

Fine tuning of vision-based prey-choice decisions by a predator that targets malaria vectors

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Abstract. *Evarcha culicivora* Wesolowska & Jackson 2003 is a jumping spider (Aranea: Salticidae) that has the distinction of being the only predator known to express an active preference for the vectors of human malaria (i.e., the mosquito genus *Anopheles*) and to feed indirectly on blood by choosing blood-carrying female mosquitoes as prey. Here we examine this predator's preference profile in greater detail than has been achieved before. Lures (dead prey mounted in life-like posture) were made from two mosquitoes (*Anopheles gambiae* and *Culex quinquefasciatus*) and a non-biting midge (*Clinotanypus claripennis*). Testing protocols were simultaneous presentation (two prey presented simultaneously), alternate day (two prey, each presented singly but on alternate days) and alternative prey (second prey presented while test spider feeding on first prey). Pre-trial fasts were 1, 7, 15 and 21 days. Findings from this combination of variables were used to estimate strengths of preferences. Besides confirming the preference of *E. culicivora* for blood meals and for *Anopheles* in particular, we provide the first evidence of a preference, independent of blood meals, for female instead of male mosquitoes. The strength of preference, measured by its persistence despite increasingly long pre-trial fasts, shows that preference for *Anopheles* is expressed by juveniles more strongly than by adults, but preference for blood meals is expressed by adults more strongly than by juveniles.

Keywords: Foraging, mosquito, predation, prey preference, salticid, specialization

The term 'preference' acknowledges that predators favor certain kinds of prey, while 'choice' refers to behavior that is driven by preference. Data on a predator's natural diet may suggest hypotheses about preferences, and these hypotheses can be used to predict the choices a predator will make. However, data on diet alone cannot reveal a predator's choices and preferences (Huseynov et al. 2008; Nelson & Jackson 2011). Predators that have been shown with appropriate experimental evidence to express distinctive preferences are of particular interest, not only as model organisms for testing predictions derived from foraging theory, but also for research on the mechanisms underlying perception and decision-making. Owing to their ability to see fine detail (Harland et al. 2011) and their intricate vision-guided prey-capture behavior (Jackson & Pollard 1996), jumping spiders (Salticidae) are exceptionally suitable subjects for experimental studies related to predatory preferences.

Striking examples of prey-choice behavior are known (Nelson & Jackson 2011) especially for salticids that prefer other spiders as prey (araneophagic species) and for salticids that prefer ants as prey (myrmecophagic species). However, *Evarcha culicivora* Wesolowska & Jackson 2003 has the most specific preferences known for a salticid. All ages of this East African species feed indirectly on vertebrate blood by actively choosing as preferred prey blood-fed female mosquitoes, both in a laboratory setting and in nature (Wesolowska & Jackson 2003; Jackson et al. 2005). *Evarcha culicivora* also distinguishes between mosquito genera, expressing a preference for *Anopheles* in particular (Nelson & Jackson 2006). Yet research on the preferences of *E. culicivora* has been based on only a few of the experimental protocols and pre-trial fasting periods that have been routine in research on araneophagic and myrmecophagic species.

Prey preference of myrmecophagic and araneophagic salticids has been studied using three different protocols and pre-trial fasting durations (Li & Jackson 1996; Li et al. 1996, 1997,

1999; Jackson et al. 1998). Here, we apply all three protocols to identify the prey preferences of *Evarcha culicivora* and to better understand the strength of its preferences.

Of these three protocols, alternative-prey testing can be envisaged as requiring the strongest expression of preference because, in these tests, a salticid is already feeding when offered a different kind of prey. Consequently, to express a preference, a spider must release an already secured prey to capture an alternative. With simultaneous-presentation testing, a salticid is given access to two potential prey at the same time and is allowed to make a snap decision to choose one or the other. Alternate-day testing, where a spider's inclination to take each of two prey types is assessed in isolation from the other, can be envisaged as a method for discerning preference stronger than that required by simultaneous-presentation testing, but not requiring the strength of preference required by alternative-prey testing. With all three testing protocols, how fasting affects preference is also of interest because one of the most basic predictions from foraging theory is that predators will become less selective in times of prey scarcity (see Sih & Christensen 2001). This, in turn, is a rationale for predicting that longer fasts will be necessary before stronger preferences dissipate.

The alternate-day and alternative-prey protocols, and the longer pre-trial fasting durations used in experiments on myrmecophagic and araneophagic salticids, have not been used previously in research on *E. culicivora*. Yet, with the preference of *E. culicivora* appearing to be especially complex, this is a species for which data from the full range of testing protocols and fasting duration would be of particular interest. Here we consider the relative strengths of preferences for blood meals and also for *Anopheles* when neither choice had received a blood meal. We compare the preference profiles of adult females with those of juveniles of *E. culicivora*. We also investigate, for the first time, the hypothesis that *E. culicivora*, independent of blood meals, expresses a preference for female

rather than male mosquitoes, based on visual cues alone. In mosquitoes the major morphological difference between the sexes lies in the number of setae on the antennae, with male antennae appearing more plumose than those of females. The rationale for our hypothesis is the fact that males subsist primarily on nectar (Klowden 1995), while only female mosquitoes feed on blood (Clements 1999). For the spider, choosing female mosquitoes will not suffice for acquiring a blood meal. However, taking an interest in female mosquitoes, independent of whether she is seen to be carrying blood, might be advantageous in the context of predisposing *E. culicivora* to pay attention to the mosquitoes that have the potential of being blood carriers.

METHODS

Spiders used for tests were juveniles (2.0 mm) and adult females (5.5 mm) of *Evarcha culicivora* (body length accurate to nearest 0.5 mm). All test spiders were from laboratory culture (see Jackson et al. 2005), with rearing diet consisting of lake flies (i.e., non-biting midges, known locally as 'lake flies': Chironomidae) and blood-fed female mosquitoes (*Culex quinquefasciatus* and *Anopheles gambiae* s.s.; henceforth simply *Culex* and *Anopheles*, respectively) provided ad libitum three days a week. Lake flies were collected locally as needed, and mosquitoes came from laboratory culture (see Jackson et al. 2005). In culture, all mosquitoes had continuous access to glucose (6% solution). Mosquitoes used for making lures were both sexes of *Culex* and *Anopheles*. Two types of female mosquitoes were used: 'blood-fed' (received blood meal 4 h before feeding spiders or being used for making lures) or 'sugar-fed' (no blood meals). Females of *Clinotanytus clari-pennis*, a common chironomid in our field site, were also used for making lures. For standardization, all insects used for making a lure were 5.0 mm in body length (accurate to the nearest 0.5 mm). Each insect was mounted centered on a cork disc and then sprayed with a transparent plastic adhesive which served to preserve lures and prevent odor cues from affecting test outcome (for details concerning making lures, see Jackson et al. 2005).

The testing arena (walls 35 mm high, see Fig. 1 for other dimensions) was a glass box with a removable glass lid that sat centered on top of a 150 mm high Plexiglas stand. All testing was carried out between 0800 and 1400 hours (laboratory photoperiod 12L:12D, lights on at 0700 hours). No test spider or lure was used in more than one type of test. We introduced spiders into the arena through a hole in the floor (Fig. 1). This hole, situated with its closer side 10 mm from one end of the box, was plugged with a removable rubber bung.

