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Mate-odour identification by both sexes of *Evarcha culicivora*, an East African jumping spider

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ABSTRACT

Evarcha culicivora is an unusual salticid spider because each sex actively courts the other and both sexes make distinctive mate-choice decisions. Here we use olfactometer experiments for investigating the ability of each sex to identify potential mates on the basis of odour alone. Test spiders spent more time in the vicinity of opposite-sex conspecific source spiders, regardless of whether or not these source spiders had previously mated, when the alternatives were conspecific individuals of the same sex, juveniles or a control (no odour source). This trend held regardless of the test spider's and source spider's age after reaching maturity and, for male test spiders, it held regardless of the test spider's mating status. However, after females had mated they no longer expressed a preference for male odour.

1. Introduction

Chemoreception, including olfaction, widely acknowledged as the most ancient sensory modality (Davis and Ludvigson, 1995; Freeman, 1999) is used by animals in many different contexts (e.g., Conover, 2007; Wyatt, 2003). For example, chemoreception might play a particularly important role in the context of reproduction when an animal needs to discriminate mates from rivals and conspecific individuals from heterospecific individuals (Smith and Breed, 1995; Wyatt, 2003), as well as determine other characteristics such as relatedness (Brennan and Kendrick, 2006; Kelliher, 2007) and the health of a potential mate (Ferkin et al., 1997).

Although all spiders probably rely to some substantial extent on chemoreception (Huber, 2005; Schulz and Toft, 1993; Stowe, 1988; Tietjen and Rovner, 1982), jumping spiders (Araneae: Salticidae) are better known for their unique, complex eyes (Blest et al., 1990; Land, 1969a,b) and for having eyesight based on a level of spatial acuity exceeding that of other animals in their size range (Harland and Jackson, 2000, 2004). Exceptional eyesight and complex eyes might suggest that, as an adaptive trade-off, the salticid is less reliant on and less effective at using other sensory modalities, but numerous studies illustrate how salticids communicate with chemical, tactile, auditory and percussion signals, either in conjunction with or as alternatives to vision-based signals (Elias et al., 2005; Jackson and Pollard, 1997). In fact, salticids are one of the spider families for which we have the most experimental evidence of chemical communication (Jackson et al., 2002, 2005; Pollard et al., 1987). This evidence has come primarily from research on how salticids respond to silk and the numerous examples suggest that most, if not all, salticid species leave species and sex identifying chemical cues on their draglines or nest silk (i.e., 'signpost signals') (Clark and Jackson, 1995; Jackson, 1987; Pollard et al., 1987; Taylor, 1998; also see references in Huber, 2005). Findings from these studies imply that it is primarily chemical cues that mediate silk-based species and sex discrimination by salticids. In particular, males stop responding after a female salticid's silk has been washed in alcohol or left in the open for a week (Jackson, 1987).

Although there have been previous studies of olfaction-based mate-identification behaviour using other spiders (Miyashita and Hayashi, 1996; Rypstra et al., 2003; Searcy et al., 1999; Watson, 1986) detailed studies using salticids have been lacking. Here we investigate how odour from conspecific individuals affects *Evarcha culicivora*, a salticid that is known to rely especially strongly on using olfaction in the context of identifying its preferred prey, blood-carrying mosquitoes (Jackson et al., 2005). Recent research (Cross and Jackson, 2009) has also shown that, for this species, male–male and female–female aggression tends to escalate when the odour of potential mates is present. However, there have been no experimental studies designed for directly testing *E. culicivora*'s ability to discriminate between the odour of opposite-sex and same-sex conspecific individuals.

Besides having unusual prey-choice behaviour, *E. culicivora* also has an unusual mating system. Salticids in general are known for having complex courtship routines (Jackson and Pollard, 1997),

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but *E. culicivora*'s courtship behaviour is exceptionally complex even for a salticid (Cross et al., 2008) and *E. culicivora*'s mate-choice behaviour also departs considerably from the salticid norm. Typically, male salticids have a more active role at displaying during courtship whereas females are generally envisaged as placing greater emphasis on deciding whether to mate with the displaying male (Jackson and Pollard, 1997). However, both sexes of *E. culicivora* are active participants in courtship and both sexes make distinctive vision-based mate-choice decisions. There is also an interesting male–female difference. Before mating the first time, both sexes of *E. culicivora* choose larger opposite-sex individuals by vision alone. The male's preference appears not to change after mating, but mated females switch their preference to smaller males (Cross et al., 2007).

Here, instead of considering vision-based mate-identification behaviour, we investigate *E. culicivora*'s olfaction-based mate-identification behaviour with our hypothesis being that both sexes of this unusual species identify potential mates using olfaction alone. More specifically, we consider (1) whether adults of both sexes discriminate between the odour of opposite-sex and same-sex conspecific individuals, (2) whether adults of both sexes discriminate between the odour of opposite-sex conspecific adults and conspecific juveniles, (3) whether juveniles discriminate between the odour of juveniles and adults, (4) whether the odour of adults (males and females) changes after mating and (5) whether the odour of potential mates continues to be relevant for males and females after mating.

