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Ontogenetic shift in plant-related cognitive specialization by a mosquito-eating predator

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Abstract

Evarcha culicivora, an East African salticid spider, is a mosquito specialist and it is also a plant specialist, with juveniles visiting plants primarily for acquiring nectar meals and adults visiting plants primarily as mating sites. The hypothesis we consider here is that there are ontogenetic shifts in cognition-related responses by *E. culicivora* to plant odour. Our experiments pertain to cross-modality priming effects in three specific contexts: executing behaviour that we call the ‘visual inspection of plants’ (Experiment 1), adopting selective visual attention to specific visual targets (Experiment 2) and becoming prepared to respond rapidly to specific visual targets (Experiment 3). Our findings appear not to be a consequence of salient odours in general elevating *E. culicivora*’s motivation to respond to salient visual stimuli. Instead, effects were specific to particular odours paired with particular visual targets, with the salient volatile plant compounds being caryophyllene and humulene. We found evidence that prey odour primes juveniles and adults to respond to seeing specifically prey, mate odour primes adults to respond to seeing specifically mates and plant odour primes juveniles to respond to seeing specifically flowers. However, plant odour appears to prime adults to respond to seeing specifically a mate associated with a plant.

Keywords

cross-modality priming; selective attention; spider; Salticidae; *Evarcha culicivora*; *Lantana camara*

1. Introduction

It is often acknowledged that, even for vertebrates (Shettleworth, 2010; Vallortigara et al., 2010), including humans, cognitive processes such as working memory and selective attention are subject to substantial capacity limitations (Driver, 2001; Dukas, 2004; Cowan, 2010). Compared with most vertebrates, insects and spiders have nervous systems with vastly fewer, but not markedly smaller, neurons (Homberg, 2008; Eberhard and Weislo,

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2012), and this disparity makes it easy to assume a comparable disparity in cognitive ability. However, the actual consequences of having fewer neurons remain poorly understood (see: Eberhard, 2011; Eberhard and Wcislo, 2011) and there is considerable experimental evidence that insects and spiders express at least rudimentary capacities for working memory, selective attention, numerical judgment, reasoning and planning (Zhang et al., 2005; Menzel et al., 2007; Dacke and Srinivasan, 2008; Jackson and Cross, 2011; Jakob et al., 2011; Nelson and Jackson, 2012a; Cross and Jackson, 2014, 2016, in press; Giurfa, 2015; Alem et al., 2016).

Our overarching hypothesis is that highly structured cognitive specialization often underpins the complex strategies adopted by predatory arthropods (Jackson and Cross, 2013, 2015). Some of the most complex predatory strategies known for animals in general have come from research on salticids ('jumping spiders'; e.g., Nelson and Jackson, 2011; Jackson and Cross, 2013). These spiders have eight eyes and, owing to a large forward-facing pair, known as the principal eyes, they have a capacity for high-precision visual discrimination (Harland et al., 2012; Land and Nilsson, 2012; Dolev and Nelson, 2014; Menda et al., 2014). Many salticids also have a well-developed capacity for precise odour-based identification of prey and mates (Cerveira and Jackson, 2011, 2013; Nelson et al., 2012a; Uhl, 2013). However, instead of considering these two sensory modalities in isolation from each other, our objective here is to investigate how cognitive specialization is expressed by a salticid in the context of cross-modality effects of olfaction (i.e., a sense of smell) on vision-guided behaviour (see Jackson et al., 2002; Jackson and Cross, 2009a; Uhl and Elias, 2011; Tedore and Johnsen, 2013).

Cross-modality effects are known especially for *Evarcha culicivora*, a mosquito-specialist salticid from East Africa (Jackson and Cross, 2015) that feeds indirectly on vertebrate blood by actively choosing blood-carrying female mosquitoes as preferred prey (Jackson et al., 2005), and then takes prey discrimination a step further by actively choosing *Anopheles* as its preferred genus of mosquitoes (Nelson and Jackson, 2006). Both sexes of *E. culicivora* smell more attractive to mates after indirect blood meals (Cross et al., 2009) and, besides being proficient at identifying prey, mates and conspecific rivals by sight and by smell (Nelson and Jackson, 2012b; Cross and Jackson, 2013), *E. culicivora* is also attracted to human odour (Cross and Jackson, 2011) and to the odour of two particular plant species (Cross and Jackson, 2009b), *Lantana camara* and *Ricinus communis*.

We know from research in Kenya that the dominant volatile compounds emitted by one of these plants, *L. camara*, are (*E*)- β -caryophyllene, α -humulene and 1,8 cineole (hereafter referred to simply as caryophyllene, humulene and cineole) and that caryophyllene and humulene attract *E. culicivora* in olfactometer experiments (Nelson et al., 2012b). Adults, but not juveniles, are also attracted to cineole, albeit less strongly than to caryophyllene and humulene. Another juvenile-adult difference is that, after a fast, the juveniles but not the adults of *E. culicivora* become more strongly attracted to the odour of caryophyllene and humulene (Nelson and Jackson, 2013; Kuja et al., 2012), with the benefits of nectar meals for juveniles including rapid improvement in their prey-capture efficiency (Carvell et al., 2015). Although the role of plants in the biology of adults is less well understood, natural-

history observations suggest that *L. camara* might be important as a mating site (Cross et al., 2008).

This combination of findings suggests that, for *E. culicivora*, there are ontogenetic shifts in the way plant-related cross-modal effects are expressed. The ontogenetic shifts we consider here pertain to three specific contexts: eliciting vision-based plant-investigating behaviour (Experiment 1), priming selective visual attention to specific visual targets (Experiment 2) and priming a rapid response to specific visual targets (Experiment 3). By ‘priming’, we mean that exposure to particular odour sources predisposes *E. culicivora* to be more responsive to particular visual targets (e.g., see Cross and Jackson, 2009a). Our hypothesis is that, for *E. culicivora*, there is a shift in context for which plant odour is relevant, with the context for juveniles being nectar meals and with the context for adults being mating.

2. Materials and methods

2.1. General

In three experiments, we used spiders from laboratory cultures (F2 & F3 generation) derived from individuals collected at our field site in Mbita Point, Western Kenya (elevation 1200 m above sea level; latitude 0°25′S–0°30′S; longitude 34°10′E). As our methods for rearing and maintaining spiders in the laboratory corresponded closely to those in earlier studies (e.g., Cross and Jackson, 2013), only critical details are provided here.

We used chironomid midges and blood-fed mosquitoes as the standard diet for all spiders and their parents. The midges were collected from the field as needed. The mosquitoes we used for rearing, and as odour sources and mounts in experiments, were always *Anopheles gambiae* s.s. taken from stock cultures. Owing to our interest in specifically the innate predispositions of *E. culicivora*, we wanted to minimize any influence of learning and maternal effects on the outcomes of our experiments (see Jackson and Cross, 2011). With this goal as our rationale, we ensured that no test spider had prior experience with conspecific individuals and that neither the test spider nor its parents had prior experience with the apparatus, with any of the plant species or with any of the three compounds we used in experiments. No individual spider was tested more than once, and no individual spider was an experimental subject in any other research project. All experiments were carried out between 0800 and 1400 hours (laboratory photoperiod 12L:12D, lights on 0700 hours).

We used six test-spider categories: adult males (5.0 mm), adult females (5.5 mm) and four size classes of juveniles (1.5 mm, 2.5 mm, 3.5 mm and 4.5 mm). Size was body length (accurate to the nearest 0.5 mm) measured from the anterior end of the cephalothorax to the posterior end of the abdomen, exclusive of spinnerets. These measurements were made on the day of testing, which was always 1 day after the spider’s last meal. All adult test spiders had matured during a 2–3 week period preceding use in experiments. All juvenile test spiders had moulted at least 3 days before use in experiments and none moulted again for at least another 3 days. The expressions ‘large’ and ‘small’ were used for juveniles that were 4.5 mm or 1.5 mm in body length, respectively. The different test-spider categories used in different experiments are specified below. Body lengths of *E. culicivora* and *An. gambiae* used for making mounts were also accurate to the nearest 0.5 mm.

The apparatus was specific to each of the three experiments (Figs. 1–3), but there were also some basic similarities. The test spider was always confined to a central chamber (the ‘arena’) from which it could view, but not enter, display chambers. Many components of the apparatus were made from glass, with all glass pieces being transparent and 3 mm thick. For all experiments, there was an odour chamber in which an odour source could be housed. Using a pump and an airflow meter (Matheson FM-1000 set at 1500 mL/min), air was pushed through the odour chamber and then through the arena. Silicone tubing connected the airflow meter and the pump to various glass tubes which, in turn, were connected to the arena.

The entire apparatus was lit by a 100-W incandescent lamp centred 400 mm overhead, with fluorescent ceiling lamps providing additional ambient lighting. Between trials, the arena and odour chamber were dismantled and wiped clean with 70% ethanol and then with distilled water, after which they were dried. Removable lids on the arena and the odour chamber facilitated cleaning. All netting, stoppers and silicone tubing were replaced after each trial.

3. Experiment 1: Odour priming of vision-based plant investigating behaviour

For this experiment, our primary hypothesis was that plant-related odour elicits vision-based inspection of plants by the adults and the juveniles of *E. culicivora*. We considered whether these odour effects are expressed specifically in the context of seeing *L. camara* or whether they might be expressed in the context of seeing a wider range of plants. Based on earlier findings (Nelson et al., 2012b), we predicted that caryophyllene and humulene, but not cineole, would mediate these effects. We also proposed that it is specifically plant-related odour that elicits visual inspection of plants instead of it being the case that salient odours in general elevate *E. culicivora*’s motivation to inspect plants (i.e., we predicted that we would find no effect of prey or mate odour).

3.1. Materials and methods

3.1.1. Apparatus—There were two versions of the apparatus, ‘large’ for testing *E. culicivora* adults (Fig. 1) and ‘small’ for testing *E. culicivora* juveniles (Fig. 2). The spider could not leave the apparatus during trials because there was nylon netting over the arena’s inflow and outflow holes. Glass tubes were inserted in these holes, with the distal end of the outflow tube being open, but with a rubber stopper being in the distal end of the inflow tube.

Surrounding the arena were four display chambers, each aligned next to one of the four sides of the arena table. There were two grooves (3 mm wide, 40 mm apart) that ran from the top to the bottom of the side walls of each display chamber, and these grooves also ran across the floor of each chamber. A removable glass front was positioned in the groove closest to the arena and a removable wooden back was positioned in the other groove. The inner side of the glass front was 40 mm from the inner side of the wooden back. With this arrangement of the display chambers, the arena was surrounded by glass that rose to 115 mm above the top of the arena table.

A hole (diameter 12 mm) was centred in the bottom of each display chamber and, correspondingly, in the display chamber table. A plant cutting (stem, leaves and flowers) protruded through the hole in one of the four chambers (designated as 'display chamber 1'), with this chamber being chosen at random for each trial. The other chambers remained empty and the holes in these three chambers were plugged with rubber stoppers. The top of the cutting was about 10 mm below the top of the chamber and its cut end sat in a pot of water below the chamber. The plants we used were *Lantana camara* (Verbenaceae), *Bougainvillea glabra* (Nyctaginaceae), *Lippia kituensis* (Verbenaceae), *Parthenium hysterophorus* (Asteraceae) and *Senna didymobotrya* (Fabaceae), all of these plants being common in *E. culicivora*'s habitat.

