nelson et al. 2012). Between trials, the apparatus was dismantled and cleaned with 80 % ethanol followed by distilled water and then oven dried.

To begin testing, the holding chamber containing a quiescent spider in the distal portion of the chamber was connected between the odor and exit chambers. Netting prevented entry into the odor chamber, so the only way out of the holding chamber was via the opening into the exit chamber. We recorded the time elapsing between the test beginning and the spider entering the exit chamber ('retention time'). As test duration was 1 h, we recorded retention time as 60 min whenever this time elapsed with the spider still in the holding chamber. Similar procedures were used when spiders were tested using the stems, leaves and flowers of a living plant as an odor source (see Nelson et al. 2012 for details).

All compounds were tested with the prescribed volume set at 1.0 μL added to 1 g of petroleum jelly as a 'carrier' (see Nelson et al. 2012 for details), with the carrier alone used as a no-odor control. For the compounds (β-caryophyllene, α-humulene and 1,8 cineole) known to be salient to *E. culicivora* adults (Nelson et al. 2012), we determined dose–response characteristics by setting the prescribed volume at 0.5 and at 4.0 μL.

For juveniles, all data sets were normally distributed, so paired *t* tests were used to analyze each individual's retention time when exposed to odor (experimental) compared with the no-odor control. We also carried out unpaired *t* tests to compare the experimental retention time of the hungry spider to that of sated juvenile spiders. In addition, as β-caryophyllene, α-humulene and 1,8 cineole were tested at different concentration levels, a 'difference score' was calculated for each test spider by subtracting its experimental retention time from its retention time when tested with the no-odor control. The resulting scores were used to run ANOVAs for the different concentration levels of these compounds separately for both hungry and sated spiders (using Tukey tests for pairwise comparisons).

Retention times were not normally distributed for adults, but the difference scores were. Correspondingly, we carried out Wilcoxon tests to compare the individual retention times in the paired odor and control tests, and used ANOVA on the difference scores to compare responses to *L. camara* odor at different hunger levels.

Table 1 *T* test results for comparisons to spider response to control (no odor) and different odors, both when sated and when hungry, and comparisons between retention times with odor when sated compared with when hungry ($N = df$ plus 1 in all cases)

	Paired *t* tests (retention time in experimental and control comparison)				Unpaired *t* tests (sated/hungry comparison)	
Odorant	Sated *t*, *df*	Sated *P*	Hungry *t*, *df*	Hungry *P*	*t*, *df*	*P*
Live *Lantana camara*	**4.170**, **29**	**0.0003**	**8.400**, **28**	**<0.0001**	**2.891**, **57**	**0.005**
α-Terpineol	0.472, 18	0.642	0.355, 23	0.726	0.197, 41	0.845
Aromadendrene	0.292, 17	0.774	0.604, 15	0.555	0.217, 32	0.829
δ-3-Carene	0.284, 13	0.781	0.512, 23	0.614	0.227, 36	0.822
Linalool	0.612, 18	0.548	0.477, 21	0.639	0.708, 39	0.483
Myrcene	0.623, 21	0.540	0.315, 20	0.756	0.208, 41	0.836
E-Nerolidol	0.611, 20	0.548	0.778, 15	0.449	0.680, 35	0.501
E-Ocimene	0.371, 20	0.714	0.173, 23	0.864	0.451, 43	0.654
Thujene	0.331, 19	0.744	0.595, 17	0.560	0.225, 36	0.823
1,8 Cineole (0.5 μL)	0.668, 23	0.512	0.856, 30	0.399	0.305, 53	0.762
1,8 Cineole (1.0 μL)	0.391, 22	0.700	0.308, 28	0.760	0.163, 50	0.871
1,8 Cineole (4.0 μL)	0.623, 23	0.540	0.800, 31	0.430	0.734, 54	0.466
β-Caryophyllene (0.5 μL)	0.612, 23	0.546	0.655, 29	0.518	0.607, 52	0.546
β-Caryophyllene 1.0 μL	**2.778**, **21**	**0.011**	**8.545**, **28**	**<0.0001**	**3.231**, **49**	**0.002**
β-Caryophyllene (4.0 μL)	**4.085**, **27**	**0.0004**	**9.161**, **30**	**<0.0001**	**3.020**, **57**	**0.004**
α-Humulene (0.5 μL)	0.767, 26	0.500	0.914, 19	0.372	1.439, 45	0.157
α-Humulene (1.0 μL)	**3.270**, **24**	**0.003**	**7.111**, **28**	**<0.0001**	**3.257**, **52**	**0.002**
α-Humulene (4.0 μL)	**2.878**, **25**	**0.008**	**8.863**, **35**	**<0.0001**	**2.737**, **60**	**0.008**

