



Effects of phytochemicals on predatory decision making in a spider

Ximena J. Nelson^{a,*}, Andrew J. Pratt^b, Stephen Abok Aluoch^c, Robert R. Jackson^{a,c}

^a School of Biological Sciences, University of Canterbury, Christchurch, New Zealand

^b School of Physical and Chemical Sciences, University of Canterbury, Christchurch, New Zealand

^c International Centre of Insect Physiology and Ecology, Thomas Odhiambo Campus, Mbita Point, Kenya

ARTICLE INFO

Article history:

Received 25 November 2020

Initial acceptance 2 February 2021

Final acceptance 10 March 2021

Available online 22 May 2021

MS. number: 20-00855

Keywords:

arthropod–plant interaction

attention

cognitive capacity limitation

cross-modality priming

phytochemical

salticid

Animals with small nervous systems may be prone to limitations in processing ability when confronted with a diversity of stimuli, especially if these involve multiple sensory modalities. We investigated the effect of the odour of the plant *Lantana camara* and its dominant volatile compound, β -caryophyllene, on the prey choice decisions of *Evarcha culicivora*, a jumping spider (Salticidae) that frequently visits *L. camara* as a site to mate and to obtain nectar. *Evarcha culicivora* expresses a strong preference for blood-fed female *Anopheles* mosquitoes as prey. We tested juvenile and adult *E. culicivora* in experiments involving live prey, lures made from dead prey and virtual prey, when presented with no odour, odour from *L. camara* and β -caryophyllene. We found that, in the presence of the plant-related odours, *E. culicivora*'s prey preference was no longer expressed. This effect seems to be a consequence of the plant-related odours reducing the extent to which *E. culicivora* relies on a pre-attack pause and visual fixation routine ('stationing') when seeing prey. Stationing may be critical for adequate visual scanning, and thus correct identification of the prey, as we found that the longer the spider was stationed in front of prey before being subjected to a disruption, the more strongly *E. culicivora* expressed a preference for blood-fed female mosquitoes. This seems to be an unusual example of a specific modulation of visually based prey choice behaviour being mediated by an environmental chemical cue not pertinent to the prey.

© 2021 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Cognition pertains to how information is acquired and translated into behaviour, for example through decision making (Dukas, 2002; Shettleworth, 2010). Animals may 'know how' to do something without necessitating decision making (procedural knowledge) or may rely on cognitive processes that allow for cost/benefit evaluation of a given decision (declarative knowledge), allowing them to adjust to unpredictable scenarios. We define assessment as how animals evaluate perceived stimuli and convert these evaluations to information that can be used to deduce the benefit of a given decision (Blumstein & Bouskila, 1996). Thus, decision making is the cognitive process interfacing assessment and discernible behaviour (Blumstein & Bouskila, 1996). Decision making and assessment can be guided, and sometimes improved, by multi-sensory information (Dall, Giraldeau, Olsson, McNamara, & Stephens, 2005; Partan & Marler, 1999, 2005). Such use of multiple channels of sensory information can affect decisions regarding predation risk, communication and navigation (e.g. De Agrò et al., 2020; Hebets et al., 2014; Hebets & Uetz, 2000; Martin, Fodrie, Heck, & Mattila, 2010; Ward & Mehner, 2010), among others.

Known as 'jumping spiders', there are over 6200 described species in the family Salticidae (World Spider Catalog, 2020), most of which capture prey without the use of a web and tend to feed on a wide variety of insects, generally showing no preference for specific types of prey. However, there are distinctive exceptions, which include species that express active preference for highly specific prey categories (Nelson & Jackson, 2011a). Like tiny cats, salticids visually locate and identify prey from a distance, carefully stalk the prey, often pausing for some time while intently looking at the prey (which we refer to as 'stationing') before attacking by pouncing (Forster, 1985).

Although, as suggested by their common name, many salticids are exceptionally good at jumping, the most distinctive characteristics of these spiders are their unique, complex eyes and the intricate vision-based strategies they deploy when capturing their prey (e.g. Aguilar-Argüello, Gerhard, & Nelson, 2020; Cross & Jackson, 2016; Jackson & Cross, 2011; Jakob, Skow, & Long, 2011; VanderSal & Hebets, 2007). However, for salticids, exceptional eyesight has not precluded exceptional abilities pertaining to other senses, including the use of multiple sensory modalities at the same time (Echeverri, Morehouse, & Zurek, 2017; Elias, Hebets, Hoy, Maddison, & Mason, 2006; Girard, Kasumovic, & Elias, 2011; Nelson, Warui, & Jackson, 2012).

* Corresponding author.

E-mail address: ximena.nelson@canterbury.ac.nz (X. J. Nelson).

Salticids have four pairs of camera-type eyes (Land, 1985), but their unusual visual system is largely due to one pair of these: the large forward-facing principal eyes, which have spatial acuity vastly exceeding that of any other animal with a similar eye size (Blest, O'Carroll, & Carter, 1990; Land, 1981, 1985; Williams & McIntyre, 1980). Briefly, the principal eyes consist of the corneal lens, a long eye tube and a boomerang-shaped retina lying at the end of the eye tube (Land, 1969a). The retina allows for colour discrimination ability, varying from dichromatic to tetrachromatic, depending on the species (Harland, Li, & Jackson, 2012; Zurek et al., 2015). These eyes have a field of view of only 3–5°, but they compensate for this by using muscles attached to the eye tube that move the retina side-to-side and up-and-down by as much as 35°. These muscles can also rotate the retina on its axis and, by intricate combined rotary and displacement movement of the retinae, the salticid can perform detailed scanning of the visual object of interest (Land, 1969b), somewhat akin to the active vision described in bees (e.g. Nityananda, Skorupski, & Chittka, 2014).

Visually mediated preferences for narrowly defined microhabitat (e.g. bromeliads, de Omena & Romero, 2010) and prey categories are known from research on many salticids (Nelson & Jackson, 2011a), with *Evarcha culicivora* being perhaps the most extreme example. This East African salticid feeds indirectly on vertebrate blood by targeting blood-carrying females of anopheline mosquitoes as its most preferred prey (Jackson, Deng, & Cross, 2016; Jackson, Nelson, & Sune, 2005). However, as this is a predator that does a lot of categorizing, it may be more useful to focus on *E. culicivora* having a preference profile based on its own classification system (Jackson et al., 2016; Nelson & Jackson, 2006). Blood-carrying female anopheline mosquitoes are the most preferred, followed by other mosquitoes (*Anopheles* males and blood-carrying culicine females), and *E. culicivora* also expresses a preference for nonbiting midges (Chaoboridae and Chironomidae) when the alternatives are not mosquitoes (Jackson et al., 2016).

A distinctive resting posture with the abdomen tilted upwards is characteristic of *Anopheles* (Clements, 1999), and this posture appears to be a primary criterion by which *E. culicivora* identifies this specific prey (Nelson & Jackson, 2006), although other details, including features of the antennae, are also used (Nelson & Jackson, 2012). The odour of blood-fed *Anopheles gambiae* also primes selective attention to visual cues from this specific prey type (Cross & Jackson, 2009a; Dolev & Nelson, 2014).

Evarcha culicivora is also attracted to the odour of the plant *Lantana camara*, and especially to its dominant volatile component, β -caryophyllene (Cross & Jackson, 2009b; Nelson & Jackson, 2013; Nelson, Pratt, Cheseto, Torto, & Jackson, 2012). Yet the complex ways in which *L. camara* is relevant to *E. culicivora* are still poorly understood. This plant appears to be relevant to *E. culicivora* juveniles primarily as a source of supplementary nectar meals (Kuja et al., 2012) and to adults primarily as mating sites (Cross, Jackson, & Pollard, 2008). However, it seems unlikely that attraction to *L. camara* and β -caryophyllene might pertain to *E. culicivora* finding preferred prey because there is no evidence to suggest that mosquitoes are a source of β -caryophyllene (Caputo et al., 2005) and, although *A. gambiae* may visit *L. camara* for nectar meals (Impoinvil et al., 2004; Manda et al., 2007), this tends to occur at night, when *E. culicivora* becomes quiescent and is not responsive to prey (Deng, Cross, & Jackson, 2017). There are also ontogenetic changes in the way *E. culicivora* is affected by the odour of *L. camara* and β -caryophyllene: in the presence of odour, juveniles are primed to detect by sight and approach specifically flowers (for a nectar reward), whereas adults are primed to detect the plant by sight and approach it specifically because of the potential to find a mate associated with the plant (Carvell, Jackson, & Cross, 2017).

Here, for the first time, we investigated how the odour of *L. camara* and β -caryophyllene might influence *E. culicivora* in the context of making prey choice decisions, and we also consider how the effects on prey choice decisions might be mediated by an effect of these odours on this predator's prey capture sequence. Specifically, we investigated the effect of β -caryophyllene odour on *E. culicivora*'s decision to adopt stationing when seeing prey. In general, this pause and visual fixation before attacking has received little attention in the literature and it may be common for salticids in pursuit of highly motile prey to station for no more than a few seconds, but stationing by *E. culicivora* tends to be distinctively long, sometimes lasting for over an hour. Our hypothesis is that stationing time is critical for adequate visual scanning and thus correct identification of the prey. As spiders were less likely to adopt stationing with the odour of β -caryophyllene compared to without odour, we considered whether this odour could be construed as a disturbance, leading spiders to quickly attack prey rather than to correctly assess prey and make correct decisions (i.e. shorter stationing duration in the presence of β -caryophyllene might explain lowered expression of preferences). On this basis, we investigated the choice of prey attacked by *E. culicivora* when presented with either a visual or a percussive disturbance after different intervals once the spider had stationed in front of prey.

METHODS

General

Spiders were from laboratory cultures (F1–F3 generation) derived from individuals collected at our field site. Testing was performed in a laboratory at the Thomas Odhiambo Campus (TOC) of the International Centre of Insect Physiology and Ecology (ICIPE) in Mbita Point, Western Kenya, and in a laboratory at the University of Canterbury in New Zealand. The methods we adopted for rearing and maintenance of spiders were as in earlier studies (e.g. Cross & Jackson, 2009b).

