Imperfect signal transmission and female mate choice in surface- and cave-dwelling Atlantic mollies, *Poecilia mexicana* (Poeciliidae, Teleostei)

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**ABSTRACT**

Female choice for large male body size is widespread among livebearing fishes (Poeciliidae). Here, we observed female association preferences relative to male body size in surface- and cave-dwelling Atlantic mollies (*Poecilia mexicana*) in simultaneous choice tests. When females could evaluate the stimulus males on the basis of multiple cues (visual plus non-visual cues were available), females from both populations preferred large males. When only non-visual cues were allowed in darkness, only the cave form showed a preference. Cave molly females discriminated between large and small males even when only a reduced amount of non-visual information could be perceived. Our findings suggest that even in an apparently little cave-adapted, presumed young cave-dweller, strong directional selection works on the non-visual sensory properties to compensate for the absence of visual communication. We discuss the role of selection generated by female choice on the evolution of sensory properties in the cavernicolous population of *P. mexicana*.

Key words: sexual selection, female choice, sensory shift, cave fish, lateral line

**INTRODUCTION**

**Mate choice in Cave mollies**

Animals shifting from surface- to cave-living offer a unique tool to study sensory and/or behavioural adaptations to life in the absence of visual communication. Once a surface-dwelling eyed species moves to underground living, behaviour patterns that have formerly been triggered by visual cues may be reduced because the releasing signals are lacking. However, if the behaviour is under selection, a novel releasing mechanism is likely to evolve, substituting the former visual signal.

We examined the evolution of female mating preference in a cave-dwelling fish, the Mexican livebearer *Poecilia mexicana*. The cave form of *P. mexicana* (the Cave molly) inhabits a sulphurous creek in a southern Mexican limestone cave (Cueva del Azufre, syn. Cueva de Villa Luz, Gordon and Rosen 1962). While many cave fishes show a pronounced reduction of eyes and pigmentation (Weber et al 1998 for a review), the Cave molly is only slightly less pigmented than surface-dwelling *P. mexicana* and eyes are only slightly reduced in diameter (Peters et al 1973; Parzefall 2001 for a review). Eyes are capable of vision; for example, Cave mollies respond to hand movements in front of the aquarium. Due to the absence of pronounced regressive features, Peters et al (1973) hypothesised that the Cave molly is a phylogenetically young cave fish.

Some mating preferences of surface-dwelling poeciliids necessarily rely on visual information from males, for example the preference for certain male colour ornaments like bright colouration in guppies, *P. reticulata* (Houde 1997 for a review) or the preference for symmetrical vertical black bars in Sailfin mollies, *P. latipinna* (Schlüter et al 1999). Such traits cannot be detected by females in the absence of light. However, male traits associated with differences in body shape such as body length (Körner 1999; Parzefall 2001; Arndt et al 2004; Plath et al 2003a, 2004) or differences in the expenditure of the abdomen due to different nutritional states (Plath et al 2005) have been shown to be evaluated by cave-dwelling *P. mexicana* females even in the absence of light.

As typical for mollies, mature Cave molly males show a pronounced polymorphism in body size (Plath et al 2003b). Given the choice between a large and a small male, Cave molly females preferentially associated with the larger male during tests in which multiple cues (visual plus non-visual) from the two males were available, but also when solely visual information was available and even in darkness, where only non-visual information could be perceived by the females (Plath et al 2004). Under naturally dark conditions, the determination of male body size appears to be taken over by the mechanosensory lateral line system in cave-living *P. mexicana* (Plath et al 2004).
Female choice and restricted information transmission

Under natural conditions, the transmission of signals (e.g., non-visual signals from molly males) is almost always imperfect. Besides spreading losses, a propagated signal loses energy due to medium absorption and scattering (Bradbury and Vehrencamp 1998). Furthermore, naturally occurring obstacles like stones limit the unrestricted transmission of signals. Do Cave molly females also discriminate between a large and a small male when the amount of non-visual information is restricted? In the present study, we gave surface- and cave-dwelling Atlantic molly females an opportunity to associate with a large or a small male in a simultaneous choice situation, where females could perceive varying amounts of far-field information from the males. Body contact between the three fish was prevented. Hence, close-range communication was not possible.