All compounds at 1.0 μL unless stated otherwise. Significant differences in bold

Results

Regardless of whether juvenile test spiders were sated or hungry, retention times when tested with *L. camara*, and when tested with 1.0 μL of β-caryophyllene or α-humulene were significantly longer in experimental (odor) than in control trials, but this was not the case with any of the other compounds. Moreover, when tested with living plants, β-caryophyllene or α-humulene, retention times for hungry spiders were significantly longer than for sated spiders (Table 1; Fig. 1).

When hungry, juvenile responses to the odor of β-caryophyllene ($F_2 = 17.83$, $P < 0.0001$) and α-humulene ($F_2 = 11.36$, $P < 0.0001$) differed depending on volume, with difference scores being significantly higher for 1.0 and 4.0 μL than for 0.5 μL. When sated, this effect was significant with α-humulene ($F_2 = 5.790$, $P = 0.005$), but was not quite significant for β-caryophyllene ($F_2 = 2.859$, $P = 0.064$). However, when the compound was 1,8-cineole, varying volume had no evident effect on difference scores of sated ($F_2 = 0.369$, $P = 0.693$) or hungry ($F_2 = 0.354$, $P = 0.703$) spiders.

Retention times of sated adults when tested with living *L. camara* were significantly longer in the experimental than in the control trials ($W = 214$, $P = 0.001$), as was the case when they were 10 ($W = 221$, $P = 0.002$) and 20 days hungry ($W = 225$, $P = 0.002$). However, no significant differences ($F_2 = 0.069$, $P = 0.933$) were evident when difference scores were used to compare sated spiders and spiders subjected to any fasting duration [mean (±SE) for sated spiders, 15.52 ± 3.81; 10 day fast, 17.56 ± 4.47; 20 day fast, 15.80 ± 4.33].

Discussion

Many volatile compounds are known from the headspace of *L. camara* (see Andersson et al. 2002), but earlier work provided evidence of only three of its principal components (β-caryophyllene, α-humulene and 1,8 cineole) being attractive to *E. culicivora* adults (Nelson et al. 2012). Having now carried out comparable experiments on *E. culicivora* juveniles, we found that juveniles did not respond to any of the compounds to which adults showed no response. However, while significant attraction by juveniles was detected for β-caryophyllene and α-humulene, we found no evidence of response by juveniles to 1,8 cineole, a compound to which adults responded less strongly than to β-caryophyllene and α-humulene.

In addition, juveniles, unlike adults (Nelson et al. 2012), did not respond to β-caryophyllene and α-humulene preparations with volume set at 0.5 μL. This suggests that the

