Evidence that olfaction-based affinity for particular plant species is a special characteristic of *Evarcha culicivora*, a mosquito-specialist jumping spider

Running title: Plant affinity in a jumping spider

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ABSTRACT.

*Evarcha culicivora*, an East African jumping spider (family Salticidae), was shown in an earlier study to have an affinity for the odor from two particular plant species, namely *Lantana camara* and *Ricinus communis*. The olfactometer used in the earlier study was designed for choice testing. Here we focus on *L. camara* and, by using a second olfactometer method (retention testing), add to the evidence that the odor of this plant is salient to *E. culicivora*. Another 17 East African salticid species, all from different genera, were investigated using the same two olfactometer designs as used when investigating *E. culicivora*. The number of individuals of each of these 17 species that chose *L. camara* odor was not significantly different from the number that chose a no-odor control and, for each species, the latency to leave a holding chamber (retention time) in the presence of *L. camara* odor was not significantly different from retention time in the presence of a no-odor control. Based on these findings, we conclude that, rather than being a widespread salticid characteristic, an affinity for the odor of *L. camara* is a special characteristic of *E. culicivora*. 
Many insects that specialize at feeding on nectar and pollen associate with particular plant species (e.g., Chittka et al. 1999; Waser & Ollerton 2006; Díaz et al. 2007), and many insects are known to rely on specific blends of plant-derived volatile compounds for identifying the particular plant species they exploit as sites for feeding or oviposition (e.g., Pichersky & Gershenzon 2002; Bruce et al. 2005; Anfora et al. 2009; Karlsson et al. 2009). There are also examples of spiders that associate with particular types of plants, especially pitcher plants (Cresswell 1993) and bromeliads (Romero & Vasconcellos-Neto 2004, 2005). Besides offering opportunity for nectar and pollen meals (Vogelei & Greissl 1989; Pollard et al. 1995; Jackson et al. 2001; Taylor & Pfannenstiel 2008, 2009; Taylor & Bradley 2009), associating with plants may reward spiders with opportunity to feed on other plant products (Meehan et al. 2009) and on insects that land on the plants (Ruhren & Handel 1999; Whitney 2004). In some instances, the benefits of associating with plants may include opportunity to feed on insects ensnared by the plant’s sticky glandular hairs (Vasconcellos-Neto et al. 2007).

Little is known about the chemical cues by which spiders might identify specific plant species, but spiders are known to make use of chemosensory information regarding the sex, maturity, virgin-mated status, and fighting ability of conspecific individuals (Pollard et al. 1987; Clark et al. 1999; Roberts & Uetz 2005). Chemical cues are also known to be used by some spiders for detecting prey (Blanke 1972; Persons & Rypstra 2000; Clark et al. 2000a,b; Jackson et al. 2002, 2005) and predators (Persons et al. 2002; Li & Lee 2004; Li & Jackson 2005), and for determining the individual attractiveness of potential mates (Searcy et al. 1999; Roberts & Uetz 2005; Cross et al. 2009).
Two studies in particular suggest that further research is needed on how spiders might make use of plant-derived volatile compounds when identifying particular plant species. One of these studies showed associative learning by ‘ghost spiders’ (Hibana futilis Banks 1898, Anyphaenidae) when artificial odor was paired with artificial nectar (Patt & Pfannenstiel 2008). The other study showed that *Evarcha culicivora*, an East African salticid, responds in olfactometer experiments to the odor of two particular plant species on which it is commonly found, *Lantana camara* and *Ricinus communis* (Cross & Jackson 2009). That these two plant species might have a role in the mating system of *E. culicivora* has been suggested by other research (Cross et al. 2008) in which it was shown that, when on these plants, the courtship behavior of *E. culicivora* is more variable in display sequencing, more active, and more persistent. These effects are not evident during intraspecific interactions on a variety of other plant species (RRJ unpubl. data).

