

Cross-modality priming of visual and olfactory selective attention by a spider that feeds indirectly on vertebrate blood

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SUMMARY

Evarcha culicivora, a jumping spider from East Africa, specialises in feeding indirectly on vertebrate blood by choosing blood-carrying mosquitoes as preferred prey. Previous studies have shown that this predator can identify its preferred prey by sight alone and also by odour alone. Here we investigate how vision and olfaction work together. Our findings show that, for *E. culicivora*, cross-modality priming in the context of preying on blood-carrying mosquitoes works in two directions. However, we found no evidence of priming in the context of predation on less preferred prey (midges). When the spider's task was, by sight alone, to find a cryptic lure, it found mosquitoes significantly more often when the odour of mosquitoes was present than when this odour was not present. When the spider's task was to find masked odour, it found mosquitoes significantly more often after previously seeing mosquitoes than when it had not previously seen mosquitoes. When the spider's task was to find conspicuous lures or unmasked odour, the identity of the priming stimulus appeared to be irrelevant. Results were similar regardless of the spider's previous experience with prey and suggest that *E. culicivora* has an innate inclination to adopt vision-based search images specifically for mosquitoes when primed by mosquito odour and to adopt olfaction-based search images specifically when primed by seeing mosquitoes.

Key words: Salticidae, cognition, olfaction, predation, search images, vision.

INTRODUCTION

Research on attention, like most cognition research, has mainly been human based (e.g. Pashler, 1998) but, independent of this tradition in psychology, biologists who study the behaviour of non-human animals have also laboured over the topic of attention, but largely by another name, 'search images'. This is a term that can be traced back to von Uexküll (von Uexküll, 1934) (see Bond, 2007) but is now most often associated with Tinbergen and the hypothesis he used for explaining his field-based data on insectivorous birds (Tinbergen, 1960).

Tinbergen envisaged search images as perceptual changes, the idea being that the predator, after discovering a particular type of prey, 'gets an eye for' or 'learns to see' this particular type of prey (Tinbergen, 1960). In other words, having previous experience with a particular type of prey might prime a predator to become selectively attentive to specific features of this particular prey. This is the context in which the term 'search images' has been used in the more critical research following on from Tinbergen's classic paper (see Blough, 1991; Bond and Kamil, 2002; Dawkins, 1971; Langley, 1996).

However, Tinbergen's search-image hypothesis has also been the source of considerable confusion (see Guilford and Dawkins, 1987), as researchers sometimes blur the distinction between selective attention and preference. Intuitively, a dietary 'preference' refers to what an animal would like to eat (i.e. something that is expressed by choice behaviour). Search images, however, are shifts in selective attention (Cross and Jackson, 2006; Shettleworth, 1998). A crucial criterion for making this distinction is to compare experimental outcomes from trials in which prey is difficult to detect ('cryptic') with experimental outcomes from trials in which prey is easily

detected ('conspicuous'). We expect selective attention to matter especially when prey is cryptic. When prey is conspicuous, we predict that the influence of selective attention will not be so emphatic and that the animal's preferences will instead be most evident.

Jumping spiders (Salticidae) are particularly suitable subjects for research concerned with vision-based prey identification because they have unique, complex eyes and vision based on a level of spatial acuity that is unrivalled by other animals in their size range (Harland and Jackson, 2004; Land, 1969). Salticids can be tested with immobile lures instead of living prey (Jackson and Tarsitano, 1993), which means we can ascertain whether these predators have found potential prey in the absence of movement cues and without the actions of the prey individual confounding interpretation of experimental outcome. However, besides having exceptional eyesight, many salticids are known to make considerable use of chemical cues (Jackson and Pollard, 1996; Jackson and Pollard, 1997), which suggests that salticids may also be especially suitable subjects for research on cross-modality priming (i.e. research on the mechanisms by which information from one sensory modality causes attentional changes in another modality) (see Calvert et al., 2004; Spence and Driver, 2004).

Here we consider the role of selective attention in the predatory strategy of *Evarcha culicivora* Wesolowska and Jackson, a salticid from the Lake Victoria region of East Africa. This salticid is unusual because it specialises in feeding on vertebrate blood, gaining access to blood indirectly by choosing as preferred prey blood-carrying mosquitoes (Jackson et al., 2005). For *E. culicivora*, satisfying a highly precise predatory preference may be particularly challenging. Mosquitoes, although plentiful in its habitat, are vastly outnumbered

by other mosquito-sized dipterans, with non-biting midges, known locally as ‘lake flies’, from the families Chaoboridae and Chironomidae (Okedi, 1992) being especially common. Although *E. culicivora* eats lake flies as well as mosquitoes, the majority of its prey in nature is blood-carrying mosquitoes (Wesolowska and Jackson, 2003).

Knowing that *E. culicivora* can identify its unusual prey by sight alone and by odour alone (Jackson et al., 2005), our objective was to consider how vision and olfaction work together. Our hypothesis was that *E. culicivora* relies strongly on cross-modality priming of selective attention, with a stimulus in one sensory modality (vision or olfaction) triggering an innate search image in another modality (olfaction or vision). This departs from the tradition in the search-image literature of emphasising same-modality priming (i.e. instances of a stimulus in one sensory modality triggering selective attention in the same modality), where the sensory modality considered is usually vision. Another tradition in the search-image literature has been to base experiments on repeatedly exposing a predator to a particular type of prey, with an underlying hypothesis being that search images are acquired by perceptual learning. However, our hypothesis was that *E. culicivora* uses a system based on innate triggering of selective attention (i.e. we predict that, for the predator, prior experience with the priming cue is unnecessary). As another departure from tradition, our hypothesis was that, for *E. culicivora*, cross-modality priming works in two directions (i.e. we proposed that odour primes selective visual attention, and vision primes selective olfactory attention). We also proposed that *E. culicivora* is predisposed to cross-modality priming effects in the specific context of encounters with its preferred prey (i.e. blood-carrying mosquitoes).