Inside the arena, there were four aluminium partitions extending 40 mm from each of the four corners of the arena toward the centre of the arena. The height of each partition matched the inside height of the arena, meaning that the partitions created five sectors within the arena. 'Side 1' of the arena faced the plant cutting in display-chamber 1 and sector 1 was the space within a truncated triangle created by side 1 and the two partitions that intersected side 1. Comparable definitions applied to sectors 2–4. The centre sector was the circular space at the centre of the arena beyond the extent of the partitions; whenever the test spider moved from one side sector to another, it had to pass through the centre sector.

The odour chamber remained empty during no-odour control trials, but it housed an odour source during experimental trials. Two holes (diameter 20 mm), each on an opposite side of the odour chamber, were plugged with rubber stoppers. There was a hole in each of these stoppers, as well as in the stopper situated in the arena's inflow hole. A small glass tube (length 45 mm, diameter 4 mm) was positioned in each stopper hole. For airflow into the arena, one silicone tube connected the air pump to one of the glass tubes in the odour chamber, and another silicone tube connected the other glass tube in the odour chamber to the glass tube in the arena. The inner side of each stopper in the odour chamber was covered with nylon netting.

3.1.2. Procedures—Depending on the experiment, the odour source was 10 *Anopheles* females (prey) that had fed on blood 4–5 hours before being used, one opposite-sex conspecific individual (mate) or a sample of a specific compound (caryophyllene, humulene or cineole). The sample was prepared by adding 4 µl of the compound to 1.0 g of petroleum jelly that was situated in the centre of an open glass Petri dish (diameter 30 mm). Each sample was prepared 24 h earlier and kept in a refrigerator (Petri dish, with lid in place, wrapped in aluminium foil) until 60 min before a trial began, when it was transferred to the odour chamber. Prey and mates were also put into odour chambers 60 min before trials began. For ventilating the arena with the specific odour, the odour chamber was connected to the airflow system during the 60-min pre-trial period.

Spiders from six test-spider categories were presented (see above for definitions) with different combinations of a visual stimulus (i.e., a plant cutting in display-chamber 1) and an olfactory stimulus (i.e., prey items, a mate or a volatile compound in the odour chamber) (Figs. 4 and 5). At the start of each trial, the test spider was confined by rubber stoppers to a glass transfer tube (diameter 12 mm, length 25 mm) for a 5-min acclimation period after

which one stopper was removed and the open end of the transfer tube was inserted into the arena outflow hole. Whenever the spider failed to move into the arena on its own accord within 2 min, we removed the other stopper and, using a soft brush, coaxed it into the arena. Once the spider was in the arena (Figs. 1 and 2), we removed the transfer tube and returned the other glass tube (with netting) to the outflow hole. After the spider entered the arena, the trial lasted for 30 min during which time we recorded how long the test spider spent in each sector. The test spider's 'response duration' was the total time spent in sector 1.

3.1.3. Data Analysis—We used analysis of variance (ANOVA) after first determining that our data met the assumptions of ANOVA (Bartlett's tests for homogeneity of variance and Shapiro-Wilks tests for normal distribution of residuals). Using a series of six 2-factor ANOVAs from the statistics package R (R Core Team, 2013), we considered specific questions regarding the interaction of test-spider category, plant species and olfactory stimulus. In all instances, CI is the 95% confidence interval around the mean. When ANOVA outcomes revealed a significant effect, we then carried out *post hoc* Tukey's range tests. For all treatments, N = 20 (total N = 680 different spiders).

3.2. Results and Discussion

Using adults of both sexes and juveniles of all of our size classes as test spiders, we first investigated the influence of caryophyllene on response duration when the plant in display chamber 1 was *L. camara* (Fig. 4A). We found a significant effect of caryophyllene odour ($F(1,228) = 202.20$, $p < 0.001$, partial $\eta^2 = 0.470$), but no significant effect of test-spider category ($F(5,228) = 1.15$, $p = 0.336$, partial $\eta^2 = 0.025$) and no significant interaction effect between test-spider category and odour ($F(5,228) < 1$). Compared to the no-odour control, mean response duration was 8 min longer (95% CI [7.1–9.3 min]) in the presence of caryophyllene, this being a significant difference ($p < 0.001$). On the basis of these findings, we conclude that, for all test-spider categories, caryophyllene is an olfactory stimulus that influences visual inspection of *L. camara*.

For considering whether the effect of caryophyllene on the visual inspection of plants is specific to *L. camara* as the visual stimulus, we used adult males as test spiders in a series of trials in which we used other plants in place of *L. camara* (*Lippia kituensis*, *Parthenium hysterophorus* and *Senna didymobotrya*; Fig. 4B). We found a significant effect of caryophyllene on response duration ($F(1,114) = 112.67$, $p < 0.001$, partial $\eta^2 = 0.497$), but no significant effect of the particular plant species used as the visual stimulus ($F(2,114) = 1.24$, $p = 0.293$, partial $\eta^2 = 0.021$) and no significant interaction effect between plant species and type of odour ($F(2,114) < 1$). Compared to the no-odour control, mean response duration was 7 min longer (95% CI [5.9–8.6 min]) in the presence of caryophyllene, this difference being significant ($p < 0.001$). On the basis of these findings, we conclude that, at least for adult males, caryophyllene affects the visual inspection of a wider range of plant species instead of being an effect specific to the plant being *L. camara*.

We had arbitrarily chosen males to use in these trials because, as our findings were consistent for all test-spider categories when the plant was *L. camara*, it seemed unlikely that the test-spider category would matter with these other plants. We also have confidence in

concluding that test-spider category did not matter because, besides adult males, we also used adult females and small juveniles when the plant in display chamber 1 was *Bougainvillea glabra* (Fig. 4C). We found a significant effect of caryophyllene odour ($F(1,114) = 66.35$, $p < 0.001$, partial $\eta^2 = 0.368$), but not of test-spider category ($F(2,114) < 1$). Compared to the no-odour control, mean response duration was 7 min longer (95% CI [5.3–8.7 min]) in the presence of caryophyllene, this difference being significant ($p < 0.001$). There was no significant interaction effect between test spider category and odour ($F(2,114) < 1$).

Based on these findings from using five plant species, it seems likely that, for all test-spider categories, caryophyllene's effect on plant-investigation behaviour applies to a range of species wider than just *L. camara*. Of course, a lot more work will be needed for determining precisely how wide this range might be. However, it is not surprising that the effects of caryophyllene on *E. culicivora*'s vision-based plant-investigation behaviour extend to other plants. Although *L. camara* is currently a dominant plant species encountered by *E. culicivora* in the field, this is an introduced species from the American tropics (Sharma et al., 2005) whereas *E. culicivora* is known solely from East Africa (Wesolowska and Jackson, 2003). We assume that on this basis, for most of its evolutionary history, *E. culicivora* would have had no contact with *L. camara*.

In most instances, caryophyllene was the plant-related odour that we used. However, by also using humulene and cineole in a subset of our experiments (Fig. 5A), we considered whether compounds other than caryophyllene have odour effects on the visual inspection of *L. camara*. We found a significant effect of the volatile compound ($F(3,152) = 31.25$, $p < 0.001$, partial $\eta^2 = 0.382$), but no significant effect of the test-spider category ($F(1,152) < 1$) and no significant interaction effect between test-spider category and volatile compound ($F(3,152) < 1$). Compared to no-odour controls (Fig. 5A), mean response duration was 7 min longer (95% CI [4.4–10.0 min]) in the presence of humulene, a difference which was significant ($p < 0.001$); although the mean response duration in the presence of cineole was 33 s shorter (95% CI [−2.2–3.3 min]) than in control trials, this difference was not significant ($p = 0.956$). Mean response duration was 8 min longer (95% CI [4.9–10.5 min]) in the presence of caryophyllene than in the presence of cineole, a difference that was significant ($p < 0.001$); although the mean response duration in the presence of humulene was 8 s longer (95% CI [−2.7–2.9 min]) than in the presence of caryophyllene, this difference that was not significant ($p = 0.999$). Mean response duration was 8 min longer (95% CI [5.0–10.6 min]) in the presence of humulene than in the presence of cineole, a difference that was significant ($p < 0.001$). On the basis of these findings we conclude that humulene as well as caryophyllene, but not cineole, influences the visual inspection behaviour of adult test spiders when the plant is *L. camara*.

As humulene and caryophyllene are closely-related compounds that tend to be found together (Knudsen et al., 2006), it was not surprising that these compounds had similar effects. Nor was it surprising that we found no significant effect of cineole on *E. culicivora*, as this compound has previously been shown to be less attractive to *E. culicivora* than caryophyllene or humulene in olfactometer experiments (Nelson et al., 2012b). More compounds need to be used in experiments before concluding that caryophyllene and

humulene are the only plant compounds that affect visual plant investigation by *E. culicivora*. Yet, even if additional experiments reveal no evidence of other compounds being effective, this special status of caryophyllene and humulene would not, by itself, imply a special connection between *E. culicivora* and *L. camara*. This is because, instead of being specific to *L. camara*, humulene and especially caryophyllene are emitted by many plant species (Knudsen et al., 2006).

However, we were more interested in a different question. By using prey (Fig. 5B) instead of caryophyllene as the odour source, we asked whether it is specifically plant-related odour that affects *E. culicivora*'s vision-based inspection of *L. camara*. For this, the test spiders were adult males, adult females, small juveniles and large juveniles and we compared data from the no-odour control trials with data from trials during which prey odour was present. We found no significant effects of prey odour ($F(1,152) < 1$) or test-spider category ($F(3,152) = 2.43$, $p = 0.068$, partial $\eta^2 = 0.046$) on visual inspection of *L. camara* and there was no interaction effect between test-spider category and odour ($F(3,152) < 1$).

Although not significant ($p = 0.068$), there might be a suggestion that additional work would show an effect of test-spider category. Whether this is the case is, however, a secondary issue. Our primary conclusion is that there is no evidence of prey odour affecting plant-investigation behaviour.

As another step toward considering whether it is specifically plant-related odour that affects *E. culicivora*'s visual inspection of *L. camara*, we used mates (Fig. 5C) instead of caryophyllene or prey as the odour sources, with test spiders being adult males and females. When we compared data from the no-odour control trials with data from trials during which mate odour was present, we found no significant effect of mate odour ($F(1,76) = 1.36$, $p = 0.247$, partial $\eta^2 = 0.018$) or test-spider category ($F(1,76) < 1$) on the visual inspection of *L. camara*, and no significant interaction effect between test-spider category and odour ($F(1,76) < 1$). On the basis of these findings, combined with the findings from using prey odour, we conclude that it is specifically plant-related odour that motivates *E. culicivora* to spend more time visually inspecting *L. camara*.

4. Experiment 2: Odour priming of selective visual attention to specific targets

Experiment 2 resembled Experiment 1 by being designed for recording response duration as the data of interest, but with a different objective. Instead of investigating odour effects on the visual inspection of plants, our objective here was to detect odour priming of selective attention to particular visual targets associated with the plants: flowers (*Lantana camara*), prey (blood-carrying *Anopheles* females) or mates (opposite-sex conspecific individuals).