One of our objectives was to investigate specifically the innate predispositions governing *E. culicivora*'s predatory behaviour, so we adopted procedures aimed at minimizing the potential influence of variation in learning and maternal effects on experimental outcomes. For experiments 1–3, individual spiders and their parents had no prior experience with the plant species used for testing, with mosquitoes or midges or with the compounds used in the experiments; they were reared on a diet of house flies and *Drosophila* (three times a week). Test spiders in experiments 4 and 5 were maintained on a diet of chironomids and chaoborids (collected as needed from the field) and mosquitoes (*A. gambiae* females from an insectary at the TOC); these spiders had no prior experience with the compounds used in the experiments.

The lures used in experiments 1 and 5 were made from mosquitoes (*A. gambiae* or *Culex quinquefasciatus* females that had fed on blood 4–5 h beforehand) or nonbiting midges (*Chaoborus* sp.) that were first immobilized with CO₂ and then placed in 80% ethanol. The next day, each insect was mounted in a lifelike resting posture (*Anopheles* with abdomen tilted upwards; *Culex* and *Chaoborus* with abdomen parallel to cork surface) on the centre of a disc-shaped piece of cork (diameter 15 mm, thickness 2 mm). For preservation, the lure and the cork were then sprayed with a transparent plastic adhesive. See Jackson et al. (2005) for further details on the making of lures.

No individual was tested more than once. Adult spiders used in experiments had matured 2–3 weeks before being tested and had not mated; the juveniles had moulted at least 4 days before testing. All juveniles tested were subject to a 5-day fast, and all adults to a 7-day fast, before testing. All testing was carried out between 0800

and 1400 hours (laboratory photoperiod 12:12 h light:dark, lights on 0700 hours), as this is the period in which *E. culicivora* is most responsive to prey (Deng et al., 2017). Finally, between trials, all arenas and odour chambers were cleaned with 80% ethanol and allowed to dry before tests resumed.

Ethical Note

Evarcha culicivora is not endangered or protected. Hence, no permits were required to perform this research. The spiders were handled carefully to minimize stress or injury by using paintbrushes for moving spiders. Individuals were caught (using paintbrushes to brush them into vials) in the wild in Kenya, and were individually housed in large enriched containers (see Cross & Jackson, 2009b), with 80 individuals transported by air (in individual vials with a leaf to provide humidity) for testing (experiments 1–3) and rearing in a quarantine facility in New Zealand. These spiders were housed in the laboratory until dying a natural death. Spiders used in experiments 4 and 5 were tested in Kenya and were thereafter released into the wild. Behavioural testing, using 554 individuals across all five experiments, was noninvasive and no indications of stress were observed. We adhered to the ASAB/ABS guidelines for the treatment of animals.

Experiment 1: Juvenile Spider *Anopheles-Culex* Discrimination Using Lures

The odour of *L. camara* (hereafter referred to simply as 'plant odour') is known to affect *E. culicivora*'s vision-based decisions (Carvell et al., 2017), but these decisions have not been investigated in the context of prey choices. Here, we tested prey choices of juvenile of *E. culicivora* (body length 1.5–2.0 mm) in the presence ($N = 16$ successful tests) and absence ($N = 18$ successful tests) of *L. camara* odour.

During trials, the test spider had access to two mosquito lures, one made from an adult female *Anopheles gambiae* and the other made from an adult female *C. quinquefasciatus* (body length of both,

4.5 mm) placed at the end of a Y-shaped wooden ramp. The ramp was angled up by 20° (see Fig. 1 for dimensions) and was attached to a dowel connected to a wooden base. All wooden surfaces were varnished. The ramp sat within a glass tank (128 × 128 mm and 305 mm high) with a removable front panel for access to the ramp between trials. White paper covered three outer sides of the tank (i.e. not the ceiling or the removable front panel, which were used to observe experiments) to obstruct external visual stimuli.

Before each trial began, the test spider was put into a plastic vial (10 × 50 mm). Half of the vial's interior was packed with cotton wool, leaving a 5 mm length for the test spider to occupy. A stopper at the other end kept the spider inside for a 5 min acclimation period. Next, the stopper was removed, and the vial was fastened with transparent tape to the lower end of the stem of the ramp (upper half of vial sitting on ramp; cotton-packed half extending beyond the ramp). With the vial attached to the ramp, the glass front was then pushed back onto the tank.

Each trial began when the test spider walked out of the tube on to the stem of the ramp. In successful trials, the test spider moved up the stem and then on to one of the arms of the ramp and came to within at least 10 mm of a lure and stayed there for at least 30 s (during this time, spiders were usually quiescent while looking at lures, i.e. 'stationing', see below). Testing was terminated if 5 min elapsed before a test spider left the vial, if a test spider leapt off the ramp or walked underneath the ramp before coming to within 10 mm of a lure and whenever 45 min elapsed with a test spider on the top of the ramp having come to within 10 mm of a lure and stayed for 30 s.

As salticids tend to be more responsive when prey move instead of staying still, the lures were attached to a wire pedestal that protruded through a hole at the end of each arm of the ramp. The top end of the pedestal was inserted into the centre of the cork disc that held the lure. The other end of each pedestal was connected below the ramp arm to an electric 'mover' mechanism. When switched on, the mover made the pedestal rotate clockwise $6^\circ/s$. The two movers were wired to a single external controller and were turned on for 30 s with a 180 s interval. Lures were moved before

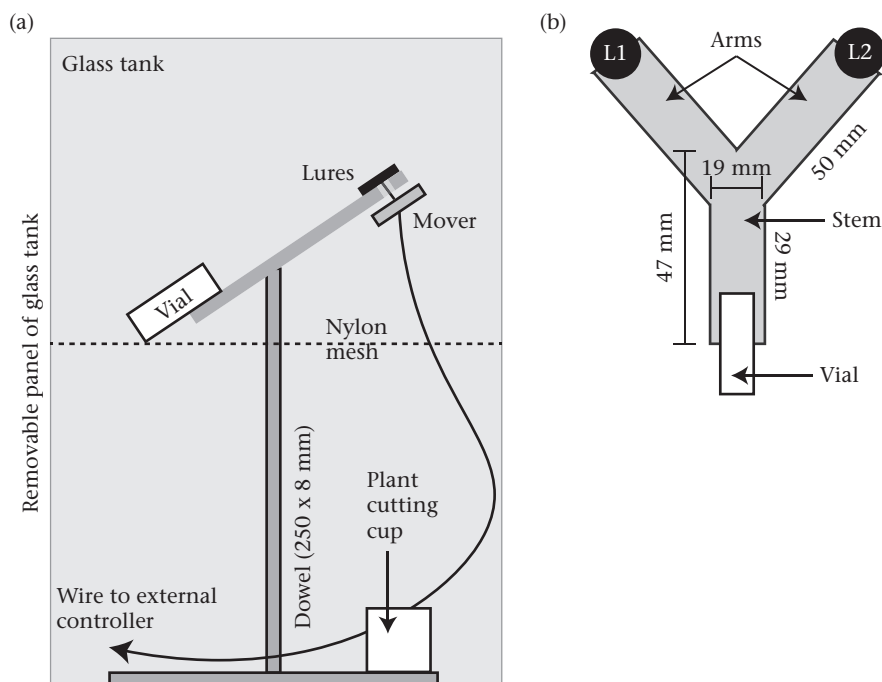


Figure 1. Apparatus used in lure prey choice testing of *E. culicivora* juveniles. (a) Side view. (b) View from above. L1: lure 1; L2: lure 2.

the test spider left the vial and then throughout the trial. The wires passed through a small gap at the bottom of the front panel of the tank. When not rotating, each lure was oriented $\pm 90^\circ$ from the stem of the ramp. This orientation was such that, for a test spider facing the lure arm, the lure's head, thorax and abdomen were all within its line of view.

During trials, a hidden cutting (stems, two leaves, two umbels) of *L. camara* was present in the tank. The cutting was placed in distilled water in a 100 mm plastic cup, with the cup and cutting covered with three layers of nylon mesh (mesh size 0.5 mm) taped to the tank walls 150 mm above the base of the tank (Fig. 1). Control trials were identical to experimental trials except that there was no plant cutting in the cup of water. The cup, with or without a cutting, was put into place 5 min before testing began. We used new netting, cuttings, distilled water and cotton wool during each trial.

We compared the prey choices made by the spider in the presence and absence of *L. camara* odour, as well as the number of successful/unsuccessful tests to determine whether treatment affected motivation to attack. Results were analysed using chi-square tests of independence, Fisher exact tests and binomial tests.

Experiment 2: Juvenile Spider *Anopheles-Culex* Discrimination Using Virtual Prey

To minimize potential issues arising from differences in shape or movement cues, we tested juvenile (body length 1.5 mm) responses to virtual prey in the presence of odour from *L. camara*, in the presence of one of its key volatile compounds, β -caryophyllene (Sigma-Aldrich, >95%), or in the absence of odour. Juveniles of *E. culicivora* are responsive to β -caryophyllene (Nelson & Jackson, 2013), which appears to prime them to search for flowers (Carvell et al., 2017), possibly as a source of a nectar meal.

As in previous work, we tested *E. culicivora* by projecting videos of virtual mosquitoes on a screen (for more detail about methods, see Nelson & Jackson, 2006, 2012). Using computer animation (3D Studio Max), drawings were made based on microscopy images of blood-fed *Anopheles* mosquitoes and motion was based on frame-by-frame copying of mosquito grooming behaviour. During testing, two side-by-side virtual mosquitoes (body length 3.2 mm) were presented to test spiders in 10 s continuously looping movies (avi format, 25 fps). Except for the abdomen, which was red to the human eye, the virtual mosquitoes were in greyscale and differed only in posture (Fig. 2a), one being in the *Anopheles* (i.e. abdomen tilted upwards) and the other in the *Culex* (i.e. abdomen parallel to the substratum) resting posture. Before each trial, whether the virtual *Anopheles* would be on the left- or right-hand side of the screen was determined at random.

