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SEX RECOGNITION IN SURFACE AND CAVE DWELLING MALE ATLANTIC MOLLIES *POECILIA MEXICANA* (POECILIIDAE, TELEOSTEI)

by

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Summary

Animals colonizing lightless subterranean habitats can no longer rely on visual signals to find mating partners. In the present study, we investigated the ability of males to recognize females in two surface and a cave dwelling population of a livebearing fish, *Poecilia mexicana*. In surface populations males discriminated between sexes with visual plus non-visual cues available and with visual stimuli only. In the cave form the ability to discriminate with solely visual stimuli is lacking. In all three populations, males did not recognize females in darkness (infrared observations), suggesting that sex recognition via far-field communication is lacking in surface and cave dwelling *P. mexicana*. Different preferences in large and small males to stay near a female or a male stimulus fish probably reflect differences concerning a trade-off between sexual and aggressive behaviour.

Keywords: sex recognition, *Poecilia*, cave fish, lateral line.

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Introduction

Species colonizing caves have to cope with dramatic changes in environmental conditions. One of the most striking differences between the former surface habitat and the underground environment is the impossibility of using vision. As shown for a wide range of taxa, the absence of light leads to the regression of or selection against the maintenance of the visual system and consequently eyes may be reduced (*e.g.* Wilkens, 1982, 1988; Langecker & Longley, 1993). Selection works on other sensory organs to compensate for the lack of visual information (Weber *et al.*, 1998; Langecker, 2000). Often, chemical cues are used to detect mating partners, which may be both attached to the substrate or water soluble (Parzefall, 1976; Parzefall *et al.*, 1980; Guillaume, 2002). For instance, in the cave salamander *Proteus anguinus*, a slowly moving aquatic animal, which is stationary for long periods when hiding in crevices or under stones, population densities are mostly very low and mature specimens are rare (Briegleb, 1962). In cave dwellers like this, chemical signals are suitable for intersexual communication, because they provide long lasting information, require little energetic costs and the sender can easily be localized. Cave animals may also improve their mechanosensory system, *i.e.* the lateral line system in fishes (*Astyanax fasciatus*: Schemmel, 1967; Montgomery *et al.*, 2001), or they possess a well-developed electric sense, *e.g.* ampullary organs in cave catfishes (Weber, 1995) or cave amphibians (*Proteus*: Istenič & Bulog, 1984).

In the present study we examined the preference to associate with individuals of the same or opposite sex in two surface and a cave dwelling population of Atlantic mollies. *Poecilia mexicana* is widely distributed in Mexican freshwaters (Rosen & Bailey, 1963; Miller, 1966). In the Cueva del Azufre near Tapijulapa (Tabasco, Mexico), *P. mexicana* has evolved a cave form (Gordon & Rosen, 1962). The Cueva del Azufre is a 300 m long limestone cave with several breaks in the ceiling in the front parts of the cave. The inner cave is lightless and the innermost isolated molly population (chamber XIII after Gordon & Rosen, 1962) constantly lives in the dark. A creek of milky sulphurous water is flowing through the cave.

In surface as well as in cave dwelling Atlantic mollies, courtship displays are almost lacking (Parzefall, 1969; Ptacek, 2002). Mating is solely initiated by males: They approach the female, followed by nipping the genital region before attempting to copulate (Parzefall, 1969). Females are receptive for

three to four days per month (Parzefall, 1973). Males prefer to mate with attractive females (Parzefall, 1969; Schlupp *et al.*, 1991), but nonetheless try to copulate almost constantly with any available female. Females avoid males and prefer to associate with females when not in the attractive stage (Plath *et al.*, 2001, in prep.).

During nipping, males touch the female genital region with their snout, thereby receiving 'substrate borne' chemical information concerning gender and the female's stage in the sexual cycle (Zeiske, 1968, 1971; Parzefall, 1969). In the Cave molly, morphological changes of the female genital region and an enhanced gustatory system in males facilitate this near-field communication (Parzefall, 1970). Males often have to follow females for considerable time before they achieve body contact (M.P., pers. obs.).