At the opposite end of the arena, there was a 'left lure hole', a 'right lure hole' and a 'central hole' (diameter of each, 5 mm). Lures were positioned outside the arena so that spiders could only see them through the arena's glass walls, and thus could not detect any odor cues. In simultaneous-presentation tests, a lure was centered on top of the right hole, and another lure was centered on the top of the left hole. In alternate-day and alternative-prey tests, a single lure was centered on the top of the central hole. The lure was placed such that it faced directly toward the side of the arena. The lure stayed in place because the diameter of the hole in the stand was narrower than the diameter of cork disc holding the lure.

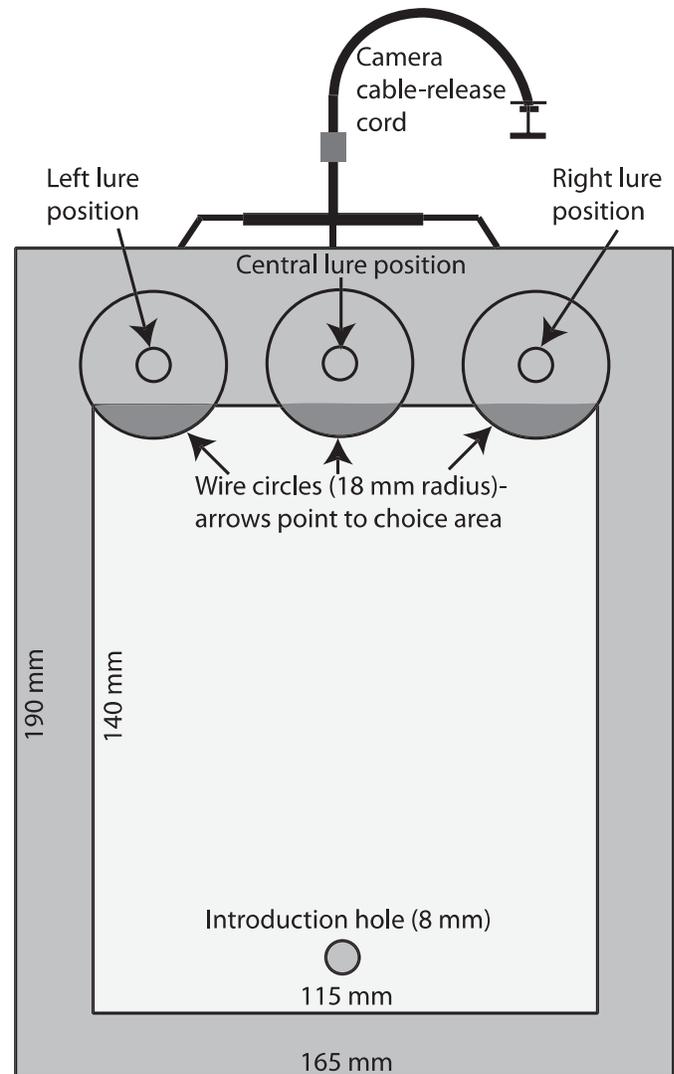


Figure 1.—Testing apparatus. Rectangular glass box (white rectangle in figure) with glass lid, sitting on top of Plexiglas stand (grey rectangle). Moving lures controlled by using a camera release cord. 'Choice area': dark grey semicircular area within wire circles. Left and right lure positions used in simultaneous-presentation tests. Central lure position used in alternate-day and alternative-prey tests.

A metal prong attached to a camera cable-release cord was connected to the underside of each of the two cork discs in simultaneous-presentation tests and to the single cork disc in alternate-day and alternative-prey tests. Pressing the cable-release moved each lure 5 mm above the floor of the arena and then, by releasing the cable, each lure moved back to the floor. As soon as the test spider entered the arena, the cable-release was pressed once every 30 s and then released immediately, causing the lure/s to move up once and down one time for each press. This ensured that salticids remained interested in the lures, as motionless prey are less effective at eliciting responses from salticids. In each simultaneous-presentation test, there were two lure types. In alternate-day and alternative-prey tests, the single lure that was present on one day was different from the single lure that was present on the other day in the pair of tests.

Table 1.—Alternative-prey testing (see text for details) of *Evarcha culicivora* adults (data outside parentheses) and juveniles (data inside parentheses). Prey presented as moving lures: *Clinotanytus claripennis* (Cc), blood-fed *Anopheles* female (Af), sugar-fed *Anopheles* female (Afs), *Anopheles* male (Am), blood-fed *Culex* female (Cf), sugar-fed *Culex* female (Cfs), *Culex* male (Cm). Note: most spiders did not drop any prey for the other (N paired tests minus sum of both columns depicting numbers of prey that were dropped). ¹ Depicts one day fasted. ⁷ Depicts seven days fasted. * $P < 0.05$.

Prey 1	Prey 2	Dropped prey 2 for prey 1	Dropped prey 1 for prey 2	N paired tests
¹ Af	Am	13* (2)	0 (1)	40 (35)
⁷ Af	Am	1 (1)	0 (1)	40 (30)
¹ Af	Afs	11* (0)	1 (0)	41 (25)
⁷ Af	Afs	0 (0)	0 (1)	30 (28)
¹ Af	Cf	0 (0)	0 (0)	40 (50)
⁷ Af	Cf	0 (0)	0 (0)	40 (50)
¹ Af	Cfs	11* (0)	1 (0)	40 (30)
⁷ Af	Cfs	0 (0)	0 (0)	40 (30)
¹ Af	Cc	13* (0)	0 (0)	60 (25)
⁷ Af	Cc	0 (0)	0 (0)	50 (29)
¹ Cf	Cm	10* (0)	0 (0)	50 (40)
⁷ Cf	Cm	0 (0)	0 (0)	50 (40)
¹ Cf	Cfs	8* (0)	0 (0)	31 (25)
⁷ Cf	Cfs	0 (0)	0 (0)	35 (25)
¹ Cf	Afs	12* (0)	2 (0)	60 (40)
⁷ Cf	Afs	0 (0)	0 (0)	50 (40)
¹ Cf	Cc	9* (1)	0 (0)	40 (25)
⁷ Cf	Cc	0 (0)	0 (0)	35 (20)
¹ Afs	Cfs	0 (0)	1 (0)	40 (40)
⁷ Afs	Cfs	0 (0)	0 (0)	40 (40)
¹ Afs	Am	1 (0)	0 (0)	40 (40)
⁷ Afs	Am	0 (0)	0 (0)	40 (40)
¹ Afs	Cm	0 (0)	1 (0)	57 (25)
⁷ Afs	Cm	0 (0)	0 (0)	40 (25)
¹ Afs	Cc	0 (0)	0 (0)	40 (30)
⁷ Afs	Cc	0 (0)	0 (0)	40 (30)
¹ Am	Cm	0 (0)	0 (0)	35 (40)
⁷ Am	Cm	0 (0)	1 (0)	35 (40)
¹ Am	Cc	0 (0)	0 (0)	35 (25)
⁷ Am	Cc	0 (0)	0 (0)	30 (20)
¹ Cm	Cc	0 (0)	0 (0)	35 (20)
⁷ Cm	Cc	0 (0)	0 (0)	35 (20)
¹ Cfs	Cm	0 (0)	0 (0)	30 (23)
⁷ Cfs	Cm	0 (0)	0 (0)	30 (25)
¹ Cfs	Am	0 (0)	0 (0)	25 (20)
⁷ Cfs	Am	0 (0)	0 (0)	25 (22)
¹ Cfs	Cc	0 (0)	0 (0)	40 (40)
⁷ Cfs	Cc	0 (0)	0 (0)	40 (40)