Using a Telex P400 LCD data projector (800 x 600 pixels), rendered movies were forward-projected on to a fine-ground matte unmarked type D Nikon F3 focusing screen (39 mm wide x 30 mm high) that was situated ca. 150 mm from the projector lens. There was a ramp (stainless steel, 15 mm wide x 150 mm long, angled up by 25°) in front of the screen (Fig. 2). We positioned the focusing screen 2 mm beyond the end of the ramp, so that spiders could not simply walk onto the screen, ensuring a clear prey choice outcome. The ramp was within a glass cylinder (diameter 300 mm, length 525 mm) with removable sealing steel end plates (diameter 200 mm) and sitting on a stainless-steel platform that spanned the length of the cylinder. A reducing lens aligned with the lens of the data projector fitted snugly in a 37 mm hole in one end plate. The screen was mounted in a bracket attached to the platform, which was located ca. 105 mm from the lens. The projector angled down by 10° and, with this configuration, spiders could walk up the ramp without entering the light path from the projector.

There were three treatments. In the plant treatment, a chamber with a cutting of *L. camara* inside was placed under the platform where it was not visible to the spider. In the treatments with compound odour, we deposited either 4 μ l of distilled water (no odour treatment) or 4 μ l of β -caryophyllene (odour treatment) on a 42 mm disc of filter paper in a 50 mm glass Petri dish, this being a volume of β -caryophyllene that *E. culicivora* juveniles can readily detect (Nelson & Jackson, 2013). The Petri dish was then placed out

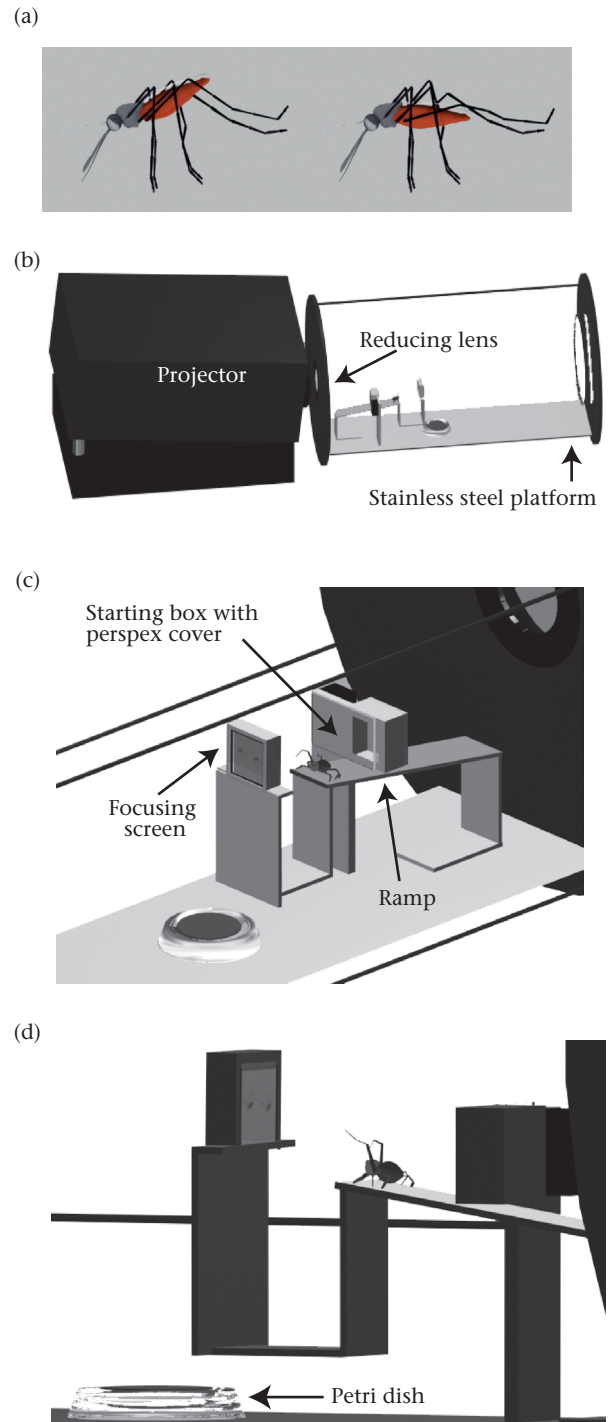


Figure 2. Stimuli and apparatus for prey discrimination by *E. culicivora* in experiment 2. (a) Stimuli used for testing. (b) Glass odour chamber and projector set-up. (c, d) Detail of methods used in prey discrimination experiments.

of the spider's line of sight on the stainless-steel platform behind the focusing screen 15 min prior to testing. A different sample was used for each trial.

When setting up a trial (and for cleaning the apparatus between trials), we removed the end plate furthest from the projector. Immediately before the trial began, the test spider was enticed on to a paintbrush and the brush was positioned so that its tip touched the ramp 10 mm from the ramp's upper end. Trials began when the spider walked off the brush on to the ramp. Each trial lasted 15 min, with a proviso that, if the test spider was stalking at 15 min, the trial continued until the end of the stalking bout. These extensions never required more than 3 min of additional time. The criterion for concluding that a test spider was 'stalking' was seeing it orient towards the prey and then begin approaching in a direct line while waving its palps.

In successful trials, the spider either leapt across the ramp–screen gap and landed directly on a virtual mosquito, or stalked a virtual mosquito, reached the end of the ramp and stayed there, oriented towards the virtual mosquito, for at least 30 s with its front pair of legs raised in typical salticid prejump posture (Forster, 1982). Tests were unsuccessful when the spider walked away from the projected image, when more than 15 min elapsed without the spider walking towards the virtual mosquito or when the spider jumped off the ramp.

In addition to the prey choices made by spiders (i.e. successful tests) under each of the three treatments, we analysed the number of unsuccessful tests in each treatment to ascertain motivation to attack. Sample sizes of successful tests were $N = 29$ (no odour), $N = 15$ (*L. camara*) and $N = 29$ (β -caryophyllene odour). Results were analysed using chi-square tests of independence and Fisher exact tests.

Experiment 3: Adult Spider *Anopheles* Discrimination Using Four Virtual Prey

Here, we designed an experiment eliciting a visually difficult decision based on the choice between three less preferred and one most preferred prey and investigated how *L. camara* and β -caryophyllene affected decision making. Owing to the task difficulty, we used adult female *E. culicivora*, as they are very responsive in prey choice tests. In each trial, three prey were *Chaoborus*, these being nonbiting midges that are similar to *Anopheles* in size and general appearance and are exceedingly abundant in *E. culicivora*'s habitat, and one was a virtual blood-fed *Anopheles* female, the most preferred prey for *E. culicivora* (Jackson et al., 2005). We used the same treatments as in experiment 2 (i.e. distilled water, β -caryophyllene or *L. camara*).

Except where stated otherwise, methods were as in the previous experiment. As we used adults, the screen was placed 5 mm (instead of 2 mm) from the upper end of the ramp. There was a stainless steel 'starting box' (width 11 mm, height 19 mm) positioned so that its closer side was 22 mm from the top of the ramp. The starting box was 22 mm deep (i.e. the rear of the box was 44 mm from the top end of the ramp). The front side of the box (11 × 19 mm) had a transparent Perspex cover that was wired to an external controller so that it could be opened remotely through the release of a spring. Before testing began, the cover was opened and a paintbrush was used to guide a test spider inside. The box was then closed and the chamber was sealed. After a 60 s acclimation period, the front of the starting box was opened. The beginning of a trial was defined by the spider walking out of the starting box and on to the ramp (always within 3 min).

All virtual prey were rendered entirely in greyscale and the same behaviour was given to all virtual prey. Two midges were situated side by side at the bottom of the screen and the top row

had a midge and a mosquito (Fig. 3). The criterion for a successful test was seeing the spider jump and land directly on one of the virtual prey.

In addition to the prey choices made by spiders in successful tests under each of the three treatments, we analysed the number of unsuccessful tests in each treatment to ascertain motivation to attack. Sample sizes of successful tests were $N = 20$ (no odour), $N = 18$ (*L. camara*) and $N = 21$ (β -caryophyllene). Results were analysed using G tests ($H_0 = 0.25$) and chi-square tests of independence.

Experiment 4: Odour Effects on Spider Preattack Behaviour Using Live Prey

Here, our objective was to determine the effect of β -caryophyllene odour on *E. culicivora*'s preattack behaviour when presented with live blood-fed female *A. gambiae*. We focused especially on *E. culicivora*'s decision to adopt stationing. When stationing, *E. culicivora* fixates its gaze on the prey and remains still, although test spiders might make localized movement so long as the principal eyes remained fixated on the mosquito. Localized movement included instances of spiders waving their palps, moving their spinnerets and repositioning their legs, while facing the prey for a minimum of 30 s.

The arena was a transparent glass box with a removable lid that sat centred on, and embedded into, a wooden platform with a square (30 × 30 mm) cut out of its centre (see dimensions in Fig. 4). Four wooden legs held the bottom of the platform 100 mm above a laboratory bench and the entire apparatus was surrounded by a 200 mm high wooden fence (painted white). Under the platform sat a glass odour chamber, an airflow regulator and a pump.

Centred on each of the four walls of the arena there was a hole plugged with a rubber stopper that fitted flush with the inner side of the arena. These holes were used to introduce the mosquito and the test spider. Additionally, there was an inflow hole and an outflow hole in the odour chamber and in the arena. The odour chamber inflow and outflow holes were centred on each of two opposite sides of the chamber. The arena inflow and outflow holes (16 mm) were in addition to the holes in the arena walls, and were centred in the arena floor and ceiling, respectively. The inner side of the stopper in the arena inflow and outflow holes were covered with nylon mesh, preventing the mosquito or test spider leaving the arena. Each inflow and outflow hole was plugged by a rubber stopper which was pierced by a glass tube (length 45 mm, diameter 4 mm). The pump drove air successively through the airflow regulator, odour chamber and arena, with silicone tubing connecting these components of the apparatus. Airflow was set at 1500 ml/min (Matheson FM-1000 airflow regulator) during trials.

