Animal Behaviour 82 (2011) 557–562

Contents lists available at ScienceDirect

Animal Behaviour

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

Effects of sensory modality on learned mate preferences in female swordtails

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ARTICLE INFO

Article history:
Received 7 April 2011
Initial acceptance 10 May 2011
Final acceptance 6 June 2011
Available online 7 July 2011
MS. number: 11-00302

Keywords:
fish
learning
mate preference
multiple modality
olfactory
reproductive isolation
swordtail
visual
Xiphophorus birchmanni

Females often base their mate selection on multiple male traits. Different cues may be weighted differently in mating decisions, and play different roles such as indicating species identity or condition. The ontogeny of preferences for each cue can differ, which may offer a proximate explanation for the differential female evaluation of multimodal traits of males. We investigated whether female preferences for the multimodal male cues of *Xiphophorus birchmanni* swordtails are learned for traits in both olfactory and visual sensory modalities. We reared *X. birchmanni* females with either conspecific adults or adults of a closely related species, *Xiphophorus malinche*. We found that both olfactory and visual preferences were learned, and that the timing of olfactory learning was different from that of visual cues.

An individual's mating decision is the outcome of a series of complex and interacting processes (Ryan et al. 2009). How mating decisions are made depends on mate recognition and preferences that are formed through development. Learning is often an important component of mate preference formation, and preferences are therefore often influenced by the phenotypic variation that individuals encounter throughout their lives (e.g. Verzijden & ten Cate 2007; Dukas 2008; Riebel 2009; Svensson et al. 2010; Kozak et al. 2011).

Mate selection is often based on multiple traits in multiple sensory modalities (Candolin 2003; Partan & Marler 2005), which may play very different roles. For example, one part of a compound signal may be more important in species recognition, while another part could be used to discriminate among conspecifics. In túngara frogs, *Engystomops pustulosus*, for example, the female preference for the 'whine' component of the male call is narrow and species specific, while the preference for the 'chuck' is permissive, directional and broadly shared across the genus (Ryan & Rand 1993).

Mate preferences for multiple traits may develop through multiple mechanisms, which can result in distinct selection gradients on each trait (Candolin 2003). Although many studies have addressed learned mate preferences, few studies have directly compared the developmental mechanisms underlying female preferences for multiple traits. Female zebra finches, *Taeniopygia guttata*, learn to prefer both male song and visual traits (ten Cate & Mug 1984; Collins et al. 1994; Riebel 2009), and preferences for both visual and olfactory traits have a learned component in wolf spiders, *Schizocosa rovneri* (Rutledge et al. 2010), sticklebacks, *Gasterosteus* spp. (Kozak et al. 2011) and zebrafish, *Danio rerio* (Engeszer et al. 2004; Gerlach et al. 2008). When preferences for multiple traits have different ontogenetic paths, this could lead to the multiple-trait preference sets contributing to mate selection in distinct ways, affecting diversification through sexual selection and reproductive isolation (Verzijden et al. 2005; Kozak & Boughman 2009; Servedio et al. 2009; Hohenlohe & Arnold 2010).

Male swordtail fish (Poeciliidae: *Xiphophorus*) court females using both olfactory and visual signals, signalling species identity, nutritional condition and social dominance (Basolo 1990; Morris et al. 1995, 2005; McLennan & Ryan 1997; Rosenthal & Evans 1998; Fisher & Rosenthal 2006; Fisher et al. 2009; Rosenthal & Ryan 2011). Females often show preferences for visual traits of heterospecifics (Ryan & Wagner 1987; Rosenthal et al. 2002; Morris

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doi:10.1016/j.anbehav.2011.06.010

et al. 2005), but, with one exception, prefer urine-borne olfactory cues (Rosenthal et al. 2011) of their own species (Crapon de Caprona & Ryan 1990; McLennan & Ryan 1997, 1999; Hankison & Morris 2003; but see McLennan & Ryan 2008). Preferences for olfactory cues can override those for visual cues (Crapon de Caprona & Ryan 1990; Morris et al. 2005). Species-specific olfactory preferences are thus dominant (sensu Partan & Marler 2005) over permissive visual preferences. This difference in female preferences may be mediated by separate developmental mechanisms for preferences in the two modalities. Learning influences preferences of *Xiphophorus* for visual traits. Male *Xiphophorus maculatus* prefer a female colour variant following a 2-month exposure period as adults (Fernö & Sjölander 1973) and female *Xiphophorus helleri* learn to prefer males with short swords over those with long swords when raised with short-sworded males (Walling et al. 2008). In this study, we investigated the role of learning in shaping the species-specific preferences of female *Xiphophorus birchmanni* for conspecific visual and olfactory traits.