Two circles made from thin copper wire were situated on the platform in simultaneous-presentation tests, and one circle made from thin copper wire was situated on the platform in alternate-day and alternative-prey tests. A lure hole was at the center of each circle, and a part of each wire circle extended under the arena (Fig. 1), visible because the bottom of the arena was made of glass. The part of the circle under the arena was the 'choice area' (Fig. 1). Our operational definition of a choice was seeing the test spider fixate its gaze on a lure and then, while retaining fixation, entering the choice area. 'Fixate' refers to the corneal lenses of the salticid's large forward-facing principal eyes being held oriented toward a lure. There were rare instances (< 5%) of the 15-min test period ending with the test spider outside the choice area, but with its gaze fixated on a lure. In these instances, we extended the test period until the test spider either made its choice or turned away.

During simultaneous-presentation testing, the test spider was exposed to two lures at the same time, each being made from a different kind of prey (side for each lure determined at random). These data were analyzed using chi-square tests of goodness of fit. In alternate-day tests, the test spider was exposed to a single lure of one type on one day and a single lure of another type on the next day (which prey type presented first randomized). In alternate day tests, only those test pairs in which *E. culicivora* chose one prey, but not the other, provided evidence of preference. In alternative-prey tests, the spider was exposed to a single lure made from one prey type while feeding on another prey type. Alternative-prey testing, like alternate-day testing, was carried out in two trials, one on each of two successive days. The prey type on which the test spider was feeding on the first day was the prey type provided as a lure on the second day. Which of the two

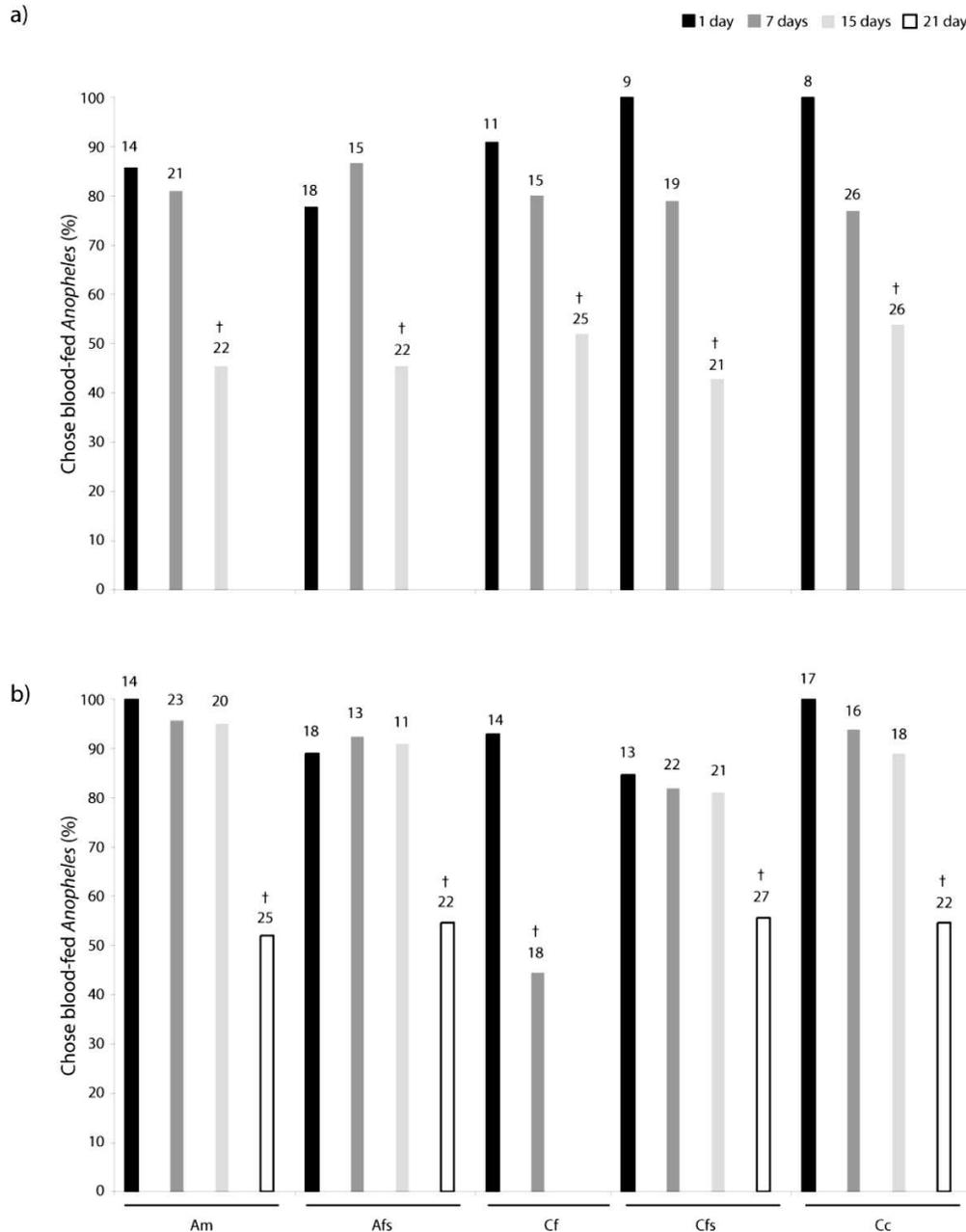


Figure 2.—Expression of preference during simultaneous-presentation tests (data analysis: test of goodness of fit, $H_0 = 50/50$) by a) juveniles and b) adults of *Evarcha culicivora*. Pre-trial fast durations indicated by different shadings. Each spider tested with blood-fed *Anopheles* female and with another prey. Data expressed as percentage of test spiders that chose *Anopheles* females (N used for statistics over bar). Other prey: *Anopheles* male (Am), sugar-fed *Anopheles* female (Afs), blood-fed *Culex* female (Cf), sugar-fed *Culex* female (Cfs), *Clinotanytus claripennis* (Cc). † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose blood-fed *Anopheles* ($P < 0.05$).

prey types served as a lure in the first-day trials was randomized.