2. Materials and methods

2.1. General

For rearing and maintenance, we adopted the standard procedures routinely used in our laboratory for salticid research (for details, see Cross et al., 2008; Jackson and Hallas, 1986). Each individual of *E. culicivora* used as a test spider or as a source spider was from the F2 or F3 laboratory generation (cultures derived from individuals collected at our field site: Mbita Point, western Kenya). Once dispersed from its egg sac, each individual was kept isolated from encounters with other conspecific individuals until used as a test spider or as a source spider, or until given access to an opposite-sex individual for mating (see below). All test and source spiders were fed three times a week on blood-carrying *Anopheles gambiae* (Culicidae) females. Laboratory-rearing environments were 'enriched' (see Carducci and Jakob, 2000) and, for humidity and drinking water, a water-logged cotton roll was always present in each spider's cage.

Experiments were carried out using an olfactometer designed for 'retention testing', the underlying rationale for this testing method being an expectation that spiders would remain near a more preferred odour source for longer than a less preferred odour. Our objective was to determine whether, on the basis of odour alone (i.e., on the basis of cues from volatile chemical compounds), *E. culicivora* can identify the odour of same- and opposite-sex conspecifics, as well as the odour of conspecifics that differ in age and mating status (virgin or already mated). We also wanted to determine whether, for test spiders, being of a particular age or mating status influenced retention time. Determining this required a series of retention tests (see Tables 1–4). However, no individual test spider and no individual source spider was used in more than one retention test.

We adopted some terminological conventions in the interest of streamlining the text. Male and female: adult males and females (both sexes 5 mm in body length). Juvenile: immature individual that had moulted 8–12 days before used, was 2 mm in body length, did not moult again in fewer than 10 days after used and, after moulting, was still immature. The rationale for including juveniles in the experiments was to have test spiders and odour sources that were effectively sexless (i.e., juveniles do not elicit courtship display from adults, nor do they perform courtship displays upon encountering a conspecific individual, whether the encountered individual be adult or juvenile, male or female). New virgin: a male or a female that had reached maturity (i.e., had undergone its final moult) 7 days earlier, had not mated and had not encountered any conspecific individuals since emerging from its egg sac. Mated: like new virgin, except that, on the seventh day after reaching maturity, it mated. There were two categories of mated individuals: young mated (tested 7 days after mating for the first time and, if it was a female, had not yet oviposited), old mated (tested 27–31 days after mating for the first time and, if it was a female, had oviposited fertile eggs). "Young virgin female" and "old virgin female": same as a "new virgin female" except for being 7 days older (i.e., same adult age as young mated) or 30 days older (i.e., same adult age as old mated), respectively. "Young virgin male": same as a "new virgin male" except for being 7 days older.

2.2. Retention tests

Retention testing was based on an alternate-day design, with each test spider being presented with odour from one source spider during a test on 1 day and with the odour of another source spider, or with no odour source ('control'), on the next or the previous day. We determined at random which of the two tests would come first. During testing, air was pushed successively through a stimulus chamber, a holding chamber and an exit chamber (Fig. 1). Airflow was always adjusted to 1500 ml/min (Matheson FM-1000 airflow regulator) and there was no evidence that this airflow setting impaired locomotion or had any other adverse effects on *E. culicivora*'s behaviour. All tests began between 08:00 and 14:00 h (laboratory photoperiod 12L:12D, lights on at 07:00 h). Between tests, the olfactometer was dismantled and cleaned with 80% ethanol, followed by distilled water, and then dried. No individual was used in more than one pair of retention tests.

One component of the apparatus (the "stimulus chamber") was a glass cube (inner dimensions, 70 mm × 70 mm × 70 mm) made from 5-mm thick glass, with a removable top. There were two holes (diameter 20 mm) in the cube that were opposite each other and each was plugged with a rubber stopper. There was a hole in each stopper through which a glass tube (diameter 4 mm) passed and air moved into and out of the stimulus box through these glass tubes. The stimulus chamber contained a source spider or else it was the control (i.e., it was empty). The source spider was put in the stimulus chamber 30 min before testing began. For cleaning, the removable top on the stimulus chamber provided access to the interior.

The holding chamber was a glass tube (length 90 mm; inner diameter 15 mm; rubber stopper in one end; other end open). The open end of the holding chamber fit snugly in the hole in the exit chamber, flush with the inner wall of the exit chamber. At the other end of the holding chamber, there was a hole in the stopper with a glass tube going through to the stimulus chamber. A nylon-netting screen over the stopper ensured that the test spider could not enter the stimulus chamber and the only way it could leave the holding chamber was via the opening into the exit chamber. New netting was used for each test. The exit chamber was a glass cube identical to the glass cube used as a stimulus chamber.

Before starting, the test spider was kept for 2 min in the holding chamber and the holding chamber was not yet connected to the stimulus chamber or the exit chamber. During this 2-min interval, the end that would be open during testing was plugged with a rubber stopper. We began tests by un-plugging the holding chamber and connecting it between the stimulus and exit chamber, with a