Ontogenetic shift in plant-related cognitive specialization by the mosquito-eating jumping spider, *Evarcha culicivora*

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A thesis submitted in partial fulfillment of the requirements of the degree of

Master of Science

March 2016

Acknowledgements

Thank you to my primary supervisor, mentor and friend, Professor Robert R. Jackson. It has been a privilege to learn the lessons of my early academic life under your guidance. Thank you to my co-supervisor, Dr. Ximena Nelson, for your clear-headed advice and technical expertise.Let me express my greatest appreciation for your ongoing patience and support.

Asante sana to Stephen Abok Aluoch for your exceedingly hard work at ICIPE, and to Aynsley McNab for yours at the Canterbury Spider Lab.

For their assistance in the preparation of the publication “Nectar Meals of a Mosquito-specialist Spider”, thanks to Godfrey Sune, Josiah Kuja and Josiah’s supervisors who are co-authors on this manuscript.

Thank you to my colleagues and friends Emily Tighe, Yinon Dolev and Dr. Fiona Cross, who supported me during the production of this thesis, for your advice and your ongoing sympathies.

Finally, thank you to my family — Mum, Dad, Josh and everyone else — for all your love throughout “The Spider Years”. Without you I would have given up a hundred times over.

Funding for this research was provided in part by National Institutes of Health (NIH) Grants R21-AI062957. (The author, not NIH, are solely responsible for the contents of this report.)

Preface

My investigation into the association that one mosquito-loving spider in East Africa has with a certain plant began with an unlikely question posed by Thomas Nagel (1970):

"What is it like to be a bat?"

Nagel does not suppose to answer this question per se, but simply to posit that there must be something that it is like to be a bat. That the sum of bat-hood includes some subjective experience, or what we call "qualia".

A philosopher of a certain style might be inclined then to argue that all living things, or perhaps, to be restrained, all living things in possession of a central nervous system — who sense the external environment in a way analogous to a human or a bat, and who process this information and respond accordingly — have something that it is like to be them.

It was set by fate then that when a man dressed in the quintessential field biologists uniform (a short sleeved shirt and slacks in khaki) who would soon become my supervisor, laid down the paraphrased question

"What is it like to be a spider?"

I would jump at the chance to investigate.

Spiders hold an unfortunate place in western culture, interpreted by evolutionary or societal pressure as hidden, dangerous, “creepy-crawlies”, with little to no value placed on their lives. At best spiders are seen as a tolerable nuisance, and given no further thought; at worst they are the subject of extreme phobia and copious bug spray.

Spiders hold an unfortunate place in behavioural biology too. Invertebrates with no obvious charisma, their genomes are not uniquely simple, their capture is not uniquely easy in the field, their husbandry not uniquely easy in the lab. For any given research question, it would seem there is an organism better suited to its study than a spider.

However, there are 45 thousand species of spider, each occupying its own niche, contributing to and depending on its local ecosystem. Each species of spider fought through eons as a surviving thread running back to the first moment of life on earth. Each species is just as successful, just as valid, just as integral a part of the world as any common research subject.

The fact remains that there must be something that it is like to be any given spider. If the goal of science is to make known that which is unknown, the question “what is it like?” is a rich and worthy target.

Abstract

The East African jumping spider, *Evarcha culicivora*, preferentially feeds on Anopheles mosquitoes. This spider carries out apparently complex cognitive processes, namely, cross-modal selective attention, to detect and locate this specific prey. Juvenile *E. culicivora* supplement their diet with nectar, primarily from *Lantana camara*, and the sugar from these nectar meals makes them more proficient at capturing their preferred prey. Both the adult and the juvenile spiders are attracted to the odour of *L.camara* among other plants. Here, I test the effects of plant odours on adult and juvenile spiders' response to visual stimuli, in order to elucidate the function of *E. culicivora*'s response to plant odours across the spider's lifetime. I found that, for juveniles, plant odours elicit selective attention to a visual stimulus consisting of *L.camara* flowers, consistent with previous research showing plants are important to juveniles in the context of nectar feeding. For adults, I found that plant odours elicit selective attention to a visual mate stimulus, in much the same way that mate odour did. Specifically, adult spiders responded strongly to a visual stimulus consisting of mates in conjunction with plants after exposure to plant odour. I discuss the implications of these findings with regards to the representation of the plant stimulus in the spider's miniature brain. I propose a model

in which the cognitive process triggered by the plant odour stimulus changes between the juvenile and adult life stages. I conclude with the suggestion that spiders use highly specialized representations of salient stimuli to perform apparently complex cognitive tasks. Moreover, my results show that these representations change between these life stages.

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Chapter 1

Introduction

1.1 *Evarcha culicivora*, the mosquito-eating jumping spider

In this thesis, I document my investigation into the behaviour of a small, grey-brown jumping spider, *Evarcha culicivora* (Salticidae) – a jumping spider with peculiar preferences and extraordinary abilities. *E. culicivora* is best known for its bizarre feeding behaviour, indirectly consuming vertebrate blood by selecting blood-fed mosquitoes as its preferred prey. Intrigued by this unusual and unusually specific behaviour, a team of researchers based in western Kenya began investigating this spider and the things it can do with its tiny brain.

As they delved further into the spider's ethology, they discovered that the unusual characteristics of *E. culicivora* extend far beyond its prey-choice decisions to include intricate mate choice decisions, a capacity to detect and find sources of human odour, and interest in plants and plant based chemicals. *E. culicivora* seemed to be repeatedly challenging the widely accepted idea that small animals, with their small brains, must live simple lives. The

wider significance of investigating this unusual predator is to contribution to our understanding of the level of behavioural complexity that can be achieved by animals with small brains and the cognitive processes that this complex behavior implies.

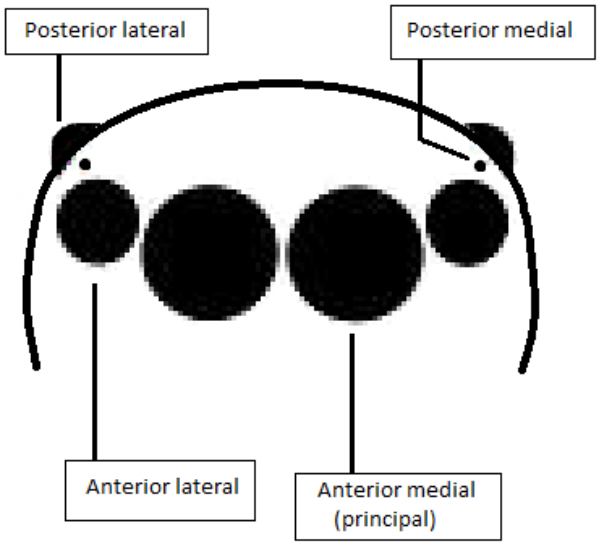
One of the overarching objectives of research on *E. culicivora* is to gain an understanding of the minds of small animals (I use the term 'mind' here with caution, sensu Minksy [1] - "minds are simply what brains do"). My own research can be characterized as an investigation into the workings of one small corner of *E. culicivora*'s mind. In the following chapters, I investigate some of the plant-related behaviour exhibited by *E. culicivora*, with an emphasis on how the role of plants changes during this spider's life cycle. I aim to integrate the resulting information into the current literature, to contribute to an ecologically relevant account of the role of plants across this jumping spider's life history.

1.2 Vision, olfaction and behavior

Although jumping is characteristic behaviour among jumping spiders, what really distinguishes salticids from other spiders is their phenomenal eyesight. Salticids can be identified by the arrangement of their eight eyes (Fig.1.1). Notable are a pair of large forward facing principal (anterior medial) eyes (AMEs), whose unique structure (Fig.1.2) grants the spider a level of spatial acuity unrivalled for an animal of their size.

Spatial acuity refers to the eye's ability to separate distinct objects in a scene, which depends in part on the arrangement of receptors in the retina. Salticids AMEs have tiered retinae, each comprising four layers of rhabdomeres (photoreceptor cells), totalling between 1000 and 10,000 individ-

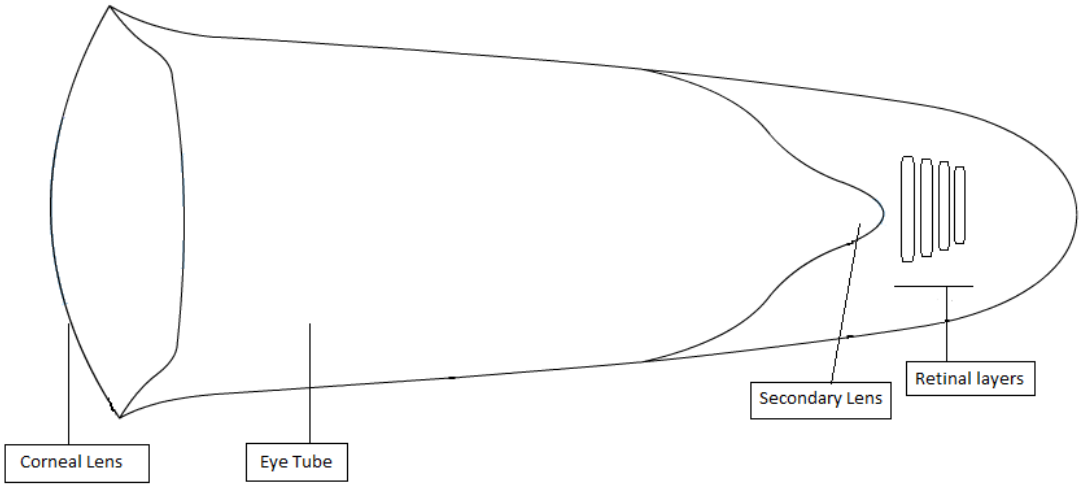
Figure 1.1: Salticid eye configuration as viewed from front of cephalothorax



ual photoreceptors per eye [2]. Each layer contains photoreceptors that are sensitive to a specific range of light wavelength, giving the spider colour vision ranging from red through to ultraviolet [2]. To put the salticid's visual acuity into context, the retinae of the AMEs have inter-receptor angles as small as 2.4 arcminutes. This is six times smaller than the most acute vision found among insects, that of the dragonfly, *Aeschna sp.*, whose minimum inter-receptor angle is 14.4 arcminutes [3], and only six times larger than the acuity of the human eye, which typically has an inter-receptor angle of 0.4 arcminutes[3].

Accordingly, many salticids have developed complex vision-based behaviour, including courtship, threat displays, navigation, and predation (eg. [4, 5, 6, 7, 8, 9, 10, 11, 12]). *E. culicivora*'s unusual predatory habits depend in large part on its exceptional vision. The specificity of *E. culicivora*'s prey choice behaviour goes beyond finding mosquitoes, these spiders pick their preferred prey, blood-fed female Anopheles mosquitoes, out of crowds of

Figure 1.2: Structure of salticid anterior medial eye as viewed from above



numerous similar insects (most commonly non-biting midges, Chaoboridae and Chironomidae, known as “lake flies”) [13].

Lake flies and mosquitoes resemble each other in general appearance and, types of mosquito, anopheline and culicine, are even more difficult to distinguish. In the laboratory, technicians must be trained to discriminate between mosquitoes and non-mosquitoes, by the presence or absence of piercing mouthparts; between anopheline and culicine mosquitoes, by body posture; and between male and female Anopheles, by the fine details of the mosquitoes’ antennae. *E. culicivora* appears to have the innate capacity to detect these differences [14]. Using choice tests with motionless, odour-free lures or virtual prey presented on a monitor, Nelson & Jackson [14] showed that *E. culicivora* are able to distinguish each of these stimuli, even when the decision must be based solely on visual information.

Salticids also make extensive use of chemoreception, that is, they process both chemotactic (taste) and olfactory (smell) stimuli (eg. [15, 16, 17, 18]). Olfactometer experiments, in which the spider is exposed to the

odour of a salient stimulus, show that *E. culicivora* can identify a wide range of odours, including the odour of mosquitoes in general, blood-fed mosquitoes specifically [19], potential mates, those mates who have recently fed on blood-fed mosquitoes [19], and importantly for the research reported here, plants and specific plant volatiles [19, 17, 20].

1.3 Selective Attention

Perhaps the most fascinating discovery to come from research on *E. culicivora* so far, is that these spiders do not use these senses independently. Rather, they integrate visual and olfactory cues to form what can be described as a representation of a salient stimulus. This means that exposure to a salient stimulus triggers activation of the representation of that stimulus, which changes the way in which further stimuli are processed. Specifically, the spider then responds more strongly to stimuli associated with the representation of the same target, and less strongly to stimuli associated with the representation of another otherwise salient target. For example, sensing mosquito odour, especially the odour of blood-fed female Anopheles, appears to "prime", or prepare, the spider to better identify a mosquito presented visually, especially when the visual stimulus is cryptic, or difficult to distinguish from its surroundings [19]. Furthermore, this cross-modal association also works in the other direction, that is, seeing a mosquito (without being exposed to its odour) appears to prime the spider to better identify cryptic mosquito odour [19].

In ethology literature, this has been referred to as use of "search image" (eg. [21, 22]), but in cognitive science the same phenomenon is called "selective attention" (eg. [23, 24, 25]). Here, I have opted to use the latter, as

the term “search image” was coined in reference to visual effects only, and I feel that its use limits our ability to acknowledge the multi-modal nature of the effect.

1.4 *Evarcha culicivora* and plants

Cross & Jackson [19] showed that the odours of two particular plant species, Lantana camara (Verbenaceae) and Ricinus communis (Euphorbiaceae), are attractive to *E. culicivora*. These plants are both prevalent in *E. culicivora*'s habitat, and *E. culicivora* is commonly found on *L.camara* in the wild [26]. Nelson et al. [20] went on to show that *E. culicivora* adults are attracted to three of the dominant volatile compounds in the headspace of *L.camara* – β-caryophyllene, α-humulene, and 1,8-cineole (hereafter referred to as caryophyllene, humulene and cineole) – and that juveniles are attracted to both caryophyllene and humulene. As caryophyllene is the most abundant volatile compound produced by *L.camara*, and *E. culicivora* respond especially strongly to it [20], further research, including some of the research presented in this thesis, has used caryophyllene as a proxy for plant odour (eg. [27]).

In investigating *E. culicivora*'s attraction to caryophyllene, Nelson & Jackson [28] found a critical difference between adult and juvenile behaviour. When juvenile, but not adult, *E. culicivora* are exposed to caryophyllene after fasting, they respond more strongly to the plant odour. That is, the juvenile spider's attraction to caryophyllene increases with increasing hunger (i.e. after fasts of increasing duration), while adults' attraction to the plant odour appears to be stable across hunger levels. In the context of this thesis, questions raised by these findings were critically important.

Spiders are often characterized as obligate predators (eg. [29]), and knowing the lengths to which they go to feed on mosquitoes, it would be tempting to assume *E. culicivora* is no different. However, it is becoming increasingly evident that spiders often supplement their predatory diet with plant material (eg. [30, 31, 32, 33, 34]). Indeed, B. kiplingi is a spider that appears to be almost entirely vegetarian [35]. Perhaps we should expect that spiders occupy an entire range of feeding niches.

With this in mind, I became involved in research related to nectar feeding by *E. culicivora*. Appendices 1 and 2 present two published manuscripts related to the work in the main body of this thesis. Appendix 1, published in Psyche as "Nectar meals of a mosquito-specialist spider" [27], demonstrates that *E. culicivora* acquires fructose from its natural diet and can ingest fructose directly from plant nectaries. We found that 53.5% of 1,215 small juveniles *E. culicivora*, but only 3.4% of 622 adults, left with plants for 24 hours, showed evidence of nectar feeding (i.e. tested positive for fructose). From these findings we concluded that fructose is especially important for early-instar juveniles of *E. culicivora*.

Appendix 2, published in Royal Society Open Science as "Rapid nectar-meal effects on *E. culicivora*'s capacity to kill mosquitoes" [36] demonstrates that juvenile *E. culicivora* benefit in a particular way from consuming nectar. Although *E. culicivora* are specialized at feeding on mosquitoes, capturing this prey can be a difficult task for the younger juveniles. These spiderlings are only 1.5-2.0 mm in body length, less than half the size of their mosquito targets, which are typically 4.5mm long. We showed that prior nectar feeding renders *E. culicivora* juveniles more proficient at capturing blood-carrying mosquitoes. The benefit appears to come from the ingestion of high concentrations of specific sugars (especially fructose and

sucrose).

The findings from these two studies help elucidate the roles played by plants in the biology of *E. culicivora* juveniles, but tell us little about the roles of plants in the biology of *E. culicivora* adults. Natural history observations [26] show that adults do encounter potential mates on plants. That plants may be important to the adult spider as a mating site is one of the hypotheses I consider in the following chapters.

1.5 Adult – Juvenile differences in plant use and plant-related cognition

The body of research shows that *E. culicivora* has an exceptionally complex lifestyle, and that plants fit into this lifestyle somehow, but precisely what roles plants play in the life history of this spider remain unclear. This gap in knowledge inspired the central research question for this thesis: Does the role of plants change over the *E. culicivora*'s lifetime?

More specifically, I investigate the way in which *E. culicivora*'s cognitive response to plants might be subject to changes over the spider's lifetime, i.e. ontogenetic shifts. I examine whether there is an ontogenetic shift in the way plant-related cross-modal effects are expressed. I consider these effects in three contexts: eliciting vision-based plant-investigating behaviour (objective 1), priming selective visual attention to specific visual targets (objective 2) and priming a rapid response to specific visual targets (objective 3).

For objective 1, my primary hypothesis is that plant-related odour elicits vision-based inspection of plants by the adults and the juveniles of *E. culicivora*. I consider whether these odour effects are expressed specifically in

the context of seeing *L.camara* or whether they might be expressed in the context of seeing a wider range of plants and I predict that caryophyllene and humulene, but not cineole, mediate these effects. I also propose that it is specifically plant-related odour that elicits visual inspection of plants, i.e., I predict that other salient odours (prey or mate odour) will have no effect on response to a plant visual stimulus.

For objective 2, I consider four hypotheses as a step toward identifying the visual targets that *E. culicivora* searches for while in the presence of particular odours.

1. For *E. culicivora* juveniles, but not adults, exposure to caryophyllene elicits selective visual attention to flowers.

2. For *E. culicivora* adults and juveniles, exposure to prey odour elicits selective visual attention to prey.

3. For *E. culicivora* adults, exposure to mate odour elicits selective visual attention to mates.

4. For *E. culicivora* adults, exposure to caryophyllene elicits selective visual attention to mates specifically in the presence of plants.

To determine whether odour affects specifically selective attention, as opposed to preference (see [37]), I vary whether the visual stimulus is cryptic (more difficult to detect and identify) or conspicuous (easier to detect and identify). I predict that the effects of plant odour will be evident when the visual stimulus is cryptic but not when it is conspicuous.

A critical prediction of the selective attention hypothesis is that selective attention to one stimulus renders the spider less able to detect and identify other, otherwise salient stimuli, than it would be even if no priming had occurred. As such, I predict that *E. culicivora* will be less attentive

to salient cryptic visual stimuli while exposed to incongruent odour than while exposed to no odour in control trials (see [19]).

The goal for objective 3 is to determine whether specific odours prepare *E. culicivora* to respond rapidly to specific visual targets. I make four specific predictions.

1. Prey odour primes *E. culicivora* to respond rapidly to seeing prey.
2. Caryophyllene odour primes *E. culicivora* to respond rapidly to seeing a plant or to seeing a mate, but not to seeing a prey item.
3. Independent of whether a plant is also in view, mate odour primes *E. culicivora* to respond rapidly to seeing a mate.
4. When a plant is also in view, caryophyllene odour primes *E. culicivora* to respond rapidly to seeing a mate.