Each simultaneous-presentation and alternate-day test began when the test spider entered the arena. However, before alternative-prey tests were initiated, the test spider was put in a Petri dish (diameter 90 mm) with a single prey item of one type. The test spider usually captured and began feeding on this prey no later than 15 min after being introduced into the Petri dish, and 30 s after beginning to feed the test spider was introduced, while still feeding, into the arena. Testing was

cancelled on the rare occasions when the test spider failed to capture the prey in the Petri dish within 15 min or failed to hold on to the prey when being introduced into the arena. These spiders were not used again. The operational definition of ‘choice’ in alternative-prey testing included an additional requirement: the spider had to drop the prey on which it was feeding, either before entering or while inside the choice area, and only spiders that dropped their prey were used for analysis. However, no spiders entered the choice area carrying prey and left it still carrying the prey, nor were there any

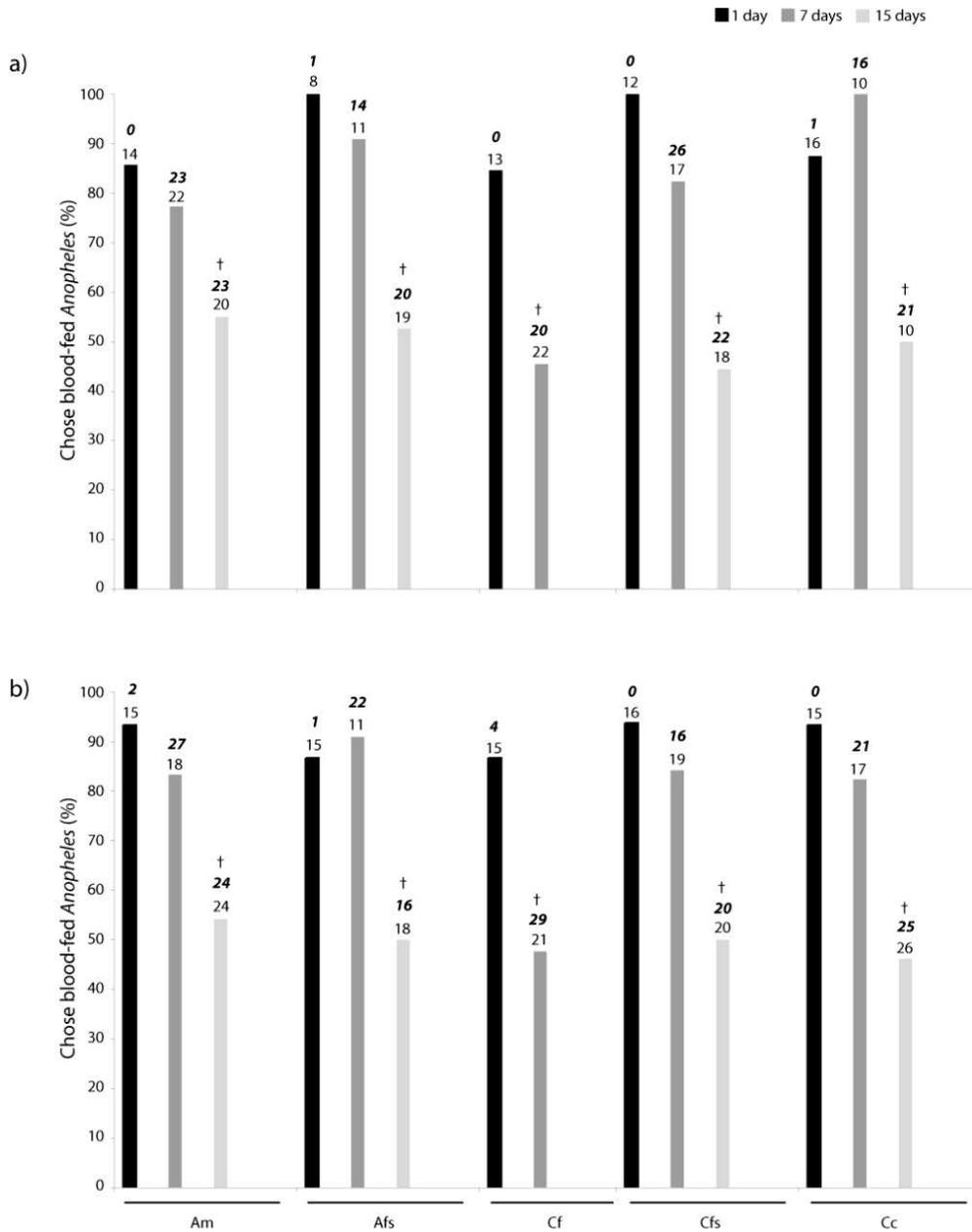


Figure 3.—Expression of preference during alternate-day tests (data analysis: McNemar tests for significance of changes) by a) juveniles and b) adults of *E. culicivora*. Pre-trial fast durations indicated by different shadings. Each spider tested with blood-fed *Anopheles* female and with another prey. Data plotted include only results from spiders that moved to the choice area for one prey and not the other (i.e., those that chose neither or both prey were omitted). Data expressed as percentage of test spiders that chose *Anopheles* females (N used for statistics over bar). Other prey: *Anopheles* male (Am), sugar-fed *Anopheles* female (Afs), blood-fed *Culex* female (Cf), sugar-fed *Culex* female (Cfs), *C. claripennis* (Cc). † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose blood-fed *Anopheles* ($P < 0.05$). The number of spiders that chose both prey is indicated in bold italics above bars.

instances of spiders outside the choice area dropping their prey without being fixated on a lure.

Four feeding regimes (pre-trial fasting durations) were adopted: 1, 7, 15 or 21 days (i.e., the test spider was fed to satiation, held without prey for the indicated fast duration and then tested). Two of these feeding regimes (1-day and 7-day fast) were adopted in all experiments. Whenever preference was still evident after a 7-day fast, testing was also carried out

after a 15-day fast and, whenever preference was still evident after a 15-day fast, testing resumed after a 21-day fast.

When used in alternate-day and alternative-prey testing, the test spider was exposed to a moving lure on two successive days and this meant that, on the second day, fasting duration was actually a day longer than stated (e.g., ‘7-day fast’ means the test spider’s last meal was 7 days before the first and 8 days before the second test in the test pair). The data analysis used

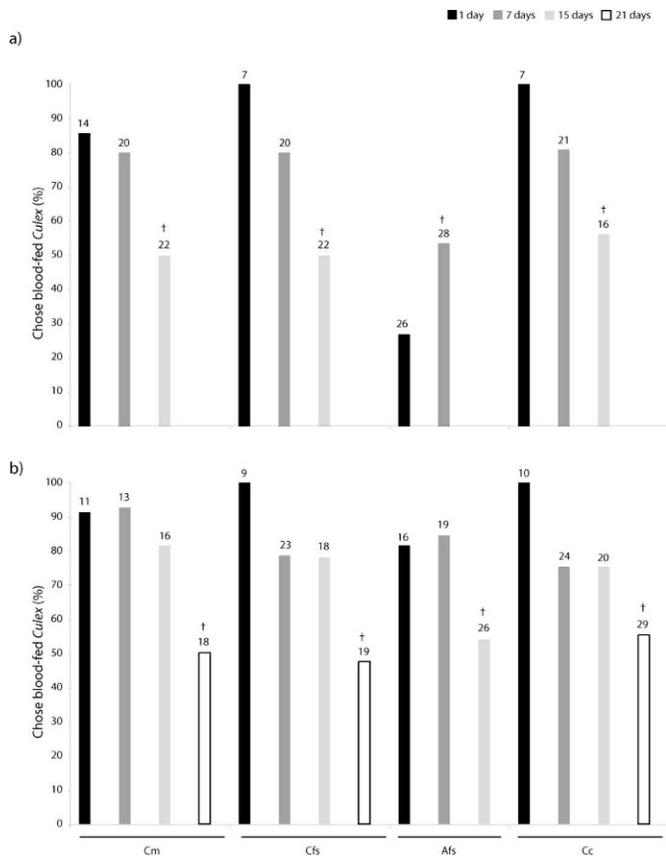


Figure 4.—Expression of preference during simultaneous-presentation tests (data analysis: test of goodness of fit, $H_0 = 50/50$) by a) juveniles and b) adults of *E. culicivora*. Pre-trial fast durations indicated by different shadings. Each individual spider tested with blood-fed *Culex* female and with another prey. Data expressed as percentage of test spiders that chose *Culex* females (N used for statistics over bar). Other prey: *Culex* male (Cm), sugar-fed *Culex* female (Cfs), sugar-fed *Anopheles* female (Afs), *C. claripennis* (Cc). † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose blood-fed *Culex* ($P < 0.05$).