We considered four hypotheses pertaining to particular odours priming selective visual attention to specific visual targets.

1. For *E. culicivora* adults and juveniles, prey odour is a stimulus that elicits selective visual attention to prey.

2. For *E. culicivora* adults, mate odour is a stimulus that elicits selective visual attention to mates.
3. For *E. culicivora* juveniles, but not adults, caryophyllene is an odour that elicits selective visual attention to flowers.
4. For *E. culicivora* adults, caryophyllene is an odour that elicits selective visual attention to mates.

Unlike in Experiment 1 where there was a plant in only one of the four display chambers, here we put a plant in each of the four display chambers and we associated a visual target with one of these chambers. We then recorded test-spider response to different visual targets in the presence of different odours, with plants always being visible in the background. However, as we were interested in detecting specifically selective visual attention, we varied whether the target was cryptic (more difficult to see and identify) or conspicuous (easier to see and identify). The rationale for having both cryptic and conspicuous visual targets in these experiments was to distinguish between an expression of a preference and the use of selective attention (see Cross and Jackson, 2010). We predicted that, if the primary effect of odour is on preference, then response to the visual target will be more strikingly evident when the visual target is conspicuous than when it is cryptic. We also predicted that, if the primary effect of odour is on selective visual attention, performance in the experiment will improve when the visual target is cryptic but, when the visual target is sufficiently conspicuous, there will be little or no scope for improvement in performance in the experiment because the visual target would already be readily seen even when the test spider is not selectively attentive to the target.

When designing Experiment 2, we also wanted to consider the hypothesis that selective attention is subject to pronounced capacity limitations (see Dukas, 2004). For this, the critical prediction was that performance by *E. culicivora* in trials with cryptic targets and incongruent odour would be poorer than in trials with the same cryptic visual target but no odour (see Cross and Jackson, 2009a). The specific hypothesis underlying this prediction is that, owing to the incongruent odour, *E. culicivora* commits its limited capacity for selective attention to the task of detecting and identifying a target different from the visual target which is present. This leaves *E. culicivora* with a shortfall in capacity for performing well at detecting and identifying the different visual target that is present in the scene.

4.1. Materials and methods

4.1.1. Apparatus—The apparatus (Figs. 1 and 2) was the same as for Experiment 1 except that there were no glass fronts on the display chambers, there was a *L. camara* cutting in each of the four display chambers instead of only one, and there were no flowers on the cuttings. However, we ensured that the cuttings did not extend beyond the grooves at the front of the chambers (i.e., the grooves where the glass fronts had been positioned for Experiment 1).

We positioned a mount between one side of the arena and the plant cutting in the display chamber that was facing this side of the chamber (display-chamber 1). We decided at random for each trial which of the four chambers would be display-chamber 1. The mount

was a ‘visual target’ (‘prey’, ‘mate’ or ‘flower’) affixed on top of a cork disc (diameter 15 mm, thickness 2 mm). The prey was always a female mosquito (body length 4.5 mm) that had fed on blood 4–5 h before being transferred to 80% ethanol. On the following day, we removed the mosquito from the ethanol and positioned it on the top of the cork disc in the resting posture characteristic of *Anopheles* (i.e., with its abdomen tilted upward; see Clements, 1999). In each instance the mate was an opposite-sex conspecific individual (males 4.5 mm in body length, females 5.5 mm) positioned on the top of the cork disc in the normal resting posture for *E. culicivora* (see Cross et al., 2008). Mate mounts were prepared in the same way as the mosquito mounts, but without the preceding blood meal. In each instance the flower was a 10-mm wide umbel of *L. camara* flowers positioned with its stem on the top of the cork disc (diameter 5 mm). The stem was cut short enough so that the umbel draped over the top of the disc.

For preservation, we used a transparent plastic adhesive (Crystal Clear Lacquer, Atsco Australia, Pty) to spray mosquitoes and mates, along with the discs on which they were mounted. However, there was no ethanol pre-treatment and no spraying when making flower mounts because we knew from preliminary work that these procedures often distort flower shape and coloration. More details concerning mount-making methods can be found elsewhere (e.g., Jackson et al., 2005).

A hole (diameter 2 mm) was centred in the removable wooden back of display chamber 1 and a metal pin extended through this hole. The pin was held horizontal by a clamp and stand situated behind display-chamber 1. The end of this pin that was closest to the arena was stuck into the side of the cork disc on which the flower, the prey item or the mate was mounted. Mounted prey and mates were oriented so that they were facing 45° to the left or right (determined at random) from directly toward the arena.

4.1.2. Procedures—We made the visual stimuli either cryptic or conspicuous by varying how close the mount was to the plant cutting in display chamber 1. Cryptic mounts were positioned with their midlines even with the front grooves of display chamber 1 (i.e., close to the plant cutting). Conspicuous mounts were positioned with their front ends even with the closest side of the glass base on which the arena sat (i.e., conspicuous mounts were out in the open instead of next to the plant cuttings). Mount odour was not a relevant factor because the mount was always outside the airtight glass arena that housed the test spider.

As with Experiment 1, ‘response duration’ was the total time the spider spent in sector 1 (i.e., the sector facing display-chamber 1). For determining whether an odour triggered selective attention to a particular visual stimulus, we compared response durations in no-odour control trials with response durations in trials where odour (from the odour chamber below the arena) was congruent with the visual target (e.g., prey odour when the visual target was a prey item). We also considered whether there were trade-off effects and, for this, we compared response durations in no-odour control trials with response durations in trials where odour was incongruent with the visual target (e.g., mate odour when the visual target was prey).

4.1.3. Data analysis—After determining that our data met the assumptions of ANOVA (Bartlett's tests for homogeneity of variance and Shapiro-Wilks tests for normal distribution of residuals), we considered three specific questions in the context of whether an odour triggered selective attention to a particular visual stimulus. First, for each of the three cryptic visual targets presented to the test spider (flower, prey and mate), we used 2-factor ANOVAs for determining interactions between test-spider category and olfactory stimulus (i.e., no odour and odour congruent to the visual target). Using a series of t tests, we then compared findings from trials in which the visual target was conspicuous with findings from trials in which the visual target was cryptic, again with the odour and visual target being congruent. After that, we used another series of t tests for comparing findings from trials in which the cryptic visual targets were paired with incongruent odours instead of with no odour. It was necessary to use t tests for these series of comparisons because not all test-spider categories were tested with conspicuous visual targets or with incongruent odour. This meant that, for consistency, we also used t tests instead of *post hoc* Tukey's range tests in Experiment 2 whenever ANOVA outcomes revealed a significant effect. The number of comparisons we made using the same data sets ranged from 1–4 and, instead of applying different alpha values (ranging from 0.05–0.013) to these different comparisons, we simply adopted 0.013, the most conservative adjusted alpha value. For all treatments, $N = 20$ (total $N = 820$ different spiders).

4.2. Results and Discussion

By ascertaining the relationship between specific priming odours and selective attention to specific visual targets, the goal of Experiment 2 can be envisaged as asking what *E. culicivora* is searching for when visually inspecting plants (Experiment 1). Selective attention is usually characterized as an effortful, capacity-limited task (Driver, 2001), and this characterization was our rationale for presenting test spiders with visual targets that were difficult to discern and identify (i.e., targets that were 'cryptic') and then comparing the findings from trials in which a specific odour was presented with the findings from trials in which the same visual target, but no odour, was presented.

When the cryptic visual targets were *L. camara* flowers (Fig. 6A) and either there was no odour (control) or there was a congruent odour (i.e., caryophyllene), test-spider category ($F(3,152) = 12.59$, $p < 0.001$, partial $\eta^2 = 0.199$) and odour ($F(1,152) = 23.77$, $p < 0.001$, partial $\eta^2 = 0.135$) were significant main effects and there was also a significant interaction effect between test-spider category and odour ($F(3,152) = 5.39$, $p = 0.001$, partial $\eta^2 = 0.096$). For juveniles, response durations were significantly longer in the presence of caryophyllene than in the no-odour control trials (small juveniles, $t(38) = 4.18$, $p < 0.001$, Cohen's $d = 1.320$; large juveniles, $t(38) = 4.01$, $p < 0.001$, Cohen's $d = 1.269$); however, the response durations recorded for adults in the presence of caryophyllene were not significantly different from the response durations recorded for adults in the no-odour control trials (males, $t(38) < 1$; females, $t(38) < 1$).

When the visual targets were cryptic prey items (Fig. 7A), and either there was no odour (control) or there was a congruent odour (i.e., prey), we found a significant main effect of odour ($F(1,152) = 52.96$, $p < 0.001$, partial $\eta^2 = 0.258$), and of test-spider category ($F(3,152)$

= 4.70, $p = 0.004$, partial $\eta^2 = 0.085$), but there was no significant interaction effect ($F(3,152) < 1$). For all test-spider categories, response durations in the presence of prey odour were significantly longer than in the no-odour control trials (males, $t(38) = 3.70$, $p < 0.001$, Cohen's $d = 1.170$; females, $t(38) = 4.77$, $p < 0.001$, Cohen's $d = 1.510$; small juveniles, $t(38) = 2.63$, $p = 0.012$, Cohen's $d = 0.830$; large juveniles, $t(38) = 3.80$, $p < 0.001$, Cohen's $d = 1.202$).

When the visual targets were cryptic mates (Fig. 8A), and either there was no odour (control) or there was a congruent odour (i.e., mate or caryophyllene), there was no significant interaction effect ($F(2,114) < 1$), but there was a significant main effect of odour ($F(2,114) = 24.20$, $p < 0.001$, partial $\eta^2 = 0.298$) and also a significant main effect of test-spider category ($F(1,114) = 13.40$, $p < 0.001$, partial $\eta^2 = 0.105$; response durations longer for adult males than for adult females). Caryophyllene and mate odour had similar effects. Response durations were significantly longer in the presence of mate odour than in the no-odour controls for males ($t(38) = 3.21$, $p = 0.003$, Cohen's $d = 1.014$) and for females ($t(38) = 2.95$, $p = 0.006$, Cohen's $d = 0.931$), and response durations were also significantly longer in the presence of caryophyllene odour than in the no-odour controls for males ($t(38) = 4.85$, $p < 0.001$, Cohen's $d = 1.533$) and for females ($t(38) = 5.20$, $p < 0.001$, Cohen's $d = 1.643$). Moreover, response duration in the presence of mate odour was not significantly different from response duration in the presence of caryophyllene odour for males ($t(38) = 1.47$, $p = 0.149$, Cohen's $d = 0.466$) or for females ($t(38) = 1.99$, $p = 0.054$, Cohen's $d = 0.628$). These findings suggest that, for the adults of *E. culicivora*, besides mate odour being congruent with seeing a mate, the odour of the plant compound caryophyllene is also congruent with seeing a mate.

As predicted by our hypothesis that odour primed selective visual attention, response durations were significantly longer in the presence of a congruent odour than in the no-odour control trials as long as the visual targets were cryptic. When juveniles were presented with cryptic flowers and when adults were presented with cryptic mates, we found significantly stronger response in the presence of caryophyllene odour than in the corresponding no-odour control trials.

