INTRODUCTION

In animals moving from surface to cave living, behaviour patterns triggered by visual cues become useless. For example, female mating preferences which rely on the perception of visual cues from males immediately become obsolete when a species colonises a dark subterranean habitat. In many surface dwelling animals, females prefer large males as mating partners (Andersson 1994). If such female choice is maintained in cave animals, females must use cues other than visual (e.g. mechanosensory or chemical cues) to determine male size. Female choice for large male body size on the basis of non-visual cues was found in two species of cave fish studied so far – in some cave populations of the Mexican tetra Astyanax fasciatus Cuvier, 1819 (Characidae; Plath et al in prep.) and in the Cave molly Poecilia mexicana Steindachner, 1863 (Poeciliidae; Körner 1999; Parzefall 2001; Plath et al 2003c, 2004). In epigean A. fasciatus or P. mexicana females, no mating preference on the basis of non-visual cues was found (P. mexicana: Körner 1999; Parzefall 2001; Plath et al 2003c, 2004). In epigean A. fasciatus or P. mexicana females, no mating preference on the basis of non-visual cues was found (P. mexicana: Körner 1999; Parzefall 2001; Plath et al 2004; A. fasciatus: Plath et al in prep.), indicating that a behavioural pre-adaptation for mate choice in darkness is absent in these species. Strong directional selection appears to work on the evolution of non-visual mate choice behaviour in these cave fishes. This requires that sensory systems that have previously not been used for the detection of male traits gain a new function in perceiving relevant signals.

The Cave molly Poecilia mexicana inhabits a sulphurous creek in a South-Mexican limestone cave (Cueva del Azufre; Gordon and Rosen 1962). Specimens caught in the cave appear whitish pale; however, light-reared specimens become greyish coloured and males develop –to some extend weaker– black and orange colour ornaments. Eyes are only slightly reduced in diameter and Cave mollies are still capable of vision. The lack of features typical for other cave animals (i.e. the reduction of eyes and/or pigmentation) may be due to young phylogenetic age of the cave population (Peters et al 1973). If this assumption holds true, progressive characters like non-visual mating preferences must have evolved relatively fast (Plath et al 2004).

Little is known about whether in live-bearing fishes (Poeciliidae) – like the Cave molly – female mating preferences change during ontogeny. In the closely related Amazon molly, P. formosa, age affects female sexual preferences: Mature females exhibit mating preferences whereas immature females do not (Landmann et al 1999). Does sexual experience also influence mating preferences? In surface P. mexicana, females might learn to avoid small males during early sexual encounters: Male mating behaviour is generally based on coercion in P. mexicana (Plath et al 2003a). However, small males exhibit considerably higher amounts of forced copulations than large males (Plath et al 2003a). Females might learn that by associating with large males they can avoid sexual harassment by small males, because large males almost constantly defend shoals of females from approaching smaller males (Parzefall 1969). By contrast, sexual harassment is lacking in the Cave molly, where sexual activity is considerably lower and small males are surprisingly in-active (Plath et al 2003a). Therefore, it is unlikely that Cave molly females acquire their mating preference for large males due to the avoidance of sexual harassment. The presence of the female preference for large male body size in the Cave molly (Körner 1999; Parzefall 2001; Plath et al 2003c, 2004) lead us to the assumption that this preference is innate rather than acquired during ontogeny. In the present study, we tested this hypothesis. In a simultaneous dichotomous choice situ-
ation, we observed females’ preferences to associate with a large male and a small male. To determine the influence of sexual experience on female choice behaviour, three groups of Cave molli females were tested: (I) Cave molli females from stock tanks comprising both sexes. They are known to exhibit a mating preference for large male body size (Körner 1999; Parzefall 2001; Plath et al 2004). (II) Cave molli females that had given birth before the tests. These females were unequivocally sexually experienced. (III) Sexually inexperienced (virgin) females. Here, two scenarios seem possible: (a) If the mating preference for large male body size is innate, these females should exhibit a preference to associate with large males, whereas (b) if females acquire this preference during sexual encounters, sexually inexperienced females should not have such a preference.

METHODS

Study organism and general housing conditions

We used Cave molli females from the innermost cave chamber (chamber XIII after Gordon and Rosen 1962) of the Cueva del Azufre near Tapijulapa in Tabasco, Mexico. Fish were maintained as large randomly outbred stocks comprising both sexes in 50 – 200 l aquaria under a 16:8 h light-dark illumination cycle at the Biocentrum Grindel in Hamburg. Temperature was at 25 – 30 °C. Fish were fed twice a day ad libitum flake food, supplemented by live Artemia naupliae, Daphnia and Tubifex worms.

Preference tests

For the preference tests, a standard test aquarium (100 x 35 x 35 cm) was filled up with water of 25-28°C (Fig. 1). We marked three equal compartments by vertical lines on the front: two lateral preference zones and one central neutral zone. We placed a cylinder (12 cm diameter) in each of the preference zones to hold one stimulus male each. Cylinders were made of green plastic grid (1 mm wire diameter; 5 mm mesh-width), so that a maximum of cues from the stimulus males could pass through and the focal females could perceive relevant cues even in the absence of light. Illumination was provided by two incandescent bulbs during the observations in light or by two 500 W infrared bulbs (>800 nm) during the observations in darkness (Fig. 1). Cave molli females do not discern infrared light (Körner 1999).