(McNemar tests for significance of changes) considers only the instances in which a test spider chose a lure on one, but not the other, of the two successive days. However, for spiders subjected to a 1-day fast, we ensured that the fast was 1 day for the first and for the second test of the test pair. This was achieved by feeding the test spider to satiation immediately after testing on the first day. The stringency of alternative prey testing made obtaining these data particularly difficult (because test spiders rarely dropped one prey to take another), leaving us with only small sample sizes for statistical analysis despite the numerous tests carried out (20 to 60 paired tests, see Table 1).

For each pair of prey types, we calculated an arbitrary preference index, this being a number that increased with the number of testing protocols and fasting durations for which significant evidence of preference had been shown. For example, if a blood-fed female *Anopheles* was chosen over a particular alternative under all three testing protocols, but not after fasts longer than 7 days (second of 4 possible fasting durations), this would have an index of $3 \times 2 = 6$.

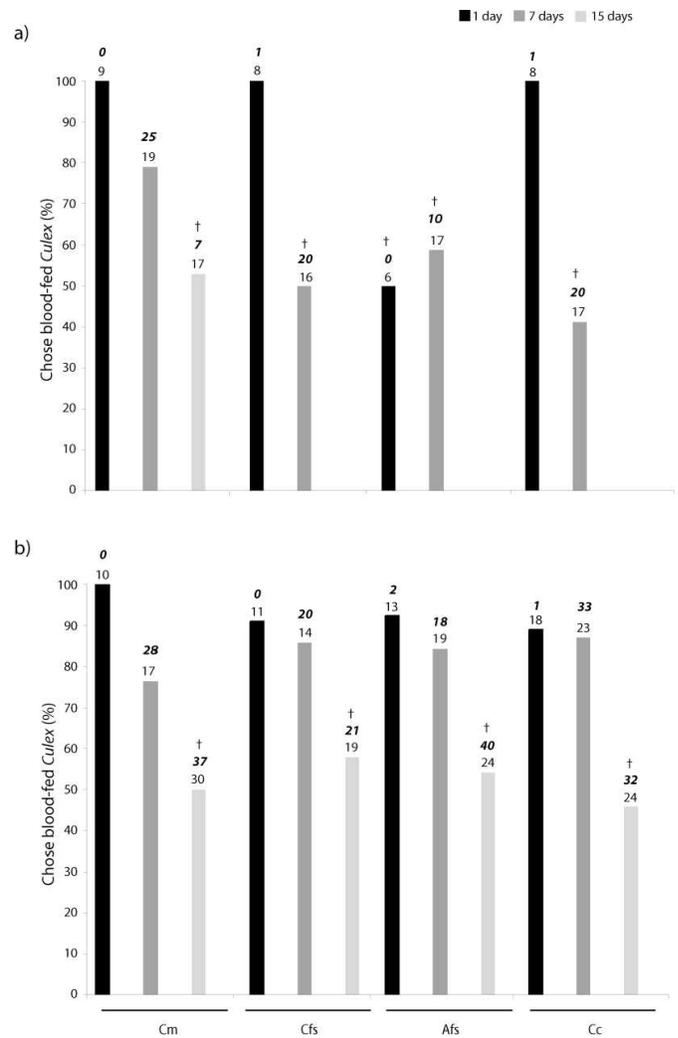


Figure 5.—Expression of preference during alternate-day tests (data analysis: McNemar tests for significance of changes) by a) juveniles and b) adults of *E. culicivora*. Pre-trial fast durations indicated by different shadings. Each individual spider tested with blood-fed *Culex* female and with another prey. Data plotted include only results from spiders that moved to the choice area for one prey and not the other (i.e., those that chose neither, or both prey were omitted). Data expressed as percentage of test spiders that chose *Culex* females (N used for statistics over bar). Other prey: *Culex* male (Cm), sugar-fed *Culex* female (Cfs), sugar-fed *Anopheles* female (Afs), *C. claripennis* (Cc). † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose blood-fed *Culex* ($P < 0.05$). The number of spiders that chose both prey is indicated in bold italics above bars.

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

RESULTS

Preference for blood.—First, we consider data from adult test spiders. In all instances and regardless of testing method, when one prey was a blood-fed female mosquito and the other was not carrying blood (i.e., the other prey was a sugar-fed female mosquito, a male mosquito, or a lake fly), preference

Table 2.—Preference indices for juveniles and adults of *Evarcha culicivora*. Preference index derived by multiplying the number of testing protocols under which significant evidence of preference was shown times the fasting durations under which significant evidence of preference was shown (see text for example). Higher values for the index indicate stronger preferences. With one exception*, preference is for prey 1. * Chose prey 2 (sugar-fed *Anopheles* female).

Prey 1	Prey 2	Juvenile index	Adult index
Blood-fed <i>Anopheles</i> female	<i>Anopheles</i> male	4	6
Blood-fed <i>Anopheles</i> female	Sugar-fed <i>Anopheles</i> female	4	6
Blood-fed <i>Anopheles</i> female	Blood-fed <i>Culex</i> female	3	2
Blood-fed <i>Anopheles</i> female	Sugar-fed <i>Culex</i> female	4	6
Blood-fed <i>Anopheles</i> female	Lake fly	4	6
Blood-fed <i>Culex</i> female	<i>Culex</i> male	4	6
Blood-fed <i>Culex</i> female	Sugar fed <i>Culex</i> female	3	6
Blood-fed <i>Culex</i> female	Sugar-fed <i>Anopheles</i> female	1*	5
Blood-fed <i>Culex</i> female	Lake fly	3	5
Sugar-fed <i>Anopheles</i> female	Sugar-fed <i>Culex</i> female	4	2
Sugar-fed <i>Anopheles</i> female	<i>Anopheles</i> male	3	2
Sugar-fed <i>Anopheles</i> female	<i>Culex</i> male	3	2
Sugar-fed <i>Anopheles</i> female	Lake fly	3	2
Sugar-fed <i>Culex</i> female	<i>Culex</i> male	0	0
Sugar-fed <i>Culex</i> female	<i>Anopheles</i> male	0	0
Sugar-fed <i>Culex</i> female	Lake fly	0	0
<i>Anopheles</i> male	<i>Culex</i> male	3	0
<i>Anopheles</i> male	Lake fly	1	0
<i>Culex</i> male	Lake fly	0	0

for the blood meal was expressed (Table 1, Figs. 2–5). However, there was variation in the fasting durations over which preferences were maintained (Figs. 2–5). With two exceptions, the preference index (see Table 2) was 6, the exceptions being a slightly lower preference index of 5 when blood-fed *Culex* females were paired with sugar-fed *Anopheles* females or with lake flies.