Our findings from using conspicuous visual targets were also as predicted by our selective-attention hypothesis. When test spiders were presented with visual targets that were easy to detect and identify, their response durations in the presence of congruent odour were not significantly different from their response durations in no-odour controls: flower as the visual target (small juveniles, $t(38) = 1.31$, $p = 0.197$, Cohen's $d = 0.415$) (Fig. 6B); prey as the visual target (adult males, $t(38) < 1$; small juveniles, $t(38) = 1.47$, $p = 0.151$, Cohen's $d = 0.463$) (Fig. 7B); mate as visual target (adult males, mate odour: $t(38) = 1.35$, $p = 0.187$, Cohen's $d = 0.425$; caryophyllene odour: $t(38) < 1$) (Fig. 8B). Likewise, when conspicuous mates were the visual targets, test-spider response durations in the presence of mate odour and test-spider response durations in the presence of caryophyllene, the other odour congruent with seeing a mate, were not significantly different (adult males, $t(38) < 1$) (Fig. 8B).

In the no-odour control trials, response duration was significantly longer when the visual target was conspicuous instead of cryptic: for small juveniles, when flowers were the visual targets ($t(38) = 4.57$, $p < 0.001$, Cohen's $d = 1.444$) (Fig. 6B); for small juveniles, when prey was the visual target ($t(38) = 2.75$, $p = 0.009$, Cohen's $d = 0.868$) (Fig. 7B); for adult males, when prey was the visual target ($t(38) = 2.91$, $p = 0.006$, Cohen's $d = 0.921$); for adult males, when a mate was the visual target ($t(38) = 5.31$, $p < 0.001$, Cohen's $d = 1.678$) (Fig. 8B). Significantly longer response durations when visual targets were conspicuous instead of cryptic appear to verify that the procedure we adopted for making the visual target 'conspicuous' really did make the visual target easier to detect and identify.

Our findings when we paired cryptic visual targets with incongruent odours are as predicted by the hypothesis (see Dukas, 2004) that selective attention is subject to pronounced capacity limitations: response duration in the presence of incongruent odour was significantly shorter than response duration in the no-odour control trials (Figs. 6A–8A). More specifically, when the cryptic visual targets were flowers (Fig. 6A), response durations of small juveniles were significantly shorter in the presence of prey odour (incongruent) than in the no-odour control trials ($t(38) = -2.75$, $p = 0.009$, Cohen's $d = 0.869$), but the response durations of large juveniles were not significantly different ($t(38) = -2.32$, $p = 0.026$, Cohen's $d = 0.734$). Similar comparisons using adults were not made because, when adults were tested with cryptic flowers, no significant differences were found when response durations in the presence of congruent odour (i.e., caryophyllene) were compared to response durations in the no-odour control trials. However, when cryptic prey was the visual target (Fig. 7A), response durations for adult test spiders were significantly shorter in the presence of mate odour (incongruent) than in the no-odour controls (males, $t(38) = 3.00$, $p = 0.005$, Cohen's $d = 0.949$; females, $t(38) = 2.82$, $p = 0.008$, Cohen's $d = 0.893$). We also found that response duration was significantly shorter in the presence of caryophyllene than in the no-odour controls when cryptic prey was the visual target for males ($t(38) = 3.75$, $p < 0.001$, Cohen's $d = 1.185$), females ($t(38) = 2.93$, $p = 0.006$, Cohen's $d = 0.926$) and small juveniles ($t(38) = 3.03$, $p = 0.004$, Cohen's $d = 0.957$), and neared significance for large juveniles ($t(38) = 2.59$, $p = 0.013$, Cohen's $d = 0.820$). When the visual target was a cryptic mate (Fig. 8A), response durations of females were significantly shorter in the presence of prey odour (incongruent) than in the no-odour controls ($t(38) = -3.02$, $p = 0.005$, Cohen's $d = 0.954$), but response durations for males were not significantly different ($t(38) = -2.38$, $p = 0.023$, Cohen's $d = 0.751$). Our primary finding was that, in many cases, our test spiders appeared to be subject to a trade-off whereby becoming selectively attentive to one visual target diminished capacity to detect and identify other visual objects.

5. Experiment 3: Odour priming of rapid response to specific visual targets

Instead of looking for evidence of selective attention by comparing performance when visual targets were cryptic with performance when visual targets were conspicuous (Experiment 2), we only used conspicuous visual targets in Experiment 3. This was because our objective in Experiment 3 was to determine whether *E. culicivora* becomes prepared to respond rapidly to specific visual targets in the presence of specific odours. We considered four hypotheses.

1. Prey odour primes *E. culicivora* for a rapid response to seeing prey.

2. Caryophyllene odour primes *E. culicivora* for a rapid response to seeing a plant or to seeing a mate, but not to seeing a prey item.
3. Independent of whether a plant is also in view, mate odour primes *E. culicivora* for a rapid response to seeing a mate.
4. When a plant is also in view, caryophyllene odour especially strongly primes *E. culicivora* for a rapid response to seeing a mate.

We only used adult males in this experiment, the rationale for this decision being that, when the task was to find a mate in Experiment 2, the response durations of males were significantly longer than the response durations of females (Fig. 8A).

5.1. Materials and methods

5.1.1. Apparatus—For this experiment (Fig. 3), there were only two display chambers instead of four and, instead of using a square arena, we used an arena that had three sectors, with the sector in the middle being narrower than the sectors at the two ends. Aligned with and touching opposite ends of the arena table, there were two wooden display chambers, chamber A facing arena side-sector A and chamber B facing arena side-sector B. We decided at random which would be chamber A. As for Experiments 1 and 2, there were grooves in the walls and in the floor of each chamber. There was no glass front in the front grooves, but we positioned a wooden back in the grooves farthest from the arena.

There was a hole (diameter 8 mm) centred in the wooden back of chamber A and a pin which extended through this hole. The pin was held in place by a clamp and stand behind chamber A. The end of the pin was pushed into the side of a cork disc holding it horizontal. A prey item or a mate was on the top of the disc, facing 45° to the left or right (decided at random) from the nearest arena wall. The top of the disc was 23 mm above the top of, and even with, the edge of the arena table. The mount was made conspicuous by being positioned so that its front end was 30 mm from the inner side of the nearest wall of arena sector A. In some trials, a *L. camara* cutting (stem, leaves & flowers) was in chamber A and, in other trials, chamber A was empty. Chamber B was always empty (i.e., there were no plant cuttings in chamber B and no associated pin or mount).

As with Experiments 1 and 2, there was an odour chamber, pump and airflow meter, as well as silicone tubing, for delivering specific odours. Glass tubes (inner diameter 11 mm, outer diameter 12 mm, length 25 mm) were positioned in the arena's inflow and outflow holes, one end being flush with the inner side of the arena and the other end extending out from the arena.

5.1.2. Procedures—During a 15-min pre-exposure period, a test spider was in the tube in the arena's outflow hole, confined there by nylon netting over each end. Air moved from the odour chamber through the arena and then through the tube with the test spider inside. To start a trial, the tube was lifted away, the nylon netting was removed from the end of the tube farthest from the spider and then this open end was inserted into the outflow hole. If the spider failed to move through the open end of the tube and into the arena on its own accord within 2 min, we removed the netting from the other side of the tube and, using a soft brush,

coaxed the spider into the arena. Once the spider was in the arena, we replaced the tube with a rubber stopper. There was a 4-mm hole in the stopper and nylon netting covered the end of the stopper closest to the arena.

After entering the arena, the test spider was only allowed 5 min in which to respond (i.e., the response had to be rapid), with our definition of ‘respond’ being that the spider moved into side sector A and remained there for 30 s. As a prerequisite for a successful trial, we stipulated that the test spider had to maintain the gaze of its principal eyes on the visual object continuously for a minimum of 15 s before responding.

5.1.3. Data Analysis—We made pairwise comparisons of response rates by carrying out chi-square tests of independence. For this, we made multiple comparisons (ranging from 4–7) using the same data sets. Instead of applying different alpha values (ranging from 0.013–0.007) to these different comparisons, we simply adopted 0.007, the most conservative adjusted alpha value and this did not affect the significance of any of our results. For all treatments, $N = 90$ (total $N = 1,800$ different spiders).

5.2. Results and Discussion

The males’ baseline response rates (i.e., their response rates in the no-odour controls in Experiment 3) were significantly greater (Table 1) when the visual target was *L. camara* with a mate (hereafter ‘*L. camara* + mate’) instead of *L. camara* by itself (*L. camara* + mate vs. *L. camara* alone, $\chi^2 = 11.31$, $p < 0.001$) or instead of *L. camara* with a prey item (*L. camara* + mate vs. *L. camara* + prey, $\chi^2 = 8.32$, $p = 0.004$). These findings appear to show that males have a default tendency to be especially responsive to seeing a mate, but not a prey item, associated with a plant. No other comparisons of baseline response rates were significant (mate alone and *L. camara* alone: $\chi^2 = 5.50$, $p = 0.019$; mate alone and *L. camara* + mate: $\chi^2 = 1.09$, $p = 0.296$; mate alone and prey alone: $\chi^2 = 0.83$, $p = 0.361$; prey alone and *L. camara* alone: $\chi^2 = 2.08$, $p = 0.149$; prey alone and *L. camara* + prey: $\chi^2 = 0.90$, $p = 0.343$; *L. camara* alone and *L. camara* + prey: $\chi^2 = 0.25$, $p = 0.620$).

Having ascertained these baseline levels of responsiveness to the different visual targets, we considered whether specific odours prepare *E. culicivora* to respond rapidly to different specific visual targets. For this, we compared the number of test spiders that responded rapidly to a particular visual target in the presence of a specific odour with the number that responded rapidly to the same visual target in no-odour control trials (Table 2). For each odour and for the no-odour control trials, we also considered whether the identity of the visual target mattered. For this, we compared the number of test spiders that responded to one particular visual target with the number that responded to another particular visual target (Table 3).

Whenever the visual target included a mate, a *L. camara* cutting or both, response rates in the presence of caryophyllene were significantly higher than the corresponding baseline response rates (a mate alone, 59 vs. 39, $\chi^2 = 8.96$, $p = 0.003$; *L. camara* alone, 51 vs. 24, $\chi^2 = 16.66$, $p < 0.001$; *L. camara* + mate, 81 vs. 46, $\chi^2 = 32.76$, $p < 0.001$). When the visual target was prey alone, response rates in the presence of caryophyllene were not significantly different from baseline (26 vs. 33, $\chi^2 = 1.24$, $p = 0.266$) (Tables 1 and 2); however, when the

visual target was *L. camara* + prey, response rate in the presence of caryophyllene was significantly higher than in the corresponding control (51 vs. 27, $\chi^2 = 13.03$, $p < 0.001$). These findings are as predicted by our hypothesis that caryophyllene odour prepares male test spiders to respond rapidly to seeing mates and to seeing *L. camara*, but not to seeing prey.