Test spiders were assigned at random to one of two groups: experimental (β -caryophyllene in the odour chamber) or control (no odour in the odour chamber). The apparatus was set up 30 min before testing began: all parts of the apparatus were connected, the pump was turned on and the odour sample was placed in the odour chamber. The sample was situated in the centre of an open 30 mm glass Petri dish placed in the centre of the odour chamber. The sample was prepared by adding either 4 μ l of β -caryophyllene (odour) or distilled water (no odour) to 1.0 g of petroleum jelly (the 'carrier'). Each sample was prepared 24 h earlier, covered with a lid and wrapped in aluminium foil, and placed in a refrigerator until 60 min before a trial began, at which time it was transferred to the odour chamber.

During each trial, there was one living blood-fed *A. gambiae* with one adult female *E. culicivora* in the arena. The mosquito was put in the arena and, once it had been quiescent for 2 min, we waited a further 15 min before introducing the test spider into the arena.

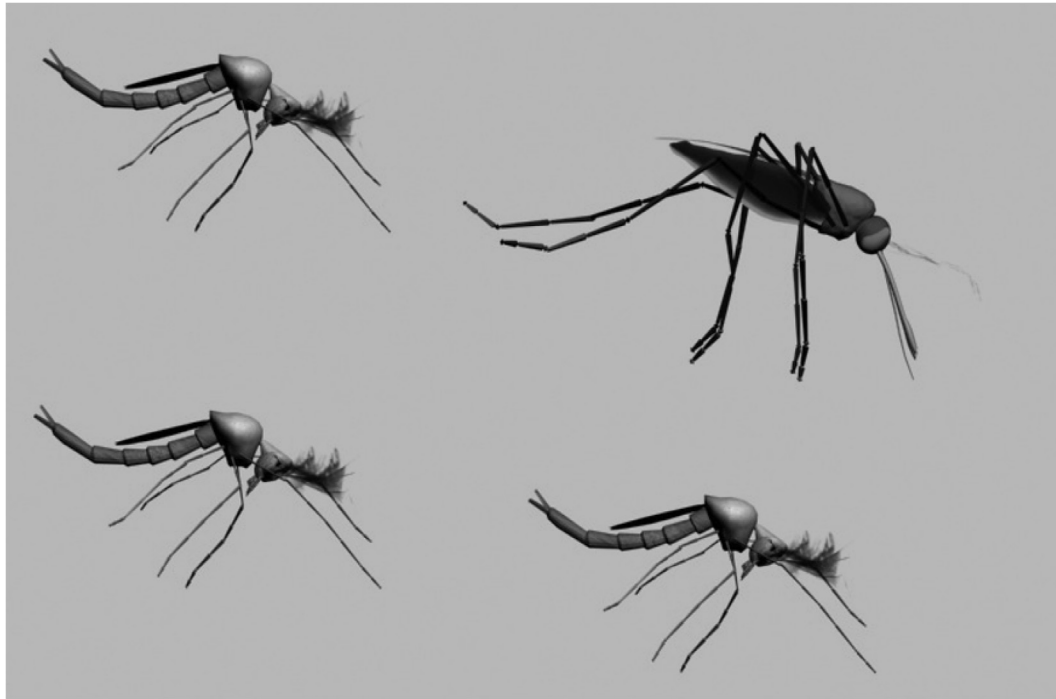


Figure 3. Stimulus set (three chaoborid midges and one mosquito) used for prey discrimination by *E. culicivora* in experiment 3.

Testing was terminated if this criterion was not met within 60 min. Tests were also terminated if the mosquito became active (including grooming) while the test spider was being introduced into the arena or before the test spider in the arena adopted stationing or attacked.

To introduce a mosquito into the arena, the stopper was removed from a hole in one of the walls of the arena, an aspirator was used to gently blow a mosquito through the hole and the stopper was replaced. Before the mosquito was put into the arena, the test spider was taken into a glass tube (length 20 mm, diameter 16 mm) and kept there with a rubber stopper in each end until the criteria to begin a trial were met. At this point, the stopper was removed from the arena hole most distant from the mosquito and a stopper in the tube was removed. The open end of the tube was inserted into the hole in the arena (open end of tube flush with the inside wall of the arena). Usually, the test spider moved spontaneously out of the tube and into the arena. If 5 min elapsed without this happening, the stopper was removed from the other end of the tube and a soft brush was used to prod the test spider, after which it promptly moved into the arena. As soon as the test spider entered the arena, we removed the tube and plugged the hole with the stopper, and trials began. All netting, stoppers and silicone tubing were replaced after each trial.

Being interested specifically in whether β -caryophyllene had an effect on the test spider's inclination to adopt stationing, we only recorded whether a test spider adopted stationing or whether it attacked and captured the quiescent mosquito without first stationing. In all cases, trials ended whenever 30 min elapsed without the test spider responding (i.e. without stationing or attacking); whenever a spider adopted stationing before 30 min elapsed, the trial continued until stationing ended. Stationing ended when the mosquito moved away, when the mosquito remained quiescent and the spider attacked, or else moved away from the mosquito, or when the spider pivoted its body so that its gaze was no longer on the mosquito. Data were analysed using chi-square tests of independence.

Experiment 5: Disturbance Effects on Prey-Based Behaviour

Preliminary observations had suggested that *E. culicivora* often seems to be inclined to attack prey when there is a sudden disturbance. Here we tested whether a disturbance ('prod'), or its timing, affected the test spider's behaviour once it had adopted preattack stationing. For this, it was necessary to use lures, instead of living prey. We tested adult female *E. culicivora*, and for lures we used *Chaoborus* sp. midges and blood-fed female *A. gambiae* (body length 4.5 mm) presented simultaneously at a fixed distance on a disconnected platform. Lure position (left or right side of the platform) was determined at random; different lures were used in each trial, and no spider was tested more than once.

The test apparatus was designed to encourage spiders to quickly adopt stationing (while simultaneously viewing both lures). The apparatus consisted of two plastic platforms, two wooden tables and four parallel wooden walls (see dimensions in Fig. 5). One platform was the 'display platform' and the other was the 'spider platform'. The display platform, on which the lures were situated, fitted in a slot in one of the walls ('display wall'). The spider platform, on which the test spider stood, fitted in a slot in another wall ('spider wall') 20 mm away. The display platform had four sectors (or 'targets'). A lure was positioned on each of the two middle sectors. Two kinds of prodding were used, percussive (a 22 g weight (an eraser) suddenly dropping on the spider platform behind the test spider) or visual (the two lures on the display platform suddenly moving). A measuring tape was used as a retraction spool which, when a spring button was pressed, caused it to release. When released, the retractable ribbon from the spool either dropped the weight onto the spider platform or pulled the lures back by 10 mm. Lures were connected to the retraction spool by a wire attached to each lure forming a T-junction; this junction, in turn, was connected via a wire to the retractable ribbon (Fig. 5). These two types of prodding disturbances were delivered at three intervals (0, 2.5 and 5 min), resulting in six randomly allocated treatments.

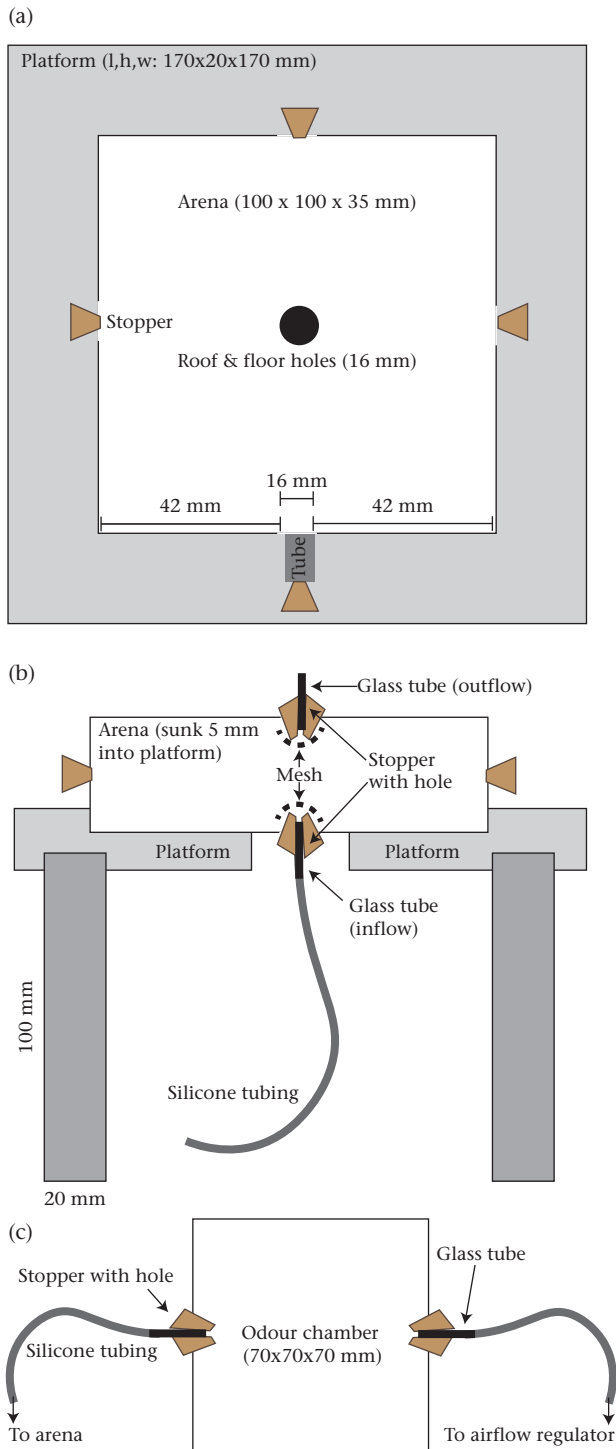


Figure 4. Live prey preattack behaviour arena. The arena, connected to the odour chamber, was embedded within the platform. (a) View from above. (b) Side view. (c) Odour chamber seen from above. Measurements as given as length × width × height.