Next we consider data from juveniles. When the blood meal was a *Culex* female and the no-blood meal was an *Anopheles* female (i.e., when preference for *Anopheles* clashed with preference for blood meals), preference for *Anopheles* was expressed in preference to blood (Fig. 4). However, as the expression of this preference dissipated after a 7-day fast, the preference index was only 1. In all other instances of simultaneous-presentation and alternate-day testing, preference for blood meals was expressed, but the fasting durations over which preference was maintained varied (Figs. 2–5), and no preference was expressed in alternative prey testing (Table 1). When the blood meal was an *Anopheles* female, the preference index was typically 4, which was slightly higher than the preference index characteristically expressed for blood-fed *Culex* females (Table 2).

Preference for *Anopheles*.—Except in alternative-prey tests (Table 1), adult and juvenile test spiders expressed preference for *Anopheles* when both prey were blood-fed mosquitoes (adult index 2, juvenile index 3; Figs. 2, 3) and when both were sugar-fed mosquitoes (adult index 2, juvenile index 4; Figs. 6, 7). When the two mosquitoes were males, preference for *Anopheles* was expressed by juveniles (index 3), but not by adults (Fig. 8, Table 2).

Preference for female mosquitoes.—When tested with two mosquitoes, one being a male and the other being a female, with neither carrying blood, preference for the female was expressed when the female was *Anopheles* (Figs. 6, 7), regardless of whether the male was *Anopheles* or *Culex* (for both mosquito species: preference index was 2 for adults and 3 for juveniles). However, no preference was evident when the

female was *Culex* (Fig. 9), regardless of whether the male was *Anopheles* or *Culex* (Table 2).

Preference for mosquitoes when the alternatives are lake flies.—When one prey was a lake fly and the other was a mosquito that was not carrying blood, adults expressed preference for *Anopheles* females (Figs. 6, 7; preference index 2), but not for *Culex* females (Fig. 9) and, with the exception of juveniles that had fasted for only 1 day in simultaneous-presentation tests (Fig. 8), not for males of either mosquito species (Table 2). Adult and juvenile spiders expressed preference (Table 2) for *Anopheles* females (Figs. 6, 7), and juveniles expressed a weak preference for males (Fig. 9), but neither juveniles nor adults expressed preference for either sex of *Culex* when tested against lake flies (Figs. 8, 9).

Response levels.—There was a significant effect of fasting time on the number of juveniles ($H_2 = 36.34$, $P < 0.001$) and adults (Kruskal-Wallis, $H_3 = 35.33$, $P < 0.001$) that responded in simultaneous-presentation tests, as well as in alternate-day tests (juveniles, $H_2 = 27.18$, $P < 0.001$; adults, $H_2 = 33.73$, $P < 0.001$). In both cases, the effect was based on a sharp drop in the number of spiders that failed to choose between 1 and 7-day fasts (Fig. 10).

In alternative prey tests spiders did not respond at all beyond 7-day fasting periods (no spiders ever dropped their prey for the alternative), so instead of using Kruskal-Wallis tests, these data were analyzed using Mann-Whitney U tests for 1-day and 7-day fasting data. However, the number of juveniles ($U = 178.5$, $P = 0.964$) and the number of adults ($U = 147.5$, $P = 0.335$) that failed to choose (i.e., did not drop one prey for the other) did not differ after a 1 or a 7-day fast and instead remained consistently high (Table 1, Fig. 10).

DISCUSSION

The choices made by *Evarcha culicivora* in these experiments reveal the prey preferences of this unusual predator. The prey-preference profile of *E. culicivora* appears to be structured in

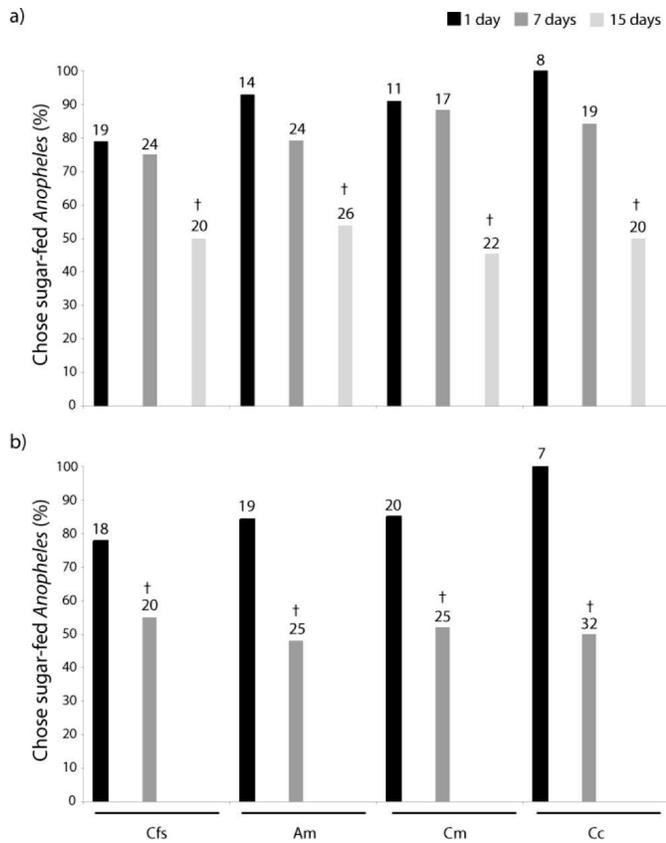


Figure 6.—Expression of preference during simultaneous-presentation tests (data analysis: test of goodness of fit, $H_0 = 50/50$) by a) juveniles and b) adults of *E. culicivora*. Pre-trial fast durations indicated by different shadings. Each individual spider tested with sugar-fed *Anopheles* female and with another prey. Data expressed as percentage of test spiders that chose *Anopheles* females (N used for statistics over bar). Other prey: sugar-fed *Culex* female (Cfs), *Anopheles* male (Am), *Culex* male (Cm), *C. claripennis* (Cc). † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose sugar-fed *Anopheles* ($P < 0.05$).

tiers of prey choice decisions. This is more complex than the way spider preferences for prey are normally envisaged, and unusual for predators in general. With each of the three testing protocols, we always reached a fasting duration after which preference was no longer detected, but the required duration varied with testing protocol and with whether the spider was an adult or a juvenile. Juveniles and adults expressed preferences for the same prey types, but there were differences in strength and priority. When test spiders were adults, the highest priority was consistently for blood meals, with a preference for *Anopheles* independent of acquiring a blood meal seeming to be superimposed as a secondary preference. These findings, based on more testing protocols and fasting durations than previously used, confirm basic conclusions from earlier studies (Jackson et al. 2005; Nelson & Jackson 2006).