Moreover, in the presence of caryophyllene odour, significantly more test spiders responded rapidly to seeing *L. camara* + prey than to seeing prey alone (51 vs. 26, $\chi^2 = 14.18$, $p < 0.001$), but the number that responded to *L. camara* + prey in the presence of caryophyllene was not significantly different from the number that responded to *L. camara* alone (51 vs. 51, $\chi^2 = 0.00$, $p = 1.000$) (Tables 1 and 3); this suggests that the effect of caryophyllene odour pertains only to seeing *L. camara* in these trials. We also found that, in the presence of caryophyllene odour, significantly more test spiders responded rapidly to seeing *L. camara* + mate than to seeing a mate alone (81 vs. 59, $\chi^2 = 15.56$, $p < 0.001$), but we found no significant difference between the number of males that responded rapidly to seeing a mate alone and the number that responded rapidly to seeing *L. camara* alone in the presence of caryophyllene odour (59 vs. 51, $\chi^2 = 1.50$, $p = 0.221$). On this basis, we propose that caryophyllene odour has an effect of making *E. culicivora* males especially strongly prepared to respond rapidly to seeing a mate specifically when the mate is associated with the plant.

As with baseline response rates, significantly more males in trials with caryophyllene odour present responded rapidly to seeing *L. camara* + mate than to seeing *L. camara* alone (81 vs. 51, $\chi^2 = 25.57$, $p < 0.001$) and to seeing *L. camara* + mate than to seeing *L. camara* + prey (81 vs. 51, $\chi^2 = 25.57$, $p < 0.001$). We also found that, in the presence of caryophyllene, significantly more males responded rapidly to seeing a mate alone than to seeing prey alone (59 vs. 26, $\chi^2 = 24.27$, $p < 0.001$) and significantly more males responded rapidly to seeing *L. camara* alone than to seeing prey alone (51 vs. 26, $\chi^2 = 14.18$, $p < 0.001$) (Tables 1 and 3).

In the presence of prey odour, the number of test spiders that responded rapidly was significantly greater than the corresponding baseline response rates when the visual target was prey alone (61 vs. 33, $\chi^2 = 17.46$, $p < 0.001$) or *L. camara* + prey (54 vs. 27, $\chi^2 = 16.36$, $p < 0.001$), but not when the visual target was a mate alone (31 vs. 39, $\chi^2 = 1.50$, $p = 0.221$), *L. camara* alone (28 vs. 24, $\chi^2 = 0.43$, $p = 0.511$) or *L. camara* + mate (43 vs. 46, $\chi^2 = 0.20$, $p = 0.655$) (Tables 1 and 2). We also found that, in the presence of prey odour, significantly more test spiders responded rapidly to seeing prey alone than to seeing a mate alone (61 vs. 31, $\chi^2 = 20.01$, $p < 0.001$), to seeing prey alone than to seeing *L. camara* alone (61 vs. 28, $\chi^2 = 24.20$, $p < 0.001$) and to seeing *L. camara* + prey than to seeing *L. camara* alone (54 vs. 28, $\chi^2 = 15.14$, $p < 0.001$), but there was no significant difference between seeing prey alone and seeing *L. camara* + prey (61 vs. 54, $\chi^2 = 1.18$, $p = 0.277$), seeing a mate alone and seeing *L. camara* alone (31 vs. 28, $\chi^2 = 0.23$, $p = 0.634$), seeing a mate alone and seeing *L. camara* + mate (31 vs. 43, $\chi^2 = 3.30$, $p = 0.069$), seeing *L. camara* alone and seeing *L. camara* + mate (28 vs. 43, $\chi^2 = 5.23$, $p = 0.022$) or seeing *L. camara* + prey and seeing *L. camara* + mate (54 vs. 43, $\chi^2 = 2.71$, $p = 0.100$) (Tables 1 and 3). These findings suggest that the effect of prey odour is specifically to prepare males to respond rapidly to seeing prey, with it being irrelevant whether *L. camara* is visible with the prey. Moreover, prey odour appears to have

no effect on *E. culicivora*'s preparedness to respond rapidly to mates alone or to *L. camara* alone.

Response rates of test spiders in the presence of mate odour were significantly higher than baseline response rates when the visual target was a mate alone (70 vs. 39, $\chi^2 = 22.35$, $p < 0.001$) or *L. camara* + mate (65 vs. 46, $\chi^2 = 8.48$, $p = 0.004$), but not when the visual target was prey alone (29 vs. 33, $\chi^2 = 0.39$, $p = 0.530$), *L. camara* alone (34 vs. 24, $\chi^2 = 2.54$, $p = 0.111$) or *L. camara* + prey (30 vs. 27, $\chi^2 = 0.23$, $p = 0.631$) (Tables 1 and 2). We also found that, in the presence of mate odour, significantly more test spiders responded rapidly to seeing a mate alone than to seeing prey alone (70 vs. 29, $\chi^2 = 37.73$, $p < 0.001$), to seeing a mate alone than to seeing *L. camara* alone (70 vs. 34, $\chi^2 = 29.51$, $p < 0.001$), to seeing *L. camara* + mate than to seeing *L. camara* alone (65 vs. 34, $\chi^2 = 21.57$, $p < 0.001$) and to seeing *L. camara* + mate than to seeing *L. camara* + prey (65 vs. 30, $\chi^2 = 27.31$, $p < 0.001$). However, we found no significant differences in response rates when visual targets were a mate alone vs. *L. camara* + mate (70 vs. 65, $\chi^2 = 0.74$, $p = 0.389$), prey alone vs. *L. camara* alone (29 vs. 34, $\chi^2 = 0.61$, $p = 0.435$), prey alone vs. *L. camara* + prey (29 vs. 30, $\chi^2 = 0.03$, $p = 0.874$) or *L. camara* alone vs. *L. camara* + prey (34 vs. 30, $\chi^2 = 0.39$, $p = 0.533$) (Tables 1 and 3).

On the whole, these findings are as we predicted by our hypothesis that *E. culicivora* becomes prepared to respond rapidly to specific visual targets when primed by congruent odour sources. When we compared responsiveness to a visual target in the presence of a particular odour with responsiveness to the same visual target in no-odour control trials (Table 2), we found a clear trend. Significantly more test spiders responded rapidly to seeing prey while in the presence of prey odour than in no-odour control trials, but there was no significant effect of prey odour when test spiders viewed a scene that did not include prey. We also found that significantly more test spiders responded rapidly when they viewed a mate while in the presence of mate odour than in no-odour control trials, but we found no significant effects of mate odour when test spiders viewed a scene that did not include a mate. It was especially interesting that there was no significant effect of mate odour when the visual target was *L. camara* with no mate present.

As in Experiment 2, but now in a different context, the effects of caryophyllene odour were consistent with our hypothesis that this plant-derived compound is cognitively relevant to *E. culicivora* males specifically in the context of encountering potential mates. In the presence of this odour, we found that test spiders were significantly more responsive when they viewed a scene that included *L. camara* or a mate, but not when they viewed a scene that included a prey item alone. We also found that, in the presence of caryophyllene, response rates to *L. camara* + mate was significantly higher than response rates to seeing a mate alone or *L. camara* alone, suggesting that the odour of caryophyllene, but not mate odour, prepares *E. culicivora* adults for encounters with mates associated with plants.

6. General discussion

An interest in cognitive specialization was the rationale for how we designed our experiments, but the term 'specialization' needs to be used carefully. In ecology, for

example, there often seems to be a deeply ingrained tradition of equating predatory specialization with instances of a predator feeding primarily, or exclusively, on a narrow subset of the available prey in its natural habitat (e.g., Futuyma and Moreno, 1988; Pagani-Nunez et al., 2016). This is a confusing practice because the appropriate terms for diet breadth are ‘stenophagy’ when a predator’s natural diet is narrow and ‘euryphagy’ when its diet is wide. We need the expression ‘predatory specialization’ in a different context, namely when we are discussing the extent to which specific characteristics of a predator are well adapted with respect to specific kinds of prey (West-Eberhard, 2003; Jackson and Cross, 2015). This is an especially important conceptual distinction when we are discussing salticids because many examples from these predators illustrate how specialization and natural diet breadth can vary independently of each other (Jackson and Pollard, 1996; Nelson and Jackson, 2011; Jackson and Cross, 2013).

Evarcha culicivora is highly specialized as a predator of mosquitoes (Jackson and Cross, 2015), but the basis for saying *E. culicivora* is a ‘mosquito specialist’ is the particular ways in which this spider has become adapted to mosquitoes as prey, not the fact that it eats a lot of mosquitoes in the field (Wesolowska and Jackson, 2003). There is also evidence suggesting that *E. culicivora* is specialized with respect to using plants. In laboratory olfactometer experiments (Cross and Jackson, 2009b), adult females, adult males and juveniles from all size classes were attracted to the odour of two plant species, *L. camara* and *R. communis*. The juveniles of *E. culicivora* are especially inclined to feed on nectar when they visit plants (Kuja et al., 2012) and there is a specialized relationship between nectar meals and predation on mosquitoes: after nectar meals, the juveniles of *E. culicivora* become more effective at subduing the mosquitoes they attack (Carvell and Jackson, 2015). However, we propose that mating instead of feeding is the context in which *E. culicivora* adults make specialized use of plants.

With three experiments, we investigated specifically plant-related cognitive specialization by *E. culicivora* and found evidence that plant odour has cognitive effects in three specific contexts: executing behaviour that we call the ‘visual inspection of plants’ (Experiment 1), adopting selective visual attention to specific visual targets (Experiment 2) and becoming prepared to respond rapidly to specific visual targets (Experiment 3). We considered a possible alternative explanation for *E. culicivora*’s behaviour, which we will call the ‘general-motivation hypothesis’. According to this hypothesis, salient odours in general elevate *E. culicivora*’s motivation to respond to salient visual stimuli in general. We could readily consider this hypothesis because we already knew that *E. culicivora* juveniles and adults respond to prey odour (Jackson et al., 2005) and that *E. culicivora* adults respond to mate odour (Cross and Jackson, 2009c). However, contrary to the general-motivation hypothesis, we only found effects when particular odours were presented with particular visual targets.

Lantana camara was the plant species in the display chamber in most of the experiments for Experiment 1 and in all of the experiments for Experiments 2 and 3. However, by using another four plant species in a subset of the trials in Experiment 1, we determined that caryophyllene effects on *E. culicivora* are not specific to instances in which the plant in view

is *L. camara*. In this part of Experiment 1, we found that caryophyllene had significant effects on the visual inspection of all the plant species we used.

However, questions pertaining to whether there is a special relationship between *E. culicivora* and *L. camara* were secondary to our primary objective of investigating ontogenetic shifts related to cognition. Major ontogenetic shifts are well known for arthropods in a different context. Some insects progress through substantial morphological changes ('complete metamorphosis'), with these morphological changes often being accompanied by substantial changes in lifestyle, behaviour and physiology. Mosquitoes are striking examples. After complete metamorphosis, the adults of these holometabolous insects emerge as individuals that only remotely resemble the wingless, legless, aquatic insects they had been as juveniles.

The morphological transitions of spiders as they advance from juvenile to adult are less substantial, but remarkable instances are known from spiders of predatory strategies being subject to ontogenetic shifts. Aggressive mimicry as practised by *Mastophora hutchinsoni* (family Araneidae) is an example. By mimicking the female sex pheromones of moths, *M. hutchinsoni* adults attract their prey, male moths (Lepidoptera), but these moths are too large for small *M. hutchinsoni* juveniles. Juveniles also practice aggressive mimicry, but they specialize at preying on a smaller prey, the males of moth flies (Diptera, Psychodidae); they mimic the sex pheromones of these insects instead of the sex pheromones of the moths (Yeargan and Quate, 1996). Examples of ontogenetic shifts in predatory strategy are also known for salticids. For example, the smaller juveniles of *Portia africana* adopt a highly specialized method of capturing a specific type of prey (oecobiid spiders) but this oecobiid-specific prey-capture method is dropped from the predatory repertoire of the larger juveniles and adults (Jackson et al., 2008).