We carried out three different choice experiments where the amount of information females could perceive was reduced successively. The first two experiments are similar to a previous study (Plath et al 2004). Here, we tried to replicate the results from our previous experiments on mate choice with “unrestricted” signal transfer. In experiment (1), the females perceived both visual and non-visual cues from the males. Hence, the focal females could choose on the basis of a maximum of multiple cues (see Jonstone 1996). Based on our previous study (Plath et al 2004), we predicted that both the surface and the cave-dwelling females would show a preference for large males.

In experiment (2), the two stimulus males were presented in darkness. Here, we predicted that only the cage form would show a preference for large males (Plath et al 2004).

In experiment (3), we reduced the amount of non-visual information available to the females. This choice situation mimicked restricted signal transmission, for example by naturally occurring obstacles. Do Cave molly females discriminate between two males that differ in size when unrestricted signal transmission by non-visual cues is impossible?

METHODS

The study populations and their maintenance

All test fish used in this study were offspring of laboratory reared fish. Stocks were derived from animals collected from two localities in southern Mexico (Tabasco), near Tapijulapa. One population was collected in an epigean river, the Rio Oxolotan, near the cave (surface form). The other population originated from the innermost cave chamber of the Cueva del Azufre (chamber XIII after Gordon and Rosen 1962; approximately 300 m from the cave mouth). Fish were maintained as large aquarium stocks comprising both sexes in 50 – 200 l aquariums at 25

– 30°C at the ‘Biozentrum Grindel’ in Hamburg. Both populations were maintained under “surface conditions” regarding photoperiods: In addition to diffuse natural daylight, artificial illumination provided a 16:8 hour light: dark regime. Hence, even the cave fish were familiar with light. Previous studies have shown that light-reared Cave molly females readily show mating preferences when transferred into the dark (Arndt et al 2004; Plath et al 2003a, 2004). Fish were fed twice a day ad libitum with commercially available flake food and live Artemia naupliae, Daphnia and Tubifex worms. Prior to the tests, females were isolated for four days in 25 – 50 l aquaria as small all-female groups to standardise motivations. All fish tested were mature and had had the opportunity to interact with the opposite sex before the tests.

Mate choice experiments

For the preference tests we used a standard test tank (100 x 35 x 35 cm), filled to two thirds with aged tap water of 24 – 28°C. Black gravel covered the base. Three sides of the aquarium were covered with dark plastic foil. We marked two vertical lines on the front to visually separate three equal compartments (a central neutral zone and two lateral preference zones). Each preference zone contained a cylinder (12 cm in diameter), placed upright in the centre of the compartment, to hold one stimulus male. Hence, females could swim around the male, but body contact was prevented.

Typically, sexual preferences are measured as association times (e.g. Schlüter et al 1998; Landmann et al 1999; Plath et al 2004). Recently, Gonçalves and Oliveira (in press) have demonstrated that the time female peacock bennies (Salarias pavo) spend associating with a male appears to be a good indicator of their mating preferences. In P. mexicana, males do not show any courtship – even in the surface form – (Parzefall 1969; Ptacek 2002) and a typical mating sequence consists of a male approaching the female, then nipping at her gonopore and finally attempting to copulate (Parzefall 1969). Throughout the year, males of both forms almost always try to mate upon finding a female. In the cage form, male sexual activity is considerably lower, but typically Cave molly males also try to mate with unfamiliar females within the first few minutes (Plath et al 2003b). Hence, a P. mexicana female that associates with a certain male is likely to attract the attention of this particular male. In summary, it seems reasonable to measure association preferences of a P. mexicana female as an indicator of her mating preferences (but see Gabor 1999 for a different view in Sailfin mollies, P. latipinnia).

Prior to the tests, a pair of stimulus males was taken from the stock tanks and placed into one of the cylinders each. Stimulus males matched the population of the focal female. They were exchanged between the trials. Stimulus males differed in size by at least 3 mm (mean size difference: 6.7±1.2 mm). We waited until the males
were swimming freely in their cylinders. Trials in which the males did not start to swim freely were terminated. Then, a focal female was gently introduced into the neutral zone. Measurement was initiated when the focal female started to swim. A trial was terminated if the female did not start to swim after 15 minutes.

We measured the time the female spent in the preference zones near either the large and the small male during 10 minutes. Then, the cylinders were gently exchanged from left to right and vice versa. When all fish were swimming freely again, measurement was repeated. This procedure enabled us to detect side-biases. Females were tested only once per experiment. However, some females were tested both in experiment (1) and (2).