Here we focus on *L. camara* in particular and test 17 additional East African salticids with the odor of this plant species. Our hypothesis is that an affinity for the odor of *L. camara* is a special characteristic of *E. culicivora*. The rationale for testing other salticids is to consider, as an alternative hypothesis, the possibility that having an affinity for *L. camara* odor is a widespread salticid characteristic. Besides using choice-test olfactometers with these 17 salticid species (as adopted in Cross & Jackson’s (2009) study on *E. culicivora*), we also use retention-test olfactometers in experiments with these 17 salticids and with *E. culicivora*. Retention-test olfactometers are used for determining how long a test spider will remain in a small holding chamber when exposed to specific odors. This type of testing has been used in earlier research with *E. culicivora* (Cross et al. 2009), but never before specifically for examining response to plant odor.
METHODS

General.- Olfactometer testing was carried out using salticids from laboratory cultures (F2 and F3 generation). Rearing methods, as well as the basic procedures used in olfactometer experiments, were as in earlier research (Cross & Jackson 2009; Cross et al. 2009) and only essential details are provided here.

For rearing and maintenance, each spider was fed to satiation three times a week on blood-carrying female mosquitoes (Anopheles gambiae s.s. from laboratory culture) and ‘lake flies’ (Nilodorum brevibucca, Chironomidae; collected as needed from field). Hunger level was standardized by subjecting each test spider to a 7-day pre-trial fast.

Two olfactometer methods were used (choice testing and retention testing), with the odor source being a plant cutting held in an odor chamber (glass-cube box). Each cutting was two Lantana camara umbels (clusters of flowers) with accompanying leaves and stems (no flowers senescent) taken from the field immediately before setting up for an experiment; median weight/umbel (1st and 3rd quartiles) = 364 (329 and 384) mg, (n=10). Disposable surgical gloves were worn while collecting and handling plant material.

Testing was carried out between 0800 h and 1400 h (laboratory photoperiod 12L-12D, lights on at 0700 hr). Between trials, olfactometers were dismantled and cleaned with 80% ethanol followed by distilled water and then dried in an oven. Airflow in the olfactometers was adjusted to 1500 ml/min (Matheson FM-1000 airflow regulator) and there was no evidence that this setting had any adverse effects on the salticid’s locomotion or other behavior. The spiders used in choice tests were different from the spiders used in retention tests, but no test spiders had prior experience with plants. No spider was used in more than one choice test or more than one pair
(experimental one day, control another day) of retention tests. All test spiders were adults that matured 2-3 weeks before being tested and none had mated. Both sexes of all species were used in choice testing, but only males were used in retention testing.

**Choice testing.**- Y-shaped glass olfactometers were used for choice testing (Fig. 1a). The two ends of the Y were the ‘choice arms’, with each choice arm being connected to an odor chamber. Which of the two odor chambers contained the plant cutting was determined at random. Air was pumped separately into the two odor chambers and then through the choice arms before converging at the stem of the Y (‘test arm’).

Before testing began, the test spider (N = 70 per sex and species) was confined for 2 min in a holding chamber at the far end of the test arm. While in the holding chamber, the test spider’s access to the test arm was blocked by a removable metal grill that fit within a slit in the chamber roof. Testing began by lifting the grill. When the spider entered a choice arm and remained there for 30 sec, we recorded the arm entered as the test spider’s choice. The spider was allowed 30 min within which to make a choice and the number of spiders that failed to make a choice was, for each species, always fewer than 5% of the spiders tested.

**Retention testing.**- During retention testing, air was pushed successively through an odor chamber, a holding chamber and an exit chamber (Fig. 1b). The holding chamber was a glass tube (rubber stopper in one end, other end open). The open end of the holding chamber fit securely in the hole in the glass cube that formed the exit chamber, flush with the inner wall of the exit chamber. At the other end of the holding chamber, there was a hole in the stopper with a glass tube going through to the odor chamber, which was identical in size to the exit chamber (see Fig. 1b for dimensions). A
nylon-netting screen over the stopper (new netting for each test) ensured that the test spider could not enter the odor chamber, the only way out of the holding chamber being via the opening into the exit chamber. The exit chamber was another glass cube identical to the odor chamber.

The test spider (N = 20 for each species) was first kept in the holding chamber for 2 min, with the holding chamber not yet connected to the stimulus and exit chambers. The end of the holding chamber that would go into the exit chamber was plugged with a rubber stopper. To begin a test, this stopper was removed and the holding chamber was positioned between the stimulus and exit chamber, but with a prerequisite being that the test spider had to be in the half of the holding chamber distal to the exit chamber. If this prerequisite was not met at the end of the 2-min pre-test period, the beginning of the test was delayed until the spider moved on its own accord into the distal half of the chamber and remained there for 2 min. Testing was aborted if this criterion was still not met after waiting 15 min, but aborted tests were rare (< 5% for any given species).