The hypothesis we propose for juveniles may seem intuitive, as our findings are consistent with plants being important to juveniles in the context of gaining nectar meals. Caryophyllene is a sesquiterpene, and terpenes in general are synthesized by plants but not typically by animals (Schiestl, 2010). Although caryophyllene is not known to be present in the nectar of *L. camara* (Alm et al., 1990) and is not known to be a normal component of the nectar of plants in general (Nicolson and Thornburg, 2007; Brandenburg et al., 2009), the odour of this compound would be a good indicator of the presence of a plant and plants are normally where nectar can be found. However, for the adults of *E. culicivora*, our findings are consistent with plants being important in a different and less obvious context. Our hypothesis is that plants are a place where potential mates can be found and that plant odour prepares adults not for nectar meals but instead for mating opportunities.

A different possibility is that *E. culicivora* adults sequester and store caryophyllene (see Opitz and Müller, 2009) when visiting plants, and later release this compound as a sex pheromone. There is already evidence (Cross et al., 2009) that *E. culicivora* adults use a diet-derived odour (i.e., an odour derived from feeding on blood-carrying mosquitoes) to attract mates. Moreover, males of euglossine bees store volatile compounds from orchid flowers and use this odour to attract females (Lunau, 1992; Mitko et al., 2016). If it were the case that *E. culicivora* relies on storing and then using caryophyllene from its own body to attract

potential mates, then there would be an alternative explanation for our experimental findings. It could be argued that the *E. culicivora* adults normally respond to caryophyllene as olfactory signals coming directly from potential mates instead of from the plants on which mates tend to be associated. On the basis of this hypothesis, we would have predicted different findings in Experiment 3: that the level of responsiveness to seeing a mate by itself in the presence of caryophyllene odour would be higher than to seeing *L. camara* by itself in the presence of caryophyllene odour. However, contrary to this prediction, no significant difference was found (Table 3). We also found in Experiment 3 that, in the presence of caryophyllene odour, rapid response by test spiders was significantly more common when the visual stimulus was seeing a mate associated with *L. camara* instead of seeing a mate that was not associated with *L. camara*. On the whole, our findings suggest that the caryophyllene odour relevant to *E. culicivora* adults normally comes directly from plants, rather than from the spiders (potential mates).

Moreover, the effects of caryophyllene and mate odour in Experiment 3 were different. In the presence of mate odour, the number of test spiders that responded rapidly to seeing a mate by herself was significantly higher than the number that responded rapidly to seeing *L. camara* by itself; however, in the presence of mate odour, the number of test spiders that responded rapidly to seeing a mate associated with *L. camara* was not significantly different from the number that responded rapidly to seeing a mate by herself (Table 3).

Our hypothesis is that, for *E. culicivora* adults, *L. camara* is relevant specifically in the context of mating. That mate odour prepares *E. culicivora* to respond rapidly to seeing a mate, but that caryophyllene odour prepares *E. culicivora* to respond rapidly to seeing more specifically a mate associated with a plant, is consistent with this hypothesis. Our findings from Experiment 1 are also consistent with this hypothesis; adult test spiders appeared to become especially motivated to inspect a plant when in the presence of caryophyllene odour but not when in the presence of mate odour. This is in addition to the evidence from Experiment 2 that, for the adults of *E. culicivora*, caryophyllene odour and mate odour are both stimuli that elicit selective visual attention to mates. The critical evidence implicating selective attention was response in experimental trials being stronger than in controls when the visual stimulus was cryptic but not when it was conspicuous.

By contrast, there was no evidence in Experiment 3 of caryophyllene affecting how many test spiders responded rapidly to seeing prey (Table 2). Moreover, in the presence of caryophyllene, significantly more test spiders responded rapidly to scenes that included *L. camara* or a mate than to scenes that included prey by itself (Table 3). These findings are as predicted by our hypothesis that caryophyllene odour elevates responsiveness to seeing *L. camara* and to seeing mates in a scene, but has no effect on responsiveness to seeing prey in a scene.

It is not surprising that caryophyllene had no significant effect on responsiveness to prey. Based on our current understanding of mosquito behaviour, there is no clear rationale for proposing that *L. camara*, or other plants that release caryophyllene, would be relevant to *E. culicivora* as sites for encountering preferred prey (i.e., blood-carrying female anopheline mosquitoes). Although both sexes of *Anopheles gambiae* are known to visit plants and feed

on nectar (Foster, 1995; Manda et al., 2007), they do so primarily at night (Müller et al., 2010; Nyasembe et al., 2012) and blood-carrying females of anthropophilic *Anopheles* species tend to rest on the walls of human dwellings while digesting blood (Clements, 1999) instead of visiting plants for nectar or for resting.

7. Conclusions

When discussing predatory specialization in ecology, it is conventional to use the expression ‘specialists’ for predators with especially narrow or restricted natural diets and to envisage these predators as being subject to severe adaptive trade-offs. However, we mean something very different when we say that *E. culicivora* is a ‘specialist’. Determining the extent to which *E. culicivora*’s natural diet is restricted to mosquitoes is the basis for conclusions about stenophagy, not specialization (Jackson & Cross 2015). ‘Mosquito specialist’ is an expression we use when discussing the ways in which *E. culicivora* is adapted with respect to specifically mosquitoes as prey.

We have been especially interested in understanding the ways *E. culicivora* might be cognitively specialized with respect to mosquitoes. Another way of saying this is that we want to know what mosquitoes mean to this predator. ‘Plant specialist’ is another convenient expression when discussing *E. culicivora* but progress at understanding what plants, especially *L. camara* and *R. communis*, mean to this spider has been slow in comparison to advances in our understanding of specialization on mosquitoes. Earlier research suggests that visiting plants has different functions depending on whether the spider is an adult or a juvenile, with juveniles using plants primarily as food sources (Kuja et al., 2011; Carvell et al., 2015) and adults using plants as mating sites (Cross et al., 2008). However, our experiments here were designed to examine what plants mean to *E. culicivora* from a different perspective.

Instead of a direct focus on function, we investigated whether this predator relies on specialized plant-related cognitive structuring and we proposed differences in the way adults and juveniles are cognitively specialized with respect to plant odour. Consistently across three different experiments, we found support for our hypothesis that, for juveniles, plant odour pertains cognitively to nectar meals and that, for adults, plant odour pertains cognitively to mating opportunities.

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References

Alem S, Perry CJ, Zhu X, Loukola OJ, Ingraham T, Søvnik E, Chittka L. Associative mechanisms allow for social learning and cultural transmission of string pulling in an insect. *PLoS Biology*. 2016; 14:e1002564.doi: 10.1371/journal.pbio.1002564 [PubMed: 27701411]

- Alm J, Ohnmeiss TE, Lanza J, Vriesenga L. Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. *Oecologia*. 1990; 84:53–57. DOI: 10.1007/BF00665594 [PubMed: 28312774]
- Brandenburg A, Dell’Olivo A, Bshary R, Kuhlmeier C. The sweetest thing: advances in nectar research. *Curr Opin Plant Biol*. 2009; 12:486–490. DOI: 10.1016/j.pbi.2009.04.002 [PubMed: 19447671]
- Carvell GE, Kuja JO, Jackson RR. Rapid nectar-meal effects on a predator’s capacity to kill mosquitoes. *R Soc Open Sci*. 2015; 2:140426.doi: 10.1098/rsos.140426 [PubMed: 26064651]
- Cerveira AM, Jackson RR. Interpopulation variation in kairomone use by *Cyrtba algerina*, an araneophagic jumping spider from Portugal. *J Ethol*. 2011; 29:121–129. DOI: 10.1007/s10164-010-0233-1
- Cerveira AM, Jackson RR. Love is in the air and on the ground: olfactory and tactile cues elicit visual courtship behavior by *Cyrtba* males (Araneae: Salticidae). *J Arachnology*. 2013; 41:374–380. DOI: 10.1636/Hi12-42.1
- Clements, AN. The biology of mosquitoes: sensory reception and behaviour. CABI Publishing; Oxon: 1999.
- Cowan N. The magical mystery four: how is working memory capacity limited, and why? *Curr Dir Psychol Sci*. 2010; 19:51–57. DOI: 10.1177/0963721409359277 [PubMed: 20445769]
- Cross FR, Jackson RR. Cross-modality priming of visual and olfactory selective attention by a spider that feeds indirectly on vertebrate blood. *J Exp Biol*. 2009a; 212:1869–1875. DOI: 10.1242/jeb.028126 [PubMed: 19483005]
- Cross FR, Jackson RR. Odour-mediated response to plants by *Evarcha culicivora*, a blood-feeding jumping spider from East Africa. *NZ J Zool*. 2009b; 36:75–80. DOI: 10.1080/03014220909510142
- Cross FR, Jackson RR. Mate-odour identification by both sexes of *Evarcha culicivora*, an East African jumping spider. *Behav Processes*. 2009c; 81:74–79. DOI: 10.1016/j.beproc.2009.02.002 [PubMed: 19429199]
- Cross FR, Jackson RR. The attentive spider: search-image use by a mosquito-eating predator. *Ethology*. 2010; 116:240–247. DOI: 10.1111/j.1439-0310.2009.01731.x
- Cross FR, Jackson RR. Olfaction-based anthropophily in a mosquito-specialist predator. *Biol Lett*. 2011; 7:510–512. DOI: 10.1098/rsbl.2010.1233 [PubMed: 21325304]
- Cross FR, Jackson RR. The functioning of species-specific olfactory pheromones in the biology of a mosquito-eating jumping spider from East Africa. *J Insect Behav*. 2013; 26:131–148. DOI: 10.1007/s10905-012-9338-4
- Cross FR, Jackson RR. Specialised use of working memory by *Portia africana*, a spider-eating salticid. *Anim Cogn*. 2014; 17:435–444. DOI: 10.1007/s10071-013-0675-2 [PubMed: 23982622]
- Cross FR, Jackson RR. The execution of planned detours by spider-eating predators. *J Exp Anal Behav*. 2016; 105:194–210. DOI: 10.1002/jeab.189 [PubMed: 26781057]
- Cross FR, Jackson RR. Representation of different exact numbers of prey by a spider-eating predator. *Interface Focus*. In press.
- Cross FR, Jackson RR, Pollard SD. Complex display behaviour of *Evarcha culicivora*, an East African mosquito-eating jumping spider. *NZ J Zool*. 2008; 35:151–187. DOI: 10.1080/03014220809510112
- Cross FR, Jackson RR, Pollard SD. How blood-derived odor influences mate-choice decisions by a mosquito-eating predator. *Proc Natl Acad Sci USA*. 2009; 106:19416–19419. DOI: 10.1073/pnas.0904125106 [PubMed: 19887641]
- Dacke M, Srinivasan MV. Evidence for counting in insects. *Anim Cogn*. 2008; 11:683–689. DOI: 10.1007/s10071-008-0159-y [PubMed: 18504627]
- Dolev Y, Nelson XJ. Innate pattern recognition and categorization in a jumping spider. *PLoS ONE*. 2014; 9:e97819.doi: 10.1371/journal.pone.0097819 [PubMed: 24893306]
- Driver J. A selective review of selective attention research from the past century. *Brit J Psychol*. 2001; 92:53–78.
- Dukas R. Causes and consequences of limited attention. *Brain, Behav Evol*. 2004; 63:197–210. [PubMed: 15084813]