MATERIALS AND METHODS

General

Our field site and laboratory were at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology (Mbita Point) in western Kenya. Standard spider-laboratory procedures were adopted (Cross et al., 2008; Jackson and Hallas, 1986) and all trials were carried out between 08:00 h and 13:00 h (laboratory photoperiod 12 h:12 h, L:D, lights on at 07:00 h).

We adopted some shorter terms for lures, odour and prey. ‘Mosquitoes’ were always blood-carrying females of *Anopheles gambiae* ss (Culicidae). ‘Lake flies’ were always *Nilodorum brevibucca* (Chironomidae). All spiders were fed to satiation three times a week on one of three diet regimes: mosquito diet, lake-fly diet or mixed diet (i.e. a diet of lake flies and mosquitoes). The spiders were always adult females of *E. culicivora* (virgin, matured 2–3 weeks before used in trials) and no individual spider was used more than once. We decided to use females instead of males because female salticids may generally be, compared with males, more strongly motivated to feed (Givens, 1978; Jackson and Pollard, 1997). As in an earlier study (Jackson et al., 2005), a short pre-trial fast (7 days) was adopted, the rationale for this being to ensure that the test spiders would be motivated to feed during the trials and to standardise the hunger level of test spiders. The mosquitoes used for feeding *E. culicivora*, for making lures and for odour sources in experiments had been given human blood 4–5 h before being used. Lake flies were collected from the field immediately before use.

Insects used for making lures were first immobilised with CO₂ and then placed in 80% ethanol. The next day, each insect was mounted in a life-like posture on the centre of a disc-shaped piece of cork. For preservation, the lure and the cork were then sprayed with a transparent plastic adhesive.

Rationale

In previous research (Jackson et al., 2005), when a wide range of prey types were used in prey-choice experiments, *E. culicivora* consistently chose blood-carrying mosquitoes more often than other prey, and there was no suggestion of variation in how *E. culicivora* responded to the other prey. On this basis, we decided to standardise our priming experiments by using only mosquitoes and lake flies as prey.

There were two experimental designs (Fig. 1), one where *E. culicivora* was presented with the task of finding prey (a lure) by sight while being primed with prey odour (Experiment 1) and one where *E. culicivora* was presented with the task of finding prey by olfaction after having been primed by seeing prey (Experiment 2). The rationale for having two different experimental designs was to determine whether, for *E. culicivora*, cross-modality priming goes in both directions. Features common to both experiments will be described first, followed by details specific to each of the two experiments.

To distinguish between effects of selective attention and effects of preference, there were two trial types, ‘cryptic’ and ‘conspicuous’, in each experiment. In the cryptic trials of Experiment 1, *E. culicivora* was presented with the task of finding a lure (Fig. 1A) that was behind nylon netting and accompanied by ‘distractors’ (i.e. cork discs on which no lure was mounted). In the cryptic trials of Experiment 2, *E. culicivora* was presented with the task of finding prey odour that was accompanied by a masking odour [i.e. there was a potentially distracting odour in the ‘cryptic’ (‘masked’) trials (Fig. 1B)]. For the masking odour, we used *Lantana camara*, a highly aromatic plant that is common in *E. culicivora*’s habitat. *E. culicivora* associates with this plant species (Cross et al., 2008) and is attracted to its odour (Cross and Jackson, 2009). The masking-odour source was put in chambers (‘masking chambers’) positioned in front of a control chamber (empty) and in front of a stimulus chamber that contained prey. We also included an extra chamber (‘transition chamber’) through which *E. culicivora* had to pass before getting close to an experimental odour source. The rationale for using the transition chamber was to make the task of finding the masked prey more difficult for *E. culicivora*.

For both experiments, we also had other trials (conspicuous and unmasked) which were like the cryptic and masked trials except for the absence of the features intended to make prey difficult to find [i.e. in Experiment 1 (Fig. 1A), there was no netting and no distractors and, in Experiment 2 (Fig. 1C), there was no masking odour and no transition chamber].

Experimental setup

In both experiments, there was a ‘stimulus chamber’. The stimulus chamber contained prey (either 10 mosquitoes or 10 lake flies) or, in Experiment 1, it was sometimes empty (‘control’). In each trial in Experiment 2, there was always a stimulus chamber (contained prey) and a ‘control chamber’ (empty) and a ‘priming chamber’ (i.e. a chamber used for allowing *E. culicivora* to see a particular prey type before being given an opportunity to locate prey odour). In masked trials of Experiment 2, there were also two masking chambers and a transition chamber.

Each chamber had two holes opposite each other. In both experiments, air moved into and out of stimulus, control and masking chambers through glass tubes (diameter 4 mm) inserted into rubber stoppers that plugged the holes. Airflow between components of the apparatus was bridged by silicone tubes that were connected to the glass tubes.