Before a trial began, the test spider was in a holding chamber connected to a tube secured on the spider platform. There were two slots in the holding chamber with a removeable metal screen in each slot and before testing, the spider was confined to the space between the two screens for 1 min, after which the screen between the holding chamber and the tube was lifted away, giving the test spider access to the tube. The test spider was then allowed 2 min to move, on its own, into the tube. If the spider was still inside the

holding chamber after 2 min, the screen at the opposite side of the holding chamber was removed and a paintbrush was used to gently prod the spider until it moved into the tube. Once the test spider was in the tube, the screen was replaced on the side of the holding chamber closest to the tube.

Trials began when the test spider moved out of the holding chamber and into the tube and then out of the tube on to the spider platform, facing the display platform. The test spider had no access to the spider wall behind the spider wall (all gaps were plugged with cotton). A blind between the spider platform and display platform, which hid the lures from the test spider's view, was lifted as soon as the test spider moved on to the top of the spider platform, allowing a view of the lures. If the test spider did not adopt stationing within 10 min and did not continue stationing for the extra time required before the prod, testing was terminated. Thus, for the 'immediate' condition, the prod was delivered immediately after the spider had remained stationary facing the lures for 30 s, while for the 2.5 min condition, the prod was delivered 150 s after the test spider had initiated stationing (i.e. 3.0 min being stationary, including the 30 s time criterion for being stationary), and for the 5 min condition, the prod was delivered 300 s after the test spider had initiated stationing. Trials were terminated whenever these specifications were not met, and we continued testing until 25 successful tests for each treatment had been completed.

The outcome of interest was whether an attack was made after a prod. We were interested in whether the prey's identity mattered and whether the spider was affected by stationing time prior to the prod. We defined attacking as leaping from the spider platform and landing on one of the four sectors of the display platform, either one of the two lures or one of the two empty side sectors (i.e. 'attack lure' or 'attack empty'). Data were analysed using a generalized linear model (GLM) with a binomial distribution with prey choice (attack the mosquito or the midge) as the dependent variable and time (continuous) and prod type (categorical) as the predictor variables.

RESULTS

Experiment 1: Juvenile Spider *Anopheles-Culex* Discrimination Using Lures

There was a significant difference between the prey choices *E. culicivora* made in the presence versus the absence of *L. camara* flowers ($\chi^2 = 4.142$, $N = 34$, $P = 0.042$). In the absence of odour from flowers, spiders chose lures made from *Anopheles* mosquitoes (72%) somewhat more often than they chose lures made from *Culex* mosquitoes, although this was not statistically significant ($N = 18$, $P = 0.096$). In the presence of odour, *E. culicivora* did not appear to discriminate between lures of *Anopheles* (38%) and *Culex* ($N = 16$, $P = 0.454$).

There was no evidence of the presence of odour affecting the number of successful trials. The number of unsuccessful trials in the presence of odour (4/20 tests, 20%) did not differ significantly ($N = 41$, $P = 0.29$) from the number of unsuccessful trials in the absence of odour (3/21 tests, 14%).

Experiment 2: Juvenile Spider *Anopheles-Culex* Discrimination Using Virtual Prey

There were no significant differences between the choices made by spiders towards virtual mosquitoes in the presence of β -caryophyllene and *L. camara* odour ($N = 44$, $P = 1$). Choices with β -caryophyllene ($N = 58$, $P = 0.007$) and *L. camara* ($N = 44$, $P = 0.019$) were significantly different from choices in the no odour control. In the absence of odour, the number of spiders that chose the mosquito in the *Anopheles* posture ($N = 26$) was significantly higher ($P = 0.001$) than the number that

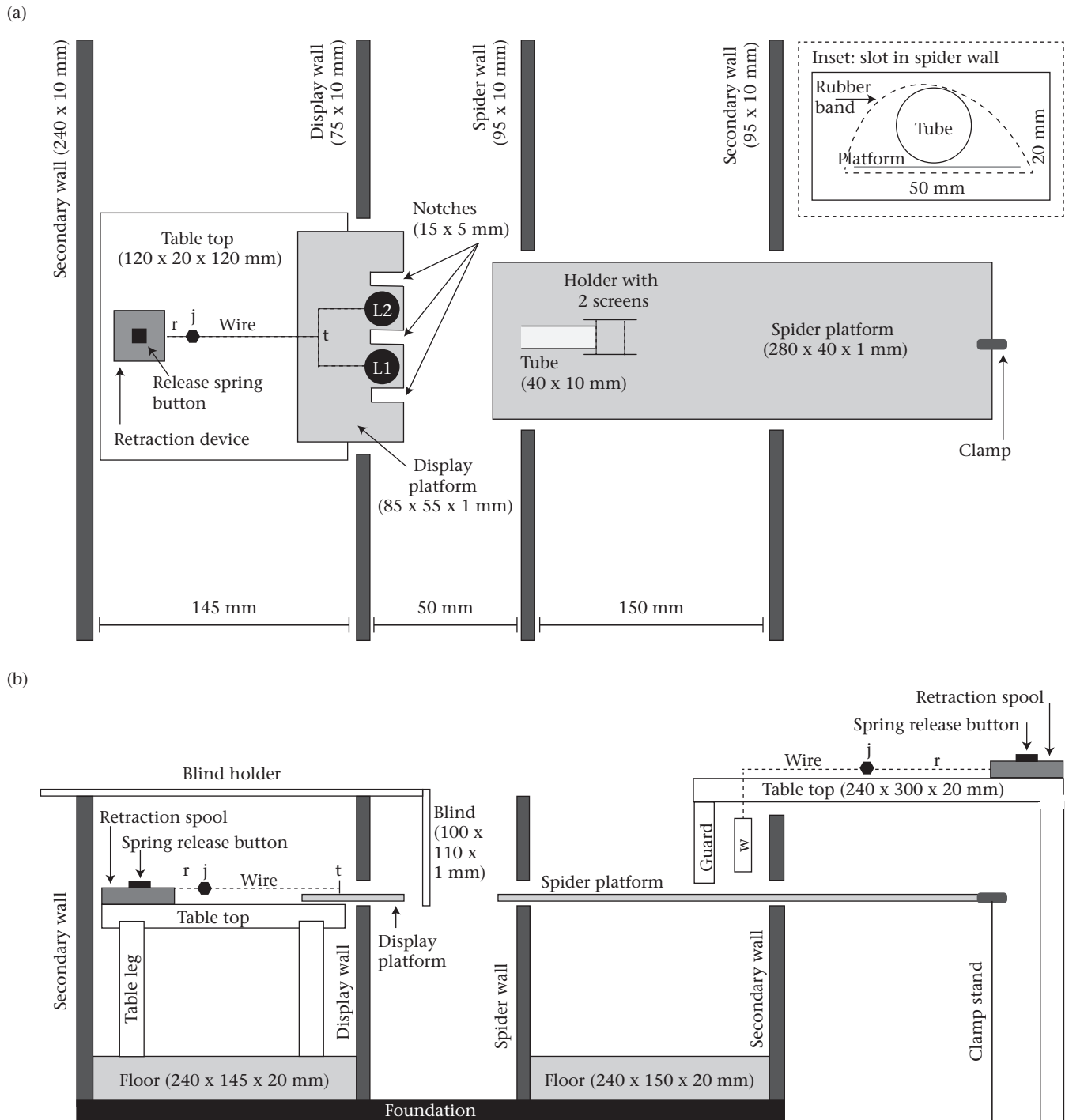


Figure 5. Platform arena for disturbance testing. (a) View from above. Upper level (blind/holder and weight table) is not shown. (b). Side view. Upper level shown. L1: lure 1; L2: lure 2; r: retractable ribbon (retracts into spring-loaded retraction spool); j: junction between retractable ribbon and wire; t: T-junction for lure fork; w: weight (in elevated position; attached to wire through hole drilled through weight table). Wires were aligned through 30 × 15 mm eye screws in the tables. The display wall has a slot (15 mm high, 40 mm wide) through which lures on the display platform are visible when the blind is removed. The slot in the spider wall is depicted in the upper right inset. Measurements as given as length × width × height. Not to scale.

chose the mosquito in the *Culex* posture ($N = 3$). However, there was no significant difference between the number of spiders that chose either kind of mosquito in the presence of β -caryophyllene (*Anopheles* posture: 16; *Culex* posture: 13; $P = 0.711$) or *L. camara* (*Anopheles* posture: 8; *Culex* posture: 7; $P = 1.00$). Additionally, there was no difference in the number of successful tests between treatments (no odour: 29/43 (67%); β -caryophyllene: 29/44 (66%); *L. camara*: 15/31 (48%); $\chi^2_2 = 3.259$, $P = 0.196$).

Experiment 3: Adult Spider *Anopheles* Discrimination Using Four Virtual Prey

Overall, there was a significant difference in prey choice between treatments ($\chi^2_6 = 13.20$, $N = 59$, $P = 0.04$). Based on an expected probability of 0.25, spiders chose virtual *Anopheles* significantly more often than they chose the virtual midges when tested without odour ($N = 12$ chose the mosquito versus $N = 8$

chose midges; $G = 9.501$, $N = 20$, $P = 0.002$), but there was no difference in the choice of prey type in the presence of the odour of *L. camara* ($G = 0.074$, $N = 18$, $P = 0.786$; $N = 4$ chose the mosquito versus $N = 14$ chose midges) or of β -caryophyllene ($G = 0.016$, $N = 21$, $P = 0.9$; $N = 5$ chose the mosquito versus $N = 16$ chose midges). Furthermore, choices did not differ significantly between the *L. camara* and β -caryophyllene treatments ($\chi^2_3 = 3.11$, $N = 39$, $P = 0.38$).