In surface Atlantic mollies, males can use visual cues for sex recognition (Körner, 1999; Plath *et al.*, in prep.). Thus, in surface populations males can minimize the energetic costs of unnecessary approaches. In the cave population, the sex ratio is about 1:1 (Heubel *et al.*, unpubl. data), suggesting that no sex specific predation occurs (Godin & Donoughe 2003). Hence, without far-field sex recognition only 50% of the fish a Cave molly male starts to follow would be females. Consequently, the evolution of far-field sex recognition would enable males to save energetic and time costs for unnecessary approaches.

Relatively little is known about far-field communication in mollies apart from visual communication. Therefore we asked, if Cave molly males use non-visual information for sex-recognition. The Cueva del Azufre is energy-rich (Langecker *et al.*, 1996), and in some cave chambers Cave mollies occur in densities of up to 100-200 individuals per m² in contrast to 2-50 animals per m² in surface habitats (Parzefall, 1979, 1993). However, in chamber XIII, the origin of the cave fishes used for this study, densities are relatively low with approximately 10-12 animals per m² (after Körner, 1999; Heubel *et al.*, unpubl. data).

Water borne pheromones from different females would soon be mixed by the current of the creek. Hence, the localization of the sender might be difficult. In a previous study, male Atlantic mollies from the cave entrance did not discriminate between water from a tank containing conspecific females and control water (Wenzel, 1997). In this population, vision is restricted due to the high content of colloidal sulphur in the water, which gives the water a milky appearance.

Cave molly females have evolved the ability to determine male traits associated with body shape differences (body size and nutritional state) in darkness (Körner, 1999; Parzefall, 2001; Plath *et al.*, in prep.). They probably use their modified mechanosensory lateral line system (Parzefall, 1970) to detect these traits. The improved lateral line system may also be involved in the detection of mating partners in male Cave mollies. Communication using electrical or acoustical senses is unknown for Poeciliids. No evidence indicates it evolved in the cave form.

Another aspect we simultaneously considered in this study is how male body size interacts with male preferences to associate with either sex. *P. mexicana* males reach maturity at different ages. Since body growth almost stops at the age of maturity (Snelson, 1989), males show a wide continuous range in body size with small males often maturing at less than 25% of the body size of the largest males (see Menzel & Darnell, 1973). There are no discrete size classes as shown for swordtails (genus *Xiphophorus*), where a genetic basis for the size dimorphism has been found (Kallman & Borkoski, 1978; Ryan *et al.*, 1992). In many Poeciliids, males exhibit size dependent alternative mating behaviour (Farr, 1989).

In surface populations of *P. mexicana* body length and rank are positively correlated: larger males (LM) dominate smaller ones (Parzefall, 1974). They become intensely coloured and try to monopolize females (Parzefall, 1969). In the presence of LM, small males (SM) remain cryptically coloured. SM are more submissive in response to LM aggressive behaviour and attempt to copulate by means of forced copulations in the absence of LM (Parzefall, 1969; Plath *et al.*, in press). Even in the absence of competing LM, male body size is negatively correlated with sexual activity: SM exhibit considerably more sexual behaviour than LM (Plath *et al.*, in press). Apparently, size-dependent alternative mating behaviour is not determined by the presence of larger/smaller rivals.

In the cave form, size-dependent alternative mating tactics have not been found (Parzefall, 1969; Plath *et al.*, in press). Aggressive behaviour is strikingly reduced (Parzefall, 1974, 1979; Parzefall *et al.*, 1997) and male-male competition for females is absent. Sexual activity was found to be lower in Cave mollies and LM even showed slightly higher sexual activity than SM (Plath *et al.*, in press). Consequently, we expected the amount of time males spent near females (sexual behaviour) and amount of time associating with males (aggressive behaviour) to differ between surface and Cave

mollies and between LM and SM. Therefore, they were tested as separate groups: one consisting of males obviously larger and one group of males obviously smaller than the stimulus fish.

In this study we asked whether male Cave mollies discriminate between a male and a female in the absence of body contact. Does information obtained by the lateral line enable them to recognize females? In the Cave molly eye size is only slightly reduced, presumably because it is phylogenetically young (Peters *et al.*, 1973) and eyes are still capable of vision. Therefore, we were able to compare the reaction of surface and cave dwelling Atlantic mollies to visual and non-visual cues, using three different experimental set-ups: treatments allowing for the use of (a) the visual system only, (b) all sensory channels or (c) non-visual channels only. Simultaneously we addressed the question whether preferences interact with male body size. More specifically, we tested (1) whether males of three *P. mexicana* populations discriminate between stimulus fish of different gender, (2) whether non-visual sensory pathways (the lateral line) can be used for sex recognition in darkness, and (3) whether males of different sizes relative to the stimulus fish differ in their preferences.