Chapter 2

Methods

2.1 General

We used spiders from laboratory cultures (F2 & F3 generation) derived from individuals collected at our field site in Mbita Point, Western Kenya (elevation 1200 m above sea level; latitude 0°25'S–0°30'S; longitude 34°10'E)). As our methods for rearing and maintaining spiders in the laboratory corresponded closely to those in earlier studies (e.g., [38]), only critical details are provided here.

Chironomid midges and blood-fed mosquitoes were the standard diet for all spiders and their parents. The midges were collected from the field as needed and the mosquitoes that were used for rearing and in experiments were always Anopheles gambiae s.s. taken from stock cultures. No test spider had prior experience with conspecific individuals, nor did it or its parents have prior experience with any plant species, any of the three compounds used in experiments or the apparatus. No individual spider was tested more than once, and no individual spider was an experimental subject in any other research project. All experiments were carried out

between 0800 and 1400 hours (laboratory photoperiod 12L:12D, lights on 0700 hours).

We recognized six test-spider categories: adult males (5.0 mm), adult females (5.5 mm) and four size classes of juveniles (1.5 mm, 2.5 mm, 3.5 mm and 4.5 mm). Size was body length (accurate to the nearest 0.5 mm) measured from the anterior end of the cephalothorax to the posterior end of the abdomen, exclusive of spinnerets. These measurements were made on the day of testing, which was always 1 day after the spider's last meal. All adult test spiders had matured during a 2–3 week period preceding use in experiments. All juvenile test spiders had moulted at least 3 days before use in experiments and did not moult again for at least another 3 days. The expressions 'large' and 'small' were used for juveniles that were 4.5 mm or 1.5 mm in body length, respectively. The different test-spider categories used in different experiments are specified below.

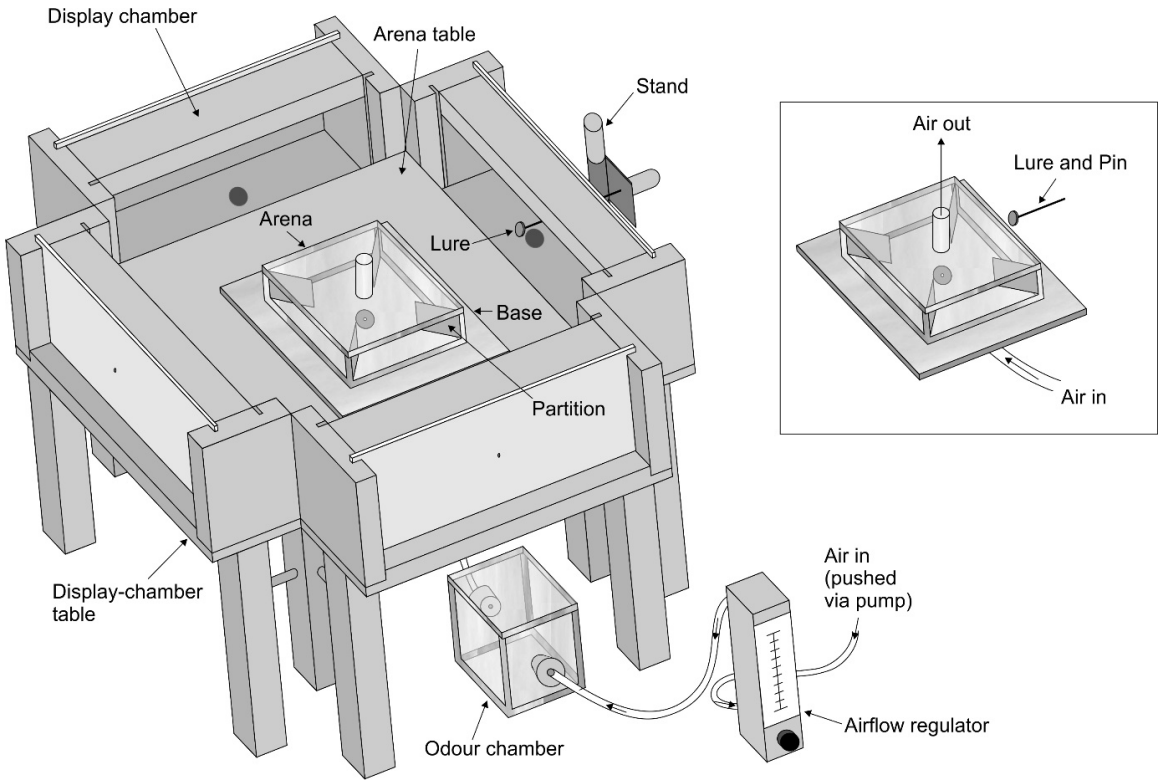
2.2 Apparatus

Here, I describe the apparatus and the procedures for objective 1. Details pertaining to how the apparatus and procedures differed for objectives 2 and 3 can be found in the sections on those objectives. However, for all objectives, there were some basic similarities: the test spider was confined to a central chamber (the 'arena') from which it could view, but not enter, display chambers; for all objectives, there was an odour chamber in which an odour source could be housed; using an airflow meter (Matheson FM-1000 set at 1500 mL/min) and pump, air was pushed through the odour chamber and then through the arena.

There were two versions of the apparatus for objective 1, 'large' for test-

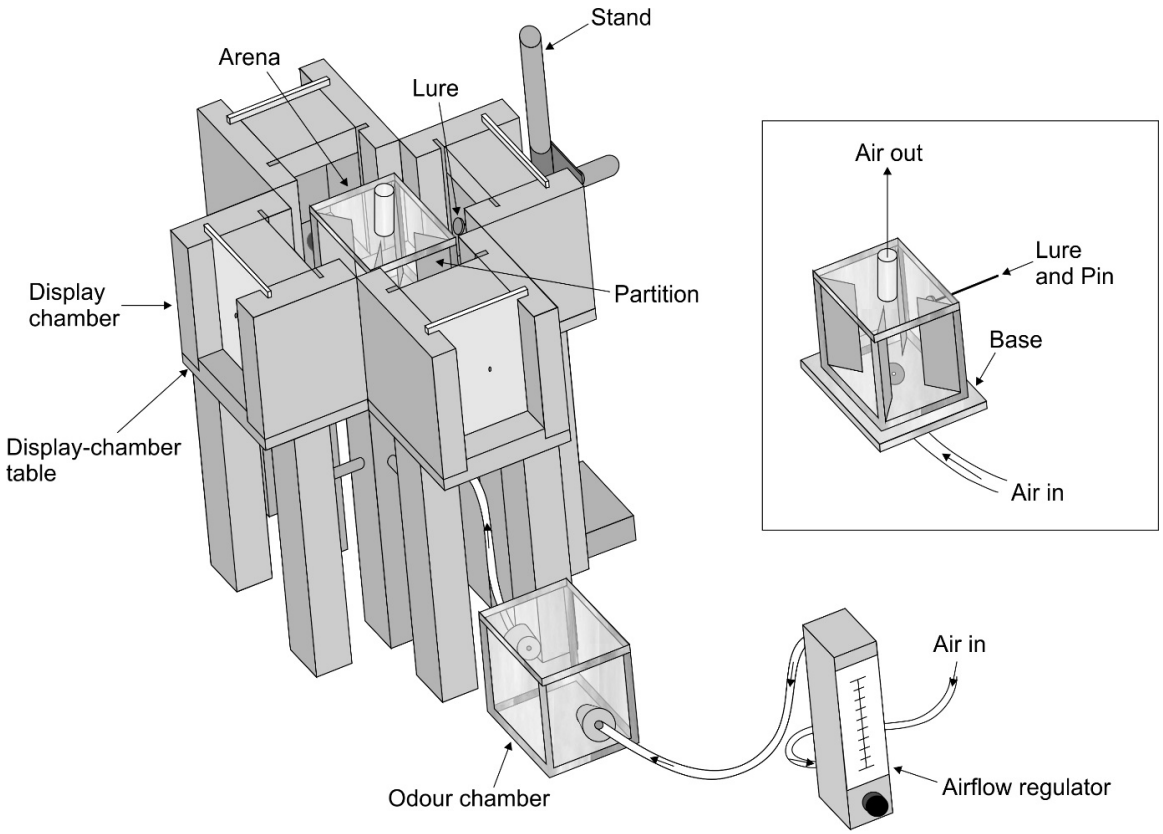
ing *E. culicivora* adults (Fig.2.1) and 'small' for testing *E. culicivora* juveniles (Fig.2.2). The arena was a glass box (internal dimensions: large, 100 mm × 100 mm, 37 mm high; small, 50 mm × 50 mm, 37 mm high) centred on the top of a glass base (large, 140 × 140 mm; small 56 mm × 56 mm). The glass base was in turn centred on the top of a purpose-built wooden table ('arena table'; large, 300 mm × 300 mm; small, 90 mm × 90 mm; wood thickness for both 17 mm), supported by four legs (each leg 40 mm × 40 mm in cross section and 250 mm in height). All glass pieces were transparent and 3 mm thick.

Figure 2.1: Apparatus for objectives 1 and 2 (see text for details) when test spiders were adult males and females of *Evarcha culicivora*



Air entered through a hole centred in the arena table top and through a corresponding inflow hole in the bottom of the arena, and air left the arena through an outflow hole centred in the arena lid (hole diameters 12 mm).

Figure 2.2: Apparatus for objectives 1 and 2 (see text for details) when test spiders were juveniles of *Evarcha culicivora*



During trials, nylon netting over the inflow and outflow holes confined the test spider to the arena. There was a glass tube in the outflow hole and another glass tube in the inflow hole (inner diameter of each tube 11 mm, outer diameter 12 mm, length 20 mm). The distal end of the outflow tube was open, but there was a rubber stopper in the distal end of the inflow tube.

There were four display chambers, each aligned next to one of the four sides of the arena table. Metal braces held each chamber tightly against the table top, ensuring that there were no open spaces at the corners of the table. Each display chamber had two wooden side walls (80 mm wide, wood thickness 20 mm) and there were two grooves (3 mm wide, 40 mm

apart) which ran from the top to the bottom of each side wall and across the floor of the chamber. Each display chamber had a removable glass front positioned in the grooves closest to the arena and a removable wooden back positioned in the other grooves. The inner side of the glass front was 40 mm from the inner side of the wooden back.

Each display chamber was centred on a wooden table ('display-chamber table'; wood 20 mm thick; large: length 300 mm, width 95 mm; small: length 90 mm, width 80 mm). Each display-chamber table was supported by a pair of wooden legs (height 220 mm) and, for stability, each of these legs was bolted to the nearest leg of the arena table. With this arrangement of the display chambers, the arena was surrounded by glass that rose to 115 mm above the top of the arena table.

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

Side 1 of the arena faced the plant cutting in display-chamber 1. Inside the arena, there were four aluminium partitions extending 40 mm from each of the four corners of the arena toward the centre of the arena. The

height of each partition matched the inside height of the arena, meaning that the partitions created five sectors within the arena. Sector 1 was the space within a truncated triangle created by side 1 and the two partitions that intersected side 1. Comparable definitions applied to sectors 2–4. The centre sector was the circular space at the centre of the arena beyond the extent of the partitions; whenever the test spider moved from one side sector to another, it had to pass through the centre sector.

The odour chamber was a glass cube (inner dimensions 70×70×70 mm) that sat under the arena table. This chamber remained empty during no-odour control trials, but it housed an odour source during experimental trials. Depending on the experiment, the odour source was 10 *Anopheles gambiae* females (prey) that had fed on blood 4–5 hours before being used, one opposite-sex conspecific individual (mate) or a sample of a specific compound - caryophyllene, humulene or cineole. The sample was prepared by adding 4 µl of the compound to 1.0 g of petroleum jelly that was situated in the centre of an open glass Petri dish (diameter 30 mm). Each sample was prepared in a petri dish 24 h earlier and kept with lid in place, wrapped in aluminium foil, in a refrigerator. 60 min before a trial began, the appropriate sample was transferred to the odour chamber and allowed to adjust to the ambient temperature. Prey and mates were also put into odour chambers 60 min before trials began. To perfuse the arena with the specific odour, the odour chamber was connected to the airflow system during the 60-min pre-trial period.

Two holes (diameter 20 mm), each on an opposite side of the odour chamber, were plugged with rubber stoppers. There was a hole in each of these stoppers, as well as in the stopper situated in the arena's inflow tube, and there was a small glass tube (length 45 mm, diameter 4 mm) positioned

in each stopper hole. The inner side of each stopper in the odour chamber was covered with nylon netting. For airflow through the apparatus, there was silicone tubing connecting the various glass tubes, the airflow meter and the pump.

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

2.3 Objective 1: Odour priming of vision-based plant investigating behaviour

In experiments, six test-spider categories were presented with different combinations of a visual stimulus, i.e., a plant cutting in display-chamber 1, and an olfactory stimulus, i.e., prey items, a mate or a volatile compound in the odour chamber. 680 spiders were used across 34 conditions (N=20 per condition). Specific stimulus combinations are listed in Tables ref and ref. At the start of each trial, the test spider was transferred to a glass transfer tube (diameter 12 mm, length 25 mm, closed with rubber stoppers) for a 5-min acclimation period after which one stopper was removed and the open end of the transfer tube was inserted into the arena outflow hole. The spider was allowed 2 min to move into the area of its own accord. If the spider failed to move during this time, we removed the other stopper and, using a soft brush, coaxed it into the arena. Once the spider was in the

arena, we removed the transfer tube and returned the original glass tube (with netting) to the outflow hole. After the spider entered the arena, the trial lasted for 30 min. We recorded how long the test spider spent in each sector. The test spider's 'response duration' was the total time spent in sector 1.

2.4 Objective 2: Odour priming of selective visual attention to specific visual targets

Our objective here was to determine whether there was cross-modality priming of selective visual attention. We recorded test-spider responses to different visual targets, presented in front of a plant cutting, in the presence of different odours. 580 spiders were used in total, 400 in part 1 across 20 conditions, and 180 in part 2 across 9 conditions, (N=20 per condition). The apparatus was the same as for objective 1 (Figs. 2.1 & 2.2) except that there were no glass fronts on the display chambers, there was a *L.camara* cutting in each of the four display chambers instead of only one, and there were no flowers on the cuttings. We ensured that the cuttings did not extend beyond the grooves at the front of the chambers (i.e., the grooves where the glass fronts had been positioned for objective 1).

We positioned a "visual target" between one side of the arena and the plant cutting in the facing display chamber (display-chamber 1). For each trial, which of the four chambers would be chamber 1 was decided at random. The visual target was either 'prey', 'mate' or 'flower', and consisted of an object affixed on top of a cork disc (diameter 15 mm, thickness 2 mm). Prey was always a female mosquito (body length 4.5 mm) that had fed on blood 4–5 h before being transferred to 80% ethanol. On the follow-

ing day, we removed the mosquito from the ethanol and positioned it on the cork disc in the resting posture characteristic of Anopheles (i.e., with its abdomen tilted upward; see [39]). Mate was an opposite sex conspecific individual (males 4.5 mm in body length, females 5.5 mm) positioned in the normal resting posture for *E. culicivora* (see [26]). Mates were prepared in the same way as prey, but without the preceding blood meal. Flower was a 10-mm wide umbel of *L.camara* flowers positioned with its stem on a cork disc (diameter 5 mm). The stem was cut short so that the umbel draped over the top of the disc.

For preservation, we used a transparent plastic adhesive (Crystal Clear Lacquer, Atsco Australia Pty) to spray mosquitoes and mates, along with the discs on which they were mounted. However, there was no ethanol pre-treatment and no spraying when making flower mounts because we knew from preliminary work that these procedures often distort flower shape and coloration. More details concerning mount-making methods can be found elsewhere (e.g., [40]).

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

As our objective was to look for evidence of specifically selective attention instead of preference (see [7]), we made the visual stimuli either cryptic or conspicuous. We achieved this by varying how close the target was to the plant cutting in display chamber 1. Cryptic mounts were positioned with

their midlines even with the front grooves of display chamber 1 (i.e., close to the plant cutting). Conspicuous targets were positioned with their front ends even with the closest side of the glass base on which the arena sat (i.e., conspicuous targets were out in the open instead of next to the plant cuttings).

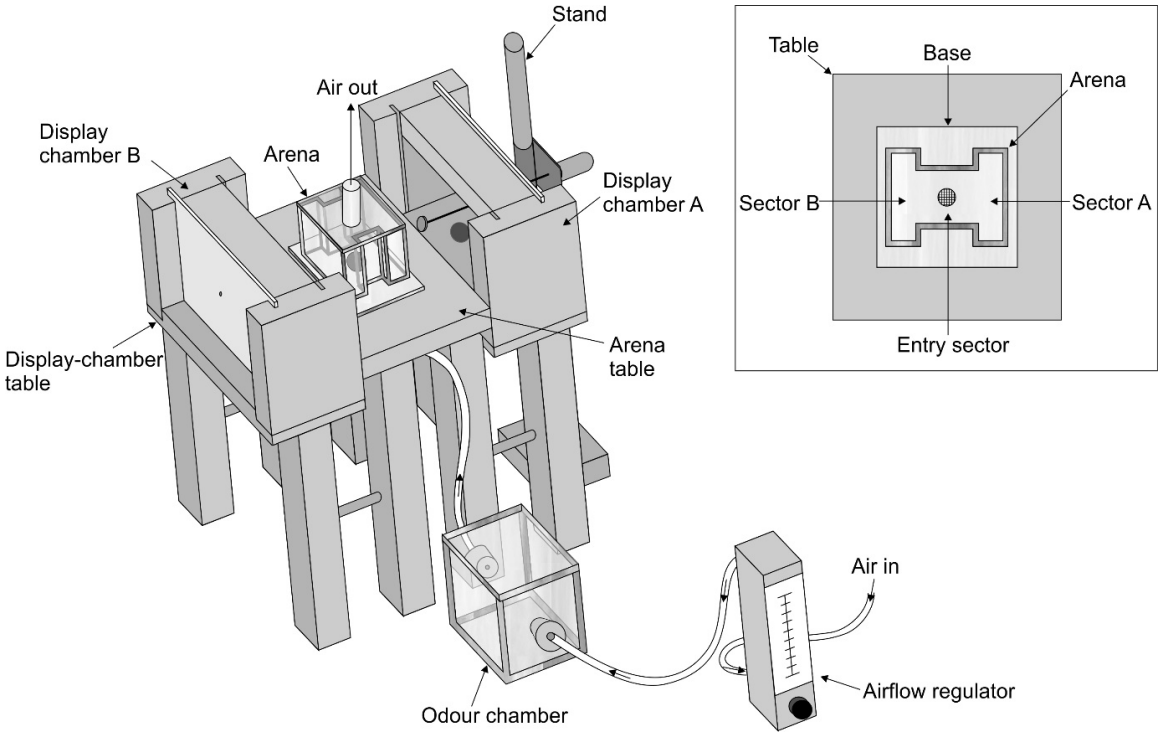
We considered whether an odour triggered selective attention to a particular visual stimulus by comparing test-spider response in no-odour control trials with test-spider response in the trials where odour was congruent with the visual target (e.g., prey odour when the visual target was a prey item). We also considered whether there were trade-off effects by comparing responses in no-odour control trials with responses in trials where odour was incongruent with the visual target (e.g., mate odour when the visual target was prey).

2.5 Objective 3: Odour priming of rapid response to specific visual targets

For this objective, there were two display chambers the arena consisted of three sectors (Fig. 2.3), where the centre sector was narrower (25 mm wide) than the two side sectors (50 mm). The side sectors were designated at random as A and B. The arena's inside height was 37 mm and its total inside length was 70 mm (centre sector 30 mm, each side sector 20 mm).