There was no evidence that the presence of odour affected the number of tests that were successful. In the absence of odour, 45% (20/44) of tests were successful, in the presence of odour from *L. camara* 55% (18/33) of tests were successful and in the presence of β -caryophyllene 57% (21/37) of tests were successful ($\chi^2_3 = 1.173$, $N = 114$, $P = 0.556$).

Experiment 4: Odour Effects on Spider Preattack Behaviour Using Live Prey

When comparing β -caryophyllene to no odour trials, our interest was to ascertain whether odour affected the inclination to attack or station in response to prey. Of trials in which spiders stationed or attacked prey, a significantly larger proportion of spiders ($\chi^2 = 15.241$, $P < 0.0001$) adopted stationing (instead of simply attacking without stationing) in the absence of the odour of β -caryophyllene: 59% of spiders stationed with no odour compared to 17% with β -caryophyllene odour. Additionally, marginally more spiders stationed or attacked in the absence of odour (47/65) than with the odour (34/65) of β -caryophyllene ($\chi^2 = 5.535$, $P = 0.019$).

Table 1
Number (and percentage) of attacks by *E. culicivora* ($N = 25$ /treatment) subjected to a preattack percussive (P) or visual (V) disturbance ('prod') at different time periods (i.e. after different periods of adopting stationing behaviour)

Prod type	Prod time (min)	Attack mosquito	Attack midge	Attack empty
V	0	9 (36)	7 (28)	9 (36)
V	2.5	18 (72)	5 (20)	2 (8)
V	5	22 (88)	2 (8)	1 (4)
P	0	13 (52)	8 (32)	4 (16)
P	2.5	12 (48)	3 (12)	10 (40)
P	5	19 (76)	2 (8)	4 (16)

Experiment 5: Disturbance Effects on Prey-Based Behaviour

The choice of prey became markedly more skewed towards mosquitos with longer stationing times, such that when the prod was delivered immediately, prey choice appeared random, with longer stationing time leading to an increasing tendency towards prey choices for mosquitoes (Table 1, Fig. 6). Corroborating this, the GLM showed an effect of stationing time on the spider prey choice behaviour, but neither prod type nor the interaction between time and prod type affected prey choice (Table 2).

DISCUSSION

As in earlier research (Jackson et al., 2005; Cross & Jackson, 2009a,b), here we have evidence of specific olfactory cues (odours) affecting *E. culicivora*'s proficiency at identifying and locating preferred prey, but with the important difference that the olfactory cues were derived from plants instead of from prey. Another difference is that here, instead of facilitating the identifying of preferred prey, the effect of the olfactory cues was to render *E. culicivora* less proficient at discriminating between prey of a more preferred and a less preferred type. This is what we found irrespective of whether the individuals of *E. culicivora* used in experiments were juveniles or adults, irrespective of whether we tested the spiders with lures made from dead prey or with virtual prey projected from a computer, and irrespective of whether the preferred prey type was paired with one or with three prey of another type (experiments 1–3).

Table 2
Results of a binomial GLM for prey choice depending on prod time (0, 2.5, 5 min) and prod type (percussive or visual)

Predictor	Estimate	CI	SE	Z	P
Intercept	0.55789	-0.276–1.456	0.43568	1.280	0.200
Prod type	-0.31873	-1.588–0.937	0.63887	-0.499	0.618
Prod time	0.38150	0.057–0.770	0.17752	2.149	0.032
Interaction (type*time)	0.04508	-0.454–0.532	0.24756	0.182	0.856

Significant difference is indicated in bold. Confidence interval (CI) at 95%; $df = 1$.

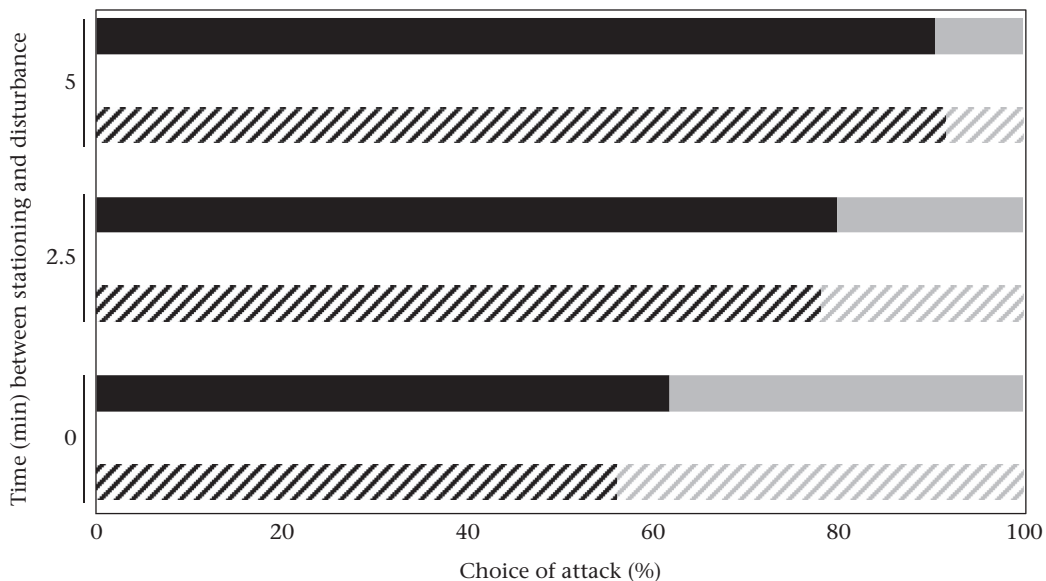


Figure 6. Stacked histogram depicting percentage of attacks by *E. culicivora* ($N = 25$ per bar) on mosquitoes (black), midges (dark grey) when subjected to a preattack visual (thatched bars) or percussive (solid bars) disturbance ('prod') after three different periods of adopting stationing behaviour.

While salticids use information from multiple sensory modalities, given the restrictions imposed by having a small number of neurons with which to process information, we might expect some cognitive limitations; when engaged in demanding tasks, even vertebrates, with their considerably larger brains, may be limited to selectively attending to only a single item at a time. (Franconeri, Alvarez, & Cavanagh, 2013). Research on humans and other mammals has shown that input from one sensory modality can affect information processing in another (see Calvert, Spence, & Stein, 2004), and it seems likely that this would be especially true in animals with tiny nervous systems, including salticids. Indeed, experiments 1–3 suggested that simultaneous processing of conflicting visual and olfactory information may distract the spider, impair its ability to accurately identify prey or alter prey selectivity, possibly due to conflicting priorities, as adults use plants, including *L. camara*, as mating sites (Cross et al., 2008), and juveniles use these plants as a source of nectar (Carvell, Kuja, & Jackson, 2015; Kuja et al., 2012).

As there was no evidence of these olfactory cues simply suppressing *E. culicivora*'s motivation to attack prey (i.e. how many spiders attacked a prey in the presence of the odour was not significantly different from how many attacked a prey in the absence of the odour), we might conclude that a plant-derived odour specifically impairs *E. culicivora*'s capacity to identify its preferred prey. However, being impaired in this way does not necessarily imply that *E. culicivora*'s behaviour is maladaptive when in the presence of this odour and we might get a different perspective when we consider the time required for accurate discrimination between two prey types. In experiments 4 and 5, we specifically tested prey discrimination proficiency, finding that the time required to make a decision was important for accurate assessment, and that this was impaired by disturbances, including visual, percussive and odour (β -caryophyllene). As we found that the longer the spider had been stationed in front of the prey before being disturbed, the stronger the choice for its preferred prey type, stationing may be a key aspect of *E. culicivora*'s prey assessment, and thus of making the correct decision about the type of prey to attack. These experiments can be envisaged as testing for a relationship, or indeed a trade-off, between choosing accuracy and speed in an animal with a small nervous system with which to process information (e.g. Nityananda et al., 2014). Indeed, stationing duration in salticids correlates with decision making and forward planning (Aguilar-Argüello et al., 2020; Cross & Jackson, 2016; Tarsitano & Andrew, 1999).

We assume that time spent stationing corresponds to time spent in visual inspection but, strictly speaking, our data for stationing duration do not simply reveal how long *E. culicivora* was engaged in visually inspecting the prey, which requires details pertaining to retinal movement. However, it is at least a likely hypothesis that there is a positive correlation between the duration of stationing and the duration of visual inspection, with more time for visual inspection allowing *E. culicivora* to get a detailed assessment of the identity of the prey. This hypothesis is especially suggested by the results from experiment 5, in which stationed spiders 'prodded' by disturbances at different intervals showed an increase in prey choice proficiency with increasing time spent stationing. Here, prodding was a mechanism used to explore speed–accuracy trade-offs, by 'forcing' the spider to immediately 'choose' upon prodding or face missing out on a meal. Experiments 4 and 5 suggest that, when faced with the task of integrating sensory cues from both the environment and the prey, the spider's prey identification behaviour is affected. Seeing prey while in the presence of β -caryophyllene odour may challenge *E. culicivora* with a problem of how to adjust to conflicting priorities, with the odour providing the conflict of prioritizing finding mates (*E. culicivora* adults) or flowers

(*E. culicivora* juveniles) against prioritizing prey identification and capture. *Evarcha culicivora*'s response to this challenge may be to modulate reliance on stationing. We suggest that the apparently negative effect of β -caryophyllene on *E. culicivora*'s performance of a prey identification task is a consequence of this odour rendering *E. culicivora* less motivated to engage in a task of visually inspecting the prey long enough to accurately identify it. Thus, the time and effort needed to ensure that the preferred prey category is chosen seems to become a lower priority when in the presence of β -caryophyllene, possibly due to *E. culicivora*'s priorities having shifted away from ensuring that preferred prey is chosen and instead towards ensuring that any mates (for adults) or flowers (for juveniles) present in the vicinity will be detected. Consequently, when in the presence of β -caryophyllene or *L. camara*, *E. culicivora* seems to be willing to engage in prey capture if prey comes into view, but without being willing to make the extra effort that would be needed for determining which prey individual belongs to the preferred type.