Material and methods

Study organisms

We used two different populations of surface dwelling *P. mexicana*. One originated from animals living in coastal brackish waters near Tampico, (Tamaulipas, Mexico), collected in 1995, the other came from a river (Rio Oxolatan) near the cave and was collected in 1995 and 1998. For the cave form we used a stock originating from the innermost chamber of the Cueva del Azufre (Tabasco, Mexico; Gordon & Rosen 1962), first collected in 1970 and repeatedly refreshed in 1975, 1982 and 1996. Most of the trials with the Tampico males were carried out in 1998, those with the Oxolatan males were all carried out in 2002 (Table 1). Most of the trials with the cave form were carried out in 1998. Therefore, at the time of the trials, the stocks had been maintained in captivity for roughly the same number of generations. We randomly outbred stocks in tanks of 50-200 l at the Zoological Institute and Museum of the University of Hamburg under artificial daylight with a 16 : 8 h light : dark cycle. Temperature was kept between 25-30°C. Fish were fed *ad libitum* twice a day with commercially available flake food, *Artemia*-naupliae, water fleas and *Tubifex* worms. Test males were separated from females and maintained in 25 l-aquaria for two days before the experiment to standardize motivation. Male Atlantic mollies strongly increase the number of sexual behaviours after sexual deprivation (Franck, 1975).

TABLE 1. *The number (N) of trials conducted with large males (LM) and small males (SM) in three populations of Atlantic mollies during the three treatments (tr. 1-3)*

Population	Size category	Tr. 1: Plexiglas cylinders, light	Tr. 2: wire mesh cylinders, light	Tr. 3: wire mesh cylinders, darkness
Surface, Tampico	LM	7 (2002)	6 (1998, SB: 1, RI: 1)	7 (1998, SB: 1) 3 (2002)
	SM	7 (2002)	7 (1998, SB: 1, RI: 1)	7 (1998, SB: 1, RI: 1)
Surface, Rio Oxolatan	LM	7 (2002, SB: 1)	7 (2002)	7 (2002, SB: 1)
	SM	10 (2002, SB: 1)	9 (2002, SB: 2)	9 (2002)
Cave, Cueva del Azufre	LM	13 (1998, SB: 1)	7 (1998, SB: 1) 3 (2002, SB: 1)	5 (1998, SB: 2, RI: 1) 4 (2002)
	SM	13 (1998, SB: 1)	12 (1998, SB: 1)	13 (1998)

In parentheses: the year in which the trials were carried out and the number of excluded trials. Exclusion criteria were side biases (SB) and low response indices (RI).

Choice tests

We used a 100 × 35 × 35 cm test aquarium, filled up to two thirds with water. The bottom was covered with black gravel. Temperature was maintained at 28-30°C. Three equal compartments were marked by lines drawn on the front of the tank. In both outer compartments (preference zones), a cylinder (12 cm in diameter) for the stimulus fish was placed. The focal males could swim around these cylinders. A randomly chosen male and a female, matched for size, were introduced into one of the cylinders each. They were given five minutes of acclimatization before the experiment. Stimulus fish came from the respective population of the test fish. To avoid pseudo-replication, stimulus individuals were exchanged for each trial. Test males were used only once per treatment. The order of the treatments was randomised to avoid order effects and males were not tested again before at least one week after the last trial.

Treatments were performed with three different combinations of cylinders and illumination conditions: (a) clear Plexiglas cylinders in light, (b) wire mesh cylinders (wire diameter 1 mm, mesh-width 5 mm) in light and (c) wire mesh cylinders in darkness. For the treatments in light the experimental tank was illuminated by two 60 W-incandescent bulbs from 28 cm above the preference zones. For the experiments in absence of light visible to the fish we used two 500 W infrared-bulbs (wavelength > 800 nm), installed above the preference zones. *P. mexicana* does not see in the infrared (Körner, 1999). During the tests in light, the observer was sitting quietly about two meters in front of the tank. The tests in darkness were recorded by an infrared video camera and monitored in a neighbouring room.