There was a glass base (80 mm × 80 mm, thickness 3 mm) on which the arena was centred and this base was centred on a wooden table (140 mm × 130 mm). Aligned with and touching opposite ends of the table, there were two wooden display chambers, chamber A facing arena side-sector A and chamber B facing arena side-sector B. Each display chamber

Figure 2.3: Apparatus for objective 3 (see text for details). Test spiders were always adult males of *Evarcha culicivora*



(inner dimensions 100 mm × 100 mm) had two side walls (80 mm wide, wood thickness 20 mm). As for objectives 1 and 2, there were grooves in the walls and the floor of each chamber. There was no glass front in the front grooves, but there was a wooden back in the grooves farthest from the arena.

Each display chamber sat on a display-chamber table, with the height of the arena-table legs and display-table legs arranged so that the top of each chamber was 73 mm above the arena-table top. There was a hole centred in the wooden back of chamber A and a pin, held in place by a clamp and stand behind chamber A, extended through this hole (diameter 8 mm). The end of the pin was pushed into the side of a cork disc holding a prey or mate target, keeping the disc horizontal. The prey or mate was on top of the disc, facing 45o to the left or right (decided at random) from the nearest

wall of the arena. The top of the disc was 23 mm above the top of, and even with the edge of, the arena table. The target was made conspicuous by being positioned so that its front end was 30 mm from the inner side of the nearest wall of arena sector A. In some trials, a *L.camara* cutting (stem, leaves & flowers) was in chamber A and, in other trials, chamber A was empty. Chamber B was always empty (i.e., there were no plant cuttings in chamber B and no associated pin or target).

As with objectives 1 and 2, there was an odour chamber, pump, airflow meter and silicone tubing to deliver specific odours in experiments. An 'inflow hole' was centred in the floor of the arena, with a corresponding hole in the table top, and an 'outflow hole' was centred in the arena lid (diameter of each hole 12 mm). A glass tube (inner diameter 11 mm, outer diameter 12 mm, length 25 mm) was positioned in each arena hole, and one end was flush with the inner side of the arena, while the other end extended out from the arena.

1800 spiders were used across 20 conditions (N=90). During a 15-min pre-exposure period, a test spider was confined to the tube in the outflow hole, with nylon netting over each end. Air moved from the odour chamber through the arena and then through the tube with the test spider inside. At the start of the trial, the nylon netting was removed from the end of the tube farthest from the spider and then this open end was inserted into the outflow hole. If the spider failed to move through the open end of the tube and into the arena of its own accord within 2 min, we removed the netting from the other side of the tube and, using a soft brush, coaxed the spider into the arena. Once the spider was in the arena, we replaced the tube with a rubber stopper.

After entering the arena, the test spider was only allowed 5 min in which

to respond. Response was defined as the spider moving into side sector A and remaining there for 30 s. As a prerequisite for a successful trial, the test spider had to maintain the gaze of its principal eyes on the visual target continuously for a minimum of 15 s.

2.6 Data analysis

For the time-related data from objectives 1 and 2, we used analysis of variance (ANOVA) after first determining that our data met the assumptions of ANOVA (Bartlett's tests for homogeneity of variance and Shapiro-Wilks tests for normal distribution of residuals). In all instances, CI is the 95% confidence interval around the mean. As we made multiple comparisons using the same data, p-values were adjusted using Bonferroni correction. The statistics package R [41] was used for all analysis.

Objective 1

For objective 1, we considered specific questions regarding the interaction of test-spider category, plant species and olfactory stimulus by using a series of six 2-factor ANOVAs. When ANOVA revealed a significant effect, we then carried out *post hoc* Tukey's range tests.

Objective 2

Three specific questions pertaining to the interaction of test-spider category, olfactory stimulus and visual target were considered with the data from objective 2. First, we performed an ANOVA on the data that came from trials in which the visual targets were cryptic and then, for all in-

stances in which ANOVA revealed significant effects, we used t-tests for *post hoc* comparisons. We also used t-tests to compare data from trials in which visual targets were conspicuous with data from trials in which visual targets were cryptic. We used another series of t-tests to compare data from trials in which the visual target and the odour were incongruent with data from the corresponding no-odour control trials.

Objective 3

For objective 3, we first fit a logistic regression with logit links to the binary response data. We used likelihood ratio testing to compare the best-fit regression model with alternative models. Finally, we made *a priori* pairwise comparisons of response rates by carrying out χ^2 tests of independence.

Chapter 3

Results

3.1 Objective 1

Using adults of both sexes and juveniles of all size classes as test spiders, we investigated the influence of caryophyllene on response duration when the plant in display chamber 1 was *L.camara* (Table 3.1). We found a significant effect of caryophyllene odour ($F_{1,228} = 202.200, p < 0.001$), but no significant effect of test-spider category ($F_{5,228} = 1.174, p = 0.336$) and no significant interaction effect between test-spider category and odour ($F_{5,228} = 0.791, p = 0.557$). Compared to the no-odour control, mean response duration was 8 min (CI : 7.1–9.3$min, p < 0.001$) longer in the presence of caryophyllene. On the basis of these findings, we conclude that, for all test-spider categories, caryophyllene increases visual inspection of *L.camara*.

To determine whether the effect of caryophyllene on visual inspection of plants is specific to *L.camara* as the visual stimulus, we used adult males as test spiders in a series of trials with alternative plants in place of *L.camara* (Table 1 3.1). We found a significant effect of caryophyllene on response duration ($F_{1,190} = 151.829, p < 0.001$), but no significant effect

Table 3.1: Objective 1. Response duration (mean +/-SEM) in trials using no odour (control) and caryophyllene odour. Plants used as visual stimuli vary. Juveniles specified by body length (mm). N = 20 for each row

Visual stimulus	Test spider	Response duration (control)	Response duration (caryophyllene)
Lantana camara	Adult male	7.3 +/- 0.80	14.4 +/- 1.17
	Adult female	7.1 +/- 1.14	14.2 +/- 1.06
	Juvenile (1.5)	5.2 +/- 0.65	13.8 +/- 1.19
	Juvenile (2.5)	6.1 +/- 0.59	16.5 +/- 0.92
	Juvenile (3.5)	6.2 +/- 0.65	13.7 +/- 1.44
	Juvenile (4.5)	5.1 +/- 0.72	13.7 +/- 1.22
Lippia kituensis	Adult male	8.0 +/- 0.65	14.6 +/- 1.05
Parthenium hysterophorus	Adult male	6.8 +/- 0.73	13.9 +/- 0.81
Senna didymobotrya	Adult male	7.5 +/- 0.70	15.7 +/- 1.00
Bougainvillea glaba	Adult male	8.1 +/- 0.82	14.3 +/- 1.11
	Adult female	8.8 +/- 0.91	15.3 +/- 1.44
	Juvenile (1.5)	6.7 +/- 0.92	15.0 +/- 1.00

of the plant species used as the visual stimulus ($F_{4,190} = 0.574, p = 0.682$) and no significant interaction effect between plant type and odour type ($F_{4,190} = 0.313, p = 0.869$). Compared to the no-odour control, mean response duration was 7 min ($CI : 5.9–8.1 min, p < 0.001$) longer in the presence of caryophyllene. On the basis of these findings, we conclude that, for adult males, caryophyllene increases visual inspection of not only *L.camara*, but also a wider range of plant species.

When the plant in display chamber 1 was Bougainvillea glaba instead of *L.camara*, we used adult females and small juveniles, as well as adult males (Table 1 3.1), and found a significant effect of caryophyllene odour ($F_{1,114} = 66.353, p < 0.001$), but not of test-spider category ($F_{2,114} = 0.680, p = 0.509$). Compared to the no-odour control, mean response duration was 7 min ($CI : 5.3–8.7 min, p < 0.001$) longer in the presence of caryophyllene.

There was no significant interaction effect between test spider category and odour ($F_{2,114} = 0.559, p = 0.573$). Based on these findings, we conclude that, for small juveniles and adult females, as well as for adult males, caryophyllene's effect on plant-investigation behaviour applies to a range of species wider than just *L.camara*.

To determine whether compounds other than caryophyllene have odour effects on the visual inspection of *L.camara*, we used adult males and females in trials in which, besides caryophyllene, we used cineole and humulene as the odour source (Tables 3.1 and 3.2). We found a significant effect of the compound ($F_{3,152} = 31.246, p < 0.001$), but no significant effect of the test-spider category ($F_{1,152} = 0.009, p = 0.923$) and no significant interaction effect between test-spider category and compound ($F_{3,152} = 0.078, p = 0.972$). Compared to no-odour controls (Table 3.1), mean response duration was significantly longer ($7min, CI : 4.4$–$10.0min, p < 0.001$) in the presence of humulene; mean response duration in the presence of cineole was not significantly different from the no odour control ($33s$ shorter, $CI : -2.2$–$3.3min, p = 0.956$). Mean response duration was significantly longer ($8min, CI : 4.9$–$10.5min, p < 0.001$) in the presence of caryophyllene (Table 3.1) than in the presence of cineole (Table 3.2). Mean response duration in the presence of humulene (Table 3.2) was not significantly different from mean response duration in the presence of caryophyllene (Table 3.1) ($8s, CI : -2.7$–$2.9min, p = 0.999$). Mean response duration was significantly longer ($8min, CI : 5.0$–$10.6min, p < 0.001$) in the presence of humulene than in the presence of cineole. On the basis of these findings, we conclude that humulene as well as caryophyllene, but not cineole, influences the visual inspection behaviour of adult test spiders when the plant is *L.camara*.

Using prey (Table 3.2) instead of caryophyllene as the odour source,

Table 3.2: Objective 1. Response duration (mean +/- SEM) in trials using odours other than caryophyllene. Visual stimulus always Lantana camara. Juveniles specified by body length (mm). N = 20 for each row

Odour	Test spider	Response duration
Cineole	Adult male	6.3 +/- 1.01
	Adult female	6.9 +/- 0.95
Humulene	Adult male	14.6 +/- 1.42
	Adult female	14.2 +/- 1.08
Prey	Adult male	6.7 +/- 0.62
	Adult female	7.1 +/- 0.97
	Juvenile (1.5)	5.2 +/- 0.90
	Juvenile (4.5)	7.9 +/- 0.73
Mate	Adult male	8.6 +/- 0.83
	Adult female	7.9 +/- 0.88

we considered whether odour effects on the visual inspection of *L.camara* are specific to plant-related odour. We used adult males, adult females, small juveniles and large juveniles as test spiders and compared data from the no-odour control trials with data from trials during which prey odour was present (Table 3.1). We found no significant effects of prey odour ($F_{1,152} = 0.116, p = 0.734$) or test-spider category ($F_{3,152} = 0.992, p = 0.398$) on visual inspection of *L.camara* and no interaction effect between test-spider category and odour ($F_{3,152} = 1.711, p = 0.167$).

As another step toward considering whether odour effects on visual inspection of *L.camara* are specific to plant-related odour, we used a mate (Table 3.2) as the odour source. We used adult males and females as test spiders and compared data from the no-odour control trials with data from trials during which mate odour was present (Table 3.1). We found no significant effect of mate odour ($F_{1,76} = 1.360, p = 0.247$) or test-spider category ($F_{1,76} = 0.294, p = 0.589$) on visual inspection of *L.camara*, and no significant interaction effect between test-spider category and odour ($F_{1,76} = 0.074, p = 0.787$). On the basis of these findings, we conclude that the odour that

motivates *E. culicivora* to spend more time visually inspecting *L.camara* is specifically an odour from a plant.

3.2 Objective 2

Objective 2 determined whether odour primes selective attention. In these experiments, there was a plant in each of the four display chambers, with an additional visual target associated with only one of these chambers. First we considered instances in which specific odours are congruent with the visual targets, where the visual target is cryptic. Next, we compared trials in which the visual target is conspicuous with trials in which the visual target is cryptic, and, the odour and visual target are congruent. Finally, we considered trials in which the visual targets and odours are incongruent.

When the cryptic visual targets were *L.camara* flowers (Table 3.3) and either there was no odour (control) or there was a congruent odour source (i.e., caryophyllene), test-spider category ($F_{3,266} = 12.068, p < 0.001$) and odour ($F_{3,266} = 28.741, p < 0.001$) were significant main effects and there was also a significant interaction effect between test-spider category and odour ($F_{7,266} = 4.378, p < 0.001$). For juveniles, response durations were significantly longer in the presence of caryophyllene than in the no-odour control trials (small juveniles: $t_{34} = 4.175, p < 0.001$; large juveniles: $t_{38} = 4.013, p < 0.001$). However, the response duration of adults when they were in the presence of caryophyllene was not significantly different from their response durations in the no-odour control trials (males: $t_{37} = 0.315, p = 0.755$; females: $t_{31} = 0.722, p = 0.475$).

When the cryptic visual targets were prey items (Table 3.4), and either there was no odour (control) or there was a congruent odour source (i.e.,

Table 3.3: Objective 2. Response duration (mean +/- SEM) in trials using cryptic flowers. Odour sources vary. Juveniles specified by body length (mm). N = 20 for each row

Odour	Test spider	Response duration
No odour (control)	Adult male	7.5 +/- 1.02
	Adult female	6.6 +/- 0.80
	Juvenile (1.5)	7.9 +/- 0.78
	Juvenile (4.5)	8.7 +/- 0.98
Caryophyllene	Adult male	7.9 +/- 0.88
	Adult female	7.2 +/- 0.48
	Juvenile (1.5)	13.7 +/- 1.13
	Juvenile (4.5)	14.3 +/- 1.01
Prey (incongruent)	Juvenile (1.5)	4.7 +/- 0.88
	Juvenile (4.5)	5.6 +/- 0.89

prey), there was a significant main effect of odour ($F_{3,266} = 80.844, p < 0.001$), but not of test-spider category ($F_{3,266} = 2.390, p = 0.069$), and there was no significant interaction effect ($F_{7,266} = 0.458, p = 0.865$). After pooling data for all test-spider categories, we found that response durations in the presence of prey odour were significantly longer than in the no-odour control trials ($t_{158} = 7.082, p < 0.001$).

When the cryptic visual target was a mate (Table 3.5), and either there was no odour (control) or the odour source was either mate or caryophyllene, there were no significant interaction effects ($F_{3,152} = 0.110, p = 0.954$), but there was a significant main effect of odour ($F_{3,152} = 44.590, p < 0.001$) and a significant main effect of test-spider category ($F_{1,152} = 18.140, p < 0.001$). Mean response durations were longer for adult males than for adult females (No odour: $\Delta = 54s$, caryophyllene: $\Delta = 42s$). Caryophyllene and mate odour had similar effects, that is, response durations were longer in the presence of mate odour than in the no-odour controls for males ($t_{38} = 3.208, p = 0.003$) and for females ($t_{36} = 2.945, p = 0.006$). Response

Table 3.4: Objective 2. Response duration (mean +/- SEM) in trials using cryptic prey. Odour sources vary. Juveniles specified by body length (mm). N = 20 for each row

Odour	Test spider	Response duration
No odour (control)	Adult male	11.9 +/- 1.23
	Adult female	10.8 +/- 1.02
	Juvenile (1.5)	9.1 +/- 1.26
	Juvenile (4.5)	8.7 +/- 1.21
Prey	Adult male	18.1 +/- 1.08
	Adult female	17.6 +/- 1.01
	Juvenile (1.5)	13.9 +/- 1.36
	Juvenile (4.5)	14.7 +/- 0.99
Caryophyllene (incongruent)	Adult male	6.1 +/- 0.87
	Adult female	7.1 +/- 0.73
	Juvenile (1.5)	4.6 +/- 0.77
	Juvenile (4.5)	5.2 +/- 0.66
Mate (incongruent)	Adult male	7.6 +/- 0.67
	Adult female	6.9 +/- 0.91

durations were also longer in the presence of caryophyllene odour than in the no-odour controls for males ($t_{38} = 4.847, p < 0.001$) and for females ($t_{37} = 36.553, p < 0.001$). Moreover, response duration in the presence of mate odour was not significantly different from response duration in the presence of caryophyllene odour for males ($t_{38} = 1.474, p = 0.149$) nor for females ($t_{38} = 1.985, p = 0.054$). This is evidence that caryophyllene, like mate odour, is congruent when the visual stimulus is a mate.

When the visual targets were cryptic, we found in all instances that response duration was significantly longer in the presence of a congruent odour than in the no-odour control trials. This was as predicted by our

Table 3.5: Objective 2. Response duration (mean +/- SEM) in trials using cryptic mate. Odour sources vary. N = 20 for each row

Odour	Test spider	Response duration
No odour	Adult male	12.1 +/- 1.09
	Adult female	9.3 +/- 0.86
Mate	Adult male	17.1 +/- 1.11
	Adult female	13.4 +/- 1.08
Caryophyllene	Adult male	19.3 +/- 1.02
	Adult female	16.4 +/- 1.05
Prey (incongruent)	Adult male	8.6 +/- 0.96
	Adult female	6.0 +/- 0.70

selective attention hypothesis. Our findings when using conspicuous prey as visual targets were also as predicted by our selective attention hypothesis. When visual targets were conspicuous (i.e., when they could be easily detected and identified), response duration in the presence of congruent odour and response duration in no-odour controls were not significantly different (Table 3.6): flower as the visual target (small juveniles: $t_{38} = 1.313, p = 0.197$); prey as the visual target (adult males: $t_{37} = 0.256, p = 0.793$; small juveniles: $t_{37} = 1.466, p = 0.151$); mate as visual target (adult males, mate odour: $t_{37} = 1.345, p = 0.187$; caryophyllene odour: $t_{38} = 0.776, p = 0.442$). When conspicuous mates were the visual targets, we also found no significant differences in test spider response duration in the presence of mate odour and test spider response duration in the presence of caryophyllene odour ($t_{37} = 0.521, p = 0.605$).

In the no-odour control trials, response duration was significantly longer when the visual target was conspicuous instead of cryptic: for small juveniles, when flowers were the visual targets ($t_{34} = 4.567, p < 0.001$) (Tables 3.3 and 3.6); for small juveniles, when prey was the visual target ($t_{38} = 2.746, p =$

Table 3.6: Objective 2. Response duration (mean +/- SEM) in trials using conspicuous visual stimuli. Odour sources vary. Juveniles were small (1.5 mm). N = 20 for each row

Visual stimulus	Test spider	Odour	Response duration
Flower	Juvenile	No odour	14.2 +/- 1.13
		Caryophyllene	16.2 +/- 1.02
Prey	Adult male	No odour	16.4 +/- 0.87
		Prey	16.1 +/- 0.99
	Juvenile	No odour	13.8 +/- 1.17
		Prey	16.1 +/- 1.04
Mate	Adult male	No odour	18.7 +/- 0.60
		Caryophyllene	18.0 +/- 0.58

0.009) (Tables 3.4 and 3.6); for adult males, when prey was the visual target ($t_{33} = 2.912, p = 0.006$); for adult males, when a mate was the visual target ($t_{30} = 5.307, p < 0.001$) (Tables 3.5 and 3.6). These findings corroborate that the procedure we adopted for making the visual target 'conspicuous' made the visual target easier to find.