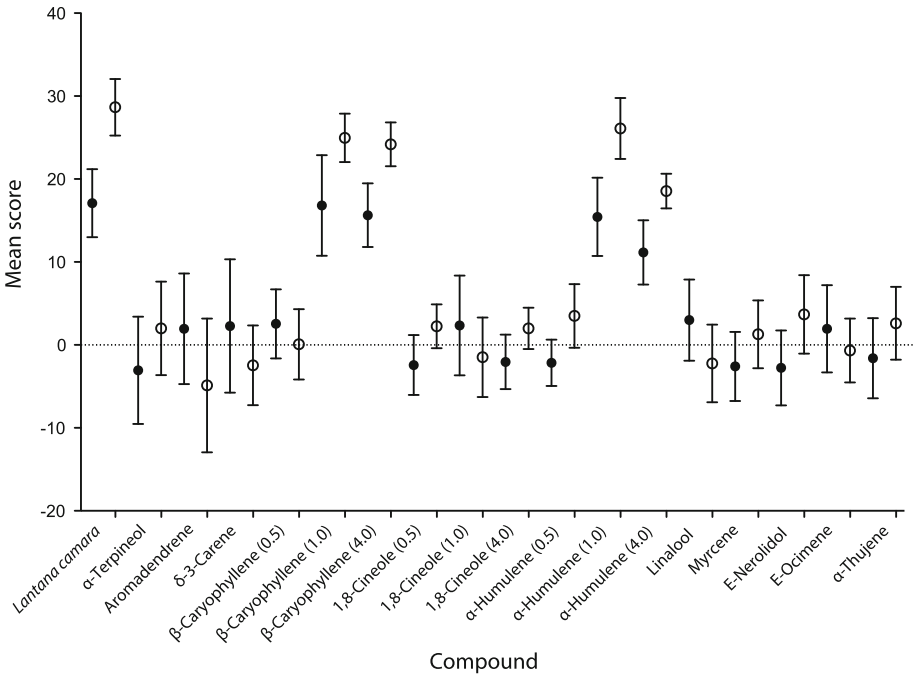

Fig. 1 Mean (±SE) of difference score of retention time of both hungry (*open circles*) and sated (*closed circles*) juveniles of *Evarcha culicivora* with living *Lantana camara* and with different compounds (and concentrations *indicated in parentheses*) found in the headspace of *L. camara*. Scores calculated by subtracting latency to leave holding chamber when tested with control from latency to leave holding chamber when tested with odor compound or with a live plant (positive: spider spent longer within holding chamber with odor; negative: spider spent longer within holding chamber with no-odor control; *dashed line* indicates equal time spent in both odor and no-odor treatments). Only live *L. camara*, and β-caryophyllene and α-humulene (at concentrations ≥1.0 μL) elicited significantly higher retention times between experimental and control trials

response threshold is higher for early-instar *E. culicivora* juveniles than for adults, perhaps as a consequence of that small spiders have fewer, or less well-developed, olfactory chemoreceptors than adults.

For olfaction, spiders probably rely on tarsal organs, which are small pits, or sometimes rods, on the dorsal side of each leg tarsus (Foelix and Chu-Wang 1973; Dumpert 1978). Whether juvenile spider tarsal organs differ from those of adults is unknown. Nevertheless, with contact chemoreceptors, Vallet et al. (1998), found that while the number of receptors on the legs of *Tegenaria atrica* (Agelenidae) increased as spiders got larger, their density remained similar. Furthermore, there was no electrophysiological evidence of differential responses to contact chemicals between juvenile and adult *T. atrica* (Vallet et al. 1998), failing to support the hypothesis that capacity for contact chemoreception of small juvenile spiders is inferior to that of adults.

Although field-collected adults of *E. culicivora* test positive for fructose, this is considerably less often than that found for small juveniles (Kuja et al. 2012), suggesting that it is primarily the early-instar juveniles of *E. culicivora* that feed on nectar. As predicted, we found that, compared to sated juveniles, hungry juveniles responded more strongly to the odor of living *L. camara* and to β-caryophyllene and α-humulene, suggesting that response to these odors functions for juveniles as a mechanism for finding food. In contrast, our finding that there was no significant effect of fasting on the response of adults to the odor of living *L. camara* provides support for our hypothesis that attraction to *L. camara* by adults may function primarily to locate these plants as sites for mating, rather than for obtaining nectar meals. This is the first study to reveal that attraction to plant odor by spiders may have different functions depending on life history stage.

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