Animal Behaviour 92 (2014) 33–43

Contents lists available at ScienceDirect

Animal Behaviour

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

Discrimination of mates and intruders: visual and olfactory cues for a monogamous territorial coral reef butterflyfish

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ARTICLE INFO

Article history:
Received 17 October 2013
Initial acceptance 21 January 2014
Final acceptance 28 February 2014
Published online
MS. number: 13-00873

Keywords:
Chaetodon multicinctus
Chaetodontidae
Hawaii
mate recognition
pebbled butterflyfish
territoriality

Recognition of conspecifics is essential for territorial and monogamous animals in order to maintain pair bonds, mate-guard and defend territories. However, cues required for mate discrimination are essentially unknown in monogamous fishes, despite the importance of recognition behaviour that promotes this mating system. This field study tested the role of visual and olfactory cues in the discrimination of mates and unfamiliar conspecifics in the territorial, socially monogamous pebbled butterflyfish, *Chaetodon multicinctus*. A series of model bottle field experiments presented cues from a mate and an unfamiliar nonmate to a focal resident fish within its feeding territory. When both visual and olfactory cues were first matched, the resident spent more time and engaged in more agonistic displays near the bottled intruder. In contrast, when olfactory cues of bottled fish were first mismatched, the resident fish spent equivalent time at each bottle stimulus. When scent cues were then reversed to a matched odour condition (odour released next to bottled fish), the resident again spent more time with the nonmate. Resident fish spent equivalent time with the mate and nonmate in the absence of any associated odour stimuli. In addition, resident fish did not show a differential response when the mate's odour was associated with only one of two intruder fish. Thus both visual and olfactory cues are necessary for butterflyfish to discriminate their mate from intruders within feeding territories. These results indicate that mate recognition in animals with long-term pair bonds may require multimodal stimuli and future studies on mate recognition should address multiple sensory channels.

Individual recognition of mates is of profound importance for animals that maintain long-term social bonds and is especially important for territorial species. Recognition of specific individuals is important for social interactions (Mateo, 2004; Tibbetts & Dale, 2007; Wiley, 2013) and is well documented for many invertebrates (Gherardi & Tiedemann, 2004; Karavanich & Atema, 1998; Müller, Eggert, & Elsner, 2003), fish (Griffiths, 2003; Griffiths & Ward, 2011), amphibians (Bee & Gerhardt, 2002), reptiles (Carazo, Font, & Desfilis, 2008; Husak & Fox, 2003), birds (Clark, Boersma, & Olmsted, 2006; Robertson, 1996; Wanker, Apcin, Jennerjahn, & Waibel, 1998; Wascher, Szipl, Boeckle, & Wilkinson, 2012) and mammals (Charrier & Harcourt, 2006; Koren & Geffen, 2011; Miller & Thomas, 2012). Cues and signals used for recognition of mates in monogamous species are important to maintain pair bonds, yet only a few studies have rigorously investigated multimodal cues used to distinguish mates from unfamiliar conspecifics (Tibbetts & Dale, 2007). When monogamous individuals encounter conspecifics after a period of separation from their mate, rapid discrimination of the mate from a territorial conspecific intruder can be favoured. Failure to distinguish a mate from another territorial conspecific could weaken the pair bond and result in an injurious attack or loss of mate and territory.

The evidence for discrimination of familiar from unfamiliar conspecifics by fishes comes primarily from laboratory studies on shoaling behaviour (Griffiths, 2003; Griffiths & Ward, 2011) in nonreproductive contexts. In addition, several freshwater or estuarine fish species recognize and discriminate familiar from unfamiliar nonkin (Brown & Colgan, 1986; Brown & Smith, 1994; Croft, Arrowsmith, Webster, & Krause, 2004; Edenbrow & Croft, 2012; Ward, Duff, Horsfall, & Currie, 2008). Discrimination and individual recognition is associated with visual (Fricke, 1973), acoustic (Myrberg & Riggio, 1985), electrosensory (Painter & Kramer, 2003), olfactory or possible multiple cues and signals (Hojesjo, Johnsson, Petersson, & Järvi, 1998; Noble & Curtis, 1939; Sogabe, 2011). However, the relevant stimuli used for mate discrimination are not

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http://dx.doi.org/10.1016/j.anbehav.2014.03.022
0003-3472/

well defined and may be especially important for the maintenance of monogamous and haremic social systems.

Monogamy is relatively rare among teleost fishes (Barlow, 1984; Whiteman & Côté, 2004); however, individuals must discriminate mates from nonmates after periods of separation in order to maintain the pair bond. In addition, mate recognition may be of value for haremic species in which males defend several females in a social group (Aldenhoven, 1986; Gaisner, 2005; Hourigan, Stanton, Motta, Kelley, & Carlson, 1989; Neudecker & Lobel, 1982). Few studies have examined the proximate cues required for discrimination of mates from nonmates in monogamous fishes in natural settings. The butterflyfishes (family Chaetodontidae) are speciose (>120 spp.) and well represented on Indo-Pacific coral reefs (Nelson, 2006). Members of this family exhibit no parental care, lack obvious sexual dimorphisms, and many are socially monogamous and territorial (Hourigan, 1989; Reese, 1975; Roberts & Ormond, 1992; Whiteman & Côté, 2004). Some species maintain long (up to 10 years) socially monogamous pairings (Hourigan, 1989) and thus are able to discriminate between mates and nonmates that enter feeding territories (Driscoll & Driscoll, 1988; Sutton, 1985; Tricas, 1989a; Yabuta, 2000). The efficient discrimination of familiar mates from unfamiliar intruder conspecifics could reduce territory incursions, enhance the efficiency of aggressive behaviours and eliminate misdirected aggression towards mates. Thus, because of their long-term monogamous pairing and site-attached territorial behaviours, butterflyfish are an excellent taxon to examine the in situ proximate cues used for discrimination of familiar and unfamiliar conspecifics.