Findings when we used incongruent odours (Tables 3.3, 3.4 & 3.5) were as predicted by our hypothesis that tasks requiring selective attention are accompanied by substantial trade-off effects. When the cryptic visual targets were flowers (Table 3.3), response durations of juveniles were significantly shorter in the presence of prey odour (incongruent) than in the no-odour control trials (small juveniles: $t_{38} = -2.748, p = 0.009$; large juveniles: $t_{38} = -2.322, p = 0.026$). Similar comparisons using adults were not made because, for adults tested with cryptic flowers, no significant differences were found when response durations in the presence of congruent odour (i.e., caryophyllene) were compared to response in the no-odour control trials. When cryptic prey was the visual target (Table 3.4), response durations for adult test spiders were significantly shorter in the presence of mate odour (incongruent) than in the no-odour controls (males:

$t_{28} = 3.002, p = 0.006$; females: $t_{38} = -7.872, p = 0.008$). Also, when cryptic prey was the visual target and we used pooled data for all test-spider categories, we found that response duration was significantly shorter in the presence of caryophyllene than in the no-odour controls ($t_{135} = 6.089, p < 0.001$). When the visual target was a cryptic mate (Table 3.5), response durations of adults were significantly shorter in the presence of prey odour (incongruent) than in the no-odour controls (males: $t_{37} = -2.375, p = 0.023$; females: $t_{36} = -3.017, p = 0.005$).

3.3 Objective 3

Baseline response rates, established from the no odour control conditions, show that adult male spiders responded most strongly to the Mate + *L.camara* visual stimulus (51.1%), and with decreasing strength to Mate (43.3%), Prey (36.7%), Prey + *L.camara* (30.0%) and *L.camara* (26.7%) when no odour was presented.

The best-fit logistic model was

$$P(response|OxV) = \frac{logit(p)}{1 + logit(p)}$$

where $logit(p)$ is given by

$$logit(p) = e^{O+V+O_xV-1.01}$$

and $P(response)$ is the proportion of spiders that would be expected to respond to a visual stimulus, V, in the presence of an odour, O. e is the base of the natural logarithm, $_O$ is the coefficient of O, $_V$ is the coefficient of V, and $_{OxV}$ is the coefficient of the interaction between O and V (for

coefficients, see Table 3.7).

Using this model, we determined that odour ($deviance_{df=3} = 44.64, p < 0.001$) and visual target ($deviance_4 = 74.43, p < 0.001$ are significant predictors of response rate (main effects). We also found a significant interaction effect ($deviance_{12} = 130.97, p < 0.001$), indicating that the specific combination of odour and visual target influenced test-spider response rate.

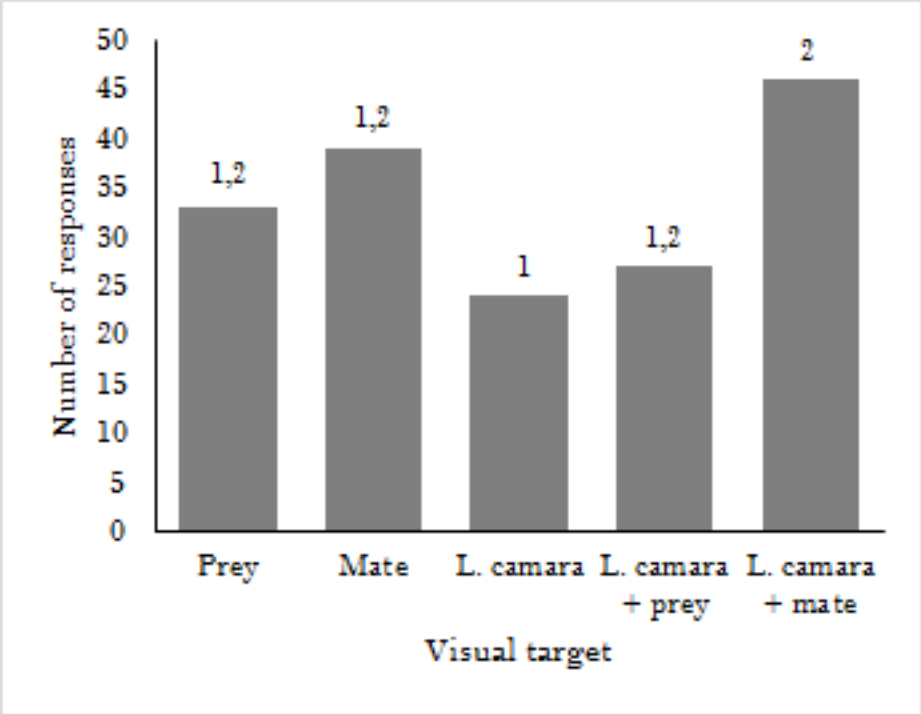
Table 3.7: Coefficients from for best-fit logistic regression (objective 3). SE: standard error of coefficient.

Factor	Term	Coefficient	SE
Odour source	Nil (intercept)	-1.01	0.24
	Caryophyllene (Car.)	0.27	0.21
	Mate	-0.50	0.22
	Prey	-0.79	0.23
Visual target	Plant+mate	1.06	0.32
	Plant+prey	0.16	0.33
	Mate	0.74	0.32
	Prey	0.47	0.32
Interaction	O: Car., V: plant+mate	0.87	0.52
	O: Mate, V: plant+mate	0.40	0.45
	O: Prey, V: plant+mate	-0.35	0.44
	O: Car., V: plant+prey	-0.16	0.45
	O: Mate, V: plant+prey	-0.36	0.46
	O: Prey, V: plant+prey	1.04	0.46
	O: Car., V: mate	-0.37	0.44
	O: Mate, V: mate	1.01	0.46
	O: Prey, V: mate	-0.59	0.45
	O: Car., V: prey	-1.63	0.45
	O: Mate, V: prey	-0.71	0.45
	O: Prey, V: prey	1.07	0.46

For no odour control trials (Fig. 3.1), there were no significant differences between adjacent pairs of visual stimulus (when ranked by response strength): *L.camara* + mate and Mate ($\chi^2 = 2.179, p = 0.140$), mate and prey ($\chi^2 = 1.629, p = 0.202$), prey and *L.camara* + prey ($\chi^2 = 1.723, p = 0.189$), prey + *L.camara* and *L.camara* ($\chi^2 = 0.476, p = 0.490$). However, there were signifi-

cant differences between mate + *L.camara* and prey ($\chi^2 = 7.515, p = 0.006$), mate and prey + *L.camara* ($\chi^2 = 6.516, p = 0.011$), prey and *L.camara* conditions ($\chi^2 = 3.876, p = 0.049$), indicating a slight increase in response across these stimuli.

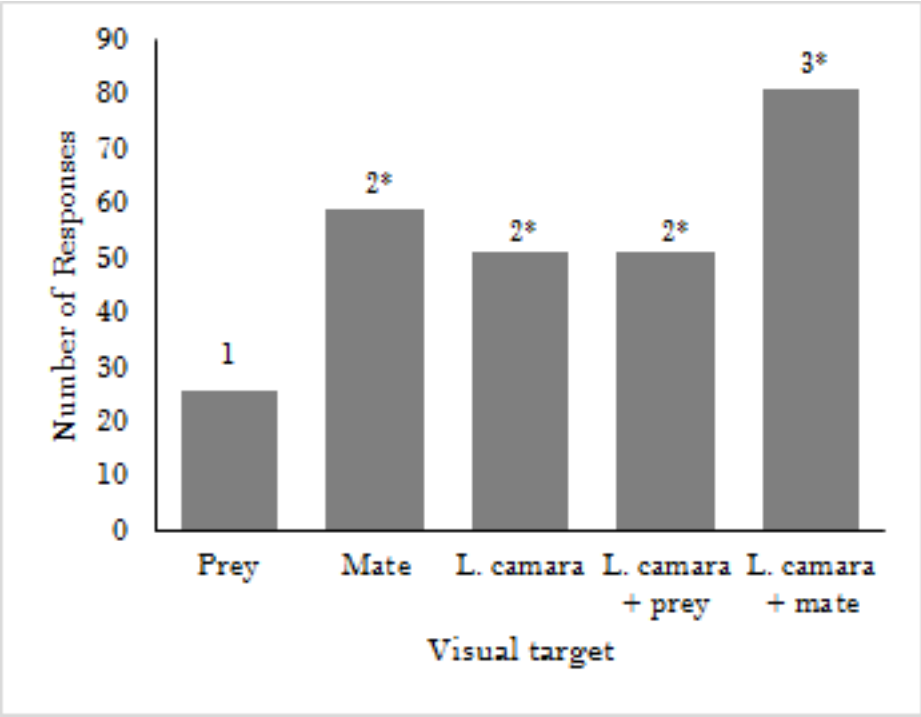
Figure 3.1: Objective 3: Number of responses to each visual target in no odour control trials. Homogenous subsets are indicated by numbers above each bar, i.e., number of responses to visual stimuli in the same subset were not significantly different from one another.



For each visual target, we found that, whenever the visual target included a mate, a *L.camara* cutting or both, response rates in the presence of caryophyllene (Fig. 3.2) were significantly greater than the corresponding baseline response rates (mate alone: $\chi^2 = 18.1, p < 0.001$; *L.camara* alone: $\chi^2 = 41.42, p < 0.001$; *L.camara* + prey: $\chi^2 = 30.476, p < 0.001$; *L.camara* + mate: $\chi^2 = 54.471, p < 0.001$), but response rates in the presence of caryophyllene were not significantly different from baseline when the visual target was prey alone ($\chi^2 = 2.345, p = 0.126$). Moreover, in the presence of

caryophyllene, significantly more test spiders responded to *L.camara* + mate than to mate alone ($\chi^2 = 59.753, p < 0.001$) and significantly more responded to *L.camara* + prey than to prey alone ($\chi^2 = 28.281, p < 0.001$), but the number that responded to *L.camara* + prey was equal to the number that responded to *L.camara* alone ($\chi^2 = 0.000, p = 1.000$). These findings indicate that caryophyllene prepares male test spiders to respond rapidly to mates and to *L.camara*, but not to prey.

Figure 3.2: Objective 3: Number of responses to each visual target in caryophyllene odour trials. Homogenous subsets are indicated by numbers above each bar, * indicates that number of responses in odour trial was significantly different from the corresponding no odour control.



Using caryophyllene odour (Fig. 3.2), we found no significant difference between the number of males that responded rapidly to a scene in which there was only a mate and the number that responded rapidly to a scene in which there was only *L.camara* ($\chi^2 = 3.149, p = 0.076$) but significantly more males responded rapidly to *L.camara* + mate than to a scene in which

L.camara was by itself ($\chi^2 = 111.110, p < 0.001$).

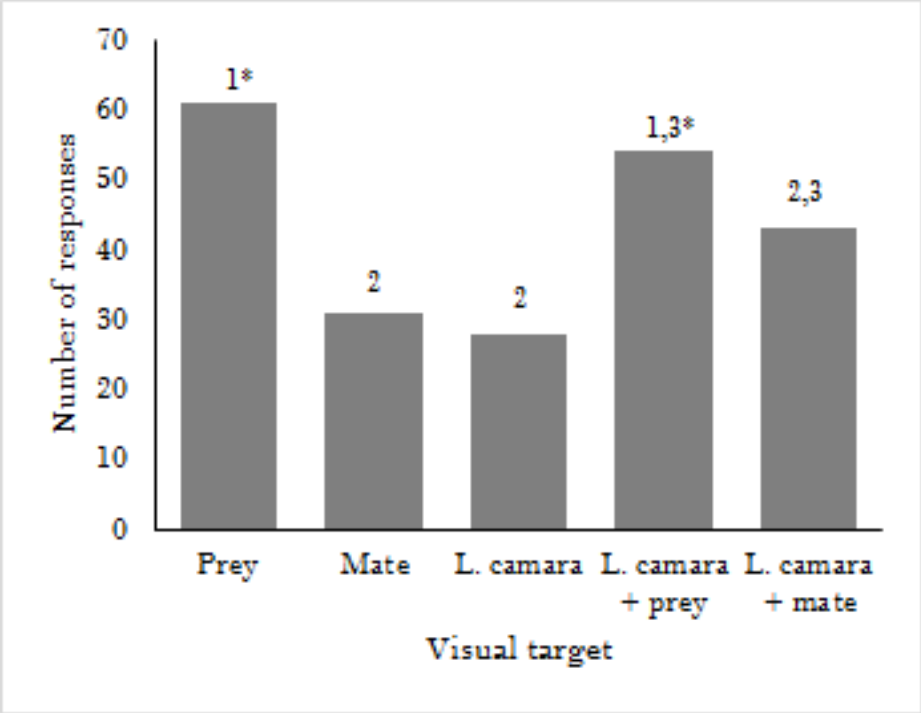
The number of test spiders that responded rapidly in the presence of prey odour (Fig. 3.3) was significantly greater than the corresponding baseline response rates when the visual target was prey alone ($\chi^2 = 37.512, p < 0.001$) or *L.camara* + prey ($\chi^2 = 38.571, p < 0.001$), but not when the visual target was a mate alone ($\chi^2 = 2.896, p = 0.089$), *L.camara* alone ($\chi^2 = 0.909, p = 0.340$) or *L.camara* + mate ($\chi^2 = 0.400, p = 0.527$). These findings suggest that prey odour prepares males to respond rapidly to prey, but whether the prey is with *L.camara* is irrelevant. Moreover, these findings suggest that prey odour has no effect on preparedness to respond rapidly to mates or to *L.camara* alone.

In the presence of prey odour (Fig. 3.3), significantly more test spiders responded rapidly when the visual target was *L.camara* + mate than when the visual target was a mate alone ($\chi^2 = 6.413, p = 0.011$) but there was no significant difference between prey alone and *L.camara* + prey ($\chi^2 = 2.493, p = 0.114$) or a mate alone and *L.camara* alone ($\chi^2 = 0.443, p = 0.506$).

Compared to baseline response rates, significantly more test spiders responded rapidly in the presence of mate odour (Fig. 3.4) when the visual target was a mate alone ($\chi^2 = 43.484, p < 0.001$), *L.camara* alone ($\chi^2 = 5.682, p = 0.017$) or *L.camara* + mate ($\chi^2 = 16.052, p < 0.001$), but not when it was a prey item ($\chi^2 = 0.766, p = 0.382$) or *L.camara* + prey ($\chi^2 = 0.476, p = 0.49$).

In the presence of mate odour (Fig. 3.4), significantly more test spiders responded rapidly to *L.camara* + mate than to *L.camara* alone ($\chi^2 = 53.225, p < 0.001$), but there were no significant differences for prey alone compared to *L.camara* + prey ($\chi^2 = 0.050, p = 0.823$), prey alone compared to *L.camara* alone ($\chi^2 = 1.182, p = 0.277$), *L.camara* alone compared to *L.camara* + prey ($\chi^2 = 0.756, p = 0.385$) or mate alone versus *L.camara* + mate ($\chi^2 =$

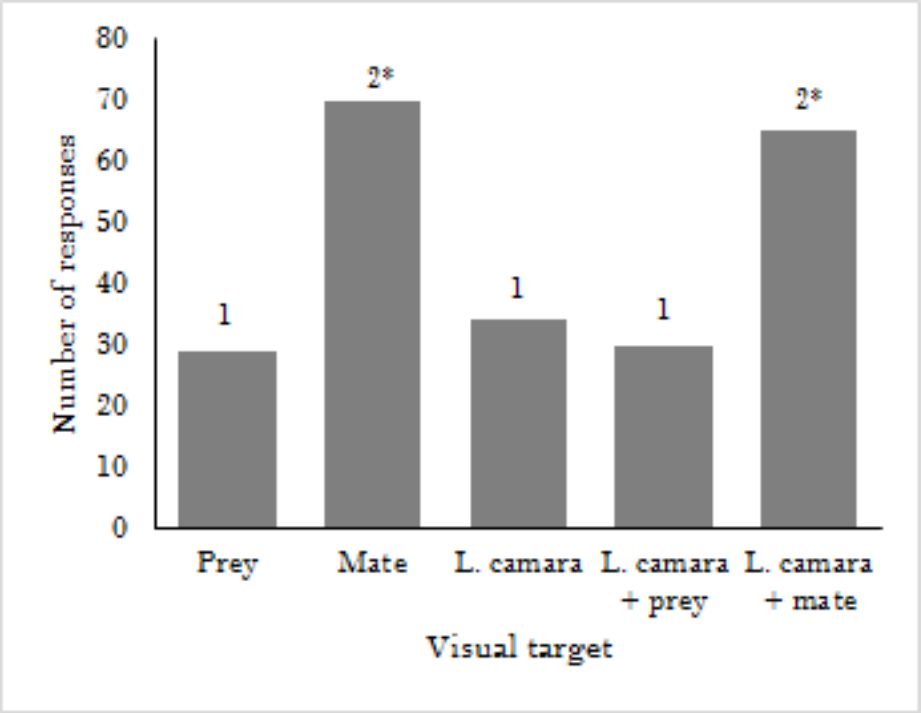
Figure 3.3: Objective 3: Number of responses to each visual target in prey odour trials. Homogenous subsets are indicated by numbers above each bar, * indicates that number of responses in odour trial was significantly different from the corresponding no odour control.



$1.607, p = 0.205$).

On the whole, these findings confirm our predicitons. Odour congruent with the visual target prepares adult males to respond rapidly to the visual target, and caryophyllene prepares adult males to respond rapidly to mates and especially mates in the presence of a plant. Evidence that caryophyllene odour is congruent with mate visual stimulus show that the odour of plants is innately linked with mate finding in the context of selective attention.

Figure 3.4: Objective 3: Number of responses to each visual target in mate odour trials. Homogenous subsets are indicated by numbers above each bar, * indicates that number of responses in odour trial was significantly different from the corresponding no odour control.



Chapter 4

Discussion

Natural scenes contain more information than any sensory system can process at a given time. As such, animals must make efficient use of their capacity for sensory processing. *E. culicivora* uses selective attention to overcome the cognitive limitations associated with using an especially small brain to interact with a vastly complex environment. In particular, adult *E. culicivora* demonstrate selective attention to mates after exposure to mate odour [19], and both adults and juveniles show selective attention to prey after exposure to prey odour [40].

It is clear from natural history observations that *E. culicivora* makes use of plants. Adults frequently encounter opposite sex conspecifics – potential mates – on the leaves of *L.camara*. Experimental results have shown that juveniles feed on nectar, and that this nectar feeding is particularly beneficial in that it makes the spider more effective at subduing the mosquitoes they attack. All life-cycle stages of this spider are attracted to the odour of at least two plant species in laboratory olfactometer experiments [19].

Here, we have extended our understanding of plant use by *E. culicivora* in a way that pertains to cognition. We demonstrated, for the first time, that

plant odour has priming effects in three specific contexts: visual inspection of plants, i.e. moving toward and directing gaze toward plants (objective 1), selective visual attention to specific visual targets (objective 2) and rapid response to specific visual targets (objective 3).

A possible explanation for *E. culicivora*'s behaviour is that salient odours in general elevate *E. culicivora*'s motivation to respond to salient visual stimuli in general. We know that *E. culicivora* juveniles and adults respond to prey odour [40] and that *E. culicivora* adults respond to mate odour [19]. This hypothesis would predict increased response to all salient visual stimuli after exposure to any salient odour. On the contrary, I only found increased responses when particular visual targets were presented with particular odours.

Caryophyllene, humulene and cineole are the most abundant odour compound in the headspace of *L.camara* in Kenya [20]. In olfactometer experiments, *E. culicivora* is known to detect and move toward these compounds [20], but not the other dominant compounds from *L.camara*'s headspace. In this study we found that exposure to caryophyllene, or humulene, but not to cineole increased visual inspection of *L.camara*. That caryophyllene and humulene had similar effects is unsurprising, as humulene and caryophyllene are closely-related compounds that tend to be found together [42]. Both of these compounds, especially caryophyllene, are commonly found in plants in general. Although adult *E. culicivora* appears to be interested in cineole, lack of evidence for a priming effect by cineole suggests that caryophyllene and humulene may be the only compounds that *E. culicivora* associates with *L.camara*.