We found that preference for *Anopheles* over *Culex* is stronger in juveniles than in adults. It is particularly notable that, independent of blood, juveniles expressed a preference for *Anopheles* over *Culex* that was sustained through longer

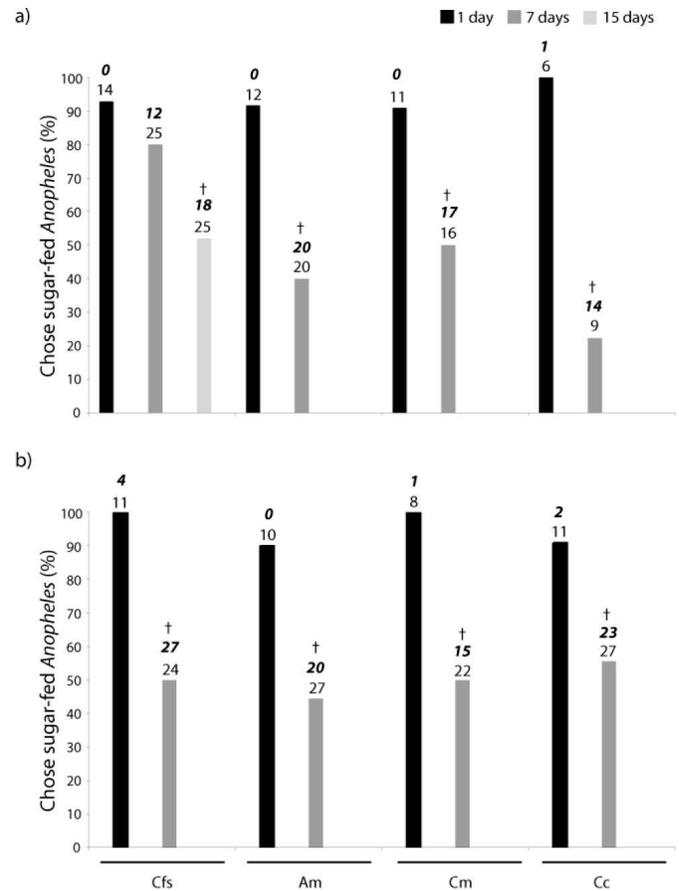


Figure 7.—Expression of preference during alternate-day tests (data analysis: McNemar tests for significance of changes) by a) juveniles and b) adults of *E. culicivora*. Pre-trial fast durations indicated by different shadings. Each individual spider tested with sugar-fed *Anopheles* female and with another prey. Data plotted include only results from spiders that moved to the choice area for one prey and not the other (i.e., those that chose neither or both prey were omitted). Data expressed as percentage of test spiders that chose *Anopheles* females (N used for statistics over bar). Other prey: sugar-fed *Culex* female (Cfs), *Anopheles* male (Am), *Culex* male (Cm), *C. claripennis* (Cc). † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose sugar-fed *Anopheles* ($P < 0.05$). The number of spiders that chose both prey is indicated in bold italics above bars.

pre-trial fasts than was the case for adults. Another striking finding from simultaneous-presentation testing was that, when fast duration was only 1 day, juveniles chose sugar-fed *Anopheles* in preference to blood-fed *Culex*. In this instance, the juvenile's priority appeared to be for *Anopheles* instead of a blood meal. This was a sharp contrast with the findings from adults, where blood meals were the highest priority whenever preferences were expressed.

Yet another preference seems to be superimposed on preference for blood meals and for *Anopheles*. Juveniles and adults of *E. culicivora* expressed a preference for female instead of male mosquitoes independent of acquiring a blood meal, but only if the female mosquito was *Anopheles*. In the absence of blood, there was no evidence that *E. culicivora* discriminated between male and female *Culex*.

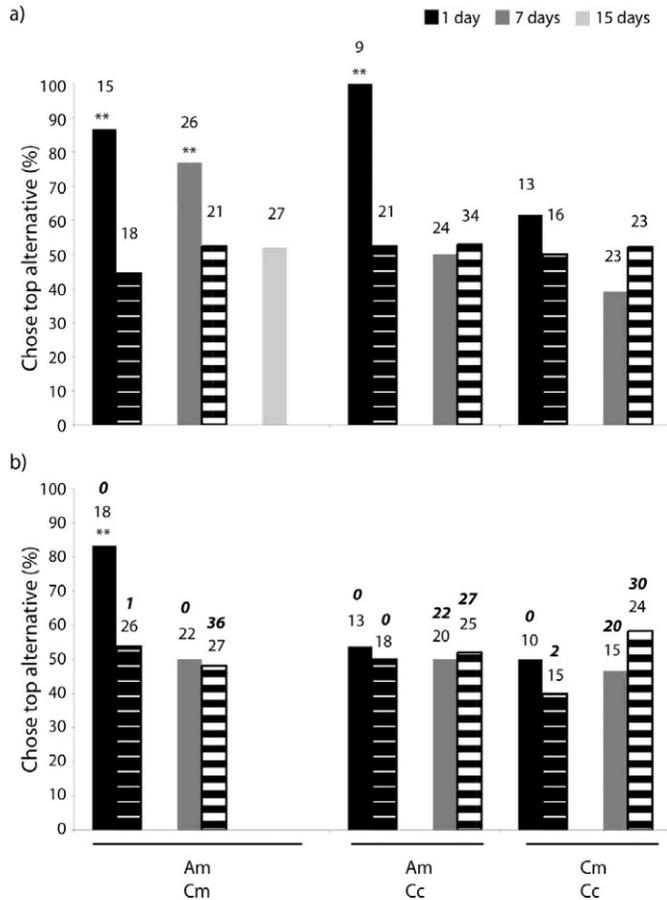


Figure 8.—Expression of preference by juveniles (solid bars) and adults (hatched bars) of *E. culicivora*. Pre-trial fast durations indicated by different shadings. Each individual spider tested with male mosquitoes (*Anopheles* and *Culex*) (percent choice of top alternative prey type depicted in bar) and other prey (both prey types in text underneath line below graphs) (N used for statistics over bar). a) Simultaneous-prey tests (data analysis: test of goodness of fit, $H_0 = 50/50$). b) Alternate-day tests (data analysis: McNemar tests for significance of changes). Prey: *Anopheles* male (Am), *Culex* male (Cm), *C. claripennis* (Cc). Results from tests all ns unless stated otherwise above bar (** $P < 0.01$). In alternate-day tests the number of spiders that chose both prey is indicated in bold italics above bars.

Why the *Anopheles-Culex* distinction matters to the small juveniles of *E. culicivora* may be explained by the small size of these spiders. The smallest instars of *E. culicivora* juveniles are only 1–2 mm in body length and yet these mite-size spiders prey on the much larger mosquitoes. This is not a trivial undertaking because the mosquito sometimes takes flight and shakes the spider off. However, small juveniles adopt *Anopheles*-specific prey-capture behavior (Nelson et al. 2005) that critically relies on the mosquito’s resting posture. *Culex* rests with its body parallel to the substrate, but *Anopheles* rests with its body at an angle to the substrate (head down and abdomen elevated: Clements 1999). Larger spiders attack mosquitoes from almost any orientation, but the mite-size juveniles maneuver so that they approach the resting *Anopheles* from behind, moving slowly under the mosquito’s elevated abdomen before leaping up and grabbing hold of the mosquito close to the junction between its thorax and