- Eberhard WG. Are smaller animals behaviourally limited? Lack of clear constraints in miniature spiders. *Anim Behav.* 2011; 81:813–823. DOI: 10.1016/j.anbehav.2011.01.016
- Eberhard WG, Weislo WT. Grade changes in brain-body allometry: morphological and behavioural correlates of brain size in miniature spiders, insects and other invertebrates. *Adv Insect Physiol.* 2011; 40:155–214. DOI: 10.1016/B978-0-12-387668-3.00004-0
- Eberhard WG, Weislo WT. Plenty of room at the bottom? *Am Sci.* 2012; 100:226–233. DOI: 10.1511/2012.96.226
- Foster WA. Mosquito sugar feeding and reproductive energetics. *Ann Rev Entomol.* 1995; 40:443–474. DOI: 10.1146/annurev.en.40.010195.002303 [PubMed: 7810991]
- Futuyma DJ, Moreno G. The evolution of ecological specialization. *Ann Rev Ecol Syst.* 1988; 19:207–233. DOI: 10.1146/annurev.es.19.110188.001231
- Giurfa M. Learning and cognition in insects. *WIREs Cogn Sci.* 2015; 6:383–395. DOI: 10.1002/wcs.1348
- Harland, DP., Li, D., Jackson, RR. How jumping spiders see the world. In: Lazareva, O., Shimizu, T., Wasserman, EA., editors. *How animals see the world: comparative behavior, biology, and evolution of vision.* Oxford University Press; New York: 2012. p. 133–164.
- Homberg U. Evolution of the central complex in the arthropod brain with respect to the visual system. *Arthropod Struct Dev.* 2008; 37:347–362. DOI: 10.1016/j.asd.2008.01.008 [PubMed: 18502176]
- Jackson RR, Clark RJ, Harland DP. Behavioural and cognitive influences of kairomones on an araneophagic jumping spider. *Behaviour.* 2002; 139:749–775. DOI: 10.1163/156853902320262808
- Jackson RR, Cross FR. Spider cognition. *Adv Insect Physiol.* 2011; 41:115–174. DOI: 10.1016/B978-0-12-415919-8.00003-3
- Jackson RR, Cross FR. A cognitive perspective on aggressive mimicry. *J Zool.* 2013; 290:161–171. DOI: 10.1111/jzo.12036
- Jackson RR, Cross FR. Mosquito-terminator spiders and the meaning of predatory specialization. *J Arachnology.* 2015; 43:123–142. DOI: 10.1636/V15-28
- Jackson RR, Nelson XJ, Sune GO. A spider that feeds indirectly on vertebrate blood by choosing female mosquitoes as prey. *Proc Natl Acad Sci USA.* 2005; 102:15155–15160. DOI: 10.1073/pnas.0507398102 [PubMed: 16217015]
- Jackson RR, Pollard SD. Predatory behavior of jumping spiders. *Ann Rev Entomol.* 1996; 41:287–308. DOI: 10.1146/annurev.en.41.010196.001443 [PubMed: 15012331]
- Jackson RR, Pollard SD, Salm K. Observations of *Portia africana*, an araneophagic jumping spider, living together and sharing prey. *NZ J Zool.* 2008; 35:237–242. DOI: 10.1080/03014220809510119
- Jakob, EM., Skow, CD., Long, S. Plasticity, learning and cognition. In: Herberstein, ME., editor. *Spider behaviour: flexibility and versatility.* Cambridge University Press; Cambridge: 2011. p. 307–347.
- Knudsen JT, Eriksson R, Gershenzon J, Ståhl B. Diversity and distribution of floral scent. *Bot Rev.* 2006; 72:1–120. DOI: 10.1663/0006-8101(2006)72[1:DADOFS]2.0.CO;2
- Kuja JO, Jackson RR, Sune GO, Karanja RNH, Lagat ZO, Carvell GE. Nectar meals of a mosquito-specialist spider. *Psyche.* 2012; 2012:898721. doi: 10.1155/2012/898721
- Land, MF., Nilsson, D-E. *Animal eyes.* 2. Oxford University Press; Oxford: 2012.
- Lunau K. Evolutionary aspects of perfume collection in male euglossine bees (Hymenoptera) and of nest deception in bee-pollinated flowers. *Chemoecology.* 1992; 3:65–73. DOI: 10.1007/BF01245884
- Manda H, Gouagna LC, Nyandat E, Kabiru EW, Jackson RR, Foster WA, Githure JI, Beier JC, Hassanali A. Discriminative feeding behaviour of *Anopheles gambiae* s.s on endemic plants in western Kenya. *Med Vet Entomol.* 2007; 21:103–111. DOI: 10.1111/j.1365-2915.2007.00672.x [PubMed: 17373953]
- Menda G, Shamblé PS, Nitzany EI, Golden JR, Hoy RR. Visual perception in the brain of a jumping spider. *Curr Biol.* 2014; 24:2580–2585. DOI: 10.1016/j.cub.2014.09.029 [PubMed: 25308077]

- Menzel, R., Brembs, B., Giurfa, M. Cognition in invertebrates. In: Kaas, JH., editor. Evolution of Nervous Systems. Vol. 1. Academic Press; Oxford: 2007. p. 403-442.
- Mitko L, Weber MG, Ramirez SR, Hedenstrom E, Weislo WT, Eltz T. Olfactory specialization for perfume collection in male orchid bees. *J Exp Biol.* 2016; 219:1467–1475. DOI: 10.1242/jeb.136754 [PubMed: 27207952]
- Müller GC, Beier JC, Traore SF, Toure MB, Traore MM, Bah S, Doumbia S, Schlein Y. Field experiments of *Anopheles gambiae* attraction to local fruits/seedpods and flowering plants in Mali to optimize strategies for malaria vector control in Africa using attractive toxic sugar bait methods. *Malar J.* 2010; 9:262.doi: 10.1186/1475-2875-9-262 [PubMed: 20854666]
- Nelson XJ, Jackson RR. A predator from East Africa that chooses malaria vectors as preferred prey. *PLoS ONE.* 2006; 1:e132.doi: 10.1371/journal.pone.0000132 [PubMed: 17205136]
- Nelson, XJ., Jackson, RR. Flexibility in the foraging strategies of spiders. In: Herberstein, ME., editor. Spider behaviour: flexibility and versatility. Cambridge University Press; Cambridge: 2011. p. 31-56.
- Nelson XJ, Jackson RR. The role of numerical competence in a specialized predatory strategy of an araneophagic spider. *Anim Cogn.* 2012a; 15:699–710. DOI: 10.1007/s10071-012-0498-6 [PubMed: 22526693]
- Nelson XJ, Jackson RR. Fine tuning of vision-based prey-choice decisions by a predator that targets malaria vectors. *J Arachnology.* 2012b; 40:23–33. DOI: 10.1636/Hill-61.1
- Nelson XJ, Jackson RR. Hunger-driven response by a nectar-eating jumping spider to specific phytochemicals. *Chemoecology.* 2013; 23:149–153. DOI: 10.1007/s00049-013-0130-5
- Nelson XJ, Pratt AJ, Cheseto X, Torto B, Jackson RR. Mediation of a plant-spider association by specific volatile compounds. *J Chem Ecol.* 2012b; 38:1081–1092. DOI: 10.1007/s10886-012-0175-x [PubMed: 22914959]
- Nelson XJ, Warui CM, Jackson RR. Widespread reliance on olfactory sex and species identification by lyssomanine and spartaeine jumping spiders. *Biol J Linnean Soc.* 2012a; 107:664–677. DOI: 10.1111/j.1095-8312.2012.01965.x
- Nicolson, SW., Thornburg, RW. Nectar chemistry. In: Nicolson, SW.Nepi, M., Pacini, E., editors. Nectaries and nectar. Springer; Dordrecht: 2007. p. 215-263.
- Nyasembe VO, Teal PEA, Mukabana WR, Tumlinson JH, Torto B. Behavioural response of the malaria vector *Anopheles gambiae* to host plant volatiles and synthetic blends. *Parasit Vectors.* 2012; 5:234.doi: 10.1186/1756-3305-5-234 [PubMed: 23069316]
- Opitz SEW, Müller C. Plant chemistry and insect sequestration. *Chemoecology.* 2009; 19:117–154. DOI: 10.1007/s00049-009-0018-6
- Pagani-Nunez E, Barnett CA, Gu H, Goodale E. The need for new categorizations of dietary specialism incorporating spatio-temporal variability of individual diet specialization. *J Zool.* 2016; 300:1–7. DOI: 10.1111/jzo.12364
- Schiestl FP. The evolution of floral scent and insect chemical communication. *Ecol Lett.* 2010; 13:643–656. DOI: 10.1111/j.1461-0248.2010.01451.x [PubMed: 20337694]
- Sharma GP, Raghubanshi AS, Singh JS. *Lantana* invasion: an overview. *Weed Biol Manag.* 2005; 5:157–165. DOI: 10.1111/j.1445-6664.2005.00178.x
- Shettleworth, SJ. Cognition, evolution, and behaviour. 2. Oxford University Press; New York: 2010.
- Tedore C, Johnsen S. Pheromones exert top-down effects on visual recognition in the jumping spider *Lyssomanes viridis*. *J Exp Biol.* 2013; 216:1744–1756. DOI: 10.1242/jeb.071118 [PubMed: 23348952]
- Uhl, G. Spider olfaction: attracting, detecting, luring and avoiding. In: Nentwig, W., editor. Spider ecophysiology. Springer-Verlag; Berlin: 2013. p. 141-157.
- Uhl, G., Elias, DO. Communication. In: Herberstein, ME., editor. Spider behaviour: flexibility and versatility. Cambridge University Press; Cambridge: 2011. p. 127-189.
- Vallortigara G, Chiandetti C, Rugani R, Sovrano VA, Regolin L. Animal cognition. *WIREs Cogn Sci.* 2010; 1:882–893. DOI: 10.1002/wcs.75
- Wesolowska W, Jackson RR. *Evarcha culicivora* sp nov., a mosquito-eating jumping spider from East Africa (Araneae: Salticidae). *Ann Zool.* 2003; 53:335–338.

- West-Eberhard, MJ. Developmental plasticity and evolution. Oxford University Press; New York: 2003.
- Yeargan KV, Quate LW. Juvenile bolas spiders attract psychodid flies. *Oecologia*. 1996; 106:266–271. DOI: 10.1007/BF00328607 [PubMed: 28307652]
- Zhang S, Bock F, Si A, Tautz J, Srinivasan MV. Visual working memory in decision making by honey bees. *Proc Natl Acad Sci USA*. 2005; 102:5250–5255. DOI: 10.1073/pnas.0501440102 [PubMed: 15795382]

Highlights

- *Evarcha culicivora*, a mosquito-specialist spider, is frequently found on plants
- There are ontogenetic shifts in responses by *Evarcha culicivora* to plant odour
- Plant odour primes juveniles to see plants for accessing nectar meals
- Plant odour primes adults to see a mate associated with a plant

Fig. 1.