Since *L.camara* is the plant on which *E. culicivora* is most commonly found in the wild, most of my experiments used *L.camara* as the visual

plant stimulus. However, when I used another four plant species as alternatives to *L.camara*, we found that caryophyllene also increased the visual inspection of these plants. These findings suggest that, for *E. culicivora*, the plant investigation effect of caryophyllene is not specific to cases in which *L.camara* is the plant. Although *L.camara* might currently be the dominant plant species encountered by *E. culicivora* in the field, I assume this is a recent development, since Lantana camara is an introduced species from the American tropics [43], whereas *E. culicivora* is only known to live in East Africa [13].

In objective 1, we found that *E. culicivora* responds to plant odour by visually inspecting plants. Objective 2, where we investigated selective attention, can be thought of as asking what *E. culicivora* is looking for when visually inspecting plants.

Selective attention is usually characterized as a capacity-limited task [44]. To test the spider's response at the upper end of its capacity to identify visual stimuli, I presented test spiders with visual targets that were difficult to discern and identify (i.e. 'cryptic'). When I compared response duration to these targets in trials in which an odour was presented with findings from trials in which no odour was presented, I found that only specific odours increased the spider's response.

I first showed that adults responded to cryptic mates more strongly in the presence of mate odour than in the no odour control, and both adults and juveniles responded more strongly to cryptic prey in the presence of prey odour than in the no odour control. These results confirm that adults selectively attend to mates after priming by mate odour, and both adults and juveniles selectively attend to prey after priming by prey odour. Additionally, this step serves to validate the methodology used here, as it differs

subtly from that used in previous studies. As is typical in selective attention research, previous studies have temporally separated the odour and visual stimuli, with the priming stimulus presented before the cryptic test stimulus(eg. [38]). In this study spiders were exposed to odour and visual stimuli concurrently. Given that our results matched those obtained using the alternate methodology, we should consider this methodology acceptable, and perhaps even a move toward more ecologically relevant testing.

The question of which cryptic visual targets the spider responded more strongly to when caryophyllene was present was of particular interest. When juveniles were presented with cryptic flowers and when adults were presented with cryptic mates, I found significantly stronger response in the presence of caryophyllene odour than in the corresponding no-odour control trials. None of these effects were evident when the visual targets were conspicuous. Responses to conspicuous visual targets were stronger than responses to cryptic visual targets, indicating that we succeeded in making our cryptic targets difficult for the spiders to find.

I found additional evidence of selective attention when we paired cryptic visual targets with incongruent odours. Consistent with attention trade-off effects demonstrated in other studies, responses to cryptic visual targets in the presence of incongruent odour were weaker than responses in the no-odour control trials. Selective attention to one target impairs the spider's ability to detect or identify incongruent, but otherwise salient, stimuli.

The results from objective 2 show a clear difference between adult and juvenile plant use. Evidence that, for juveniles, exposure to caryophyllene triggers selective attention to *L.camara* flowers, supports the hypothesis that juveniles are attracted to plants as a source of nectar, since *L.camara* nectar is obtained primarily from on and around the flowers. As a coun-

terpoint, there was no evidence for selective attention to flowers by adult spiders, indicating that adult spiders interest in plants is not related to nectar feeding.

On the other hand, evidence that, for adults, exposure to caryophyllene triggers selective attention to mates, supports the hypothesis that adults are attracted to plants as a location to encounter mates. This result is rather more unusual than any of the previously described instances of selective attention. In all other cases, the visual stimulus for which selective attention occurs is the source of the odour that triggers that selective attention, i.e. mate odour primes for selective attention to mates, prey odour triggers selective attention to prey, and odour from *L.camara* (caryophyllene) primes for selective attention to *L.camara* flowers. However, here we have found that caryophyllene primes the spider for an encounter with a potential mate, that is, the odour in this case is from a source that is distinct from the target of selective attention.

With evidence for plant odours eliciting investigation of plants, and furthermore, triggering selective attention to plants and other specific visual targets in place, we can now consider objective 3. In this study I found the presence of a particular odour affects the number of spiders that respond rapidly to visual targets and visual scenes.

When no odour was presented, more male spiders responded rapidly to a scene including *L.camara* and a mate, than to *L.camara* alone, or *L.camara* with a prey item. Consistent with the hypothesis that *E. culicivora* adults use plants as mating sites (see [26]), this suggests that adult males have a default tendency to respond rapidly to seeing a mate associated with a plant.

I found a systematic pattern in rate of response to a visual target in

the presence of a particular odour compared to rate of response to the same visual target in no-odour control trials (Figs 3.1 - 3.4). Test spiders were more responsive when they viewed prey while in the presence of prey odour instead of no odour, and I did not find any effects of prey odour when test spiders viewed a scene that did not include prey. Test spiders were also more responsive when they viewed a mate while in the presence of mate odour instead of no odour, and I found no significant effects of mate odour when test spiders viewed a scene that did not include a mate. However, the effect of caryophyllene odour was especially interesting, as test spiders were more responsive when they viewed a scene that included *L.camara* or a mate, but not when they viewed a prey item alone.

Caryophyllene is a sesquiterpene, and terpenes in general are synthesized by plants but not by animals [45]. There is evidence that some arthropods store plant derived compounds, eg. males of euglossine bees store volatile compounds from orchid flowers and use this odour to attract females [46]. We might propose that *E. culicivora* adults acquire and store caryophyllene when visiting plants, and then later release this compound as a sex pheromone. I would predict, if this hypothesis were true, that exposure to caryophyllene odour would cause spiders to be more responsive to a mate alone than to *L.camara* alone. In this study, I found no difference between these conditions. I also found that, in the presence of caryophyllene odour, rate of response to seeing a mate associated with *L.camara* was significantly higher than to seeing a mate that was not associated with *L.camara*. These findings suggest that caryophyllene odour is relevant to *E. culicivora* adults specifically in the context of seeing potential mates that are associated with plants.

Further evidence supporting the special relationship between plant and

mate stimuli is that the effects of caryophyllene and mate odour were not identical. In the presence of mate odour, spiders were more responsive to seeing a mate to seeing *L.camara*. Furthermore, the number of test spiders that responded rapidly to seeing a mate associated with *L.camara*, in the presence of mate odour, was not significantly different from the number that responded rapidly to seeing a mate by itself. The difference between the effects of caryophyllene and mate odour supports our hypothesis that for *E. culicivora* adults, *L.camara* is relevant specifically in the context of mating. This is consistent with findings from objective 1 that suggested that adult spiders become especially motivated to inspect a plant when in the presence of caryophyllene odour but not when in the presence of mate odour, and also with findings from objective 2 suggesting that, for the adults of *E. culicivora*, caryophyllene odour and mate odour are both stimuli that elicit selective visual attention to mates

By contrast, there was no evidence of caryophyllene affecting how many spiders responded rapidly to seeing prey. Based on our understanding of mosquito behaviour, there is no clear rationale for proposing that *L.camara*, or other plants that release caryophyllene, are relevant to *E. culicivora* as sites for encountering preferred prey (i.e., blood-carrying female anopheline mosquitoes). Although both sexes of Anopheles species are known to visit plants and feed on nectar [47, 48], they primarily do so at night [49, 50] and blood-carrying females of anthropophilic Anopheles species tend to rest on the walls of human dwellings while digesting blood [39] instead of visiting plants for nectar or for resting.

I take these results together to conclude that use of plants by *E. culicivora* is mediated by plant odours in a way that is consistent with use of selective attention, and the target of attention associated with plants

changes over the lifespan of the spider. Since all test spiders were naïve to the experimental stimuli, these results suggest that the *E. culicivora* have an innate capacity for selective attention with regard to their interaction with plants. While in this study I show that this selective attention occurs cross-modally, at least in one direction, previous studies have shown that cross modal selective attention by *E. culicivora* in other domains can occur bi-directionally.

Selective attention is thought to work by allowing more cognitive resources to be allocated to processes associated with a recently encountered stimulus [44, 21]. When *E. culicivora* encounters plant odour, resources are allocated to processing congruent stimuli. When the spider is in this state, it selectively attends to plant related stimuli. The difference in behavior between adult and juvenile *E. culicivora* is accompanied by a corresponding difference in the cognitive mechanism - the specific way the selective attention mechanism works. That is, as the character of *E. culicivora*'s attraction to plants shifts from hunger-dependent in juveniles, to hunger independent in adults, the target of selective attention triggered by plant odour also changes over the lifespan of the spider.

When a juvenile encounters plant odour, more resources are allocated to processes associated with plants and inflorescences. That is, exposure to plant odour causes juveniles to selectively attend to a specific target on the plant, the *L.camara* umbel. The umbel is relevant to juveniles since they are attracted to plants as a source of nectar, and *L.camara* nectar is obtained primarily from on and around the flowers. The behaviour that follows is plant investigation in the context of food seeking, leading to nectar feeding. Conversely, when an adult encounters plant odour, it becomes selectively attentive to plants and potential mates. The behavior that follows is plant

investigation in the context of mate seeking, leading to potential courtship, and eventually, copulation.

The different behavioural outcomes, obtained by adult and juvenile spiders after processing the same initial stimulus, are indicative of the ecological needs of the spider at each life stage. Published work shows that juveniles feed on nectar [27], and this nectar feeding is integral to their overall nutritional strategy by way of improved prey capture [36]. Given these findings, it is reasonable to suppose that evolutionary selection for a cognitive mechanism, linking general plant stimuli to the specific inflorescence stimulus, should occur.

Adult spiders, on the other hand, without the handicap of small size that limits juveniles, are proficient at capturing prey. Appearing not to need the additional energy provided to juveniles by nectar feeding, adults are not known to commonly feed on plants. As a result, there is no evolutionary pressure for adult spiders to have the capability to better identify inflorescences after having encountered a plant stimulus.

Adult *E. culicivora* have an impressive repertoire of courtship behaviour that comprises a complex reproductive strategy [26]. However, courtship and copulation, of course, are predicated on finding a mate. Using selective attention for plants and mates in combination should increase the likelihood that a spider on a plant will identify another spider on the same plant, which at the very least, allows for the possibility for reproductive success.

The results of this study show that use of selective attention to plant odour cues benefit the spider in two main ways. First, they are able to overcome cognitive constraints to better perform difficult search tasks, as demonstrated by objective 1, and second, they are able to detect and identify relevant salient stimuli more quickly. Although these facets of selec-

tive attention initially appear to be intertwined, they are, in fact, the results of two distinct elements of the spider's evolutionary history. Reduced processing power is an inherent limitation of operating with a small brain. With very few neurons, it is impractical to allocate computational resources to attending to many stimuli at once. The development of selective attention can mitigate the cost of reduced processing power, as it allows the spider to dedicate a larger proportion of its limited neurons to the task of attending to a single stimulus when that stimulus is likely to be encountered.

Despite the benefits *E. culicivora* gains from using selective attention, there is a cost associated with this strategy, namely, that those cognitive resources allocated to one stimulus can no longer be used to process another otherwise salient stimulus. When the spider is selectively attending to one stimulus, it becomes less proficient at detecting other, otherwise salient, stimuli. For example, after encountering plant odour, *E. culicivora* becomes less proficient at detecting prey than it would be if no odour had been encountered. Consider the scenario in which an adult spider is on a plant and there is a potential mate somewhere nearby. Triggering selective attention for mates after encountering plant odour is clearly beneficial. Even if a prey item lands nearby, the spider continues to attend to the potential mate. The spider's priority at this life stage is reproduction, so, attending to the potential mate, thereby passing up the prey item, is a satisfactory response to the available stimuli. However, when there is no potential mate nearby, the plant odour still triggers selective attention for mates, reducing the spider's ability to detect prey. In this case, when prey lands nearby, the spider fails to detect it, leaving the spider without a meal and without a mate. However, our observation of this cognitive strategy in *E. culicivora* is evidence, in itself, that the trade-off associated with selective

attention weighs in favour of the benefits gained.

In the past, researchers have been impressed by evidence for selective attention in spiders. It was surprising that the miniature nervous system was capable of flexible allocation of cognitive resources at all. The evidence presented here for an ontogenetic shift in the cognitive processes of a spider goes a step beyond even those findings. This research shows that these spiders are born with the innate capacity to use selective attention for plants to improve their ability to find food, and that as the spiders mature into adults, a change occurs, which changes the specific items that are selectively attended to when plants are encountered. The change in selective attentional mechanism is an even more nuanced display of spider cognition than we have seen before.

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Appendix A

Nectar Meals of the Mosquito-Specialist Spider, *Evarcha culicivora*

Published as: Nectar Meals of a Mosquito-Specialist Spider

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Psyche, Volume 2012, Article ID 898721

http://dx.doi.org/10.1155/2012/898721

Academic Editor: Louis S. Hesler

Accepted 8 November 2012

I was the corresponding author on this manuscript.

Abstract

Evarcha culicivora, an East African jumping spider, is known for feeding indirectly on vertebrate blood by actively choosing blood-carrying mosquitoes

as prey. Using cold-anthrone tests to detect fructose, we demonstrate that *E. culicivora* also feeds on nectar. Field-collected individuals, found on the plant *Lantana camara*, tested positive for plant sugar (fructose). In the laboratory, *E. culicivora* tested positive for fructose after being kept with *L.camara* or one of another ten plant species (*Aloe vera*, *Clerodendron magnifica*, *Hamelia patens*, *Lantana montevideo*, *Leonotis nepetaefolia*, *Parthenium hysterophorus*, *Ricinus communis*, *Senna didymobotrya*, *Striga asiatica*, and *Verbena trivernia*). Our findings demonstrate that *E. culicivora* acquires fructose from its natural diet and can ingest fructose directly from plant nectaries. However, experiments in the laboratory also show that *E. culicivora* can obtain fructose indirectly by feeding on prey that have fed on fructose, implying a need to consider this possibility when field-collected spiders test positive for fructose. In laboratory tests, 53.5% of 1,215 small juveniles, but only 3.4% of 622 adult *E. culicivora*, left with plants for 24 hours, were positive for fructose. These findings, along with the field data, suggest that fructose is especially important for early-instar juveniles of *E. culicivora*.

Introduction

Trophic switching and feeding at more than one trophic level, although often overlooked in the literature on spiders, are common themes in the evolution of arthropods [1, 2]. For example, many predatory heteropterans are known to feed facultatively on plant products [3, 4]. Spiders, however, are typically characterized as being obligate predators. The most striking known exception is *Bagheera kiplingi* [5], a Central American jumping spider (Salticidae), which is almost entirely herbivorous despite cohabiting

with edible ant species (*Pseudomyrmex spp.*). *B. kiplingi* feeds primarily on the Beltian bodies (specialized leaf tips) of the ant-acacia (*Vachellia spp.*), which also dominate the ants' diet [6, 7, 8]. Although no other spiders are known to rely as heavily on herbivory as *B. kiplingi*, many spiders do supplement a predatory diet with nectar taken from the floral or extrafloral nectaries of plants (e.g., [9, 10, 11, 12]).

Taylor and Pfannenstiel [13] and Chen et al. [14] provided evidence of fructose ingestion by one or more species from each of 13 spider families (Agelenidae, Anyphaenidae, Araneidae, Clubionidae, Corinnidae, Lycosidae, Miturgidae, Nephiliidae, Oxyopidae, Pisauridae, Salticidae, Tetragnathidae, and Thomisidae). Presence of fructose was confirmed using cold-anthrone testing, a procedure developed by Van Handel [15, 16] for detecting the presence of fructose in mosquitoes. While field and laboratory observations suggest that nectarivory might be especially prevalent among jumping spiders [12, 17], only one species (*Plexippus selipes*) has been shown to be fructose positive by cold anthrone testing [14].

Salticids have intricate vision-guided predatory strategies supported by their complex eyes [18, 19, 20], and the predatory strategy of *Evarcha culicivora* is unusually intricate even by salticid standards [21]. This species feeds indirectly on vertebrate blood by actively choosing blood-carrying female mosquitoes as preferred prey [22], a choice it can make even when restricted to using chemoreception alone. Olfactometer experiments [22, 23] have also shown that *E. culicivora* is attracted to the odour of two plant species, *Lantana camara* and *Ricinus communis*, but the role of these plants in *E. culicivora*'s biology remains largely unknown.

Here we investigate whether *E. culicivora*'s attraction to *L.camara* and *R. communis* can be explained, at least in part, by the spider acquiring

nectar meals from these plants. Using cold-anthrone testing, we confirm that some of the *E. culicivora* individuals collected from L. camara in the field have ingested fructose. We then repeat cold-anthrone testing under laboratory conditions to minimize the possibility of the spiders acquiring fructose by any means other than feeding directly on the plant's nectaries, such as feeding on other parts of the plant or on fructose-carrying prey (see: [14, 24]). Finally, we determine the specificity of *E. culicivora*'s interest in particular plants by testing for the presence of fructose in individuals that had been housed with one of ten other plant species.

Methods

General Our field site was the Thomas Odhiambo Campus (Mbita Point) of the International Centre of Insect Physiology and Ecology (ICIPE) inWestern Kenya (elevation 1200m above sea level; latitude S0°25′-S0°30′ longitude E34°10′) For the rearing and maintenance of spiders in the laboratory, we followed procedures that are standard for our salticid research (see: [25]) and summarize only essential details here.

The laboratory photoperiod was 12L : 12D, with lights coming on at 07:00 am. Except for recently hatched juveniles (see below), each individual spider was maintained in a standard cylindrical cage (diameter 45mm, height 55mm) made of transparent plastic with two holes in the top (a screen-covered hole for ventilation and another hole used for introducing prey). Each spider had continuous access to water in its cage via a cotton roll that protruded through a hole in the bottom of the cage into a water-filled pot below. All holes were 10mm in diameter. The spiders were maintained on a mixed diet of non-biting midges (Chironimidae) collected

as needed from the field and blood-fed female mosquitoes (*Anopheles gambiae s.s.*) from cultures (see: [26]). The spiders were provided with these prey three days per week (Monday, Wednesday, and Friday).

Cold-Anthrone Testing No later than 4 hours before use (see: [27]), a fresh batch of anthrone reagent was prepared by mixing 150mL of distilled water with 380mL of concentrated sulphuric acid, after which 150mg of anthrone powder was mixed with 100mL of the diluted sulphuric acid.

Each spider from the field or from an experimental trial in the laboratory (see below) was placed in a vial and stored at $-80\,^{\circ}\mathrm{C}$ to arrest enzymatic activity. After 4 hours, the frozen spider was removed and transferred to a 5mL test tube. Moisture was evaporated off the spider by holding the test tube in a hot water bath ($80\,^{\circ}\mathrm{C}$ to $90\,^{\circ}\mathrm{C}$) for 15 minutes (see: [15]). The next step in preparing the spider for cold anthrone testing was to remove cuticular wax and expose the spider's digestive tract. This was achieved by using a solution of chloroform and methanol (ratio of 1:1), which had been prepared ahead of time and stored at $-25\,^{\circ}\mathrm{C}$. Two drops of this solution were added to the test tube with the spider. 20 minutes later, the spider was gently crushed using a glass stirring rod.

Next, 0.5 mL of the anthrone reagent was added to the test tube, which was then agitated for 60 minutes on a vortex mixer held at $26\,^{\circ}\mathrm{C}$ in a water bath. We followed established procedures for preparing colorimetric standards corresponding to different fructose concentrations [28]. These standards were made by pipetting 1 µl of each of nine standard sucrose solutions (see below) into test tubes (one test tube per standard) and adding two drops of the chloroform-methanol solution and 0.5mL of anthrone reagent. The initial sucrose solution was made by dissolving 25.6 g of reagent grade

sucrose in 50mL of distilled water and adding enough water to make 100mL of solution. Next, we made eight two-fold serial dilutions (standards), as explained by Taylor and Pfannenstiel [13], each standard corresponding to a specified concentration of fructose. Standards were stored at −45 °C.