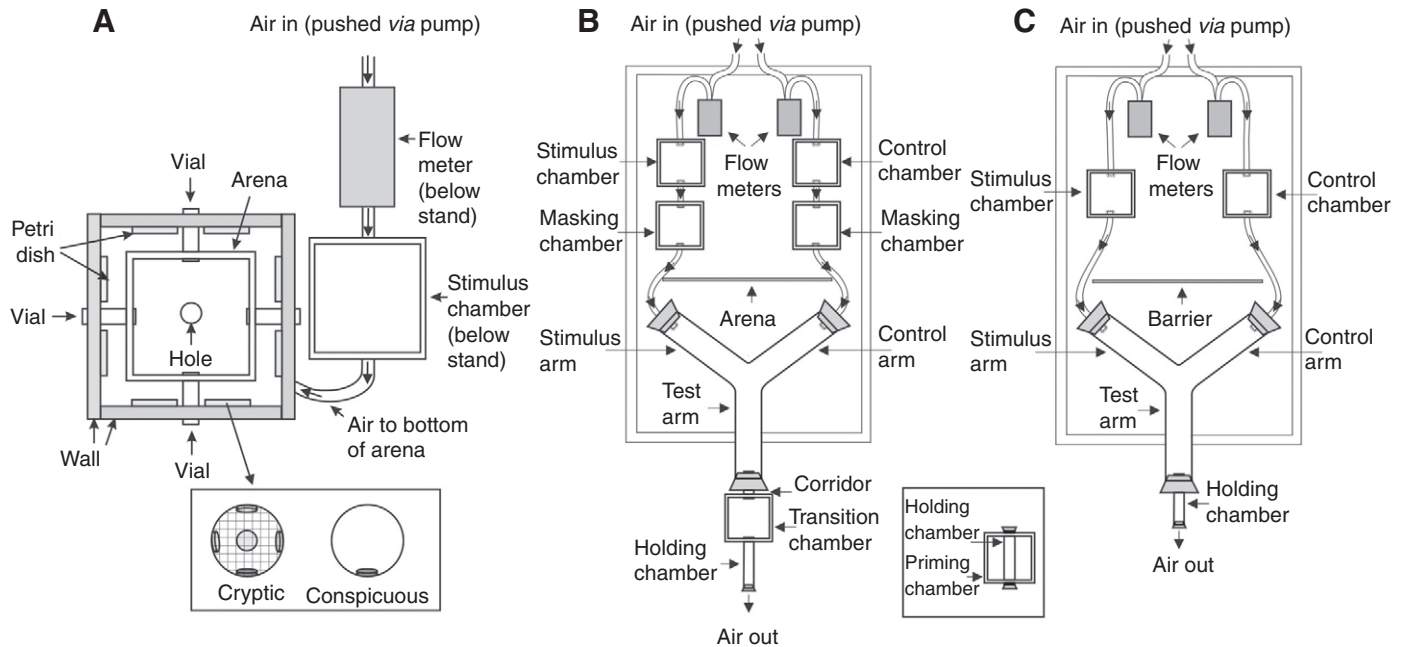


Fig. 1. Apparatus for Experiments 1 and 2. Glass chambers used in both experiments (inner dimensions 70 mm×70 mm×70 mm; thickness 5 mm; removable lid; two holes, diameter 20 mm, on opposite sides). (A) Arena for Experiment 1 (not to scale) made of glass (100 mm×100 mm, walls 35 mm high; removable lid 100 mm×100 mm). Inset: view of cork discs (diameter 10 mm; thickness 2 mm) from the perspective of a spider inside the box and facing the Petri dish. Petri dishes (diameter 35 mm, height 10 mm) were held in eight indentations (diameter 36 mm, depth 5 mm) in the wall (made of wood; each side 140 mm long, 50 mm high, 10 mm thick) surrounding the arena. The lure was placed on the shaded cork disc. Cryptic trials: dish was covered with nylon netting (1.5 mm×1.5 mm); besides the disc with the lure, another four cork discs were present (not shaded). Conspicuous trials: the cork disc with the lure was present; the other four cork discs were absent and the nylon netting was absent. There was a hole (12 mm diameter) in the lid and a hole in floor of the arena directly below (not shown). Odour entered via the hole in the floor. The test spider (in a plastic tube 65 mm long, 11 mm internal diameter) was introduced to the arena *via* the hole in the lid. Vials (50 mm long) fitted into holes (12 mm diameter) centred on four sides of the box. A wooden stand (not shown; 300 mm×300 mm; legs of stand 270 mm long) holding the arena hid the flow meter, stimulus chamber and odour source situated underneath. (B,C) Olfactometer for Experiment 2 with glass Y maze (length of each arm, 90 mm, internal diameter 20 mm). Arrows indicate direction of airflow. An opaque barrier prevented the test spider from seeing the odour source. Inset: the position of the holding chamber (90 mm long, diameter 20 mm) inside the priming chamber. (B) Masked trials. A spider entered the test arm by going through the transition chamber and corridor (40 mm long, 20 mm diameter; the inner rim was flush against the inner side of the hole in the transition chamber) and thereby gaining access to the stimulus arm and the control arm. (C) Unmasked trials. The holding chamber was inserted in the stopper, providing the spider with access to the test arm (no masking chambers, no transition chamber).

