



Audience effect alters mating preferences in a livebearing fish, the Atlantic molly, *Poecilia mexicana*

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Audience effects occur when an observing (by-standing) animal influences the behaviour of an observed individual. We examined whether audience effects influence mate choice decisions in a livebearing fish, the Atlantic molly, *Poecilia mexicana*. We gave males an opportunity to choose between a conspecific and a heterospecific, *Poecilia formosa*, female (experiment 1) or a large versus small conspecific female (experiment 2), and we determined the males' association times near the two types of females. During the second part of each test we visually presented an audience male to the focal male, and we compared male association times between the two parts. In both experiments the focal males spent less time near the initially preferred female, and spent more time near the initially nonpreferred female when we presented a conspecific audience male during the second part of the tests. When we presented a heterospecific, *Xiphophorus hellerii*, male instead, the change in male preferences was significantly weaker. Male preferences were highly consistent when we presented no audience male during the second part of the tests (control). Our study highlights that the social environment has an important effect on male mate choice decisions, and even the mere visual presence of a conspecific competitor can affect the expression of mating preferences.

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Communication events often occur in a social context, so that information exchange is not binary, but rather several individuals may interact and form communication networks (Oliveira et al. 1998; McGregor & Peake 2000; Earley & Dugatkin 2005; Matos & Schlupp 2005; Peake 2005). Even when only two individuals communicate directly, the presence of neighbouring individuals can affect the behaviour of the signaller and/or the receiver (Earley & Dugatkin 2005; Matos & Schlupp 2005). Animal decision making often includes a communication component and occurs in a social environment, such that decision making can be influenced by the behaviour of surrounding individuals (see Danchin et al. 2004 for 'public information'). Most studies on information exchange in

animal communication networks have either focused on (1) how an individual observing two or more other communicating individuals alters its behaviour in relation to the observed communication event (eavesdropping; McGregor 1993; Oliveira et al. 1998; Doutrelant & McGregor 2000; Johnstone 2001; McGregor et al. 2001; Peake et al. 2001; Naguib & Kipper 2006) or (2) how the observing ('by-standing') individual influences the behaviour of a pair of communicating individuals (audience effect; Zajonc 1965; Baltz & Clark 1997; Doutrelant et al. 2001; Oliveira et al. 2001).

In the context of mate choice, several studies have examined socially influenced (nonindependent) mate choice of an observing individual in a communication network (Pruett-Jones 1992; Kirkpatrick & Dugatkin 1994; Westneat et al. 2000; White 2004; Earley & Dugatkin 2005). For example, eavesdropping may influence mate choice decisions, whereby females learn to evaluate the quality of a male after observing male–male interactions (e.g. fighting fish, *Betta splendens*: Doutrelant & McGregor 2000;

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great tits, *Parus major*: Otter et al. 1999; chickadees, *Poecile atricapillus*: Mennill et al. 2003; Japanese quail, *Coturnix japonica*: Ophir & Galef 2003). Numerous studies have shown that individuals may also base their mate choice decisions on whether they have seen other members of their own sex sexually interact with a potential mating partner (mate choice copying; e.g. Japanese quail, *C. japonica*: Galef & White 1998), and several studies have examined mate choice copying in livebearing fish (Poeciliidae; e.g. guppy, *Poecilia reticulata*: Dugatkin 1992; Dugatkin & Godin 1992; Godin et al. 2005; sailfin molly, *Poecilia latipinna*: Schlupp et al. 1994; Witte & Ryan 1998, 2002; Hill & Ryan 2005).

To date, audience effects (i.e. the effect of an observing animal on the behaviour of another individual) have been examined primarily in a nonsexual context and in triadic interactions. For example, a female or a male audience may influence the intensity of aggressive male–male interactions (Zajonc 1965; Doutrelant et al. 2001; Oliveira et al. 2001; Matos & McGregor 2002; Matos et al. 2003; Dziewczynski et al. 2005, 2006). Whether and how the presence of an audience influences mate choice decisions has been little investigated (Baltz & Clark 1997; for reviews see Earley & Dugatkin 2005; Matos & Schlupp 2005). In this study, we examined whether the presence of an audience, a conspecific male as a potential competitor, affects male mate choice (measured as association preference) in a livebearing fish, the Atlantic molly, *Poecilia mexicana*. We gave males an opportunity to choose between two females, and we then observed whether males alter their initial association preference when another (audience) male was present. Examining the influence of an audience on mate choice decisions in this set-up includes interactions between four individuals: the choosing (focal) male, two stimulus females, and the audience male. In our set-up, all individuals could visually interact, but all except the focal male were confined to a defined location. Hence, the audience male could not approach the stimulus females to directly interact with them, so that any effect of the audience male on the focal male's behaviour would not be due to mate choice copying (see Schlupp & Ryan 1997).