No-odor control tests and odor tests were randomized. Once testing began, we recorded retention time (i.e., the test spider’s latency to leave the holding chamber, defined as the time elapsing between the beginning of a test and departure by the spider into the exit chamber; maximum time allowed, 60 min). By default, the spider’s retention time was recorded as 60 min whenever the 60-min test period ended with the test spider still in the holding chamber.

Data analysis.- Choice-test data were analyzed using tests for goodness of fit (H₀=50:50) and retention-testing data were analyzed using non-parametric Wilcoxon tests for paired comparisons (null hypothesis: latency to leave holding chamber when tested with odor source matched latency to leave holding chamber when tested with no-odor control). Retention testing data are shown based on each test spiders’ calculated absolute difference score (subtracting its latency to leave
holding chamber when tested with control from latency to leave holding chamber when tested with odor), resulting in positive scores when the spider spent more time in the holding chamber when tested with odor, and resulting in negative scores when spider spent more time in the holding chamber when tested with no odor.

Voucher specimens of all species have been deposited in the Florida State Collection of Arthropods, Gainesville, Florida, USA.

RESULTS

Choice-test data from males and females of each species did not differ in any case, so these data were pooled for simplification. In the earlier study (Cross & Jackson 2009), *Evarcha culicivora* chose *Lantana camara* odor significantly more often than the no-odor control in choice-test olfactometers (Fig. 2) and, in the present study, *E. culicivora* had a significantly longer latency to leave the holding chamber when in the presence of *L. camara* odor than when in the presence of a no-odor control. However, for the other 17 salticid species, the number of individuals that chose *L. camara* odor was not significantly different from the number that chose the no-odor control in the choice-test olfactometers. In the retention-test olfactometers the retention time in the presence of *L. camara* odor was also not significantly different, for these 17 species, from retention time in the presence of the no-odor control (Table 1, Fig. 3; note non-significant trend for *Natta rufopicta* to display greater retention in the control tests). In concordance with results from olfactometer choice tests, *L. camara* odor did induce a significant retention in *E. culicivora* (Table 1).

DISCUSSION
The earlier study (Cross & Jackson 2009) demonstrated that the odor of *Lantana camara* is salient to *Evarcha culicivora*, but left unresolved the question of whether responsiveness to *L. camara* odor by *E. culicivora* is an unusual characteristic of this particular salticid species or, alternatively, a characteristic that is widespread in the Salticidae. Here we investigated another 17 salticid species, all from different genera, from East Africa. For each of these species, when we used the same odor-based choice-testing methods and achieved the same sample sizes as in the earlier experiments with *E. culicivora*, the number of individuals that chose *L. camara* odor was not significantly different from the number that chose the no-odor control. Using the retention-testing olfactometers, we again found evidence that the odor of *L. camara* is salient to *E. culicivora* and, for retention tests, as for choice tests, the response to *L. camara* odor by each species other than *E. culicivora* was not significantly different from response to no-odor controls.

As there are more than 5,000 described species in the family Salticidae (Platnick 2010), our findings should not be construed as proving that *E. culicivora* is absolutely unique, but it seems unlikely that responsiveness to *L. camara* odor is widespread within the Salticidae.

The precise role of *L. camara* in the biology of *E. culicivora* is poorly understood. Earlier research (Cross et al. 2008) suggested that we need a better understanding of the role plants might play in the mating strategy of *E. culicivora*, but plants may also have a role in its feeding strategy. As *E. culicivora* is known to feed on nectar (RRJ unpubl. data), one hypothesis that should be considered is that responding to *L. camara* odor is related to visiting this plant species for nectar meals. This would make *E. culicivora* comparable to *Heliconius melpomene*, a butterfly that, by responding to the odor of *L. camara*, locates and feeds on the nectar of this plant (Andersson et al. 2002; Andersson & Dobson 2003). However, there is a complication with any hypothesis concerning *E. culicivora* having evolved mechanisms of exploiting specifically *L.*
*camara*. This plant species is native to the Americas and is an introduced weed in many parts of the world, including East Africa (Day et al. 2003). We need a better understanding of how *E. culicivora* responds to a wider range of plant species, including native species with which it has shared a longer evolutionary history, in addition to the primary volatile components of various plants, before we can tease apart the basis of this affinity. This large topic is the subject of ongoing research.