A pump coupled to a Matheson FM-1000 flow meter was used for pushing air through the apparatus. For permeating an arena with odour, the airflow system for Experiment 1 was similar to that used in a recent study (Cross et al., 2007). For Experiment 2, we modified a Y-shaped olfactometer used in earlier research on prey-choice decisions (Jackson et al., 2005). Airflow was set at 1200 ml min⁻¹ in Experiment 1 and at 1500 ml min⁻¹ in Experiment 2. There was no evidence that either of these airflow settings impaired locomotion or had any adverse effects on the test spider. By means of a silicone tube, air went successively into one chamber (Experiment 1) or into more than one chamber (Experiment 2; see below) and then, via another silicone tube, either into an arena (Experiment 1) or into a Y maze (Experiment 2). The silicone tubes connecting the chambers to the testing apparatus were covered with nylon netting on the end facing into the apparatus, blocking the spider's access to the chambers. Prey were put in the stimulus chambers (Experiments 1 and 2) and cuttings from *L. camara* (stems, leaves and flowers) were put in the lower half of each masking chamber (sufficient plant material added to not rise above level of inflow and outflow hole of chamber; Experiment 2 only) 30 min before trials began. The 30-min period allowed time for air to circulate evenly and ensured that air pressure was comparable throughout the apparatus. The plant material was collected from the field

60–90 min before put in the masking chamber (any visible arthropods on the material removed).

For both experiments, the entire apparatus was lit with a 200 W incandescent lamp that was positioned 400 mm overhead (additional ambient lighting from overhead fluorescent lamps). Between trials, the apparatus was dismantled and cleaned with 80% ethanol, followed by distilled water and then dried.

For trials with cryptic mosquito lures (Experiment 1) and for trials with masked mosquito odour (Experiment 2), we used test spiders that had been on each of three different diets (mosquitoes only, lake flies only and mixed). In all other trials, test spiders were on the mixed diet only.

Data for both experiments were analysed using χ^2 -tests of independence, Bonferroni adjustments being applied whenever the same data sets were analyzed more than once (see Howell, 2002). For both experiments, the relevant data were the number of spiders that found the lure or the odour. Data on latency, not being especially informative for the experimental designs we used, were not considered. For Experiment 1, $N=150$ for all conditions (i.e. 2400 individual spiders were tested). For Experiment 2, unless stated otherwise, $N=180$ for all conditions [N differed for spiders on mosquito diet and spiders on lake-fly diet (see Fig. 3B); 1781 individual spiders were tested in Experiment 2].

Experiment 1: olfactory priming of visual selective attention

The testing apparatus (Fig. 1A) was a glass arena with four glass vials that fitted into holes on each of the four sides of the arena. A wooden wall surrounding the arena had a hole (diameter 12 mm) in the centre of each side through which the glass vials protruded (open end of each vial on inside of arena; other end closed).

On either side of each hole in the wall there was an indentation, and each indentation held a small Petri dish. In cryptic trials, each Petri dish covered five cork discs (attached with double-sided adhesive tape). One disc was in the centre of the indentation in the wall. The other four discs were spaced evenly around the rim of the dish, one of these discs being positioned where the dish rim was closest to the floor of the arena ('lower rim position'). The Petri dishes were also covered with nylon netting. In conspicuous trials, there was no nylon netting and there was also only one cork disc (always in the lower rim position) per Petri dish. For both treatments, there was a lure in only one of the Petri dishes (which of the dishes would have a lure was decided at random for each trial). The disc on which the lure was mounted was always in the lower rim position and the lure was always facing into the arena.

The pump, flow meter and stimulus chamber were situated underneath the arena and wooden stand, with the stand shielding these parts of the apparatus from the test spider's view. The silicone tube connecting the stimulus chamber to the arena extended through a hole centred on the top of the wooden stand and then into the hole in the bottom of the arena (i.e. the two holes were aligned). The hole in the lid of the arena (for air outflow) was plugged with a silicone tube, with netting over the tube to prevent the spider from escaping. New netting was used for each trial.

The criterion adopted for recording that the test spider had 'found' the prey item was seeing the test spider enter the vial closest to the location of the lure and stay inside for at least 30 s. The rationale for the 30 s proviso was that, in preliminary trials, although *E. culicivora* sometimes entered a vial for a few seconds and then left, any individual that stayed in a vial for 30 s remained in this vial for at least 5 min and any that subsequently left this vial never entered and remained in another vial for as long as 30 s. We also adopted an alternative criterion: *E. culicivora* pressed its face against the side of the arena while facing directly towards the lure, but did not subsequently enter the vial. This criterion was never applicable in more than 10% of the recorded instances of finding prey for any treatment (Figs 2–3). Trials lasted until *E. culicivora* found the lure or, if *E. culicivora* did not find a lure, until 60 min elapsed.

Experiment 2: visual priming of olfactory selective attention

How the apparatus was set up depended on whether the odour was masked or unmasked, but the basic components of the apparatus were the same for the two treatments.

A Y maze made of glass was used, with the stem of the Y being the 'test arm', one of the forks of the Y being the 'control arm' and the other fork being the 'stimulus arm'. In masked trials (Fig. 1B), there was a stimulus chamber plus a masking chamber on one side of the Y and a control chamber plus a masking chamber on the other side. Air moved independently through the two chambers on the left side of the Y and through the two chambers on the right side of the Y. From the two arms of the Y, air then moved into the test arm and, from there, for the masked treatment only, through a corridor into a transition chamber and, from the transition chamber, through a holding chamber before exiting through a hole in the stopper. For the unmasked treatment (Fig. 1C), the path of air was

the same except that there was no corridor, no transition chamber and no masking chambers.

For each trial, whether the stimulus chamber was on the left or the right side was decided at random. Before trials began, a test spider was put into a glass holding chamber that was inserted through the holes in the sides of a priming chamber (Fig. 1). There were 20 lake flies or 20 mosquitoes in the priming chamber. The holding chamber was positioned so that it protruded 5 mm out from each side of the priming chamber. There was a stopper in place at each end of the holding chamber, inserted deep enough so that it confined the test spider to the part of the tube inside the priming chamber where the insects were in view.

