Animal Behaviour 79 (2010) 265–269

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Crayfish females eavesdrop on fighting males and use smell and sight to recognize the identity of the winner

Laura Aquiloni*, Francesca Gherardi

Dipartimento di Biologia Evoluzionistica 'Leo Pardi', Università di Firenze

ARTICLE INFO

Article history:
Received 19 May 2009
Initial acceptance 30 July 2009
Final acceptance 14 September 2009
Available online 4 December 2009
MS. number: 09-00334

Keywords:
bimodal communication
crayfish social eavesdropping
individual recognition
invertebrate
mate choice
Procambarus clarkii

Females of many animal species select dominant males as mates but their ability to detect the male's hierarchical status remains poorly understood. Previously, we found that females of the crayfish *Procambarus clarkii* eavesdrop on two fighting males before choosing the winner. Here, we asked whether eavesdropping females use vision together with smell to do so and whether their preference for the winner relies on a form of individual, rather than on status, recognition. When tested in a two-way choice paradigm, a bystander female visited the dominant first rather than the subordinate male, remained in his proximity for longer, and interacted with him more frequently. However, this happened only when she was offered the same individuals she had previously watched and smelled. This suggests that females recognize the winners as individuals and not as generic dominants, thus revealing unusual discrimination abilities in an invertebrate.

Animal communication generally occurs in a network of several individuals that interact with each other (McGregor & Dabelsteen 1996). Owing due to the conspicuousness of many displays, signals directed to only one individual are often picked up by other receivers: the latter can thus acquire accurate and low-cost information about the signaller and can then use such information in subsequent encounters with it (Peake 2005). This act of extracting information from the signalling interactions between conspecifics without taking part in them has been called 'social eavesdropping' (McGregor & Dabelsteen 1996).

Since its first formulation, much work on social eavesdropping has focused on vertebrates, particularly on teleost fishes (e.g. Earley & Dugatkin 2002), birds (Mennill et al. 2002), dolphins (Götz et al. 2006) and primates (Crockford et al. 2007). Conversely, this phenomenon has been seldom explored in invertebrates. The first clue of its occurrence in crustacean decapods comes from a field study (Zucker 1983), in which the females of the fiddler crab *Uca deichmanni* were described as choosing mates after having incited males to fight. More recently, Aquiloni et al. (2008) offered the first experimental evidence of social eavesdropping in an invertebrate: females of the crayfish *Procambarus clarkii* were found to choose dominant over subordinate males after having watched (and smelled) them fighting.

The occurrence of social eavesdropping in invertebrates obviously raises a number of questions. The first focuses on the sensory channels involved in gathering information. The literature is full of studies that show the role of olfaction in aquatic species, such as crabs (e.g. Bamber & Naylor 1997), lobsters (e.g. Bushmann & Atema 2000) and crayfish (e.g. Stebbing et al. 2003). The role of sight is more controversial. In some species, antennule ablation but not blindfolding (Snyder et al. 1993; Bushmann 1999) affects the animal's ability to identify the other's sex. These and other studies led us to believe that individuals do not use sight to recognize the sex of a conspecific. Conversely, the crayfish *Cherax destructor* seems to be able to recognize conspecifics using even slight visual cues (Crook et al. 2004), such as 'facial' features learned while fighting (Van der Velden et al. 2008). In *P. clarkii* (Aquiloni & Gherardi 2008a; Aquiloni et al. 2009) and other crayfish species (Acquistapace et al. 2002), male assessment by females seems to rely on both vision and olfaction. We thus expect that these two sensory modalities are also involved in social eavesdropping. Indeed, multimodality, that is, the synchronous use of different media (Rowe & Guilford 1999), offers more reliable information than unimodality (Rowe 1999) by,

---

* Correspondence: L. Aquiloni, Dipartimento di Biologia Evoluzionistica, Università di Firenze, Via Romana 17, 50125 Firenze, Italy.
E-mail address: laura.aquiloni@unifi.it (L. Aquiloni).

0003-3472/$38.00 © 2009 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.anbehav.2009.09.024

for instance, improving the receiver's ability to learn and remember the association between a given individual and its properties (Guilford & Dawkins 1991).

The second question raised here is whether the eavesdropping female recognizes a generic winner that shares its behaviour, posture or smell with other potential winners or instead identifies, remembers and uses during mate choice some features identifying that winner as an individual. That is, is this a form of status (Winston & Jacobson 1978) or of individual recognition (Barnard & Burk 1979)?

Individual recognition relies on the capacity to associate stimuli by processing different categories of information separately and then to reassemble the distilled information to identify, store and recall it (Van der Velden et al. 2008). Notwithstanding this apparent complexity, there is increasing evidence of its occurrence in invertebrates, particularly in colonial species (bees: Horridge 2005; wasps: Tibbetts & Dale 2004; ants: D'Ettore & Heinze 2005) and familiar groups (isopods: Linsenmair & Linsenmair 1971) but also in solitary crustaceans, such as stomatopods (Caldwell 1985) and decapods (hermit crabs: Gherardi & Tiedemann 2004; crabs: Vannini & Gherardi 1981; lobsters: Karavanich & Atema 1998).