Butterflyfish may employ multiple sensory cues (vision, olfaction, acoustic, mechanosensory) to facilitate rapid recognition of conspecifics and heterospecifics. Previous experimental observations on territorial behaviour in monogamous species show that aggressive territorial behaviour is initiated towards captive conspecifics and model fish placed within feeding territories (Fricke, 1986; Strang, 2005; Tricas, 1989a; Yabuta, 2008). These studies indicate that visual signals and cues are important in butterflyfish social interactions, but sound production during territorial behaviour is also involved (Tricas, Kajiura, & Kosaki, 2006). In addition, the close spatial interactions associated with agonistic behaviour and pairing may facilitate chemical communication. In this study we tested the hypothesis that both visual and olfactory cues are used for the discrimination of mate from nonmate conspecifics in a territorial and monogamous fish. A major prediction of this multimodal cue recognition hypothesis is that removal of one or more relevant stimuli should reduce the ability of a fish to distinguish its mate from a nonmate. We tested this prediction with a series of five two-choice experiments on a wild population of pebbled (multiband) butterflyfish, *Chaetodon multicinctus*, a monogamous and territorial species endemic to the Hawaiian archipelago. We assessed the responses of a free-swimming resident mate in its natural territory to presentations of olfactory and visual stimuli from a partner (its mate) and an intruder (an unfamiliar nonmate).

METHODS

Experimental Pairs and Nonmate Fish

Mate recognition experiments were conducted by scuba divers on adult pebbled butterflyfish (standard length, SL, ≥72 mm) that occur in pairs on shallow reefs along the northwest shore of the island of Hawaii. Experimental fish pairs were confirmed by direct observations for 10–15 min of pair swimming/following behaviour, feeding and common defence of territorial borders (Tricas, 1989a, 1989b). One member of the resident pair (the mate stimulus) was randomly selected, captured by hand net and held away from the territory for approximately 10–20 min during set-up of the experimental materials. The remaining individual of the pair was used as the focal individual in the experiment. A third fish (the nonmate stimulus) was collected from a distant, nonbordering territory far away from the experiment area and selected to be a similar size to the mate stimulus individual. After each experiment (except in three trials of experiment 5), the mate and nonmate fish were measured (SL to the nearest mm) and sex determined underwater by gonad catheterization (Ross, 1984). The sex of the focal resident fish (which was not catheterized) was inferred after sex determination of its mate as territorial fish form heterosexual pairs (Hourigan, 1989; Tricas, 1989a).

Stimulus Presentations

Experiments were designed to test the ability of one resident fish to discriminate between its mate and a nonmate conspecific based on associations with visual and chemical cues. The two stimulus fish (mate and nonmate) were transferred to separate glass bottles (3.8 litres, 22 cm wide) which were sealed with a metal lid fitted with two water flow tubes made of 13 mm clear vinyl tubing. Bottles with the two stimulus fish were then positioned approximately 2 m apart within the experimental arena, which consisted of two adjacent 1.5 × 1.5 m (2.25 m^2) plots that were separated by an interbottle neutral zone distance of approximately 66 cm (Fig. 1). The experimental arena was outlined with marked nails for observation and positioned within the territory of the resident fish. Chemical cues from stimulus fish were produced by a flow of fresh sea water through the bottle via Atwood Water Buster submersible bilge pumps (750 litres/h) attached to the distal end of the incurrent tube positioned 2–3 m from the arena (Fig. 1a). The excurrent tube (3 m long) collected water flow from the bottom of the bottle and released the collected water and odour stimulants at either of the two fish bottles (see experiments below). After set-up, divers ascended approximately 5–10 m above the experimental arena and recorded the time that the focal individual (the free-swimming mate) spent within or outside the square plot around each bottle. Each 15 min observation period began when the resident fish first entered one of the two square plots in the experimental arena. Divers recorded with a water-resistant stopwatch (and logged on waterproof paper) the time that the resident fish entered and exited each of the two stimulus arenas (Fig. 1). From these data we determined the total time spent at, and the number of visits to, each stimulus.

Multimodal Mate Recognition Hypothesis Predictions

A general prediction of the hypothesis that butterflyfish can discriminate mates from unfamiliar conspecifics is that fish will associate differentially with simultaneous presentations of a mate and nonmate. Several previous studies (Strang, 2005; Tricas, 1989a; Tricas et al., 2006) and pilot data showed that resident pairs were highly motivated to approach and interact with captive bottled fish that presented visual cues. Thus when necessary cues for discrimination were present, the test individual was predicted to associate differentially with nonmates and mates. The contribution of visual and olfactory cues was tested with a series of five experiments which are outlined with specific predictions below.

Experimental Trials

We conducted five experiments (detailed below) of which the first four involved two phases. Phase 1 involved approximately 7.5 min of initial observations of the behaviour of the focal fish in