The test spider was kept for 10 min inside the holding chamber, after which the holding chamber was removed from the priming chamber. The end of the holding chamber closest to the location of the test spider was plugged with a stopper. For the unmasked treatment, the open end of the holding chamber was inserted through a hole in a stopper and this stopper was inserted into the open end of the test arm of the Y. The open end of the holding chamber was flush with the end of the stopper inside the Y. For masked trials, the open end of the holding chamber was inserted into one of the holes in the transition chamber (open end flush with inside of box).

The test spider was free to walk out of the holding chamber and enter the transition chamber (masked trials) or the test arm of the Y (unmasked trials). Once the test spider entered the transition chamber, it was free to enter a corridor and then the test arm (the corridor was a tube fitted into a hole in the stopper that plugged the opening of the test arm).

Once in the test arm, the test spider was given 60 min to find the stimulus odour (i.e. to move into the stimulus arm and remain there for 30 s).

RESULTS**Does the cryptic-conspicuous distinction matter?**

Evidently the methods we used for making lures cryptic and for masking odour were effective. Regardless of the priming stimulus, spiders found conspicuous mosquito and lake-fly lures significantly more often than cryptic mosquito and lake-fly lures in Experiment 1 (Fig. 2A) and spiders found unmasked mosquito and lake-fly odour significantly more often than masked mosquito and lake-fly odour in Experiment 2 (Fig. 2B).

Does the priming stimulus matter when prey are conspicuous?

We found no evidence that the priming stimulus might matter when lures were conspicuous or when odour was unmasked. In Experiments 1 and 2, the number of spiders that found conspicuous or unmasked mosquitoes when primed with mosquitoes was not significantly different from the number of spiders that found conspicuous or unmasked mosquitoes when primed with lake flies (Fig. 2A,B) or, in Experiment 1, when there was no priming odour (control; Fig. 2A). Likewise, the number of spiders that found conspicuous or unmasked lake flies when primed with lake flies was not significantly different from the number of spiders that found conspicuous or unmasked lake flies when primed with mosquitoes (Fig. 2A,B) or, in Experiment 1, when there was no priming odour (control; Fig. 2A).

Does the priming stimulus matter when prey are cryptic?

In both experiments, it was evident that the priming stimulus mattered specifically when prey was hard to detect (cryptic lures or masked odour). In Experiments 1 and 2, significantly more spiders