To initiate a trial, a focal male was carefully released into the middle compartment. Measurements began when all three fish started to swim freely. We then measured the time the test fish spent in each of the preference zones during a ten minute observation period.

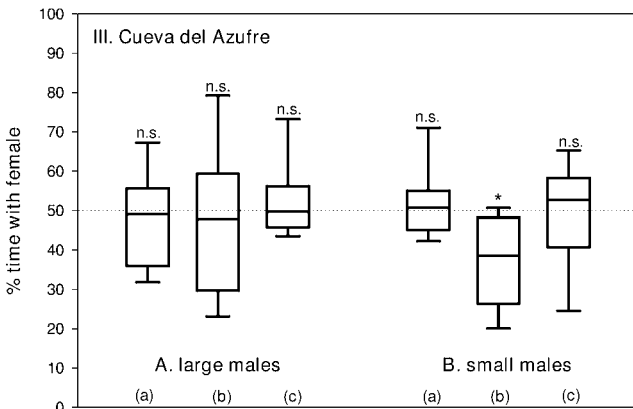
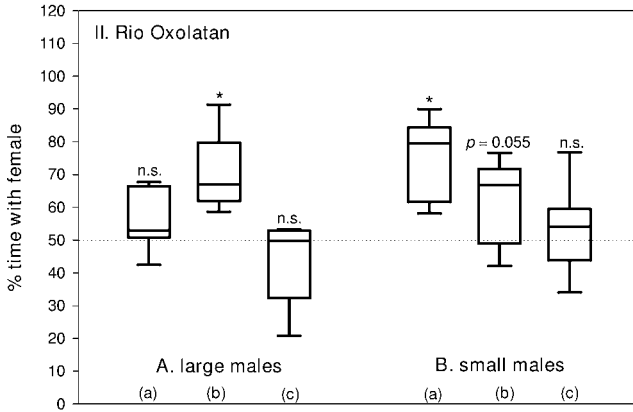
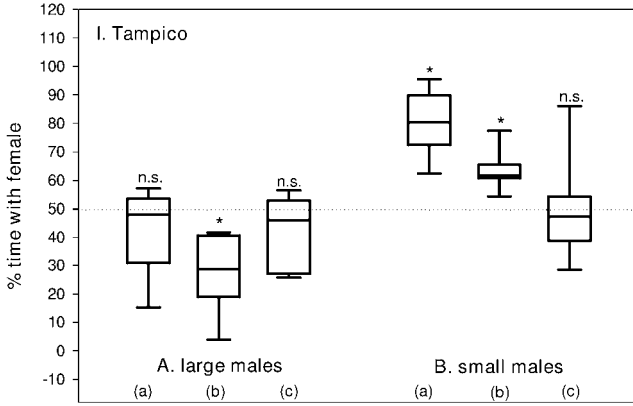
The position of the two cylinders was then reversed and measurement was repeated. Times from both trials of a test were added. Since male Atlantic mollies show little or no courtship display (Parzefall, 1969; Ptacek, 2002), the measurement of the association time near a female seems reasonable to determine their preference to mate with a female. We *a priori* decided the following criteria to exclude individual tests: Tests in which the males spent less than 50% of the total time in the preference zones were discarded due to low response. Trials in which the males spent more than 80% of their time in just one compartment were discarded as side biases (Table 1).

We used two groups of males: one group of large males (Tampico: median 34.0 mm, 4.5 mm interquartile range (IQR); Rio Oxolatan: 37.25 (5.0 IQR) mm; Cave: 33 (5.13 IQR) mm) and one of small ones (Tampico: 25.5 (1.75 IQR) mm; Rio Oxolatan: 28.0 (2.0 IQR) mm; Cave: 26.7 (4.25 IQR) mm). For the stimuli, intermediate fish were used, which differed from the test fish by at least 2 mm in size. We calculated the percentage of time the test fish spent near the male (% time near male = time near male / (time near male + time near female)) and near the female stimulus, respectively (% time near female = time near female / (time near female + time near male)). We employed non-parametric statistics to analyse the data. All *p* values are two-tailed. To estimate the statistical power of the analyses, power values from a parametric *t*-test ($\text{power}_{\text{est}}$) are shown.