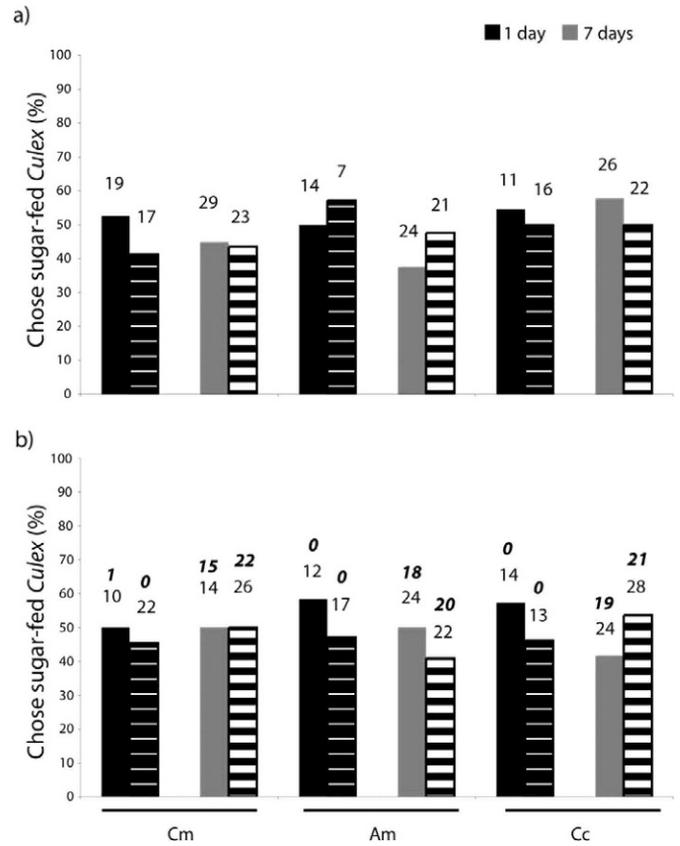


Figure 9.—Expression of preference by juveniles (solid bars) and adults (hatched bars) of *E. culicivora*. Pre-trial fast durations indicated by different shadings. Each individual spider tested with sugar-fed *Culex* female and with another prey. Data expressed as percentage of test spiders that chose sugar-fed *Culex* females (N used for statistics over bar). a) Simultaneous-prey tests (data analysis: test of goodness of fit, $H_0 = 50/50$). b) Alternate-day tests (data analysis: McNemar tests for significance of changes). Other prey: *Culex* male (Cm), *Anopheles* male (Am), *C. claripennis* (Cc). All tests ns. In alternate-day tests the number of spiders that chose both prey is indicated in bold italics above bars.

abdomen. The mosquito may take flight, but from underneath the small spider generally maintains its grip and, presumably owing to the spider’s venom taking effect, the mosquito usually soon falls to the ground with the predator on board. By expressing preference for *Anopheles*, the small juvenile targets the particular mosquitoes it is most proficient at capturing.

It is harder to explain what larger individuals of *E. culicivora* might gain by expressing a preference for *Anopheles*. Once seized by an adult or a large juvenile of *E. culicivora*, the likelihood of breaking free appears to be close to nil for a mosquito, whether it be *Anopheles* or *Culex*. On the other hand, the expression of a strong preference by juveniles and adults of *E. culicivora* for blood-carrying mosquitoes suggests that there are metabolic benefits from blood meals, this being a hypothesis that we are currently investigating. One non-metabolic role of blood meals may be that, by feeding on blood-carrying female mosquitoes, adult females and males of *E. culicivora* acquire an odor that renders them more attractive to the opposite sex (Cross et al. 2009), suggesting that

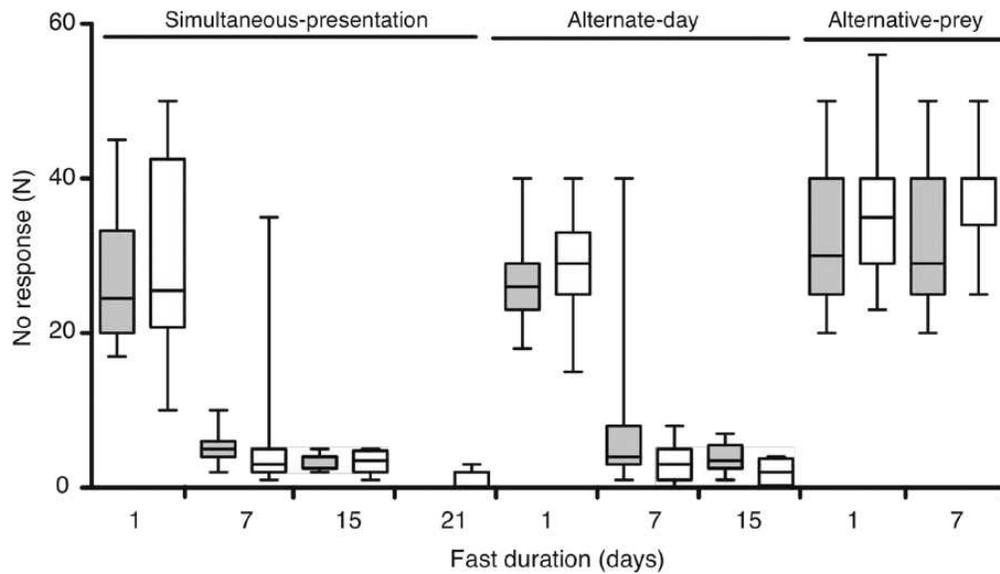


Figure 10.—Boxplots (median and quartiles) with whiskers (min and max) for numbers of juvenile (shaded) and adult *E. culicivora* that did not respond in the three testing protocols for each of the fast durations.

preference for blood meals by adults is driven, at least in part, by sexual selection. We are also investigating whether *Anopheles* is an optimal source of nutrients or of mate-attracting odor, but there is currently no evidence supporting either hypothesis. Alternatively, a preference for *Anopheles*, when expressed by larger individuals of *E. culicivora*, might be explained largely as a trait carried over as a relic from when these individuals were small juveniles.

Our findings are consistent with an important prediction from foraging models (Pyke et al. 1977; Caraco et al. 1980; Stephens & Krebs 1986; M'Namara & Houston 1990; Toft & Wise 1999), as we have shown that pronounced preference for a specific prey type veers toward indiscriminate response to prey after lengthy fasts. However, whether *E. culicivora* often experiences fasts sufficient to erode preference in the field is debatable. *E. culicivora* is a mosquito specialist, but this does not mean it feeds on mosquitoes alone. *E. culicivora* preys on other arthropods, including midges (chironomids and chaoborids) that are notoriously abundant along the shoreline of Lake Victoria (Beadle 1981).

Preferences, being cognitive attributes inherent to a predator, are knowable only by data from experiments designed specifically for determining preference. Along with araneophagic and myrmecophagic salticids, *E. culicivora* is a specialized predator that expresses preferences that are applicable to particular kinds of prey—in this case blood meals, *Anopheles* instead of *Culex* mosquitoes, and female instead of male *Anopheles*. Our results illustrate that the three testing protocols are useful to identify different levels of preference. Being so stringent, obtaining 'choice' responses during alternative prey testing was very difficult and probably only simultaneous presentation and alternate day tests with a variety of fasting periods are sufficient to extract such detailed information. By adding different hunger levels, we were able to obtain precise information about the different prey choice decisions made by juvenile and adult *E. culicivora* that would not have been illustrated without this series of

different testing protocols and fasting times. In general terms, the easier the test, the longer the fasting time required to 'break' the preference, such that invariably preference was lost in less time for alternative-prey tests than alternate-day tests, and preference in these, in turn, lost its expression before simultaneous-presentation tests. This methodology therefore can be of considerable use to determine prey-preference in animals.

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