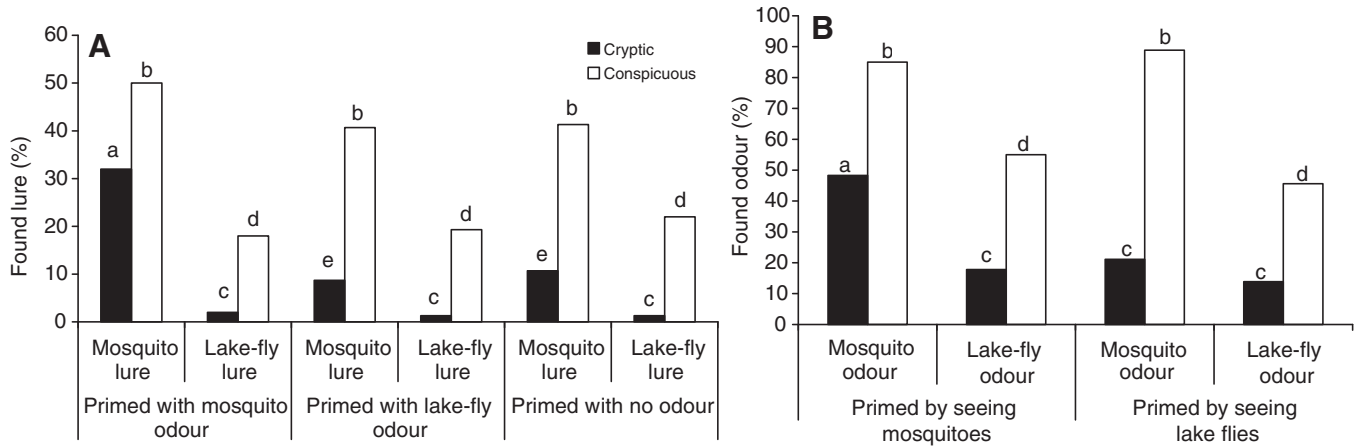


Fig. 2. (A) The influence of olfactory priming on how many spiders found the lure (cryptic or conspicuous) by sight. (B) The influence of visual priming on how many spiders found the prey (masked or unmasked) by olfaction. All spiders were maintained on the mixed diet. Letters used to denote statistical significance only for comparisons specified here. Different letters above the bars indicate significant differences ($P < 0.05$). Comparison of finding cryptic or masked vs conspicuous or unmasked prey: primed with mosquito and found mosquito (A: $\chi^2 = 10.05$, $P = 0.002$, $N = 150$; B: $\chi^2 = 54.45$, $P < 0.001$, $N = 180$), primed with mosquito and found lake fly (A: $\chi^2 = 21.33$, $P < 0.001$, $N = 150$; B: $\chi^2 = 53.87$, $P < 0.001$, $N = 180$), primed with lake fly and found mosquito (A: $\chi^2 = 26.23$, $P < 0.001$, $N = 150$; B: $\chi^2 = 43.85$, $P < 0.001$, $N = 180$), primed with lake fly and found lake fly (A: $\chi^2 = 41.33$, $P < 0.001$, $N = 150$; B: $\chi^2 = 43.21$, $P < 0.001$, $N = 180$), no priming (control) and found mosquito (A: $\chi^2 = 36.66$, $P < 0.001$, $N = 150$), no priming and found lake fly (A: $\chi^2 = 31.08$, $P < 0.001$, $N = 150$). Comparison of priming stimulus for finding conspicuous or unmasked prey: found mosquito when primed with mosquito vs lake fly (A: $\chi^2 = 2.64$, $P = 0.105$; B: $\chi^2 = 1.20$, $P = 0.274$), found lake fly when primed with mosquito vs lake fly (A: $\chi^2 = 0.09$, $P = 0.767$; B: $\chi^2 = 3.21$, $P = 0.073$), found mosquito when primed with mosquito vs control (A: $\chi^2 = 2.27$, $P = 0.132$), found lake fly when primed with lake fly vs control (A: $\chi^2 = 0.33$, $P = 0.568$). Comparison of priming stimulus for finding cryptic or masked prey: found mosquito when primed with mosquito vs lake fly (A: $\chi^2 = 25.21$, $P < 0.001$; B: $\chi^2 = 29.43$, $P < 0.001$), found lake fly when primed with mosquito versus lake fly (A: $\chi^2 = 0.20$, $P = 0.652$; B: $\chi^2 = 1.02$, $P = 0.312$), found mosquito when primed with mosquito vs control (A: $\chi^2 = 20.34$, $P < 0.001$), found lake fly when primed with lake fly vs control (A: $\chi^2 = 0.00$, $P = 1.000$). Comparison of ability to find conspicuous or unmasked mosquito vs conspicuous or unmasked lake fly: when primed with mosquito (A: $\chi^2 = 34.22$, $P = 0.001$; B: $\chi^2 = 38.57$, $P < 0.001$), when primed with lake fly (A: $\chi^2 = 16.25$, $P = 0.001$; B: $\chi^2 = 76.70$, $P < 0.001$), when not primed with odour (A: $\chi^2 = 12.96$, $P = 0.001$). Comparison of ability to find cryptic or masked mosquito vs cryptic or masked lake fly: when primed with mosquito (A: $\chi^2 = 47.84$, $P < 0.001$; B: $\chi^2 = 37.97$, $P < 0.001$), when primed with lake fly (A: $\chi^2 = 8.49$, $P = 0.004$; B: $\chi^2 = 3.25$, $P = 0.071$), when not primed with odour (A: $\chi^2 = 11.58$, $P = 0.001$).

found cryptic or masked mosquitoes when primed with mosquitoes than when primed with lake flies (Fig. 2A,B) or when there was no priming odour (control; Fig. 2A). Few spiders found cryptic or masked lake flies when primed with lake flies was not significantly different from how many spiders found cryptic or masked lake flies when primed with mosquitoes (Fig. 2A,B) or when there was no priming odour (control; Fig. 2A).

Does the identity of the prey used as a lure (Experiment 1) or for prey odour (Experiment 2) matter?

On the whole, our findings corroborate the conclusion from earlier work (Jackson et al., 2005) that mosquitoes are *E. culicivora*'s preferred prey. A bias for mosquitoes was evident in conspicuous and unmasked trials. Whether primed with mosquitoes, primed with lake flies (Fig. 2A,B) or not primed (control; Fig. 2A), significantly more spiders found mosquitoes than lake flies. A bias for mosquitoes was also evident in the cryptic and masked trials. Whether primed with mosquitoes (Fig. 2A,B) or not primed (control; Fig. 2A), significantly more spiders found mosquitoes than lake flies. When primed with lake flies in Experiment 1, significantly more spiders found cryptic mosquitoes than lake flies (Fig. 2A), but a similar trend in Experiment 2 was not significant (Fig. 2B).

Does maintenance diet matter?

Cross-modality priming by cues from mosquitoes was evident regardless of the particular diet on which *E. culicivora* was maintained. In Experiment 1, the number of spiders that found cryptic mosquitoes in the presence of mosquito odour versus in the

presence of lake-fly odour did not vary significantly depending on diet (Fig. 3A). In Experiment 2, the number of spiders that found masked mosquito odour after being primed by seeing mosquitoes versus lake flies did not vary significantly depending on diet (Fig. 3B).

Does the visual priming stimulus or identity of odour to be found affect *E. culicivora*'s inclination to enter the Y maze (Experiment 2)?

We wanted to determine whether being primed with a particular visual stimulus or being presented with a particular odour encouraged *E. culicivora* to enter the Y maze. For this, we compared the number of spiders that entered both the transition chamber and the Y maze with the number of spiders that entered the transition chamber but failed to enter the Y maze.

We found no evidence that the priming stimulus influenced the spider's inclination to enter the Y maze. When presented with masked mosquito odour, the number of spiders that entered the Y maze after seeing mosquitoes was not significantly different from the number of spiders that entered the Y maze after seeing lake flies. When presented with masked lake-fly odour, the number of spiders that entered the Y maze after seeing mosquitoes was not significantly different from the number of spiders that entered the Y maze after seeing lake flies (Fig. 4).