Effects in one sensory modality can trigger effects, such as priming, in other sensory modalities that can outlast the duration of the stimulus (Anton, Evengard, Barrozo, Anderson, & Skals, 2011). We have only recently begun investigating how background environmental stimuli affect crucial behaviours, for example through facilitation or masking of relevant stimuli (Schröder & Hilker, 2008), particularly in different sensory modalities (e.g. Skals, Anderson, Kannevorf, Löfstedt, & Surlykke, 2005). For example, sticklebacks, *Gasterosteus aculeatus*, increase handling errors and correct categorization of food versus nonfood items in acoustically noisy environments. In addition, male *Spodoptera littoralis* moths appear functionally 'deaf' to the sounds of bat cries when in the presence of strong pheromonal cues from females (Skals et al., 2005); yet, when pheromones and plant odours are simultaneously presented, the background plant odour acts as a distractor, masking the pheromone signal, and usurping some of the insect's finite attention (Party, Hanot, Büsser, Rochat, & Renou, 2013). The distractor effects on *S. littoralis*, similarly to what is suggested by our results, were chemical specific (Party et al., 2013).

Previous work has demonstrated that *E. culicivora* is attracted to the odour of *L. camara* and to β -caryophyllene (Nelson, Pratt, et al., 2012; Nelson & Jackson, 2013). Thus, when on *L. camara*, *E. culicivora* may suffer a cost in terms of accurately locating its preferred prey, because feeding on blood-fed *Anopheles* makes adults more attractive to opposite-sex conspecifics (Cross, Jackson, & Pollard, 2009) and *Anopheles* are easier for juveniles to prey on than other mosquito genera (Nelson, Jackson, & Sune, 2005). This raises the question of why this spider is attracted to a plant that impairs its prey choice behaviour. However, the potential costs for *E. culicivora* of failing to select preferred prey may be offset because β -caryophyllene may act as a proxy to locate nutritional (nectar) and mating opportunities (Carvell et al., 2015, 2017; Cross et al., 2008; Kuja et al., 2012).

The association between *E. culicivora* and *L. camara* is unusual, as other salticids found in the same habitat are not attracted to its odour (Nelson & Jackson, 2011b). That phytochemicals released by *L. camara* modulate the behaviour of *E. culicivora* is not due to specific coevolution with *L. camara*, which is not native to East Africa (Schemske, 1983). However, many plants release β -caryophyllene, and it is reasonable to assume some of these are native to *E. culicivora*'s habitat. The effect of β -caryophyllene on *E. culicivora*'s behaviour appears to be specific, although this spider is also attracted to another sesquiterpene volatile from *L. camara*, α -humulene, which may have similar (untested) effects on *E. culicivora*'s behaviour (Carvell et al., 2017; Nelson, Pratt, et al., 2012; Nelson & Jackson, 2013). However, in preliminary work, we

found no effect of the monoterpenes α -pinene or β -pinene (both compounds found in *L. camara*) on *E. culicivora*'s prey choice behaviour (i.e. choices were identical to those in the absence of odour and spiders showed a significant preference for blood-fed virtual mosquitoes in the *Anopheles* posture compared with the *Culex* posture); nor is this spider attracted to these particular odours (Nelson, Pratt, et al., 2012; Nelson & Jackson, 2013). Although β -caryophyllene is a cannabinoid, and thus may have negative effects on spider behaviour, that we found the same impairment in prey choice decision making when the spiders were disturbed by a sudden mechanical or visual stimulus suggests that this is unlikely, and, as mentioned, there are positive ramifications for *E. culicivora* to respond to β -caryophyllene (Carvell et al., 2015, 2017; Cross et al., 2008; Kuja et al., 2012).

Our work differs from other studies in that it provides an unusual example in which experiments did not involve an obvious 'trade-off' between functionally important categories (i.e. reproduction, foraging or predation) pitched against one another. Additionally, we found no evidence suggesting that, in the presence of simultaneous sensory information, the spiders only attended to one stimulus while ignoring the other stimulus (stimulus-selective attention, Dukas, 2002). Instead, adopting an expression used by Nityananda et al. (2014), *E. culicivora*'s behaviour in the presence of certain odours suggests that it cannot 'see at a glance' exactly what kind of prey is in view.

Acknowledgments

We thank Godfrey Sune for his assistance at ICIPE, David Conder for his assistance with plant rearing, and Bruce Clark, Russell Gillard and especially Aynsley MacNab and Samuel Aguilar-Argüello for technical assistance. This research was funded by the Royal Society of New Zealand (Marsden Fund (M1096, M1149) and James Cook Fellowship (E5097)), the National Geographic Society (WW-146R-17) and the US National Institutes of Health (R01-AI077722).

References

- Aguilar-Argüello, S., Gerhard, D., & Nelson, X. J. (2020). Risk assessment and the use of novel shortcuts in spatial detouring tasks in jumping spiders. *Behavioral Ecology*, 30, 1488–1498. <https://doi.org/10.1093/beheco/az105>
- Anton, S., Evenggaard, K., Barrozo, R. B., Anderson, P., & Skals, N. (2011). Brief predator sound exposure elicits behavioral and neuronal long-term sensitization in the olfactory system of an insect. *Proceedings of the National Academy of Sciences U.S.A.*, 108, 3401–3405. <https://doi.org/10.1073/pnas.1008840108>
- Blest, A. D., O'Carroll, D. C., & Carter, M. (1990). Comparative ultrastructure of layer I receptor mosaics in principal eyes of jumping spiders: The evolution of regular arrays of light guides. *Cell and Tissue Research*, 262, 445–460. <https://doi.org/10.1007/BF00305241>
- Blumstein, D. T., & Bouskila, A. (1996). Assessment and decision making in animals: A mechanistic model underlying behavioral flexibility can prevent ambiguity. *Oikos*, 77, 569–576. <https://doi.org/10.2307/3545948>
- Calvert, G. A., Spence, C., & Stein, B. E. (2004). *The Handbook of Multisensory Processes*. Cambridge, MA: MIT Press.
- Caputo, B., Dani, F. R., Horne, G. L., Petrarca, V., Turillazzi, S., Coluzzi, M., et al. (2005). Identification and composition of cuticular hydrocarbons of the major Afro-tropical malaria vector *Anopheles gambiae* ss (Diptera: Culicidae): Analysis of sexual dimorphism and age-related changes. *Journal of Mass Spectrometry*, 40(12), 1595–1604.
- Carvell, G. E., Jackson, R. R., & Cross, F. R. (2017). Ontogenetic shift in plant-related cognitive specialization by a mosquito-eating predator. *Behavioural Processes*, 138, 105–122.
- Carvell, G. E., Kuja, J. O., & Jackson, R. R. (2015). Rapid nectar-meal effects on a predator's capacity to kill mosquitoes. *Royal Society Open Science*, 2, 140426. <https://doi.org/10.1098/rsos.140426>
- Clements, A. N. (1999). *The biology of mosquitoes*. Wallingford, U.K.: CABI Publishing.
- Cross, F. R., & Jackson, R. R. (2009a). Cross-modality priming of visual and olfactory selective attention by a spider that feeds indirectly on vertebrate blood. *Journal of Experimental Biology*, 212, 1869. <https://doi.org/10.1242/jeb.028126>
- Cross, F. R., & Jackson, R. R. (2009b). Odour-mediated response to plants by *Evarcha culicivora*, a blood-feeding jumping spider from East Africa. *New Zealand Journal of Zoology*, 36, 75–80.
- Cross, F. R., & Jackson, R. R. (2016). The execution of planned detours by spider-eating predators. *Journal of the Experimental Analysis of Behavior*, 105, 194–210. <https://doi.org/10.1002/jeab.189>
- Cross, F. R., Jackson, R. R., & Pollard, S. D. (2008). Complex display behaviour of *Evarcha culicivora*, an East African mosquito-eating jumping spider. *New Zealand Journal of Zoology*, 35, 151–187.
- Cross, F. R., Jackson, R. R., & Pollard, S. D. (2009). How blood-derived odor influences mate-choice decisions by a mosquito-eating predator. *Proceedings of the National Academy of Sciences U.S.A.*, 106, 19416–19419.
- Dall, S. R. X., Giraldeau, L.-A., Olsson, O., McNamara, J. M., & Stephens, D. W. (2005). Information and its use by animals in evolutionary ecology. *Trends in Ecology & Evolution*, 20, 187–193. <https://doi.org/10.1016/j.tree.2005.01.010>
- De Agrò, M., Oberhauser, F. B., Loconsole, M., Galli, G., Dal Cin, F., Moretto, E., et al. (2020). Multi-modal cue integration in the black garden ant. *Animal Cognition*, 23, 1119–1127. <https://doi.org/10.1007/s10071-020-01360-9>
- Deng, C., Cross, F. R., & Jackson, R. R. (2017). Adaptive timing as a component of a mosquito-eating predator's specialization profile. *Journal of Insect Behavior*, 30, 695–716.
- Dolev, Y., & Nelson, X. J. (2014). Innate pattern recognition and categorization in a jumping spider. *PLoS One*, 9, Article e97819. <https://doi.org/10.1371/journal.pone.0097819>
- Dukas, R. (2002). Behavioural and ecological consequences of limited attention. *Philosophical Transactions of the Royal Society B*, 357, 1539–1547. <https://doi.org/10.1098/rstb.2002.1063>
- Echeverri, S. A., Morehouse, N. I., & Zurek, D. B. (2017). Control of signaling alignment during the dynamic courtship display of a jumping spider. *Behavioral Ecology*, 28, 1445–1453. <https://doi.org/10.1093/beheco/ax107>
- Elias, D. O., Hebets, E. A., Hoy, R. R., Maddison, W. P., & Mason, A. C. (2006). Regional seismic song differences in sky island populations of the jumping spider *Habronattus pugillis* Griswold (Araneae, Salticidae). *Journal of Arachnology*, 34, 545–556.
- Forster, L. M. (1982). Vision and prey catching strategies in jumping spiders. *American Scientist*, 70, 165–175.
- Forster, L. M. (1985). Target discrimination in jumping spiders (Araneae: Salticidae). In F. G. Barth (Ed.), *Neurobiology of arachnids* (pp. 249–274). Berlin, Germany: Springer-Verlag.
- Franconeri, S. L., Alvarez, G. A., & Cavanagh, P. (2013). Flexible cognitive resources: Competitive content maps for attention and memory. *Trends in Cognitive Sciences*, 17(3), 134–141. <https://doi.org/10.1016/j.tics.2013.01.010>
- Girard, M. B., Kasumovic, M. M., & Elias, D. O. (2011). Multi-modal courtship in the peacock spider, *Maratus volans* (O.P.-Cambridge, 1874). *PLoS One*, 6, Article e25390. <https://doi.org/10.1371/journal.pone.0025390>
- Harland, D. P., Li, D., & Jackson, R. R. (2012). How jumping spiders see the world. In O. F. Lazareva, T. Shimizu, & E. A. Wasserman (Eds.), *How animals see the world: Comparative behavior, biology, and evolution of vision* (pp. 133–163). New York, NY: Oxford University Press.
- Hebets, E. A., Aceves-Aparicio, A., Aguilar-Argüello, S., Bingman, V. P., Escalante, I., Gering, E. J., et al. (2014). Multimodal sensory reliance in the nocturnal homing of the amblypygid *Phrynus pseudoparvulus* (Class Arachnida, Order Amblypygi)? *Behavioural Processes*, 108, 123–130. <https://doi.org/10.1016/j.beproc.2014.09.014>
- Hebets, E. A., & Uetz, G. W. (2000). Leg ornamentation and the efficacy of courtship display in four species of wolf spider (Araneae: Lycosidae). *Behavioral Ecology and Sociobiology*, 47, 280–286. <https://doi.org/10.1007/s002650050667>
- Impoinvil, D. E., Kongere, J. O., Foster, W. A., Njiru, B. N., Killeen, G. F., Githure, J. I., et al. (2004). Feeding and survival of the malaria vector *Anopheles gambiae* on plants growing in Kenya. *Medical and Veterinary Entomology*, 18, 1–8.
- Jackson, R. R., & Cross, F. R. (2011). Spider cognition. *Advances in Insect Physiology*, 41, 115–174.
- Jackson, R. R., Deng, C., & Cross, F. R. (2016). Convergence between a mosquito-eating predator's natural diet and its prey-choice behaviour. *Royal Society Open Science*, 3, 160584.
- Jackson, R. R., Nelson, X. J., & Sune, G. O. (2005). A spider that feeds indirectly on vertebrate blood by choosing female mosquitoes as prey. *Proceedings of the National Academy of Sciences U.S.A.*, 102, 15155–15160.
- Jakob, E. M., Skow, C. D., & Long, S. M. (2011). Plasticity, learning and cognition. In M. E. Herberstein (Ed.), *Spider behaviour: Flexibility and versatility* (pp. 307–347). New York, NY: Cambridge University Press.
- Kuja, J. O., Jackson, R. R., Sune, G. O., Karanja, R. N. H., Lagat, Z. O., & Carvell, G. E. (2012). Nectar meals of a mosquito-specialist spider. *Psyche*, 2012, Article 898721. <https://doi.org/10.1155/2012/898721>
- Land, M. F. (1969a). Structure of the retinæ of the principal eyes of jumping spiders (Salticidae: Dendryphantinae) in relation to visual optics. *Journal of Experimental Biology*, 51, 443–470.
- Land, M. F. (1969b). Movements of the retinæ of jumping spiders (Salticidae: Dendryphantinae) in response to visual stimuli. *Journal of Experimental Biology*, 51, 471–493.
- Land, M. F. (1981). Optics and vision in invertebrates. In H. Autrum (Ed.), *Comparative physiology and evolution of vision in invertebrates* (Vol. VII, pp. 471–592). Berlin, Germany: Springer.
- Land, M. F. (1985). The morphology and optics of spider eyes. In F. G. Barth (Ed.), *Neurobiology of arachnids* (pp. 53–78). Berlin, Germany: Springer.
- Manda, H., Gouagna, L. C., Nyandat, E., Kabiru, E. W., Jackson, R. R., Foster, W. A., et al. (2007). Discriminative feeding behaviour of *Anopheles gambiae* s.s. on