In experiment (1), the males were swimming in wire-mesh cylinders, made of a green plastic grid (1 mm wire-diameter, 5 mm mesh-width). The aquarium was illuminated with visible light, provided by two 60 W incandescent bulbs above the preference zones. In this experiment, females could perceive a maximum of information from the males because the cylinder material was permeable to visual and non-visual cues. However, the perception of visual cues was slightly restricted by the wire-mesh material.

In experiment (2), the same cylinders were used, but the tests were carried out under infrared conditions. Two 500 W infrared bulbs (wavelength>800 nm) were installed above the preference zones. Infrared-sensitive photoreceptors are absent in the eye of P. mexicana (Körner 1999; Körner, Schlupp, Plath and Loew in prep.). In this experiment, the focal females perceived solely non-visual cues from the males.

With the attempt to simulate imperfect transmission of non-visual information, in another experiment (3), tests were carried out in the absence of visible light again, but the males were confined to perforated Plexiglas cylinders, where 10 small holes (5 mm diameter) were irregularly dispersed on the surface of the solid cylinders. This experiment allowed only a very restricted amount of non-visual information from the males to reach the female. Our experimental design allowed us to keep the stimulus males stationary (which is a prerequisite for choice experiments) while reducing the amount of information the females perceived.

The tests were recorded with an infrared-sensitive camera, installed in front of the tank, and the signal was transferred to a monitor in a neighbouring room and scored directly.

Experiments (1) and (2) were carried out both with surface- and cave-dwelling Atlantic mollies. Since the surface-dwelling females did not show a preference in experiment (2), they were not tested in experiment (3).

**Statistical analysis**

We summed up the times the female spent near either males during both parts of a trial. We decided a priori to exclude those trials in which the females spent less than 50 % of the total observation time in the choice zones due to low response. Trials in which the females spent more than 80 % of their choice time in only one compartment were discarded as side-biases. Side-biases occurred only infrequently [Surface form: one in experiment (2); cave form: two in experiment (1), two in experiment (2) and two in experiment (3)]. No trial was discarded due to low response. Since our data were not normally distributed, we used two-tailed Wilcoxon signed-rank tests to compare the times the females spent near either male (Siegel and Castellan 1988).

**RESULTS**

**Experiment 1: multiple cues (visual and non-visual)**

When the males were presented in wire-mesh cylinders in light, both surface- and cave-dwelling females spent significantly more time associating with the large stimulus male (Wilcoxon signed-rank test: surface: 63.4 % near the larger male; \( z = -2.49, P < 0.05, n = 11 \); cave: 58.3 %; \( z = -2.19, P < 0.05, n = 10 \); Figure 1).

**Fig 1 – The time surface- and cave-dwelling P. mexicana females spent near a large and a small male when allowed to choose between them in simultaneous choice tests.** Stimulus males were swimming in wire-mesh cylinders under illumination with visible light [above, experiment (1)] or under infrared illumination [darkness, below, experiment (2)]. Boxplots, middle line representing the median, the ends of the box representing the interquartile range (50 % of the values) and the whiskers representing the 5 % and 95 % values. Wilcoxon signed-rank test, two tailed. *\( P < 0.05 \). n.s. = non-significant.
**Experiment 2: unrestricted non-visual communication**

In the absence of visible light, surface females did not show a preference (50.0% near the large male; Wilcoxon signed-rank test: \( z=-0.31, P=0.80, n=12 \); Figure 1). By contrast, Cave molly females spent significantly more time near the larger male (54.6%; \( z=-2.43, P<0.05, n=20 \)).

**Experiment 3: reduced amounts of non-visual cues**

When the males were presented in perforated Plexiglas cylinders in darkness, Cave molly females still showed a significant preference for the larger stimulus male (56.8%; Wilcoxon signed-rank test: \( z=-2.02, P<0.05, n=20 \); Figure 2).

**DISCUSSION**

We gave females from a surface- and a cave-living population of *P. mexicana* an opportunity to associate with a large or a small male in a simultaneous dichotomous choice situation. When the females could perceive a maximum of visual plus non-visual cues [experiment (1)], both surface- and cave-dwelling females significantly preferred to associate with the larger male. When solely non-visual cues were allowed in darkness [experiment (2)], only Cave molly females showed a preference. Even when the amount of non-visual information was reduced [experiment (3)], Cave molly females still significantly preferred large males.