However, after seeing mosquitoes, significantly more spiders entered the Y maze when the masked odour was from mosquitoes instead of from lake flies. Likewise, when lake flies were the priming stimulus, significantly more spiders entered the Y maze when the masked odour was from mosquitoes instead of lake flies (Fig. 4). On the basis of this evidence, we conclude that *E. culicivora*

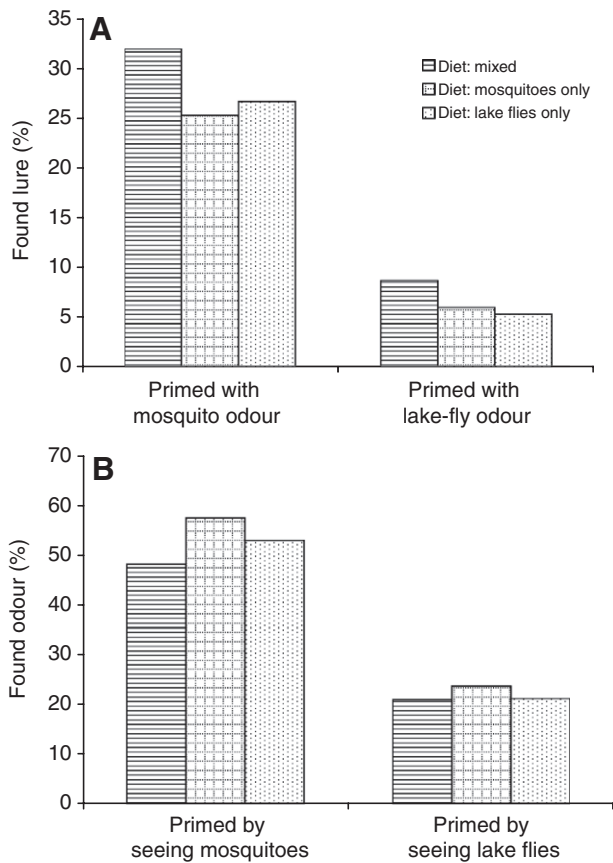


Fig. 3. The influence of diet on finding cryptic mosquito lures when primed with mosquito or lake fly odour (Experiment 1) or finding masked mosquito odour when primed by seeing mosquitoes or lake flies (Experiment 2). Comparing mixed diet and mosquito diet: finding mosquito when primed with mosquito [A: $\chi^2=1.63$, $P=0.202$, $N=150$; B: $\chi^2=2.00$, $P=0.157$, N (mixed diet)=180; N (mosquito diet)=85], finding mosquito when primed with lake fly [A: $\chi^2=0.780$, $P=0.376$, $N=150$; B: $\chi^2=0.26$, $P=0.610$, N (mixed diet)=180; N (mosquito diet)=88]. Comparing mixed diet and lake-fly diet: finding mosquito when primed with mosquito [A: $\chi^2=1.03$, $P=0.310$, $N=150$; B: $\chi^2=0.51$, $P=0.474$, N (mixed diet)=180; N (lake-fly diet)=79], finding mosquito when primed with lake fly [A: $\chi^2=1.28$, $P=0.258$, $N=150$; B: $\chi^2=0.00$, $P=0.964$, N (mixed diet)=180; N (lake-fly diet)=89]. Comparing mosquito diet and lake-fly diet: finding mosquito when primed with mosquito [A: $\chi^2=0.07$, $P=0.792$, $N=150$; B: $\chi^2=0.33$, $P=0.564$, N (mosquito diet)=85; N (lake-fly diet)=79], finding mosquito when primed with lake fly [A: $\chi^2=0.06$, $P=0.803$, $N=150$; B: $\chi^2=0.16$, $P=0.689$, N (mosquito diet)=88; N (lake-fly diet)=89].

becomes more inclined to enter the Y maze when the prey odour is from mosquitoes rather than from lake flies.

DISCUSSION

Our results from the conspicuous treatment in Experiment 1 and the unmasked treatment in Experiment 2, along with the findings from earlier research (Jackson et al., 2005), show that *E. culicivora*'s preferred prey are blood-carrying mosquitoes. Regardless of any potential priming stimuli, the number of spiders that found mosquito lures or mosquito odour was significantly higher than the number that found lake-fly lures or lake-fly odour (i.e. when prey was easy to find because it was conspicuous or unmasked, 'finding' can be understood as simply an expression of the spider's prey-choice decisions). However, when prey was harder to find (i.e. in the cryptic and masked treatments),

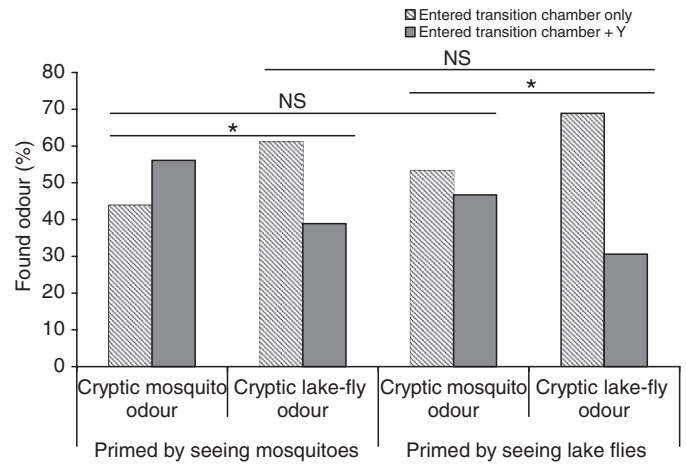


Fig. 4. The influence of the priming stimulus and of prey odour on *E. culicivora*'s inclination to enter both the transition chamber and the Y maze, rather than only the transition chamber (Experiment 2, masked treatment). Primed by seeing mosquitoes vs lake flies before finding the odour of mosquitoes ($\chi^2=3.21$, $N=180$) or lake flies ($\chi^2=2.63$, $N=180$). Spiders found the odour of mosquitoes vs lake flies after being primed by seeing mosquitoes ($\chi^2=10.70$, $N=180$) or lake flies ($\chi^2=9.61$, $N=180$). NS, non-significant; * $P<0.01$.