- endemic plants in western Kenya. *Medical and Veterinary Entomology*, *21*, 103–111.
- Martin, C. W., Fodrie, F. J., Heck, K. L., & Mattila, J. (2010). Differential habitat use and antipredator response of juvenile roach (*Rutilus rutilus*) to olfactory and visual cues from multiple predators. *Oecologia*, *162*, 893–902. <https://doi.org/10.1007/s00442-010-1564-x>
- Nelson, X. J., & Jackson, R. R. (2006). A predator from East Africa that chooses malaria vectors as preferred prey. *PLoS One*, *1*, e132. <https://doi.org/10.1371/journal.pone.0000132>
- Nelson, X. J., & Jackson, R. R. (2011a). Flexibility in the foraging strategies of spiders. In M. E. Herberstein (Ed.), *Spider behaviour: Flexibility and versatility* (pp. 31–56). New York, NY: Cambridge University Press.
- Nelson, X. J., & Jackson, R. R. (2011b). Evidence that olfaction-based affinity for particular plant species is a special characteristic of *Evarcha culicivora*, a mosquito-specialist jumping spider. *Journal of Arachnology*, *39*, 378–383.
- Nelson, X. J., & Jackson, R. R. (2012). The discerning predator: Decision rules underlying prey classification by a mosquito-eating jumping spider. *Journal of Experimental Biology*, *215*, 2255–2261. <https://doi.org/10.1242/jeb.069609>
- Nelson, X. J., & Jackson, R. R. (2013). Hunger-driven response by a nectar-eating jumping spider to specific phytochemicals. *Chemoecology*, *23*(3), 149–153. <https://doi.org/10.1007/s00049-013-0130-5>
- Nelson, X. J., Jackson, R. R., & Sune, G. (2005). Use of *Anopheles* specific prey-capture behavior by the small juveniles of *Evarcha culicivora*, a mosquito-eating jumping spider. *Journal of Arachnology*, *33*, 541–548. <https://doi.org/10.1636/05-3.1>
- Nelson, X. J., Pratt, A. J., Cheseto, X., Torto, B., & Jackson, R. R. (2012). Mediation of a plant-spider association by specific volatile compounds. *Journal of Chemical Ecology*, *38*, 1081–1092.
- Nelson, X. J., Warui, C. M., & Jackson, R. R. (2012). Widespread reliance on olfactory sex and species identification by lyssomanine and spartaeine jumping spiders. *Biological Journal of the Linnean Society*, *107*, 664–677.
- Nityananda, V., Skorupski, P., & Chittka, L. (2014). Can bees see at a glance? *Journal of Experimental Biology*, *217*, 1933–1939. <https://doi.org/10.1242/jeb.101394>
- de Omena, P. M., & Romero, G. Q. (2010). Using visual cues of microhabitat traits to find home: The case study of a bromeliad-living jumping spider (Salticidae). *Behavioral Ecology*, *21*, 690–695. <https://doi.org/10.1093/beheco/arq040>
- Partan, S., & Marler, P. (1999). Communication goes multimodal. *Science*, *283*, 1272–1273.
- Partan, S., & Marler, P. (2005). Issues in the classification of multimodal communication signals. *American Naturalist*, *166*, 231–245.
- Party, V., Hanot, C., Büsser, D. S., Rochat, D., & Renou, M. (2013). Changes in odor background affect the locomotory response to pheromone in moths. *PLoS One*, *8*, Article e52897. <https://doi.org/10.1371/journal.pone.0052897>
- Schemske, D. W. (1983). *Lantana camara*. In D. H. Janzen (Ed.), *Costa Rican natural history* (pp. 266–268). Chicago, IL: Chicago University Press.
- Schröder, R., & Hilker, M. (2008). The relevance of background odor in resource location by insects: A behavioral approach. *BioScience*, *58*(4), 308–316. <https://doi.org/10.1641/b580406>
- Shettleworth, S. J. (2010). *Cognition, evolution and behavior* (2nd ed.). New York, NY: Oxford University Press.
- Skals, N., Anderson, P., Kannevorff, M., Löfstedt, C., & Surlykke, A. (2005). Her odours make him deaf: Crossmodal modulation of olfaction and hearing in a male moth. *Journal of Experimental Biology*, *208*(4), 595–601. <https://doi.org/10.1242/jeb.01400>
- Tarsitano, M. S., & Andrew, R. (1999). Scanning and route selection in the jumping spider *Portia labiata*. *Animal Behaviour*, *58*, 255–265.
- VanderSal, N. D., & Hebets, E. A. (2007). Cross-modal effects on learning: A seismic stimulus improves color discrimination learning in a jumping spider. *Journal of Experimental Biology*, *210*, 3689–3695. <https://doi.org/10.1242/jeb.009126>
- Ward, A. J. W., & Mehner, T. (2010). Multimodal mixed messages: The use of multiple cues allows greater accuracy in social recognition and predator detection decisions in the mosquitofish, *Gambusia holbrooki*. *Behavioral Ecology*, *21*, 1315–1320. <https://doi.org/10.1093/beheco/arq152>
- Williams, D. S., & McIntyre, P. (1980). The principal eyes of a jumping spider have a telephoto component. *Nature*, *288*, 578–580. <https://doi.org/10.1038/288578a0>
- World Spider Catalog. (2020). *Version 21.5 Natural History Museum Bern*. <https://doi.org/10.24436/2>. online at <http://wsc.nmbe.ch>. (Accessed 10 November 2020).
- Zurek, D. B., Cronin, T. W., Taylor, L. A., Byrne, K., Sullivan, M. L. G., & Morehouse, N. I. (2015). Spectral filtering enables trichromatic vision in colorful jumping spiders. *Current Biology*, *25*, RR403–RR404. <https://doi.org/10.1016/j.cub.2015.03.033>