Apparatus for Experiments 1 and 2 (see text for details) when test spiders were adult males and females of *Evarcha culicivora*. Glass arena (internal dimensions: 100 mm × 100 mm, 37 mm high) was centred on top of a glass base (140 × 140 mm), which was in turn centred on top of a wooden arena table (300 mm × 300 mm; wood thickness, 17 mm) supported by four legs (each leg 40 mm × 40 mm in cross section and 250 mm in height). Metal braces held the four display chambers (inner dimensions 260 mm × 40 mm) tightly against the table top. Each display chamber had two wooden side walls (80 mm wide, wood thickness 20 mm). Each display chamber was centred on a wooden display-chamber table (length 300 mm × 80 mm; wood thickness, 20 mm), with a pair of wooden legs (height 220 mm) supporting each display-chamber table. For stability, each of these legs was bolted to the nearest leg of the arena table. A glass odour chamber (inner dimensions 70 mm × 70 mm × 70 mm) sat under the arena table. Air entered through a hole centred in the arena table top and through a corresponding inflow hole in the bottom of the arena, and air left the arena through an outflow hole centred in the arena lid (hole diameters 12 mm). There was a glass tube in the outflow hole and another glass tube in the inflow hole (inner diameter of each tube 11 mm, outer diameter 12 mm, length 20 mm).

Fig. 2.

Apparatus for Experiments 1 and 2 (see text for details) when test spiders were juveniles of *Evarcha culicivora*. Glass arena (internal dimensions: 50 mm × 50 mm, 37 mm high) was centred on top of a glass base (56 mm × 56 mm), which was in turn centred on top of a wooden arena table (90 mm × 90 mm; wood thickness, 17 mm) supported by four legs (each leg 40 mm × 40 mm in cross section and 250 mm in height). Metal braces held the four display chambers (inner dimensions 100 mm × 100 mm) tightly against the table top. Each display chamber had two wooden side walls (80 mm wide, wood thickness 20 mm). Two grooves (3 mm wide, 40 mm apart) ran from the top to the bottom of each side wall and across the floor of the chamber. Each display chamber was centred on a wooden display-chamber table (length 90 mm × width 80 mm; wood thickness, 20 mm), with a pair of wooden legs (height 220 mm) supporting each display-chamber table. For stability, each of these legs was bolted to the nearest leg of the arena table. A hole (diameter 12 mm) was centred in the bottom of each display chamber and, correspondingly, in the display chamber table. A glass odour chamber (inner dimensions 70 mm × 70 mm × 70 mm) sat out of the test spider's field of view. Air entered through a hole centred in the arena table top and through a corresponding inflow hole in the bottom of the arena, and air left the arena through an outflow hole centred in the arena lid (hole diameters 12 mm). There was a glass tube in the outflow hole and another glass tube in the inflow hole (inner diameter of each tube 11 mm, outer diameter 12 mm, length 20 mm).

Fig. 3.

Apparatus for Experiment 3 (see text for details). Test spiders were always adult males of *Evarcha culicivora*. Glass arena (inside length 70 mm, inside height 37 mm) had three sectors, with the centre sector (30 mm × 25 mm) being narrower than the two side sectors (20 mm × 50 mm). The arena was centred on top of a glass base (80 mm × 80 mm), which was in turn centred on top of a wooden arena table (140 mm × 130 mm; wood thickness, 17 mm) supported by four legs (each leg 40 mm × 40 mm in cross section and 250 mm in height). Metal braces held both of the display chambers (inner dimensions 100 mm × 40 mm) tightly against the table top. Each chamber had two wooden side walls (80 mm wide, wood thickness 20 mm). Two grooves (3 mm wide, 40 mm apart) ran from the top to the bottom of each side wall and across the floor of the chamber. Each display chamber was centred on a wooden display-chamber table (length 140 mm × width 80 mm; wood thickness, 20 mm), with a pair of wooden legs (height 220 mm) supporting each display-chamber table. For stability, each of these legs was bolted to the nearest leg of the arena table. The height of the arena-table legs and display-table legs were such that the top of each chamber was 73 mm above the arena-table top. A hole (diameter 12 mm) was centred in the bottom of each display chamber and, correspondingly, in the display chamber table. A glass odour chamber (inner dimensions 70 mm × 70 mm × 70 mm) sat out of the test spider's field of view. Air entered through a hole centred in the arena table top and through a corresponding inflow hole in the bottom of the arena, and air left the arena through an outflow hole centred in the arena lid (hole diameters 12 mm). There was a glass tube in the outflow hole and another glass tube in the inflow hole (inner diameter of each tube 11 mm, outer diameter 12 mm, length 20 mm).

Fig. 4.

Mean response duration in trials for Experiment 1 using no odour (control) and caryophyllene odour. Error bars indicate SEM. Juveniles specified by body length (mm). For all test-spider categories, N = 20 test spiders. (A) *Lantana camara* used as the visual stimulus. (B) Three other plant species used as the visual stimulus. As no male-female differences were evident with other plants (A and C), only males were used as test spiders here. (C) *Bougainvillea glabra* used as the visual stimulus.

Fig. 5.

Mean response duration in trials for Experiment 1 when only *Lantana camara* was used as the visual stimulus. Error bars indicate SEM. Note: data for caryophyllene odour and data for no odour (control) same as in Fig. 4(A). Juveniles specified by body length (mm). For all test-spider categories, N = 20 test spiders. (A) Other compounds (humulene and cineole) used as the odour source. (B) Prey used as the odour source. (C) Mates used as the odour source.

Fig. 6. Mean response duration in trials for Experiment 2 when using (A) cryptic *Lantana camara* flowers (all test-spider categories) and (B) conspicuous *Lantana camara* flowers (small juveniles only) as the visual stimulus. Odour sources vary. Error bars indicate SEM. Juveniles specified by body length (mm). For all test-spider categories, N = 20 test spiders. Note: control data for small juveniles presented with cryptic flowers in (B) the same as in (A).

Fig. 7.

Mean response duration in trials for Experiment 2 when (A) using cryptic prey (all test-spider categories) and (B) conspicuous prey (males and small juveniles only) as the visual stimulus. Odour sources vary. Error bars indicate SEM. Juveniles specified by body length (mm). For all test-spider categories, $N = 20$ test spiders. Note: control data for males and small juveniles presented with cryptic prey in (B) the same as in (A).

Fig. 8. Mean response duration in trials for Experiment 2 when using (A) cryptic mates (males and females) and (B) conspicuous mates (males only) as the visual stimulus. Odour sources vary. Error bars indicate SEM. For all test-spider categories, $N = 20$ test spiders. Note: control data for males presented with cryptic mates in (B) the same as in (A).

Table 1

Experiment 3. Number of test spiders that responded rapidly. N=90 for each row. All test spiders adult males

Odour source	Visual stimulus	Number that Responded
Nil	Prey	33
	Mate	39
	<i>L. camara</i>	24
	<i>L. camara</i> + prey	27
	<i>L. camara</i> + mate	46
Caryophyllene	Prey	26
	Mate	59
	<i>L. camara</i>	51
	<i>L. camara</i> + prey	51
	<i>L. camara</i> + mate	81
Prey	Prey	61
	Mate	31
	<i>L. camara</i>	28
	<i>L. camara</i> + prey	54
	<i>L. camara</i> + mate	43
Mate	Prey	29
	Mate	70
	<i>L. camara</i>	34
	<i>L. camara</i> + prey	30
	<i>L. camara</i> + mate	65

Summary of outcomes from Experiment 3 when comparing the number of test spiders that responded rapidly to a specific visual target in the presence of a specific odour with the number that responded rapidly to the same specific visual target in no-odour control trials.

Table 2

	Prey as visual target	Mate as visual target	<i>Lantana camara</i> as visual target	<i>Lantana camara</i> + prey as visual target	<i>Lantana camara</i> + mate as visual target
Caryophyllene as odour source	NS	More with odour	More with odour	More with odour	More with odour
Prey as odour source	More with odour	NS	NS	More with odour	NS
Mate as odour source	NS	More with odour	NS	NS	More with odour

More with odour: significantly more test spiders responded rapidly in presence of the odour than in no-odour control trials. NS; number test spiders that responded rapidly in presence of odour not significantly different than number that responded rapidly in no-odour control trials

Table 3

Summary of outcomes from Experiment 3 when comparing number of test spiders that responded rapidly to a visual target with the number that responded rapidly to another visual target, in the presence of the same odour source.

Odour source	Visual target 1	Visual target 2	Outcome
None	Prey	Mate	NS
		<i>Lantana camara</i>	NS
		<i>Lantana camara</i> + prey	NS
		<i>Lantana camara</i> + mate	X
	Mate	<i>Lantana camara</i>	NS
		<i>Lantana camara</i> + prey	X
		<i>Lantana camara</i> + mate	NS
		<i>Lantana camara</i>	<i>Lantana camara</i> + prey
	<i>Lantana camara</i>	<i>Lantana camara</i>	More with target 1
		<i>Lantana camara</i> + prey	More with target 1
Caryophyllene	Prey	<i>Lantana camara</i> + mate	X
	Mate	Prey	More with target 1
		<i>Lantana camara</i>	NS
	<i>Lantana camara</i>	<i>Lantana camara</i> + prey	X
		Prey	More with target 1
		<i>Lantana camara</i> + prey	NS
		<i>Lantana camara</i> + prey	More with target 1
	<i>Lantana camara</i> + prey	Prey	More with target 1
	<i>Lantana camara</i> + mate	Mate	More with target 1
		<i>Lantana camara</i>	More with target 1
Prey	Prey	Mate	More with target 1
		<i>Lantana camara</i>	More with target 1
		<i>Lantana camara</i> + prey	NS
		<i>Lantana camara</i> + mate	X
	Mate	<i>Lantana camara</i>	NS
		<i>Lantana camara</i> + prey	X
		<i>Lantana camara</i> + mate	NS
		<i>Lantana camara</i>	<i>Lantana camara</i> + mate
	<i>Lantana camara</i>	<i>Lantana camara</i>	More with target 1
		<i>Lantana camara</i> + mate	NS
Mate	Prey	<i>Lantana camara</i>	NS
		<i>Lantana camara</i> + prey	NS
		<i>Lantana camara</i> + mate	X
		Prey	More with target 1
	Mate	<i>Lantana camara</i>	More with target 1
		<i>Lantana camara</i> + prey	X
		<i>Lantana camara</i> + mate	NS
		<i>Lantana camara</i> + mate	NS

Odour source	Visual target 1	Visual target 2	Outcome
	<i>Lantana camara</i>	<i>Lantana camara</i> + prey	NS
	<i>Lantana camara</i> + mate	<i>Lantana camara</i>	More with target 1
		<i>Lantana camara</i> + prey	More with target 1

More with target 1: significantly more test spiders responded rapidly to visual target 1 than to visual target 2. NS: number that responded rapidly to visual target 1 not significantly different than number that responded rapidly to visual target 2. X: not compared because no clear conclusion would be implied by a significant outcome