experimental results appear to reveal how mosquitoes are salient to the spider in the context of selective attention. More spiders found cryptic mosquitoes when primed by the odour of mosquitoes than when primed by the odour of lake flies and more spiders found masked mosquitoes when primed by seeing mosquitoes than when primed by seeing lake flies. Yet there was no evidence that smelling lake flies primed selective attention to the appearance of lake flies or that seeing lake flies primed selective attention to the odour of lake flies. Moreover, these effects were evident regardless of whether spiders had been maintained, before experiments, on a diet of blood-carrying mosquitoes alone, a diet of lake flies alone or on a mixed diet. These findings suggest that *E. culicivora* is innately predisposed to becoming selectively attentive to blood-carrying mosquitoes after priming.

There is similar evidence, from research with another salticid, *Portia labiata*, of an innate system governing the way in which selective attention is deployed. Salticid species from the genus *Portia* prefer other spiders as prey (Jackson and Pollard, 1996; Jackson and Wilcox, 1998), and *Micromerys* sp. and *Scytodes* sp. are two of the spider species on which *P. labiata* is known to prey in the Philippines (Jackson and Li, 2004). In experiments, *P. labiata* adopted a search image for whichever of these two prey species had recently been encountered. The conventional context in which search-image studies are cast is of a predator acquiring a search image by perceptual learning after repeated encounters with the prey, but a single encounter suffices for making *P. labiata* selectively attentive to *Micromerys* or *Scytodes* (i.e. individuals of *P. labiata* that had no prior experience with these prey became more effective at finding *Micromerys* sp. after feeding on a single individual of *Micromerys* and more effective at finding *Scytodes* sp. after feeding on a single individual of *Scytodes*).

Yet the findings for *E. culicivora* are different because they can be explained only by cross-modal triggering of innate olfactory and visual search images (i.e. instead of *E. culicivora* having full access to the prey during priming, only visual or only olfactory cues were available). In the experiments using *P. labiata*, the predator had full access to the prey and this means that whether the priming cues

were same modality, cross modality, or both is uncertain. There is, in fact, a similar uncertainty in much of the literature on search images [for a notable exception, see Bond and Kamil (Bond and Kamil, 2002)].

However, specifically cross-modal effects have been shown for *Portia fimbriata*, another spider-eating salticid, as well as for *Habrocestum pulex*, a salticid that prefers ants as prey (i.e. in experiments using *P. fimbriata* and *H. pulex*, as in our experiments using *E. culicivora*, priming effects on selective attention were demonstrated despite there being no prior feeding on the prey). For *Habrocestum pulex* (Clark et al., 2000), chemical cues from specifically ants primed selective attention to visual cues from specifically ants. For *Portia fimbriata* (Jackson et al., 2002), olfactory cues from specifically *Jacksonoides queenslandicus*, another salticid common in the same habitat as *P. fimbriata*, primed selective visual attention to this particular prey species. The findings for *P. fimbriata* and *H. pulex*, like the findings for *E. culicivora*, reveal cross-modal priming effects that are innate, but our work with *E. culicivora* goes a step further by showing that cross-modality priming works in both directions. In Experiment 1, the odour from blood-carrying mosquitoes, but not the odour from lake flies, primed selective attention to vision-based cues from specifically blood-carrying mosquitoes. In Experiment 2, seeing blood-carrying mosquitoes, but not seeing lake flies, primed selective attention to odour-based cues from specifically blood-carrying mosquitoes. Whether cross-modality priming might also work in both directions for *H. pulex* and *P. fimbriata* has not yet been investigated.

In a recent study, VanderSal and Hebets showed that another salticid, *Habronattus dosseus*, learned to avoid colour associated with heat in the presence of a seismic stimulus, but that there was no apparent learning when the seismic stimulus was absent (VanderSal and Hebets, 2007). Although the results of this study suggest that input from one sensory modality may facilitate learning in another sensory modality, it may be more appropriate to describe the findings for *H. dosseus* as a general-arousal effect rather than an example of selective attention being triggered. This may also be the case in work with *Drosophila* where both olfactory and visual cues assist with learning to avoid a noxious heat stimulus (Guo and Guo, 2005) and where both olfactory and visual cues improve flight control, enabling an insect to fly towards an odour source (Chow and Frye, 2008).

However, showing cross-modality priming of selective attention to a particular type of prey (blood-carrying mosquitoes for *E. culicivora*, *J. queenslandicus* for *P. fimbriata* and ants for *H. pulex*) seems to be revealing something that is cognitive in a different way. One way of saying this would be that, for these three salticids, olfactory cues call up a visual representation of an expected, but not yet seen, prey and that, for *E. culicivora*, prey appearance calls up an olfactory representation of an expected but not yet smelled prey. Although an objective understanding of what these 'representations' may actually entail remains elusive, well-known studies on the European toad (*Bufo bufo*) suggest that predators may often rely on very specific features of prey appearance (Ewert, 1974). Our results with *E. culicivora* suggest that the saliency of stimuli related to the appearance of blood-carrying mosquitoes increases when the odour of this prey is present and, furthermore, that the saliency of stimuli related to this prey's odour increases after this prey is seen. One of the next steps in our research will be to determine whether, after priming, *E. culicivora* selectively attends to particular salient features of the mosquito, including particular visual features and particular volatile compounds in the odour plume.

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