Results

Surface population Tampico

- (a) Clear Plexiglas cylinders in light: LM from the surface population spent about the same time with the male and with the female stimulus fish (Wilcoxon signed-rank test, $T^+ = 9$, $T^- = -19$, $N = 7$, $p = 0.47$, $\text{power}_{\text{est}} = 0.14$). SM significantly preferred females to males (Wilcoxon signed-rank test, $T^+ = 28$, $T^- = 0$, $N = 7$, $p = 0.016$, $\text{power}_{\text{est}} = 1.0$; Fig. 1, I).
- (b) Wire mesh cylinders in light: LM spent significantly more time near the male stimulus fish than near the female (Wilcoxon signed-rank test, $T^+ = 0$, $T^- = -21$, $N = 6$, $p = 0.031$, $\text{power}_{\text{est}} = 0.80$). SM again significantly preferred females to males (Wilcoxon signed-rank test, $T^+ = 28$, $T^- = 0$, $N = 7$, $p = 0.016$, $\text{power}_{\text{est}} = 0.94$; Fig. 1, I).
- (c) Wire mesh cylinders in darkness: LM did not prefer either sex (Wilcoxon signed-rank test, $T^+ = 15$, $T^- = -40$, $N = 10$, $p = 0.23$, $\text{power}_{\text{est}} = 0.30$). They spent nearly the same time with the male and with the female. SM also spent approximately the same percentage of time with the male as with the female (Wilcoxon signed-rank test, $T^+ = 12$, $T^- = -16$, $N = 7$, $p = 0.81$, $\text{power}_{\text{est}} = 0.05$; Fig. 1, I).



In summary, in light LM spent more time near the male stimulus whereas SM preferred females. In darkness males did not discriminate.

Surface population Rio Oxolatan

- (a) Clear Plexiglas cylinders in light: In the surface population from the river near the cave, LM spent slightly more time near the female than near the male stimulus. This difference was not significant (Wilcoxon signed-rank test, $T^+ = 24$, $T^- = -4$, $N = 7$, $p = 0.11$, $\text{power}_{\text{est}} = 0.19$). SM significantly preferred females to males (Wilcoxon signed-rank test, $T^+ = 55$, $T^- = 0$, $N = 10$, $p = 0.002$, $\text{power}_{\text{est}} = 1.0$; Fig. 1, II).
- (b) Wire mesh cylinders in light: LM significantly preferred females to males (Wilcoxon signed-rank test, $T^+ = 28$, $T^- = 0$, $N = 7$, $p = 0.016$, $\text{power}_{\text{est}} = 0.96$). SM spent more time near females than near the stimulus male, but this difference was marginally not significant (Wilcoxon signed-rank test, $T^+ = 39$, $T^- = -6$, $N = 9$, $p = 0.055$, $\text{power}_{\text{est}} = 0.52$; Fig. 1, II).
- (c) Wire mesh cylinders in darkness: LM did not discriminate between females and males (Wilcoxon signed-rank test, $T^+ = 9$, $T^- = -19$, $N = 7$, $p = 0.47$, $\text{power}_{\text{est}} = 0.15$), nor did the SM discriminate (Wilcoxon signed-rank test, $T^+ = 30$, $T^- = -15$, $N = 9$, $p = 0.43$, $\text{power}_{\text{est}} = 0.05$; Fig. 1, II).

In summary, LM and SM preferred to stay near females during at least one of the treatments in light. In darkness, no discrimination could be detected.

Cave population

- (a) Clear Plexiglas cylinders in light: In both size classes Cave molly males did not show any preference. LM spent the same time with

Fig. 1. The percentage of time surface dwelling (Tampico (I), Rio Oxolatan (II)) and cave dwelling *P. mexicana* males (Cueva del Azufre (III)) spent with a female when allowed to choose between a male or a female. A. large males, B. small males. Stimulus fish in (a) Plexiglas cylinders in light, (b) wire mesh cylinders in light and (c) wire mesh cylinders in darkness. Box plots, middle line representing the median, the ends of the box representing the 25% and 75% values and the whiskers representing the 5% and 95% values. Wilcoxon signed-rank test. n.s. = non significant. * $p < 0.05$.

males and with females (Wilcoxon signed-rank test, $T^+ = 37$, $T^- = -54$, $N = 13$, $p = 0.59$, $\text{power}_{\text{est}} = 0.05$). SM also spent about the same time with males and with females (Wilcoxon signed-rank test, $T^+ = 51.5$, $T^- = -39.5$, $N = 13$, $p = 0.69$, $\text{power}_{\text{est}} = 0.05$; Fig. 1, III).