Xiphophorus birchmanni females prefer conspecific males in both visual and olfactory modalities over the closely related *Xiphophorus malinche* (Rosenthal et al. 2003; Fisher et al. 2006). By rearing *X. birchmanni* females for a long or a short time with exposure to either *X. birchmanni* or *X. malinche* adults, we investigated whether both olfactory and visual preferences are learned, and whether olfactory and visual learning are equally sensitive to the timing of experience.

METHODS

Raising and Housing

All subjects were offspring of wild-caught gravid females. Twelve clutches of *X. birchmanni* fry, from 12 *X. birchmanni* females from the Río Garces (20°56′24′ N, 98°16′54′ W), were exposed to either *X. birchmanni* or *X. malinche* adults. *Xiphophorus birchmanni* adults also originated from the Río Garces, and *X. malinche* adults were from the Arroyo Xontla near Chicayotla (20°55′30′N, 98°34′36′W). These populations are fully allopatric. In each case, fry had visual and olfactory contact with two males and two females. *Xiphophorus birchmanni* males, as in some other *Xiphophorus* species, can be of two size classes (small and large), associated with alternative mating strategies (Ryan & Causey 1989). In this experiment only large males were used as models for the fry. We used 75-litre tanks, with two perforated Plexiglas dividers, which housed a group of four adults in the middle and one clutch of fry on each end. The mean age of the fry at the time of housing with exposure models ± SD was 25.2 ± 11 days.

Six of the clutches were exposed to *X. birchmanni*, and the other six to *X. malinche* adults. Males were removed as soon as any signs of sexual differentiation were detected, to prevent focal females from mating. A timeline of exposure and preference testing is given in Fig. 1. Exposure continued for either 72 days (‘short-exposed’ treatment) or 274 days (‘long-exposed’ treatment). Following exposure, individuals were isolated from adult fish and housed in 37-litre tanks, one clutch per tank. Females in the long-exposed treatment were removed from exposure after sexual maturation. All females were monitored for gravidity, but none of the females appeared to have mated before testing. Fish were maintained on a 12:12 h light:dark cycle at 24 °C. They were fed twice daily, with Tetramin flakes (Tetra, Melle, Germany), brine shrimp and dried bloodworms (for all fish except small fry). Aquaria were enriched with plants and rocks.

Testing Olfactory Preferences

We tested whether females preferred the olfactory cues of conspecific *X. birchmanni* or *X. malinche* males. Olfactory preference tests closely followed previous studies (McLennan & Ryan 1997, 1999, 2008; Fisher et al. 2006; Fisher & Rosenthal 2006). To produce stimulus water, a group of four males was put in a glass tank containing 20 litres of water and visually exposed to females of the same population in a directly adjoining identical tank. Males and females were allowed to interact visually for at least 2 h. In total, 16 *X. malinche* males and 16 *X. birchmanni* males were used to produce stimulus water. Females were never tested with stimulus water produced by males from their own exposure treatments.

Each female was introduced to a rectangular tank (51 × 28 cm and 33 cm deep) 20 min before the trial. At the start of, and throughout, the trial, on each far end of the tank, stimulus water entered the test tank through silicone tubes that drew water from the stimulus water-holding tank. The flow rate was approximately 5 ml/min. On average per trial ± SD 47.1 ± 13.5 ml flowed in at each end. A trial started when stimulus water started dripping. The tank was divided into three equal zones along the length of the tank. The moment a female had visited each zone after the trial started, we started the observation time, 5 min, during which we measured the amount of time the female spent in each zone. Females that failed to visit all three zones within 5 min were operationally defined as unresponsive, and were retested at a later date. The water in the tanks and in the tubes leading from the stimulus container to the tank was refreshed before each trial. All females were tested twice

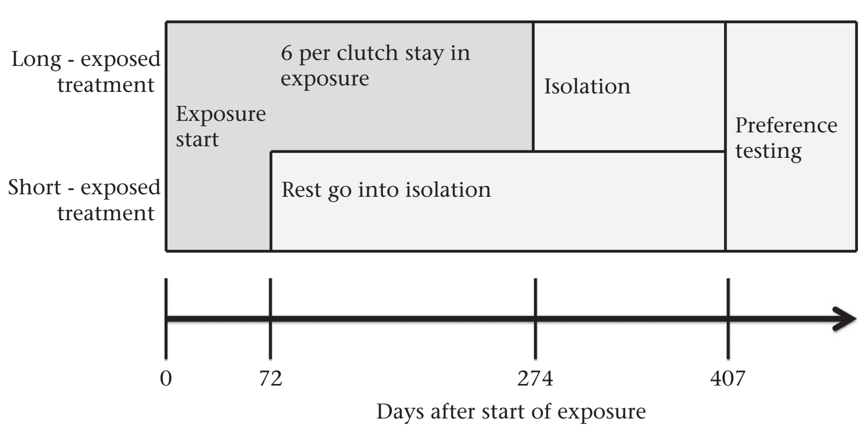

Figure 1. The timeline of the exposure treatment and preference testing of the two treatment groups.