Samples from cold-anthrone testing of spiders were evaluated by visual inspection for colour change. When fructose was present, samples turned green or blue green, but samples lacking fructose remained clear yellow. We adopted matches to the standards at above 2 µg as our criterion for recording a sample as being positive for fructose. This criterion was derived from sponge tests (see below) designed to determine how effective our cold-anthrone methods were at detecting fructose specifically in spiders (i.e., we determined the threshold match to sample above which glucose would not give a false positive for fructose). Accordingly, estimates for how many spiders ingested fructose should be envisaged as conservative. Considerable digestion of fructose might have occurred during the interval between the spider ingesting nectar and the spider being transferred to a freezer (−80 °C), and this is another factor suggesting that our estimates of numbers of spiders that ingested fructose are conservative.

Sponge Testing Earlier research [29] has shown that sponge discs soaked in honey solutions can be used for supplementing the diet of spiders. Here we used sponge discs to provide *E. culicivora* juveniles with opportunity to feed on nectar in the absence of plants. To initiate a sponge test, a clean disc (diameter 5 mm, thickness 2 mm) cut from a rubber sponge was dipped in a vial containing nectar or a sugar solution (30 % fructose or 30 % glucose) for 10 seconds, then transferred to a clean rearing cage. There was a cork, rather than a cotton roll, in the hole in the bottom of the cage and the disc

was pinned to the inside end of this cork. A spider was put into the cage at 08:00 am and a 1-hour or a 24-hour individual test (see above) was carried out. There were no plant cuttings in the cage.

The nectar came from *Leonotis nepetaefolia* grown in a field plot. We used this plant species because its flowers produce copious volumes of nectar. Nectar was squeezed by hand into plastic vials (diameter 10 mm; height 48 mm), after which the vials were stored in a freezer at −25 °C. We discovered that nectar volume was usually low in the afternoon, probably due to depletion by nectarivorous birds and insects. We avoided this problem by collecting early in the morning (06:00 – 07:00 am).

Testing Spiders for Fructose after Being Housed with Plants In the field, we collected individuals of *E. culicivora* that we found on the flowers of a particular plant species, *L.camara*, and, within 60 minutes, transferred each collected spider to a freezer (−80 °C) in preparation for cold-anthrone testing. The rationale for the focus on *L.camara* was partly that it is one of the two plants known to attract *E. culicivora* [23] and partly that it is one of the most common plant species in our field site.

For laboratory testing, we used *L.camara* and *Ricinus communis*, the two plant species known to attract *E. culicivora* [23], as well as another nine species chosen as an arbitrary sample of the numerous plants present in the study site (see: [30]). Plant cuttings collected from the field were held in a closed plastic box under 100 % carbon dioxide for 10 minutes and then examined carefully with a microscope for any arthropods (e.g. plant-eating insects) that might have remained on the plant. None were found. Next, the plant cutting was put into a cage (the size of the cutting was sufficient to almost fill the cage). The cut stem at the bottom of the cage was wedged

next to the cotton roll and extended into the water in the pot below the cage, while the rest of the cutting (flowers, stems, and leaves) was within the cage. Testing began at 08:00 am, when spiders were introduced into cages. We decided not to consider differences in how the plants responded to the treatment (e.g., drying out with exposure to CO_2) because we were primarily interested in determining qualitatively whether the spiders ingest any nectar at all from the various plants.

In the laboratory, *E. culicivora* females put their eggs in silk egg sacs situated inside cocoon-like silk nests. To acquire the juvenile spiders used for testing in the laboratory, females were removed from their cages on the day eggs were laid. After the eggs hatched and the juveniles emerged from the nest, we waited 3 days before using these juveniles in experiments. The juveniles we used had not yet fed before testing. By using recently emerged unfed juveniles, we eliminated the possibility of these spiders having acquired fructose indirectly by feeding on insects that had been feeding on plants. A 3-day waiting period was adopted because after longer fasting periods juveniles often appeared weak and, after more than 3 days,many of these spiders died. For laboratory testing, we also used adult spiders that had matured 3-4 weeks before use. Adult spiders had not mated and were fasted for 7 days before testing.

For testing spiders with plants, three protocols were adopted: 24-hour communal testing (juveniles only, all plant species), 24-hour individual testing (adults only, all plant species), and 1-hour individual testing (juveniles only, *L.camara*, *R. communis*, and *L. nepetaefolia* only). All testing began at 08:00 am. For 24-hour testing (communal and individual), spiders were left in cages with plants until 08:00 am on the following day. Communal testing included a group of about 20 spiders per cage and individual testing

included only one spider per cage.

Directly observing the behaviour by which spiders acquired fructose was not part of the protocol for field collected spiders or during 24-hour testing in the laboratory. However, we defined feeding on nectar as instances of the spider having its mouth-parts pressed against floral or extra-floral nectar and, by this definition, we saw spiders feeding on nectar during casual observations. We saw no instances of the spider having its fangs extended or making back and forth movement of chelicerae (i.e., no biting was seen).

The procedure adopted for 1-hour individual testing was to place one spider directly on the plant and then observe it continuously. Testing ended when the spider stopped feeding (i.e., when it moved its mouthparts away from the nectar for 60 seconds). We aborted the test whenever an individual had not initiated feeding after 60 minutes had elapsed. This procedure meant that, in 1-hour individual testing, we were certain the spiders we assayed using the cold-anthrone method had, according to our definition, fed on nectar and that there was no alternative means by which these spiders might have acquired fructose (i.e., none were seen with fangs extended or chelicerae making biting movements, and none were seen feeding on prey).

Mosquitoes as an Indirect Source of Fructose for Spiders For normal rearing, mosquitoes were given access to a 6% glucose solution soaked into cotton wool (see: [26]). For our experiments, instead of the normal 6% glucose solution, we used female mosquitoes that had been given access to a 6% fructose solution (via a sponge disc that had been soaked in the fructose solution). None of these mosquitoes had been fed blood. We kept each mosquito in a separate cage with a sponge disc. This was preferable to trying to feed fructose to mosquitoes in a group, as competition for

access to food would have made it difficult to ensure that most mosquitoes would receive a fructose meal during the feeding period. At 08:00 am on the following day, these fructose-fed mosquitoes were put with the spiders (each juvenile spider in a separate cage). 24 hours later, the spider was transferred to a freezer (−80 °C) in preparation for cold-anthrone testing.

Statistical Methods

Field-Collected Spiders We measured the body size (accurate to the nearest mm) of 95 field collected individuals before testing them for fructose.We then conducted a logistic regression analysis [31] and compared the resulting model to a constant only model to determine whether body size was an accurate predictor of fructose presence. We calculated Nagelkerke's R^2 [31] to assess the strength of this association and the Wald criterion [31] to determine the degree to which the predictor contributed to the strength of the model. Finally, the odds ratio [31] was calculated to show the magnitude of change across the regression.

Spider Housed with Plants or Mosquitoes When one or more adults tested positive for fructose we conducted a χ^2 test of independence [31] to compare results of the fructose tests between males and females.We conducted a further series of χ^2 tests to compare the results of the fructose tests between adult and juvenile spiders. All statistical tests were run using PASW Statistics software [32].

Results

Presence of Fructose in Field-Collected Spiders As body size of the spiders sampled from the field increased, fewer individuals tested positive for fructose (Table reftab:nf1). A test of the full model from the logistic regression against a constant only model was statistically significant, indicating that the predictor reliably distinguished between individuals that had consumed fructose and those that had not (χ^2 = 10.455, p <0.001, df = 2). Nagelkerke's R^2 was 0.168, indicating a weak relationship between prediction and grouping. Prediction success overall was 81.1%. The Wald criterion demonstrated that body size made a significant contribution to prediction (χ^2 = 7.876, p = 0.005). The EXP(B) value indicated that when body size is raised by one unit (1mm) the odds ratio becomes 0.461 times as large.

Table A.1: Cold-Anthrone results from testing field-collected *Evarcha culicivora* individuals of different sizes. All spiders collected from the plant *Lantana camara*.

Spider body length (mm)	Number positive for fructose
2 mm	10 of 29 (34.5%)
3 mm	5 of 22 (22.7%)
4 mm	2 of 18 (11.1%)
5 mm	1 of 19 (5.3%)
6 mm	0 of 7 (0%)

Sponge Testing 28 out of 35 spiders were positive for fructose after being left for 24 hours with the sponge pieces that had been soaked in a fructose solution. Three of 35 spiders left with sponge pieces that had been soaked in a glucose solution were positive after cold-anthrone testing. These samples matched the 2 µg standard. Based on these findings, we required a

match to standard above 2 µg as our criterion for recording that a spider was positive for fructose (i.e., our data from sponge testing suggest that match to a sample of 2 µg cannot be distinguished from a false positive). Although continual observation was not part of the 24- hour testing protocol, we frequently saw spiders with their mouthparts pressed against the damp pieces of sponge during casual observation. 40 out of 102 spiders were observed feeding during 1-hour continual observation trials. 37 of those 40 spiders subsequently tested positive for fructose. All spiders that were not seen feeding tested negative for fructose.

Presence of Fructose in Spiders Housed with Plants or Mosquitoes Only 21 out of 622 (3%) adult spiders tested positive for fructose after being housed with a plant cutting for 24 hours. The small number of spiders that tested positive had been housed with *Aloe vera*, *Leonotis nepetaefolia*, or *Ricinis communis*. A series of χ^2 tests comparing results between males and females for each of these groups showed no significant difference between adults of the two sexes (Table A.2). Accordingly, data from adult males and females were pooled before being compared with data from juveniles. For each plant species used, juveniles tested positive for fructose significantly more often than adults (Table A.3) after being housed with a plant cutting for 24 hours.

When housed with a nectar source and observed continually for 1 hour, those individuals that were seen with their mouthparts on the plant nectaries almost always tested positive for fructose (Table reftab:nf4). Spiders were never observed feeding from parts of the plant other than the nectaries. In the absence of plants or sugar on sponge pieces, 19 of 57 (33%) spiders tested positive for fructose after feeding on fructose-carrying

Table A.2: Inter-sexual comparisons of the numbers of *Evarcha culicivora* adults positive for fructose (cold-anthrone testing) after having been left with plants for 24 hours. There were no positive results for 8 of the 11 tested plants, so these results are omitted.

Plant species	Females positive for fructose (% positive for fructose)	Males positive for fructose (% positive for fructose)	χ^2 Test for independence, $\alpha = 0.05$
Aloe vera	1 of 40 (2.5%)	0 of 37 (0%)	0.937 ns
Leonotis nepetaefolia	5 of 35 (14.3%)	1 of 33 (3.0%)	2.675 ns
Ricinus communis	6 of 35 (17.1%)	10 of 48 (20.8%)	0.177 ns

Table A.3: Number of *Evarcha culicivora* (juveniles and pooled data for adult males and females) positive for fructose (cold-anthrone testing) after being left with plants for 24 hours. Ranked from highest to lowest percentage positive for juveniles. * indicates p<0.001

Plant species	Juveniles positive for fructose	Adults positive for fructose	χ^2 Test for independence, $\alpha = 0.05$
Lantana montevideo	39 of 45 (86.7%)	0 of 29 (0%)	53.139*
Lantana camara	155 of 195 (79.5%)	0 of 109 (0%)	176.771*
Clerodendron magnifica	43 of 62 (69.3%)	0 of 31 (0%)	39.990*
Ricinus communis	85 of 140 (60.7%)	16 of 83 (19.3%)	36.106*
Striga asiatica	26 of 43 (60.5%)	0 of 25 (0%)	24.474*
Leonotis nepetaefolia	44 of 81 (54.3%)	4 of 68 (5.9%)	39.719*
Verbena trivernia	75 of 149 (50.3%)	0 of 24 (0%)	21.326*
Senna didymobotrya	38 of 77 (49.3%)	0 of 61 (0%)	41.543*
Aloe vera	68 of 184 (37.0%)	1 of 77 (1.3%)	35.490*
Parthenium hysterophorus	51 of 154 (33.6%)	0 of 89 (0%)	37.303*
Hamelia patens	26 of 85 (30.6%)	0 of 26 (0%)	10.386*

mosquitoes.

Table A.4: Number of *Evarcha culicivora* juveniles observed feeding and number positive for fructose (cold-anthrone testing) after being left with plants for 1 hour. Spiders not seen feeding were never positive for fructose.

Plant species	Number seen feeding	Number positive for fructose
Lantana camara	12 of 25 (48.0%)	10 of 12 (83.3%)
Ricinus communis	18 of 32 (56.3%)	17 of 18 (94.4%)
Leonotis nepetaefolia	10 of 45 (22.2%)	10 of 10 (100.0%)

Discussion

Findings from cold-anthrone testing of field-collected *E. culicivora* suggest that ingesting fructose is characteristic of this spider species. As in other studies in which spiders from the field have been sampled for fructose [13, 14], we could not rule out the possibility that our spiders from the field fed from some part of the plant other than the nectaries or that they acquired fructose indirectly by feeding on fructose-carrying prey. However, our laboratory data support our hypothesis that spiders in the field acquire fructose primarily by taking nectar directly from the plants' nectaries.

Owing to pre-testing procedures, which should have removed most potential prey from the experimental plants, it is unlikely that instances of spiders being positive for fructose after 24-hour tests in the laboratory were the result of indirect acquisition of fructose from prey. Moreover, we can be especially confident that fructose was not acquired by means other than feeding directly from nectaries during the 1-hour tests, as there was continuous observation. None of these spiders were ever seen feeding on prey or feeding on any part of a plant other than the nectaries and almost every spider that was observed feeding on nectaries subsequently tested positive for fructose.

From these data, we can confidently conclude that *E. culicivora* has the capacity to ingest nectar directly from nectaries. However, after having access to mosquitoes that had been feeding on a fructose solution, many *E. culicivora* juveniles tested positive for fructose and, in these tests, the mosquito was the only fructose source that could account for the findings. This result suggests that indirect fructose acquisition should be considered as a potential contributor to our fructose-positive results when field-

collected spiders were sampled. Further research is needed to determine the relative importance of direct and indirect ingestion of plant derived nutrients by *E. culicivora*.

Examining data from field-collected spiders, we found a negative relationship between the spider's size and whether it was positive for fructose. Fructose-positive results were also considerably more common for juveniles than for adults in the 24-hour laboratory tests. Although a number of factors, such as differential fructose metabolism and how the total amount of fructose ingested is related to the spider's body size, may also play a part in explaining these results, perhaps the most interesting hypothesis suggested is that nectar meals are especially important for the smaller juveniles. As we are currently investigating this hypothesis, here we will only mention some of the factors that might be particularly relevant.

Optimal foraging models often use energy intake as a proxy for the fitness benefits gained by feeding [33]. However, numerous examples [34], including some from studies on spiders [35, 36], show that nutrient regulation, not energy maximisation, may be the more important function of feeding. Perhaps nectar meals are more relevant to the optimal nutrient balance for small juveniles than for larger *E. culicivora* individuals. Furthermore, it may be that the volume of nectar readily acquirable from *L.camara* is large enough to be significant to small juvenile *E. culicivora*, but too small to be considered by larger individuals [37, 38].

The type of benefit gained by small juveniles from nectar may also be important. Although nectar does contain other nutrients, such as amino acids, its primary component is sugar [39, 40]. Our results may indicate that sugar meals are more important to small E. culicivora than they are to larger individuals. Early-instar spiders are more vulnerable to starva-

tion than their later-instar counterparts [41, 42], which may make easily acquired sugar meals more beneficial to small juveniles than they would be to larger juveniles or adults. A sugar meal may act to sustain a small juvenile long enough that it can succeed at capturing prey and thereby acquire a more nutrient-rich meal.

Earlier olfactometer experiments [23] showed that the odours of two plant species, *L.camara* and *R. communis*, attract *E. culicivora*. Nectar meals from these plants might be particularly important, but we have shown that *E. culicivora* can acquire nectar meals not only from these two plant species but also from each of the nine other plants used in our experiments. The full significance of *L.camara* and *R. communis* to *E. culicivora* may include more than just providing nectar meals. One of our goals in ongoing research is to fully investigate the role of particular plants in *E. culicivora*'s biology.

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Appendix B

Rapid nectar-meal effects on *Evarcha culicivora*'s capacity to kill mosquitoes

Abstract

Using *Evarcha culicivora*, an East African jumping spider (Salticidae), we investigate how nectar meals function in concert with predation specifically at the juvenile stage between emerging from the egg sac and the first encounter with prey. Using plants and using artificial nectar consisting of sugar alone or sugar plus amino acids, we show that the plant species (*Lantana camara*, *Ricinus communis*, *Parthenium hysterophorus*), the particular sugars in the artificial nectar (sucrose, fructose, glucose, maltose), the concentration of sugar (20%, 5%, 1%) and the duration of prefeeding fasts (3 days, 6 days) influence the spider's prey-capture proficiency on the next day after the nectar meal. However, there were no significant effects of amino acids. Our findings suggest that benefits from nectar feeding are derived

primarily from access to particular sugars, with fructose and sucrose being the most beneficial, glucose being intermediate and maltose being no better than a water-only control.

Introduction

There has been a longstanding interest in explaining the origins and adaptive significance of omnivory (i.e. feeding at more than one trophic level). Frequently considered hypotheses include minimizing overexposure to toxins associated with otherwise superior food and surviving periods when superior food is scarce by relying on inferior food sources [1, 2, 3]. Omnivory is especially interesting when animals traditionally envisaged as being simply predators are shown also to take nutrients directly from plants [4]. For example, spiders are widely regarded as being exclusively predators, but *Bagheera kiplingi* is a striking exception [5]. This Central American jumping spider (Salticidae)cohabits with ants (*Pseudomyrmex spp.*) on ant-acacias (*Vachellia spp.*), where it sometimes captures and eats the ants, but it feeds primarily from the Beltian bodies (i.e. specialized leaf tips) that also serve as the ants' primary food [6]. No other spiders are known to express a comparable level of herbivory, but many spiders are now known to supplement a primarily predatory diet with plant products, including pollen [7, 8, 9, 10, 11, 12], honeydew [13, 14, 15] and especially nectar taken from flowers or extra-floral nectaries (EFNs) [16, 17, 18, 19, 20, 21, 22]. For two non-salticid spiders [23, 24], *Cheiracanthium mildei* (Miturgidae) and *Hibana velox* (Anyphaenidae), experiments have shown that nectarivory, when combined with feeding as a predator, improves survival, growth and fecundity.

Our research is different because we use a salticid spider and we investigate a more rapidly expressed nectar-derived benefit. Owing to the exceptional spatial acuity of their large, complex principal eyes [25, 26], salticids can readily detect and identify prey from a distance and, for some salticid species, there is experimental evidence of highly specific vision-based prey-choice decisions [27]. However, the level of specificity expressed by *Evarcha culicivora*, the species we consider here, is remarkable even by salticid standards [28]. This East African salticid feeds indirectly on vertebrate blood by actively choosing blood-carrying female mosquitoes as preferred prey [29], and *E. culicivora* chooses species from the genus Anopheles as its preferred mosquitoes [30, 31]. From a human perspective, a preference for Anopheles is particularly relevant because all human malaria vectors belong to this genus [32].