- (b) Wire mesh cylinders in light: LM showed no preference (Wilcoxon signed-rank test, $T^+ = 25$, $T^- = -30$, $N = 10$, $p = 0.85$, $\text{power}_{\text{est}} = 0.05$). SM significantly preferred male stimulus fish to females (Wilcoxon signed-rank test, $T^+ = 40$, $T^- = -74$, $N = 12$, $p = 0.003$, $\text{power}_{\text{est}} = 0.94$; Fig. 1, III).
- (c) Wire mesh cylinders in darkness: In both size classes no discrimination could be observed. LM spent about the same time near the male as near the female (Wilcoxon signed-rank test, $T^+ = 23$, $T^- = -22$, $N = 9$, $p = 1.0$, $\text{power}_{\text{est}} = 0.05$). The SM lost their initial preference observed in light and spent approximately the same percentage of time with the male and with the female (Wilcoxon signed-rank test, $T^+ = 47.3$, $T^- = -44$, $N = 13$, $p = 0.95$, $\text{power}_{\text{est}} = 0.05$; Fig. 1, III).

In summary, during experiments with wire mesh cylinders in light, LM did not spend more time near a female than near a male and SM even preferred male stimuli. With Plexiglas cylinders or in darkness, no discrimination behaviour was observed in both groups.

Discussion

In simultaneous choice tests we measured the time males from two surface populations and one cave dwelling population of *P. mexicana* spent near a male or a female stimulus fish. Under light conditions males from the epigeal populations clearly discriminated between the sexes. Large and small males exhibited different preferences for males and females. Cave molly males showed no preference when the stimulus fish were presented only visually, but SM responded when the stimulus fish were in wire mesh cylinders. In darkness, no preference was observed in any population.

Our sample size was limited by the number of adult males in our stocks, especially in the surface populations and our sample sizes are small. Consequently, statistical power is often low and negative results are discussed with

caution. Our experiments may have failed to detect an existing capability of non-visual sex recognition, but, if it existed, it must be considerably weaker than when visual cues were available.

Sex recognition in light and darkness

Using wire mesh cylinders in light to hold the stimuli, all sensory channels could be used by males to detect females. Under these conditions males of at least one size class per population did discriminate between sexes. However, only males from the surface populations discriminated with visual stimuli only (Plexiglas cylinders). In the cave form visual processing seems to have undergone regressive evolution to a certain extent. Morphological studies revealed a reduction in the number and structure of retinal cones (Peters & Peters, 1968). In phylogenetically old cave fishes, eyes may be completely lacking (Wilkens, 1982, 1988).

During the experiments in darkness, the fish obtained solely mechanosensory and potentially olfactory cues. Earlier experiments showed no role for far-field chemical communication in *P. mexicana* (Cave molly: Zeiske, 1968; cave entrance population: Wenzel, 1997). Other poeciliid fishes are known to use water soluble pheromones for species recognition (Crapon de Caprona & Ryan, 1990; McLennan & Ryan, 1997 for *Xiphophorus*) or for the determination of the female's sexual cycle (Liley, 1966; Crow & Liley, 1979; Meyer & Liley, 1982; Brett & Grosse, 1982 for *Poecilia*).

In the Cave molly, the lateral line has undergone some morphological changes during the evolution of the cave form (Walters & Walters, 1965; Parzefall, 1970): Some canals of the hyperdeveloped cephalic lateral line system remain incompletely covered so that canal neuromasts are exposed. These morphological changes may be correlated with improved mechanosensory capabilities. Some fishes are known to use the lateral line for intersexual communication (*e.g.* Salmon: Satou *et al.*, 1994). The sexes differ in body shape with females possessing a more rounded belly and shorter fins than males. Together with potential sex specific locomotion differences concerning the fin and body movement these sexually dimorphic traits might provide the basis for non-visual mechanosensory sex recognition.