METHODS

Study Organism and Fish Maintenance

Poecilia mexicana is widespread in Mexican and Middle American freshwaters. Males use their transformed anal fin, the gonopodium, to transfer sperm, and fertilization is internal (Constanz 1984). Males typically form dominance hierarchies, where dominant males (typically the largest males) aggressively defend shoals of females (Parzefall 1969). While females have a cryptic coloration, large males show conspicuous black vertical bars, and dominant males may even become completely black, with yellowish to orange margins of the dorsal and anal fins. Smaller males are typically considerably less conspicuous in coloration, and use a sneak-like mating tactic. Generally, *P. mexicana* males do not court females, and mating is initiated by males only (Plath et al., in press). *Poecilia mexicana* males almost constantly engage in either defending females

from other males, or attempting to mate (Parzefall 1969; M. Plath & I. Schlupp, personal observation in nature).

To examine whether the presence of an audience male affects male mate choice in *P. mexicana*, we carried out two simultaneous choice experiments using two different choice criteria. In each experiment we tested whether males alter their initial mating preference in the presence of another (audience) male. In the first experiment, we gave males the opportunity to choose between a conspecific female and an Amazon molly, *Poecilia formosa*. Amazon mollies are a clonal, all-female species, which depend on sperm from 'host species' (*P. mexicana*, *P. latipinna* or *Poecilia latipunctata* males) to trigger embryogenesis, but the male's genes are not used (gynogenesis; Schlupp 2005). Since mating with Amazons provides no obvious benefits to males of the host species (but see Schlupp et al. 1994 for benefits by heterospecific mate choice copying), we predicted focal males to show a preference for the conspecific female (e.g. Ryan et al. 1996; Schlupp 2005).

In our second experiment, we presented males with a large and a small female. Male mate choice for large female size has recently been shown for *P. mexicana* (Plath et al. 2006). We therefore predicted *P. mexicana* males to prefer to associate with the larger of two simultaneously presented females.

The mollies used in this study (*P. mexicana* and *P. formosa*) were descendants of fish collected in coastal brackish ditches near Tampico in central Mexico, where *P. mexicana* naturally coexists with *P. formosa*. Test fish came from large, randomly outbred single-species stocks maintained at the Institute of Biochemistry and Biology in Potsdam. We reared stocks comprising both sexes in aerated and filtered 150- to 200-litre aquaria at 27–29°C. Aquaria were well equipped with live and artificial plants and stones. Fish were fed twice daily with commercially available flake food, fish food tablets, and live red and white chironomid larvae. We isolated focal males in 25-litre tanks for 24 h before the tests to ensure that they were motivated to mate (Schlupp & Plath 2005). We tested each focal male only once; however, because of the limited number of males available from our stocks, some males were also used as audience males some days after they were used as a focal male. Heterospecific audience males (green swordtails, *Xiphophorus hellerii*) were obtained from a commercial supplier and were kept like the mollies. Visual contact between the different stock tanks was not possible.

Choice Tests

We divided the test tank (80 cm length × 30 cm width × 30 cm height) into five sections of equal size: two lateral compartments were divided by transparent Plexiglas partitions to hold the stimulus fish, the remainder was visually divided by marks drawn on the front into a central 'neutral zone' and two lateral 'preference zones' (Fig. 1). Black plastic covered all sides except the front wall. We filled the tank to 15 cm with aged tap water of 27–28°C. A 40-W incandescent lamp 35 cm above the tank and the room illumination (two 100-W fluorescent light fixtures on the ceiling of the experimental room)

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