Besides having an exceptional capacity for seeing detail, many salticids also make extensive use of chemoreception, including olfaction (e.g. [33]). The role of olfaction in the biology of *E. culicivora* is especially complex [34, 35, 36, 37, 38] and includes odour-mediated responses to plants [39]. In the field, *E. culicivora* is frequently found on *L.camara* and *Ricinus communis* [40], two of the most common plant species in its habitat [41, 42, 43] and *E. culicivora* is attracted to the odour of both of these plant species in olfactometer experiments [39].

Nectar meals may be especially important for the early instar juveniles of *E. culicivora*. β-caryophyllene and α-humulene, the dominant sesquiterpenes from the headspace of *L.camara*, attract the adults and the juveniles of *E. culicivora* [44], but pre-trial fasts make the early instar juveniles, but not the adults, of *E. culicivora* more strongly predisposed to move towards a source of these volatile compounds [45]. Evidence from cold-anthrone

testing also shows that *E. culicivora* ingests nectar from *L.camara* and other plants, but the early instar juveniles of *E. culicivora* appear to feed on nectar considerably more often than adults [46].

The hypothesis we consider here is that nectar meals make early instar juveniles more proficient at capturing mosquitoes. Part of the rationale for this hypothesis is that mosquitoes are much larger than the early instar juveniles of *E. culicivora*. After being attacked, mosquitoes sometimes shake off the early instar individuals, but larger individuals of *E. culicivora* appear to have no difficulty holding on [47].

We also consider whether prey-capture proficiency is affected by the plant species from which the nectar is derived, the dilution of sugar in solution, the particular sugars acquired by the spider or the amino acid content of nectar. We based our choice of sugars, amino acids and concentrations on current understanding of nectar chemistry. A few exceptions notwithstanding, nectar is primarily a sugar solution, with amino acids being the second most common component. Total sugar concentration varies, but around 20% to 40% solute is typical, with amino acid concentration tending to be closer to 1%. Sucrose, glucose and fructose are usually the dominant nectar sugars and other sugars, when present, are usually found at considerably smaller concentration [48, 49].

Methods

General Females of *E. culicivora* put their eggs in silk egg sacs situated inside cocoon-like nests and, after hatching, the juveniles leave the nest at roughly the same time and spread about in the cage [50]. Here, we reserve the expression 'juvenile' for these active newly emerged spiders. The

juveniles that we used were second and third-generation individuals from laboratory cultures derived from individuals collected at our field site in Mbita Point, Western Kenya (elevation 1200m a.s.l.; latitude S0°25′-S0°30′ longitude E34°10′). As our basic methods for rearing, maintaining and testing spiders were as in earlier studies (e.g. [37]), only essential details are stated here.

We isolated juveniles on the day of emergence, put them into separate maintenance cages (cylindrical, height 55mm, diameter 45mm, made from clear plastic) and then kept them without food for a specified pre-feeding interval. Each spider had continual access to water via a cotton roll ('dental wick') inserted into a hole in the bottom of the cage and positioned so that it extended into a water-filled plastic pot below the cage. There was a mesh-covered hole in the top of the cage for ventilation. A second hole in the top of the cage was plugged with a rubber stopper which could be removed when introducing prey. All holes were 8mm in diameter.

We assigned spiders at random to one of 18 meal-type groups and, after a fast of a specified duration, we gave the spider access to a first meal corresponding to the meal-type group (Table reftab:pap1). The first meal was artificial nectar (i.e. a solution of sugar or sugar plus amino acids), a plant or a water-only control (Table reftab:pap1). For the control and all artificial nectar, we used distilled water. For plants, we used *L.camara* and *R. communis*, as well as *Parthenium hysterophorus*, a species that is common in the same habitat but not known to attract *E. culicivora* in olfactometer experiments (R. R. Jackson 2008, unpublished data).

For the water-only control group, the individuals used were derived from 29 sibships (where a 'sibship' is defined as the progeny of a particular male and female). For each other group, the individuals used were derived from

8–10 sibships. No sibships contributed individuals to more than one group. The number of individuals from any one of the sibships was never more than eight or less than three. Owing to the way we took individuals from a range of sibships, we did not include sibship as a variable in our data analyses.

The sugar and amino acid content of *L.camara* nectar is known (cited in [51] as personal communication from Irene Baker to Alm et al.): sucrose 187.25 $g\,l^{-1}$, fructose 57.00 $g\,l^{-1}$, glucose 55.80 $g\,l^{-1}$, proline 0.256 $g\,l^{-1}$, glycine 0.178 $g\,l^{-1}$, serine 0.144 $g\,l^{-1}$, glutamine 0.136 $g\,l^{-1}$, threonine 0.080 $g\,l^{-1}$, alanine 0.064 $g\,l^{-1}$, asparagine 0.056 $g\,l^{-1}$, tyrosine 0.040 $g\,l^{-1}$, glutamic acid 0.048 $g\,l^{-1}$, arginine 0.032 $g\,l^{-1}$ and valine 0.016 $g\,l^{-1}$. For our experiments, we made two artificial nectar blends based on the reported ratio of the three sugars and the four dominant amino acids in this plant's nectar.

Full artificial *L.camara* nectar: sucrose 187.3 $g\,l^{-1}$, fructose 57.0 $g\,l^{-1}$, glucose 55.8 $g\,l^{-1}$, proline 0.3 $g\,l^{-1}$, glycine 0.2 $g\,l^{-1}$, serine 0.1 $g\,l^{-1}$, glutamine 0.1 $g\,l^{-1}$.

Sugar-only artificial *L.camara* nectar: sucrose 187.3 $g\,l^{-1}$, fructose 57.0 $g\,l^{-1}$, glucose 55.8 $g\,l^{-1}$.

The sugar content of *R. communis* and *P. hysterophorus* nectar is not known precisely, but the floral tissues of these plants contain sucrose, fructose, glucose and other sugars, including especially maltose [52]. We included maltose in our experiments as a sugar that may be in *R. communis* nectar, but is not known to be present in the nectar of *L.camara* or prevalent in the nectar of plants, in general.

For each meal-type group, there were two fasting-duration subgroups (3 day and 6 day): spiders kept without access to food for 3 days or 6 days before being given access to the meal corresponding to the meal-type group

(Table tab:pap1) on the 4th or 7th day.

Experimental Procedure The spider's first meal was placed in its cage at 8:00am and removed 60min later (laboratory photoperiod 12 L:12 D, lights on at 7:00am). During this feeding period, the spider was observed continuously. For each plant meal, the plant used in an experiment was a cutting taken from a living plant in the field. In each instance, the plant was held in a closed plastic box under 100% carbon dioxide for 10 min and then examined under a microscope for any arthropods that might have remained (none were found). The cut end of the stem was the only incision or wound on the plant and it remained outside the cage (i.e. the stem, positioned alongside the cotton roll, went through the hole in the bottom of the cage so that the cut end was in the pot of water below the cage). The remainder of the plant (stems, flowers and leaves) was inside, and almost filled, the cage.

When the first meal was artificial nectar or the water-only control, a disc (diameter 5mm, thickness 2mm, cut from a clean kitchen sponge) was submerged in the specified solution (or water alone for the control) for 10s at 7:30am [46]. The sponge disc was then attached by a pin to the centre top of a clean, dry cotton roll. The cotton roll that was providing water to the spider was removed at 8:00am and replaced with the clean cotton roll along with the attached solution-soaked sponge disc. The sponge disc was positioned horizontally at the top of the pin (25mm above the cage floor, 30mm below the cage ceiling and 22.5mm from the side of the cage). During each trial, the cotton roll remained dry (i.e. there was no water in the pot below the cage).

We removed any spider that failed to feed while the first meal was on

offer and replaced it with another spider. Our criterion for recording that a spider fed was seeing its mouthparts pressed against a plant (flower petal, leaf or stem), or against a sponge disc that had been soaked in artificial nectar [46].

On the following day at 7:30am, each test spider was transferred to a testing cage. Testing cages were similar to maintenance cages, but larger (height 110mm, diameter 60 mm). The larger size allowed sufficient space for mosquitoes to fly, making them harder for the spider to capture. At 8:00am, 24 h after the first meal, we removed the stopper from the hole in the top of the cage and, using an aspirator, introduced four mosquitoes (*Anopheles gambiae s.s.*), after which the stopper was returned to the hole. The mosquitoes were taken from stock cultures and had fed on blood 4 hours before being used in the experiments (for methods pertaining to mosquito culturing and feeding, see: [53].

The outcome of a trial was recorded as successful when the test spider attacked the mosquito, held on and then fed and it was recorded as unsuccessful when the spider attacked the mosquito, but failed to hold on and feed. Whenever 2 hours elapsed without the test spider attacking a mosquito, the test ended and these spiders were excluded from further analysis (i.e. all data came from instances of a test spider attacking the prey and then being either successful or unsuccessful at capturing the prey; no instances of multiple attacks were considered).

Data analysis The statistics package R [54] was used for all data analyses. We applied a logistic regression to prey capture data, with each instance of the spider capturing the prey being coded as 1 and each instance of the spider failing to capture the prey it attacked being coded as 0. Meal type

was included as a factor in the model and pre-trial fast duration (3 or 6 days) was included as a standard variable. Using the 'glm' function in the stats package, we created logit models and compared them by likelihood-ratio testing from the 'anova' function in the stats package. We made pairwise comparisons of coefficients by using Wald tests (based on χ^2) with Holm-Bonferroni corrections from the aod package [55].

Results

General The best-fit logistic model was

$$P(capture) = \frac{e^{(\beta_m - 0.21d)}}{1 + e^{(\beta_m - 0.21d)}}$$

where $P(capture)$ is prey-capture success expressed as the probability that, after making an attack, the spider will hold on and eat the prey, e is the base of the natural logarithm, d is the pre-trial fast duration in days, -0.21 ($z = 0.03$, s.e.$= 6.78$, $p < 0.001$) is the coefficient for fast duration and β_m is the coefficient for meal-type m (Table reftab:pap1).

This model was a significantly better predictor of the data than a reduced (intercept only) model (likelihood-ratio testing, $\chi^2 = 410.81$, $p < 0.001$), and it was not significantly different from an expanded model that included interaction terms ($\chi^2 = 11.26$, $p = 0.843$). There were no significant interaction effects in the expanded model and Akaike's Information Criterion (AIC) was smaller for the best-fit model than for the reduced model (Δ AIC$= 22.7$) or the expanded model (Δ AIC$= 374.8$).

There was a significant effect of pre-trial fast duration (Fig. reffig:pap1; Wald test, $\chi^2_{18} = 40.8, p < 0.001$) on the spider's success, with fewer spiders

Table B.1: Meal-type descriptions and logistic regression results for 18 meal-type groups. (H_2O, n=200. All other groups, n=50.)

Code	Meal-type	Coefficient	Std. error	z score
LC-C	*Lantana camara* cutting	3.07	0.36	8.57, $p < 0.001$
RC-C	*Ricinus communis* cutting	1.91	0.27	6.99, $p < 0.001$
PH-C	*Parthenuim hysterophorus* cutting	1.06	0.25	4.23, $p < 0.001$
LC-SAA	Full artificial *L. camara* nectar	2.71	0.32	8.39, $p < 0.001$
LC-S	Sugar-only artificial *L. camara* nectar	2.97	0.35	8.54, $p < 0.001$
Suc-20	20% sucrose solution	2.79	0.33	8.45, $p < 0.001$
Suc-5	5% sucrose solution	1.91	0.27	6.99, $p < 0.001$
Suc-1	1% sucrose solution	0.61	0.25	2.43, $p = 0.015$
Fru-20	20% fructose solution	2.49	0.31	8.14, $p < 0.001$
Fru-5	5% fructose solution	2.06	0.28	7.37, $p < 0.001$
Fru-1	1% fructose solution	0.53	0.25	2.09, $p = 0.037$
Glu-20	20% glucose solution	1.49	0.26	5.75, $p < 0.001$
Glu-5	5% glucose solution	1.06	0.25	4.23, $p < 0.001$
Glu-1	1% glucose solution	0.40	0.25	1.56, $p = 0.199$
Mal-20	20% maltose solution	0.82	0.25	3.27, $p = 0.001$
Mal-5	5% maltose solution	0.65	0.25	2.60, $p = 0.009$
Mal-1	1% maltose solution	0.35	0.25	1.38, $p = 0.167$
H_2O	Distilled water (control)	0.37	0.18	2.11 $p = 0.035$

capturing prey after the longer fast (odds ratio$= 0.81$). We also found a significant effect of meal type (Wald test, $\chi^2_{18} = 332.4, p < 0.001$).

Plants and artificial nectar compared with the water-only control We use the expression 'effect' for instances of spiders from a plant group or an artificial nectar group having significantly greater prey-capture success than spiders from the water-only control. We found an effect when spiders fed on each of the three plant species and when spiders fed on artificial *L.camara* nectar (Table reftab:pap2). When we used single-sugar solutions, we found an effect when the spiders fed on 20% and 5% solutions of sucrose, fructose and glucose. However, we found no effect for spiders that fed on 1% solutions of these sugars and no effect even at 5% or 20% when the sugar was maltose.

Figure B.1: For *Evarcha culicivora* juveniles, percentages of individuals from different meal-type group that, after attacking, succeeded in capturing mosquitoes. Abbreviations for groups defined in Table reftab:pap1. N=200 for H_2O and 50 for each other group, (a) 3 day pre-trial fast. (b) 6 day pre-trial fast. Sequence of groups on x-axis for 3 day and for 6 day fast: from highest to lowest percentage after 3 day fast. Percentages lower for 6 day than for 3 day fasts, but rankings of groups by percentage comparable for 3 day and 6 day fasts.

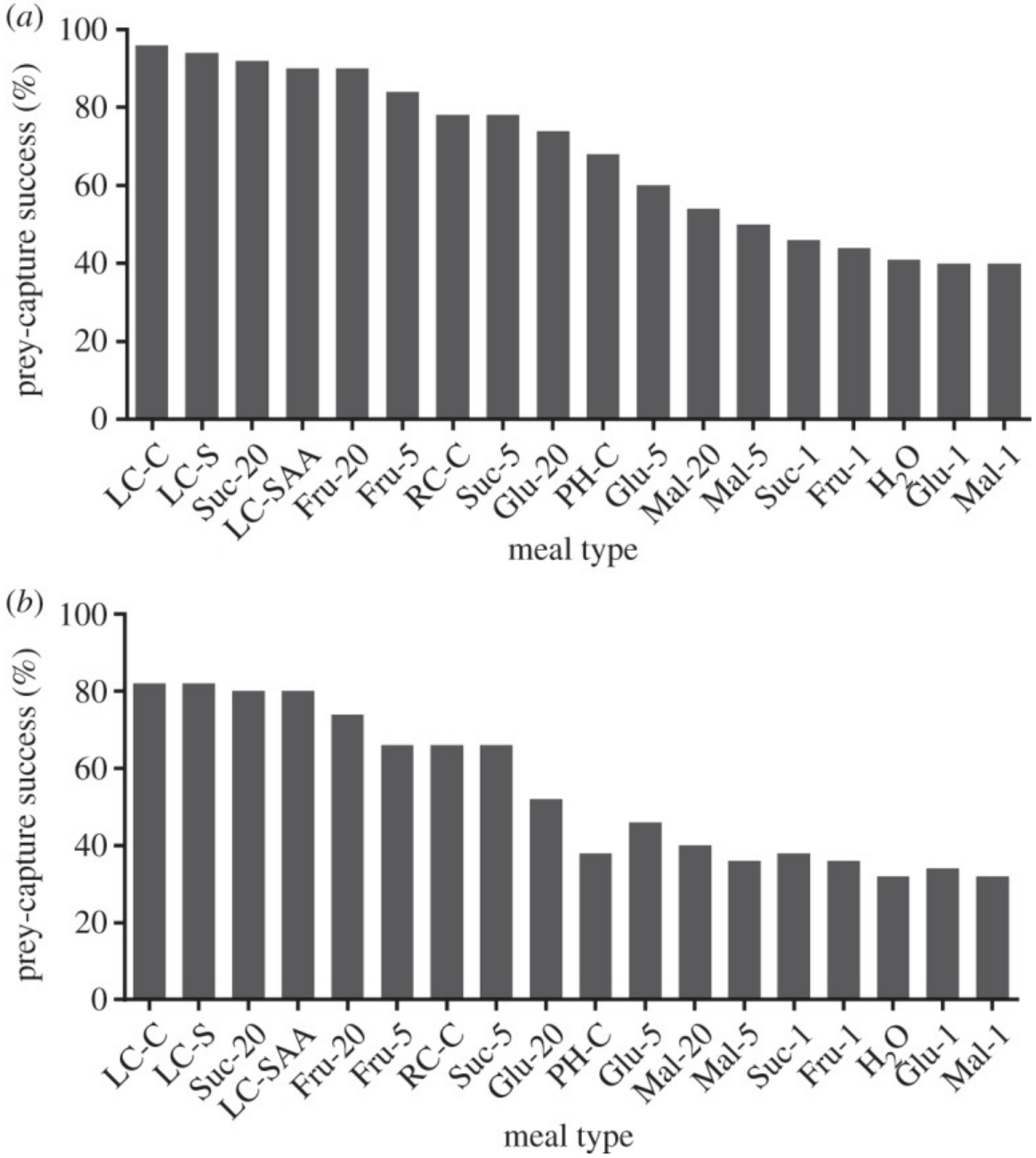


Table B.2: Pairwise comparisons (Wald test based on χ^2) difference between each meal type and the water-only control on prey-capture success

Code	Meal-type	χ^2
LC-C	*Lantana camara* cutting	63.58, $p < 0.001$
RC-C	*Ricinus communis* cutting	38.10, $p < 0.001$
PH-C	*Parthenuim hysterophorus* cutting	9.13, $p = 0.003$
LC-SAA	Full artificial *L. camara* nectar	60.23, $p < 0.001$
LC-S	Sugar-only artificial *L. camara* nectar	63.09, $p < 0.001$
Suc-20	20% sucrose solution	61.38, $p < 0.001$
Suc-5	5% sucrose solution	38.10, $p < 0.001$
Suc-1	1% sucrose solution	1.05, $p = 0.305$
Fru-20	20% fructose solution	55.98, $p < 0.001$
Fru-5	5% fructose solution	43.65, $p < 0.001$
Fru-1	1% fructose solution	0.43, $p = 0.513$
Glu-20	20% glucose solution	22.50, $p < 0.001$
Glu-5	5% glucose solution	9.13, $p = 0.002$
Glu-1	1% glucose solution	0.01, $p = 0.925$
Mal-20	20% maltose solution	3.78, $p = 0.052$
Mal-5	5% maltose solution	1.47, $p = 0.225$
Mal-1	1% maltose solution	0.01, $p = 0.925$

Plants compared Prey-capture success was significantly higher when spiders fed on *L.camara*instead of *R. communis* (Fig. reffig:pap2a); $\chi^2 = 8.79, p = 0.003$) or *P. hysterophorus* ($\chi^2 = 27.88, p < 0.001$) and also when they fed on *R. communis* instead of *P. hysterophorus* ($\chi^2 = 7.75, p = 0.005$).

***Lantana camara* and artificial *Lantana camara* nectar compared** The prey-capture success of spiders that fed on *L.camara* was not significantly different from the success of spiders that fed on either type of artificial *L.camara* nectar (Fig. reffig:pap2b): full ($\chi^2 = 0.71, p = 0.399$), sugar only ($\chi^2 = 0.05, p = 0.824$). Spiders that fed on full and sugar-only artificial *L.camara* nectar were not significantly different from each other ($\chi^2 = 0.39, p = 0.530$).