However, both the surface and the cave population were unable to recognize females in darkness. Using an experimental set-up nearly identical to

the one presented in this study, females from a phylogenetically old cave fish population of the Mexican tetra *Astyanax fasciatus* were able to discriminate between male and female stimulus fish and significantly preferred the male stimulus (Plath *et al.*, unpubl. data). By contrast, *P. mexicana* males apparently have not evolved the ability of far-field sex recognition during the evolution of a cave form. An earlier study has provided comparable results for female Cave mollies (Plath *et al.*, 2001). Information obtained by the lateral line alone seems not to be sufficient for sex recognition. This finding is in agreement with the 'multiple messages' hypothesis (Johnstone, 1996).

Nipping behaviour, *i.e.* close contact, appears to be necessary to obtain information concerning gender. Probably as an adaptation to signal transfer in Cave mollies, females have an enlarged fleshy genital pad (Walters & Walters, 1965; Parzefall, 1970). Additionally, males evolved an increased number of taste buds on the upper part of the head (Parzefall, 1970). Various alternative hypotheses, which are not mutually exclusive, can explain the lack of far-field sex recognition in male Cave mollies: The cave population may be phylogenetically young (Peters *et al.*, 1973) and not enough time has elapsed to evolve far-field sex recognition systems. Alternatively, selection pressure to evolve sex recognition mechanisms may be low or absent. The Cave molly shows no aggression (Parzefall, 1974, 1979; Parzefall *et al.*, 1997). Thus, if a male happens to get into direct contact with another male while searching for a female, it does not risk costly aggressive interactions. Furthermore, Cave mollies of various sizes, including juveniles and subadults, often gather in shallow areas, where individual distances are often less than one body length (M.P., pers. obs.). Males may try to mate in these areas where mobility is restricted and it is easy to achieve direct body contact.

Population differences in male preferences

The Oxolatan males were on average larger than males from the other stocks and very small males were not available from our stocks. However, this does not explain the observed differences between Oxolatan and Tampico males: Only LM from Tampico showed a preference for males whereas Oxolatan males uniformly behaved like SM from Tampico and preferred females. Thus, the observed behaviour differences represent inter-population differences. In the surface form from Tampico LM often try to monopolize

females and defend them against SM (Parzefall, 1969). In our experiments, aggression seems to dominate over sexual motivation, leading to more association time near the male stimulus fish. On the other hand, SM may attempt sneaky copulations with the female and furthermore avoid the LM to prevent aggressive interactions. It remains unclear why these size dependent differences have not been found in the surface form from the Rio Oxolatan. Possibly, this population is less aggressive compared to that from Tampico and in both size classes sexual behaviour dominates over the aggressive behaviour. It may be more difficult to establish territories in this stream with a considerable current than in the more stagnant waters in the coastal areas near Tampico. These two populations show a large genetic divergence, based on a comparison of mitochondrial DNA sequences, whereas the Oxolatan population appears to be closely related to the cave form (Möller, 2001). The South Mexican Oxolatan population has probably been separated from the northern Tampico population for considerable time, thus different adaptations could have evolved.

In a similar study, Gabor (1999) found that male Sailfin mollies did not discriminate between a large male and a female stimulus fish. This may be due to subtle differences in the social organisation between Sailfin and Atlantic mollies: In the Sailfin molly, male-male aggressive interactions seem to play a lesser role in determining mating success than female choice (Farr, 1989; Travis, 1994), whereas the mating system in surface Atlantic mollies is more influenced by male dominance hierarchies (Parzefall, 1969).

In the cave form, SM unexpectedly preferred to associate with the stimulus male whereas LM did not prefer either sex. Aggressive behaviour is reduced in the Cave molly (Parzefall, 1974, 1979; Parzefall *et al.*, 1997) and thus can be ruled out as explanation for this paradoxical result. Possibly, SM try to compensate for being rejected in female choice (Körner, 1999; Parzefall, 2001; Plath *et al.*, in prep.) and act as satellite males. In contrast to surface Atlantic mollies, in the Cave molly LM do not exclude SM from mating and several males may simultaneously try to copulate with one female (Parzefall, 1969). Therefore, associating with LM may increase SM's encounter rate with females (Körner, 1999; Parzefall, 2001; Plath *et al.*, in prep.).

In summary, we found that (1) surface and cave dwelling Atlantic molly males discriminate between the sexes when in a dichotomous choice situation, (2) visual cues are essential for far-field sex-recognition even in the

cave form whereas non-visual far-field sex recognition was not detected and (3) body size strongly influences male preferences, probably reflecting size-dependent alternative mating tactics.

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