**Female choice in light**

In experiment (1), visual and non-visual cues were available to the focal females. The observed preference in both populations confirms a previous study (Plath et al 2004) and suggests that mating preferences for large male body size are a common feature of *P. mexicana* females. Recent studies have shown that both surface-dwelling *P. mexicana* females and Cave molly females show a preference for large males even when solely visual information from the males is presented, for example by using video-playback or by presenting the males behind transparent Plexiglas (Plath et al 2003a, 2004). The ability of Cave molly females to discriminate on the basis of visual cues appears to be a relic behaviour, because under the permanently dark natural conditions females could not communicate visually (Plath et al 2004).

**Female choice in darkness**

In experiment (2), the females perceived solely non-visual information from the males. Surface-dwelling *P. mexicana* females were not able to discriminate between the two stimulus males, whereas Cave molly females spent significantly more time near the larger male. Again, these results are in agreement with our previous findings (Plath et al 2004). Our results demonstrate that non-visual cues provide important information for Cave molly females, but not for surface females. Surface-dwelling Atlantic mollies appear to require visual information for decision-making.

Apparently, the response to non-visual cues is a novel behaviour in Cave molly females. The ancestral (formerly visually mediated) mating preference has persisted in the cave form, but under naturally dark conditions the detection of the trait “male body size” appears to be taken over by a non-visual sensory system.

Chemical communication on the basis of water-borne substances has been reported for a variety of Poeciliids (e.g. guppies, *P. reticulata*: Shohet and Watt 2003; swordtails, *Xiphophorus*: McLennan and Ryan 1997, 1999; Hankinson and Morris 2003). By contrast, so far there is no evidence indicating that *P. mexicana* responds to chemical cues from conspecifics at all (Wenzel 1997). In this study, *P. mexicana* males from a surface population near Tampico, Tamaulipas, Mexico as well as males from the milky sulphur creek near the cave mouth did not discriminate between water from a tank containing females and water from a tank without females. Therefore, we assume that females base their choice decisions on information obtained by the mechanosensory lateral line system (see Plath et al 2004). For example, the lateral line is involved in inter-sexual vibrational communication in Blueback salmon (*Oncorhynchus nerka*, Satou et al 1994). However, chemical communication may have been improved in the Cave molly. The relative influence of chemical and/or mechanical (pressure
wave/vibrational) cues on female mate choice decisions of Cave molly females will need to be separated in future studies.

**Restricted non-visual information transmission**

In experiment (3), the amount of non-visual information that females could perceive was reduced. Pressure-wave signals (or chemical cues) could only be transmitted by ten small holes in the walls of the cylinders that contained the stimulus males. Hence, the area of the cylinder walls that allowed for information transmission was considerably smaller than in experiment (1) and (2) [app. 0.20 cm² per cylinder versus app. 7.65 cm² in experiment (1) and (2)]. Nevertheless, Cave molly females significantly preferred to associate with the larger of the two stimulus males. These results suggest that even if the transmission of non-visual information between males and females is restricted by naturally occurring obstacles (e.g. stones), or if the signal loses energy during transmission, females can evaluate males during mate choice. The sensory properties of Cave molly females appear to be particularly well adapted for mate choice in darkness. Female mate choice leads to differential mating success among males and is therefore one of the major forces of sexual selection (Andersson 1994).

At an early stage of cave colonisation, finding any mate is probably the primary challenge for a cave dweller, leading to the evolution of non-visual sex recognition systems. Simultaneously, mechanisms to evaluate potential mates will evolve, affecting both sensory systems and the higher neuronal processing. The latter scenario requires (direct or indirect) adaptive benefits of (non-visual) mate choice behaviour for females, for example by increased fertilisation success depending on male quality (e.g., Robertson 1990). We propose that selection generated by the advantages of female mate choice plays an important role in shaping the sensory capabilities of cave animals. While natural selection works mainly on those sensory and neuronal properties that facilitate orientation (e.g. cave salamander, *Proteus anguinus*: Istenič and Bulog 1984; Roth and Schlegel 1988; Durand and Parzefall 1987) or foraging in the absence of vision (e.g. Uiblein et al 1992), sexual selection should work on those sensory/neuronal properties that facilitate the perception, processing and recognition of specific characteristics of conspecifics.

In summary, we provide further evidence that Atlantic mollies have a mating preference for large male body size that is mediated by visual cues in the surface form, but is mediated by non-visual (probably mechanosensory) cues in the Cave molly. Cave molly females have evolved the ability to discriminate between males differing in size even when only a reduced amount of information is available, suggesting that strong directional selection works on their sensory properties.

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