Multiple and single-sugar nectar compared Sugar-only artificial *L.camara* nectar (Fig. reffig:pap2b) was compared with each single-sugar solution (Fig. reffig:pap3) at the highest concentration (20%). Prey-capture success was significantly higher after feeding on artificial *L.camara* nectar than after feeding on glucose alone or maltose alone: glucose ($\chi^2 = 15.79, p < 0.001$), maltose ($\chi^2 = 33.85, p < 0.001$). However, success after feeding on artificial *L.camara* nectar was not significantly different from success after feeding on sucrose alone ($\chi^2 = 0.18, p = 0.673$) or fructose alone ($\chi^2 = 1.41, p = 0.235$).

Single-sugar solutions compared When the solutions were 20% sugar, prey-capture success was significantly higher after feeding on sucrose than after feeding on glucose ($\chi^2 = 13.32, p < 0.001$; Fig. reffig:pap3a,c) or maltose ($\chi^2 = 31.10, p < 0.001$; Fig. reffig:pap3a,d), significantly higher after feeding on fructose than after feeding on glucose ($\chi^2 = 8.93, p = 0.003$; Fig. reffig:pap3b,c) or maltose ($\chi^2 = 25.40, p < 0.001$; Fig. reffig:pap3b,d) and also significantly higher after feeding on glucose instead of maltose ($\chi^2 = 5.25, p = 0.022$; Fig. reffig:pap3c,d). However, there was no significant difference between sucrose and fructose ($\chi^2 = 0.60, p = 0.439$; Fig. reffig:pap3a,b). When the solutions were 5% sugar, success was significantly higher after feeding on sucrose than after feeding on glucose ($\chi^2 = 7.75, p = 0.005$;Fig. reffig:pap3a,c) or maltose ($\chi^2 = 17.04, p < 0.001$;Fig. reffig:pap3a,d) and significantly higher after feeding on fructose than after feeding on glucose ($\chi^2 = 10.48, p < 0.001$;Fig. reffig:pap3b,c) or maltose ($\chi^2 = 20.73, p < 0.001$;Fig. reffig:pap3b,d), but there was no significant difference between 5% sucrose and 5% fructose ($\chi^2 = 0.24, p = 0.628$;Fig. reffig:pap3a,b), or between 5% glucose and 5% maltose ($\chi^2 = 2.05, p = 0.153$;Fig. reffig:pap3c,d). When the solutions were 1% sugar, there were no significant differences between

any pairs: sucrose - fructose ($\chi^2 = 0.16, p = 0.690$; Fig. reffig:pap3a,b), sucrose - glucose ($\chi^2 = 0.54, p = 0.465$; Fig. reffig:pap3a,c), sucrose - maltose ($\chi^2 = 0.77, p = 0.379$; Fig. reffig:pap3a,d), fructose - glucose ($\chi^2 = 0.19, p = 0.659$; Fig. reffig:pap3b,c), fructose - maltose ($\chi^2 = 0.35, p = 0.556$; Fig. reffig:pap3b,d), glucose - maltose ($\chi^2 = 0.02, p = 0.882$; Fig. reffig:pap3c,d).

Spiders were significantly more successful at capturing prey after feeding on 20% sucrose (Fig. reffig:pap3a) than after feeding on 5% ($\chi^2 = 5.81, p = 0.016$) or 1% sucrose ($\chi^2 = 37.70, p < 0.001$), and significantly more successful after feeding on 5% sucrose than after feeding on 1% sucrose ($\chi^2 = 81.15, p < 0.001$). For fructose (Fig. reffig:pap3b), spiders that fed from a 20% ($\chi^2 = 34.57, p < 0.001$) or 5% ($\chi^2 = 24.39, p < 0.001$) solution were significantly more successful than spiders that fed from a 1% solution, but there was no significant difference between 20% and 5% ($\chi^2 = 1.4, p = 0.226$). For glucose (Fig. reffig:pap3c), spiders were significantly more successful after feeding from a 20% ($\chi^2 = 13.53, p < 0.001$) or a 5% ($\chi^2 = 5.25, p = 0.022$) solution than after feeding from a 1% solution, but there was no significant difference between 20% and 5% glucose ($\chi^2 = 2.10, p = 0.148$). When the sugar was maltose (Fig. reffig:pap3d), there were no significant differences when concentrations were compared: 20% vs. 5% ($\chi^2 = 0.33, p = 0.565$), 20% vs. 1% ($\chi^2 = 2.54, p = 0.111$), 5% vs. 1% ($\chi^2 = 1.05, p = 0.306$).

Plants and single-sugar solutions compared The prey-capture success of spiders that fed on *L.camara* was not significantly different from the success of spiders that fed on either 20% sucrose ($\chi^2 = 0.41, p = 0.52$; Figs reffig:pap2a & reffig:pap3a) or 20% fructose ($\chi^2 = 1.97, p = 0.161$; Figs reffig:pap2a & reffig:pap3b). Prey-capture success of spiders that fed on 20% sucrose was significantly higher than the success of spiders that fed on

R. communis ($\chi^2 = 5.81, p = 0.016$; Figs reffig:pap2a & reffig:pap3a), although there was no significant difference between *R. communis* and 20% fructose ($\chi^2 = 2.84, p = 0.092$; Figs reffig:pap2a & reffig:pap3b). The success of spiders that fed on *P. hysterophorus* was significantly lower than the success of spiders that fed on either 20% sucrose ($\chi^2 = 23.82, p < 0.001$; Figs reffig:pap2a & reffig:pap3a) or 20% fructose ($\chi^2 = 18.46, p < 0.001$; Figs reffig:pap2a & reffig:pap3b).

Discussion

Numerous studies have shown that sugars and amino acids acquired by feeding on nectar can have beneficial effects on the growth, survival and reproduction of insects (e.g. [56, 57, 58, 59]), but our objective was different. We investigated rapidly expressed benefits that apply during a particular phase in a spider's life, namely the phase immediately after the spider emerges from egg sacs and before it has its first prey meal. The specific benefit we considered was plant meal derived improvement in prey-capture proficiency 1 day after the meal and the plant species we considered were *L.camara*, *R. communis* and *P. hysterophorus*. As predicted,we found that, compared with spiders from the water-only control, spiders that fed on these plants were significantly more successful at capturing prey.

The particular plant species from which the juvenile acquired its nectar meal also mattered. In our experiments, and in an earlier study [46], we never saw a spider enter or bite into flowers and instead we saw spiders feed by pressing their mouthparts against petals, leaves and stems of the plant and, when the plant was *R. communis*, drops of nectar from EFNs. Conspicuous EFNs are characteristic of *R. communis* [60, 61], but

not characteristic of *L.camara* or *P. hysterophorus*. However, many plants have EFNs and EFNs are not always conspicuous [6]. Even *R. communis* has, besides its large, conspicuous EFNs, additional EFNs that are evident only with magnification [62].

It is unlikely that the spider fed on phloem or plant tissue instead of nectar. Although nectar is derived primarily from phloem, fructose is characteristic of nectar, whereas phloem is dominated by sucrose alone [63]. Moreover, cold-anthrone testing from an earlier study [46] confirmed that *E. culicivora* ingests fructose when pressing its mouthparts against the surface of the three plant species we used.

It has been suggested that the volume of nectar provided by *P. hysterophorus* is especially low [64], and yet cold-anthrone testing showed that *E. culicivora* acquires fructose from this plant [46] and we have now shown that, after feeding on *P. hysterophorus*, spiders become significantly more successful than the control spiders at capturing prey, but not as successful as spiders that fed on *L.camara*. If nectar volume matters, then spiders from the *L.camara* group being significantly more successful than spiders from the *P. hysterophorus* group is as expected [63]. However, spiders from the *L.camara* group were also significantly more successful than spiders from the *R. communis* group, despite the copious secretion of nectar from EFNs being characteristic of *R. communis*. These findings suggest that, for the plant species we used, the primary influence on prey-capture success is something other than simply variation in the nectar volume available to *E. culicivora*.

Our findings also suggest that the presence of amino acids (or at least the four dominant amino acids) in nectar was not a primary influence on prey-capture success, but that the particular sugars present in a solution,

and their concentrations, did matter. The prey-capture success of spiders from each of our 1% single-sugar groups (sucrose, fructose, glucose and maltose) was not significantly different from the success of spiders from the water-only control, nor were there any significant differences between spiders that fed on the different sugars at 1%. However, findings from using 5% and 20% solutions revealed a ranking of the four sugars: maltose lowest, glucose intermediate, sucrose and fructose tied for highest.

Spiders that fed on sucrose and fructose at concentrations of 5% or 20% were significantly more successful than spiders that fed on glucose or maltose at the same concentrations. We also found that, when the single-sugar concentration was 5% or 20%, spiders that fed on sucrose and spiders that fed on fructose became significantly more successful than spiders from the water-only control, but there was no significant difference between the sucrose and fructose groups when concentration was 5% or 20%.

A combination of findings implies that glucose was intermediate between maltose and sucrose/fructose. Spiders that fed on 20% glucose alone were significantly less successful than spiders that fed on 20% sucrose or 20% fructose, but they were significantly more successful than spiders that fed on 20% maltose or spiders from the water-only control. However, the success of spiders that fed on 5% glucose, although significantly better than the success of spiders from the control group, was not significantly different from the success of spiders that fed on 5% maltose.

Fructose and glucose are monosaccharides, but sucrose and maltose are disaccharides. The hydrolysis of sucrose releases fructose and glucose, but maltose hydrolysis releases only glucose. Spiders from the 20% glucose group were significantly more successful than spiders from the 20% maltose group and, at all concentrations, the success of spiders that fed

on maltose alone was not significantly different from the success of spiders from the water-only control. This combination of findings suggests that the spider has little capacity for maltose hydrolysis and also suggests that acquiring glucose in addition to fructose from sucrose is of little or no advantage over solely acquiring the fructose (i.e. there was no significant difference between 20% sucrose group and the 20% fructose group).

Sucrose, fructose and glucose are known to be present in roughly comparable ratios in the floral nectar of *L.camara* (Irene Baker cited in [51]) and the EFN of *R. communis* [60], and the same sugars in similar ratios might be expected for the nectar of *P. hysterophorus* [49, 52]. The explanation for *L.camara* being ranked best, *R. communis* intermediate and *P. hysterophorus* worst might have more to do with interspecific variation in sugar concentration instead of interspecific variation in the ratios of the available sugars, but testing this hypothesis will probably be especially difficult. The concentration of sugar in nectar is known to be sensitive to relative humidity, time of day and other environmental factors [65, 66, 67], and estimating the sugar concentration encountered by a spider when it presses its mouthparts on the surfaces of the different plant species might be especially difficult. However, we can propose how the spider's access to sucrose and especially fructose might vary across the plant species we used in our experiments.

Finding no significant difference between the success of spiders that fed on 20% sucrose or 20% fructose and the success of spiders that fed on *L.camara* suggests that, on *L.camara*, *E. culicivora* juveniles can gain access to one or both of these sugars at an optimal concentration. That the spiders we let feed on 20% sucrose or 20% fructose were significantly more successful at prey-capture than the spiders we let feed on *R. commu-*

nis or *P. hysterophorus* suggests that, on these two plant species, *E. culicivora* juveniles cannot readily gain access to either of these sugars at the concentration available from *L.camara*. However, there are alternative hypotheses we cannot rule out at this stage. For example, we cannot rule out a hypothesis that unknown non-sugar compounds from *R. communis* and *P. hysterophorus*, but not *L.camara*, had negative effects on prey-capture success [68].

Spiders were less successful at capturing prey after a 6 day fast than after a 3 day fast, suggesting that longer fasting weakened the spider. Yet, the distribution of success rates across groups followed much the same pattern irrespective of fasting duration. These findings suggest that, although hungrier spiders benefit more from plant-derived nutrients, the benefit-related ranking of the nutrient sources is stable across hunger level.

Nectar and other plant-derived nutrients may often be important in the natural diets of spiders and predatory insects and, when the predators kill agricultural pests, there is an impetus to determine whether ensuring the availability of plant meal sources might make predators more effective in the biological control of the pest species [69, 70]. When discussing agricultural systems, the most frequently considered beneficial effects of nectar meals include the sustaining of predator populations during periods of prey scarcity, giving predators access to nutrients not available from prey and reducing the level of competition between predators that target the same prey (e.g. [71]). These benefits would normally be expressed over a considerable timespan and comparable long-term benefits may apply to *E. culicivora*. However, the benefits implied by our findings are expressed the next day after a nectar meal and it might be of interest to investigate whether similar rapid benefits apply to other predators, including preda-

tors that target agricultural pests.

Figure B.2: For *Evarcha culicivora* juveniles, predicted prey-capture success (i.e. probability of capture success after attacking mosquito) plus 95% confidence intervals after 3 day and 6 day fast. Predictions derived from logistic model (see text). Abbreviations for groups defined in Table reftab:pap1. (a) Spiders that fed on different plant species. (b) Spiders that fed on *L.camara* or on artificial *L.camara* nectar.

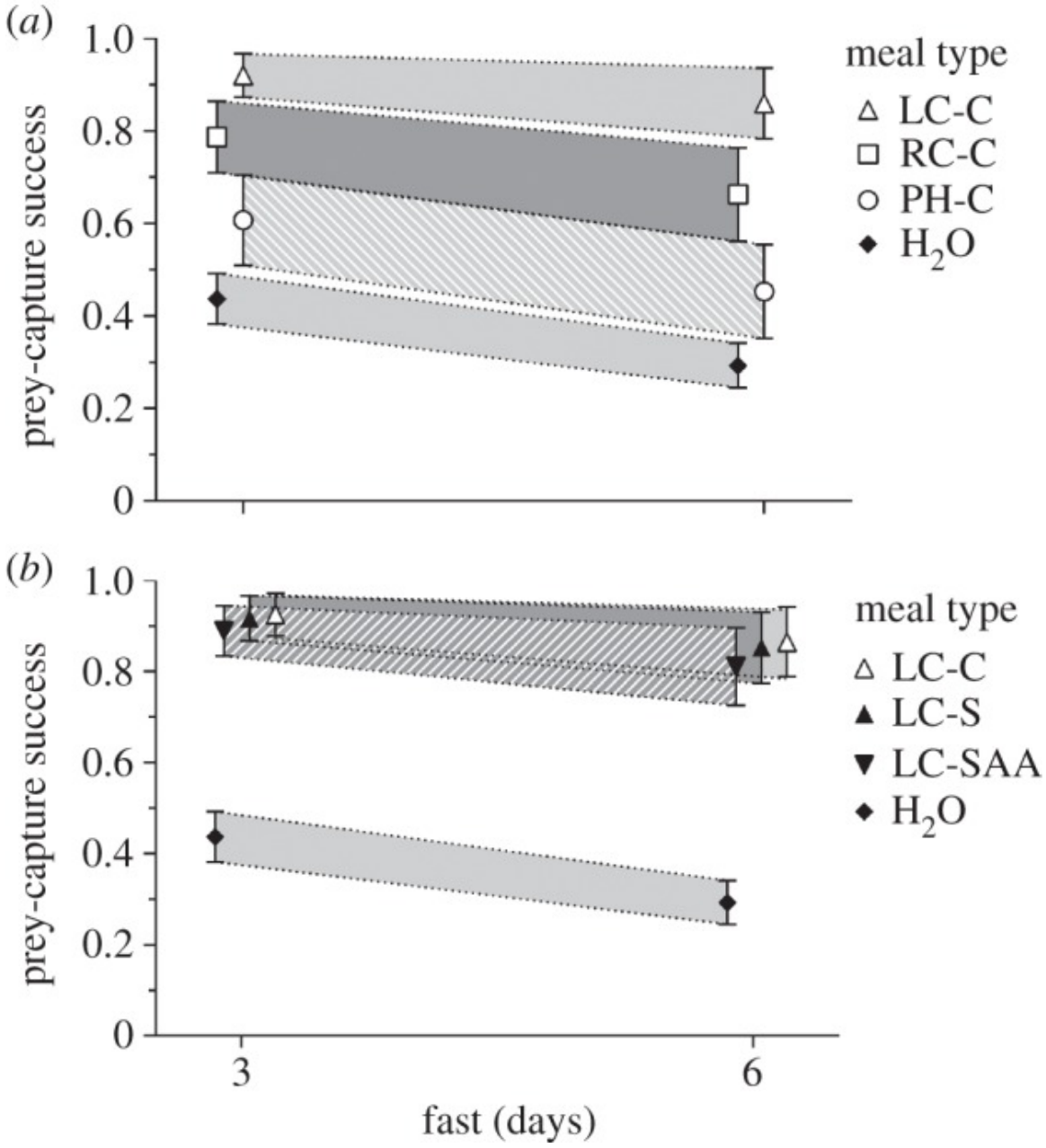
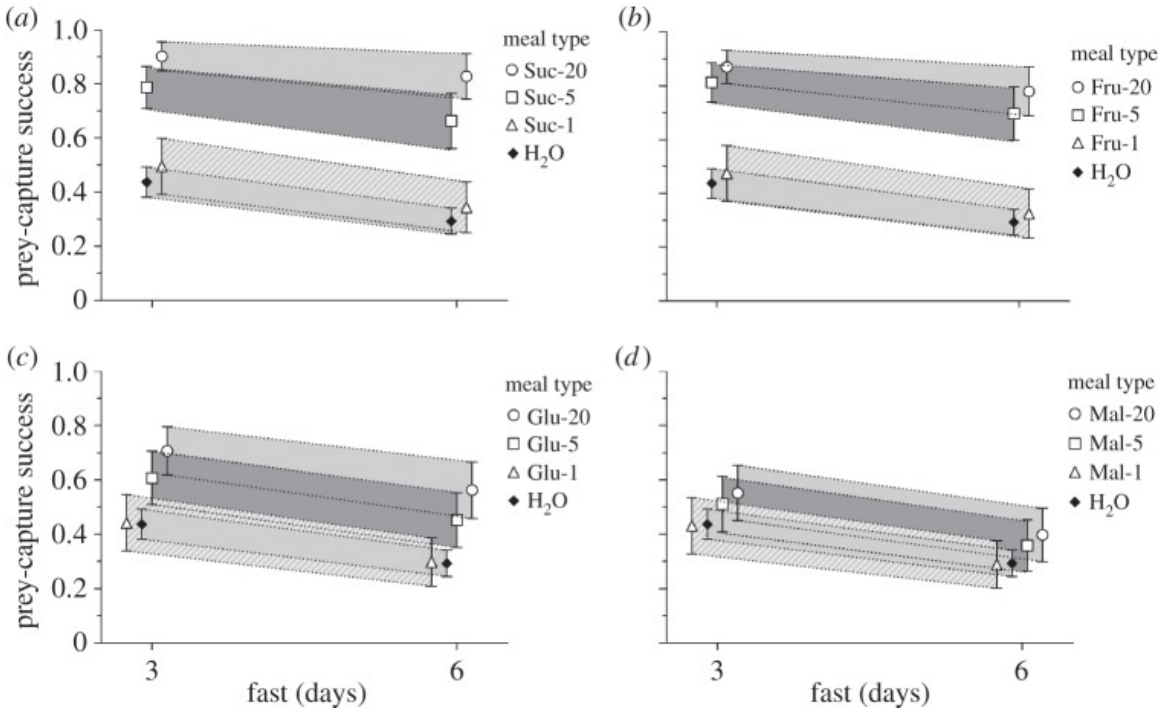


Figure B.3: For *Evarcha culicivora* juveniles, predicted prey-capture success (i.e. probability of capture success after attacking mosquito) plus 95% confidence intervals after 3 day and 6 day fast. Predictions derived from logistic model (see text). Abbreviations for groups defined in Table reftab:pap1. Spiders fed on different concentrations of (a) sucrose, (b) fructose, (c) glucose and (d) maltose.



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