*Evarcha culicivora* has an unusual predatory strategy, as its preferred prey are blood-carrying mosquitoes (Jackson et al. 2005). An alternative hypothesis is that this mosquito-specialist spider locates its prey by visiting *L. camara* or other plants. Only female mosquitoes feed on blood (Clements 1999). Male mosquitoes feed primarily on nectar, but *E. culicivora* is proficient at discriminating between males and females, has an active preference for female mosquitoes as prey and chooses *Anopheles* in preference to other mosquitoes (Nelson & Jackson 2006). However, it is now well established that visiting plants for nectar meals is important not only for the male but also for the female of a variety of mosquito species, including *Anopheles* species (McCrae et al. 1969, 1976; Gujral & Vasudevan 1983; Clements 1999; Foster & Takken 2004; Impoinvil et al. 2004; Manda et al. 2007a,b). However, it is unlikely that encounters between *E. culicivora* and female mosquitoes, including *Anopheles*, often happen on *L. camara* or other plants, as *E. culicivora*, like most salticids (Richman & Jackson 1992), appears to be active as a predator during daylight hours (RRJ unpubl. data) while its prey, the female mosquito, probably feeds from plants primarily at night. This problem notwithstanding, *E. culicivora* might find mosquitoes during the daytime resting post-feeding in the vicinity of the plants to which the spider and the mosquito have been attracted, albeit at different times.
ACKNOWLEDGMENTS

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REFERENCES


Figure 1.- Olfactometers used for: a. choice testing (view of odor source obstructed by opaque barrier) and b. retention testing (view of odor source obstructed by black paper taped to outside of odor chamber wall that faced holding chamber). Dashed arrows indicate direction of airflow. Not drawn to scale.
Figure 2.- Pooled results from olfactometer choice-tests. *Evarcha culicivora* chose odor arm significantly more often control arm (data from Cross and Jackson 2009). For all other salticid species, number of individuals that chose odor not significantly different from number that chose control. Dashed line denotes 50%. N = 140. $\chi^2$ = test of goodness of fit. *** P < 0.0001.
Figure 3.- Boxplots (median and quartiles) with whiskers (min and max) for retention testing for all species. Score calculated by subtracting latency to leave holding chamber when tested with control from latency to leave holding chamber when tested with odor (positive score: spider spent more time in the holding chamber when tested with odor; negative score: spider spent more time in the holding chamber when tested with control). N = 20.
Table 1. Test results for Wilcoxon-tests comparing latency to leave holding chamber in control or experimental tests. The difference in time between these is depicted in Fig. 3. All spiders sourced in Kenya except *Parajotus cinereus* (from Uganda).

<table>
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<tr>
<th>Test spider species</th>
<th>W</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td><em>Evarcha culicivora</em> Wesolowska &amp; Jackson 2003</td>
<td>176.0</td>
<td>0.0004</td>
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<td><em>Asemonea murphyae</em> Wanless 1980</td>
<td>-26.0</td>
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<td><em>Cyrba ocellata</em> (Kroneberg 1875)</td>
<td>70.0</td>
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<td><em>Goleba puella</em> (Simon 1885)</td>
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<tr>
<td><em>Hasarius adansoni</em> (Savigny et Audouin 1825)</td>
<td>3.0</td>
<td>0.97</td>
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<td><em>Heliophanus</em> sp.</td>
<td>51.0</td>
<td>0.24</td>
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<td><em>Holcolaetis vellerea</em> (Simon 1909)</td>
<td>-21.0</td>
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<td><em>Hyllus</em> sp.</td>
<td>50.0</td>
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<td><em>Menemerus congoensis</em> Lessert 1925</td>
<td>28.0</td>
<td>0.56</td>
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<td><em>Myrmarachne melanotarsa</em> Wesolowska &amp; Salm 2002</td>
<td>73.0</td>
<td>0.15</td>
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<td><em>Natta rufopicta</em> (Simon 1909)</td>
<td>82.0</td>
<td>0.08</td>
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<td><em>Pachyballus cordiformis</em> Berland et Millot 1941</td>
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<td>19.0</td>
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<td>1.00</td>
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<tr>
<td>Species</td>
<td>Percentage</td>
<td>Abundance</td>
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<td>-------------------------</td>
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<td><em>Plexippus sp.</em></td>
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<td><em>Portia africana</em> (Simon 1885)</td>
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<td><em>Pseudicius sp.</em></td>
<td>40.0</td>
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