Here, we asked whether *P. clarkii* females that eavesdrop on fighting males use both vision and smell to recognize the winner and whether these two media carry the same (i.e. redundant) or a different (i.e. nonredundant) information. We also tested the hypothesis that the female's choice of the winner relies on a form of individual, rather than of status, recognition.

METHODS

Collection and Holding Conditions

About 400 individuals (females and reproductive males) were collected using baited traps from the Lake Trasimeno, Umbria, Italy, in June 2008, before the onset of reproduction. Once in the laboratory, we measured the cephalothorax length (from the tip of the rostrum to the posterior edge of the carapace) of each individual using a vernier calliper. Crayfish were individually marked on their carapace with a waterproof paint. Sexes were kept apart in tanks (80 × 60 cm and 60 cm high) containing clay pots as refuges at a density of 15 per m^2. They were maintained in a natural light:dark cycle at room temperature (28 °C) and fed ad libitum with live *Calliphora* sp. larvae. Water was changed weekly. The experiment was conducted in July 2008 during 0800–1400 hours.

Criteria for Choosing Experimental Crayfish

We selected hard-shelled, intact and sexually responsive animals. Crayfish were defined as sexually responsive when, once placed with a female, the male tried to turn the female over for copulation. The mean ± SE cephalothorax length was 44.4 ± 0.4 mm for males and 45.3 ± 0.6 mm for females. Since male dominance increases with body size in crayfish (Bovbjerg 1953) and *P. clarkii* females prefer larger mates (Aquiloni & Gherardi 2008b), fighting pairs were size matched (±2 mm difference in cephalothorax length). Test crayfish were kept in isolation in opaque plastic aquaria (25 × 15 cm and 25 cm high) for at least 1 week. In no case did the animals in each trial meet each other prior to the experiment, so that we can exclude any effect of previous social experience (Bergman & Moore 2005). All crayfish were used only once to avoid pseudoreplication.

Experimental Design

The experiment was planned in two phases, the 'fight' and the 'choice' phase. In the first, a pair of size-matched males fought in the presence of a female. The apparatus was designed so that a bystander female could smell (treatment C), see (treatment V) or see and smell (treatment CV) the fighting males, whereas a naïve female was unable to see and smell the males (Control). In the choice phase, either a bystander from treatments C, V and CV or a naïve female from the Control was allowed to choose between the dominant and the subordinate male. An additional treatment (RCV) was run by presenting a bystander female from treatment CV with two males she had not previously seen and/or smelled. This pair of males was composed of a dominant and a subordinate crayfish which, during the fight phase, had interacted with each other in the presence of a female other than the bystander.

Between the two phases, the relative position of males with respect to the female was randomly switched. At the end of each trial, the males and females we had used were again tested for their sexual responsiveness. In the case of lack of responsiveness, the corresponding trial was excluded from the analysis. Similarly, the trials in which a hierarchy was not clearly established were excluded. A total of 20 replicates per treatment was reached.

Experimental Apparatus

We used two elliptical plastic aquaria (65 × 40 cm with water level 10 cm), the 'fight arena' in the first phase and the 'choice arena' in the second one. During the acclimation period (10 min) the crayfish were visually isolated from each other by a T-shaped opaque wall that divided both arenas into three equal compartments: one occupied by the female and the other two by one male each. The experiments started with the removal of the wall.

In the fight arena, the two males were free to move whereas the female was placed into a container (female box: 10 × 4 cm and 22 cm high) that precluded any physical contact between her and the two males. If the female was a bystander, the female box was (1) transparent in treatment V, (2) opaque and finely drilled with holes (diameter and density: 3 mm and 4 per cm^2) in treatment C, or (3) transparent and drilled as in (2) in treatment CV. If the female was naïve (Control), the female box was opaque and not drilled.

In the choice arena, the two males were fastened with a string, each at the opposite end of the arena in an area (the choice area) delimited by an imaginary circle (diameter 20 cm), whereas the female was free to interact at will with them. The males were prevented by the string from moving out of the choice area. Between trials, the experimental apparatus was thoroughly washed with clean tap water.

Data Collection

Both phases of each experiment ran for 30 min during which crayfish behaviour was videotaped using a Samsung digital camera (VP-L800). A blind analysis of videotapes was subsequently performed by an unbiased observer, who was well experienced in the description of crayfish behaviour but unaware of the experimental design and our predictions.

The observer recorded the following data in the fight phase.

(1) Total number and duration of fights. A fight began when one opponent approached the other and ended when one of the two individuals ran away, backed off or tail flipped away from the other at a distance longer than one body length for at least 10 s. Tail flipping away is the typical backward swimming response of crayfish.