Before a trial, two males, visibly differing in size, were randomly taken from the stock tanks and then randomly introduced into one of the cylinders each (large males: median 26.0 mm [5 interquartile range (IQR)]; small males: 22 mm [1 IQR]). Trials were started only when both males were equally swimming freely in their cylinders. Trials in which the males were lying motionless on the bottom were terminated, assuming that in this case the focal female could not perceive cues that are related to male movement. Trials were recorded by an infrared-sensitive video camera, installed in front of the test tank, and the signal was recorded on a video recorder in a neighbouring room (Fig. 1). Therefore, fish were not influenced by the observer. Video tapes were later analysed on a monitor.

To initiate a trial, a female was introduced into the middle compartment. Measurement was started when the female was swimming freely. We measured the times the females spent in either preference zones during 10 minutes. Then, the position of the cylinders was reversed from left to right and vice versa and measurement was repeated. This procedure enabled us to detect side-biases. Each female was tested under two different illumination conditions (Körner 1999; Parzefall 2001): First, the test tank was illuminated by visible light. Then, the female was observed in darkness using infrared illumination. The test in light was carried out because mating preferences were found to be more pronounced when visual information was available (Körner 1999; Parzefall 2001; Plath et al in press; Plath et al 2003c, 2004).

Statistics

We decided a priori to exclude those trials in which the females spent more than 70 % of their time in only one preference zone (side biases) and those trials in which the females spent less than 50 % of the whole observation time in the preference zones (low response index). During the experiments with females from the stock tanks, one trial was discarded due to side bias; during the experiments with virgin females, four side biases occurred. Response indices were in no case below the limit.

Association times near either stimulus males from both parts of a trial were added. We tested for differences in the times spent near the larger and the smaller stimulus male, respectively. Since data was not normally distributed, non-parametric tests (Wilcoxon matched pairs signed-rank tests) were employed throughout. All P-values are two-tailed.

RESULTS

Sexually experienced females

Females from the stock tanks

When the experiment was carried out in visible light, seven females preferred to associate with the large male, whereas only one female preferred the small male. Females spent median 498 s [269 IQR; 67 % of the time in both
preference zones] near the large male, but only 245 s (102 IQR) near the small male (Fig. 2). This difference was significant (Wilcoxon signed-rank test: \( P < 0.05; n=8 \)).

During the treatment in the absence of visible light, six females spent more time near the large male, one female preferred to associate with the small male and one spent the same amount of time near either males. The association times near the large male [489 s (105 IQR); 54 %] and near the small male [411 s (176 IQR)] differed significantly (Wilcoxon signed-rank test: \( P < 0.05; n=8; \) Fig. 2).

**Females after parturition**

During the treatment in light, five females that had given birth before the tests preferred to associate with the large male, three females preferred the small male. The time females spent in the presence of the large male [400 s (148 IQR); 57 %] or in the presence of the small male [299 s (158 IQR)] did not differ significantly (Wilcoxon signed-rank test: \( P > 0.1; n=8; \) Fig. 2).

Under infrared conditions, seven females spent more time near the cylinder comprising the large male and only one female spent more time near the small male. The difference in the association times near either males was significant [near large male: 458 s (166 IQR; 62 %); near small male: 284 s (134 IQR); Wilcoxon signed-rank test: \( P < 0.01; n=8; \) Fig. 2].

**Influence of sexual experience: Virgin females**

In light, nine virgin females preferred the large male and two females preferred the smaller one. The times spent near the larger stimulus male [488 s (190 IQR); 54 %] and near the small male [407 s (90 IQR)] were significantly different (Wilcoxon signed-rank test: \( P < 0.01; n=11; \) Fig. 2).

In darkness, 10 females spent more time near the large male and one female spent the same amount of time near the larger and the smaller stimulus male. The females spent 381 s (137 IQR; 55 %) near the large male and 314 s (161 IQR) near the smaller one (Fig. 2). The difference in the association times was significant (Wilcoxon signed-rank test: \( P < 0.01; n=11 \)).

**DISCUSSION**

**Female choice in light and in darkness**

We measured the time Cave molly females spent with a larger and a smaller stimulus male in a simultaneous choice situation. Discrimination behaviour occurred in light as well as in darkness. The tests in darkness resemble the natural conditions inside the cave, where visual communication is not possible. In this choice situ-
Sexual experience and mate choice in Cave mollies

During the tests in light, additional to non-visual cues from the males, visual cues were also available. In contrast to other cave fishes which have highly reduced eyes (see Weber et al. 1998 for a review), the Cave molly still possesses functional eyes. Cave molly females clearly discriminated when solely visual information from males that differ in size was presented (Plath et al. 2003c, 2004). They even discriminated when video images showing a large and a small male were presented (Plath et al. 2003c). The ability to discriminate on the basis of solely visual cues represents a relic behaviour (Plath et al. 2003c).

Surface dwelling *P. mexicana* females exhibit discrimination behaviour only when visual cues are available (Körner 1999; Parzefall 2001; Plath et al. 2004). Hence, the response to non-visual cues represents a novel behaviour trait in Cave molly females, where the ancestral (formerly visually mediated) mating preference is maintained but the male trait must be detected by a non-visual sensory system. *P. mexicana* appears not to use water soluble chemical cues to communicate (Wenzel 1997), hence male size is probably not determined by chemical cues. We assume that females discriminate between males of different sizes on the basis of information obtained by the mechanosensory lateral line system (Körner 1999; Parzefall 2001; see also Plath et al. 2003c).

In light, Cave mollies can differentiate between the sexes, but not in darkness – at least not in our experiments, because body contact is prevented (Plath et al. 2001; 2003b). Therefore, females have no knowledge about the sex of the stimulus fish during the tests in darkness. Nevertheless, females readily discriminate between large males and small males. Does the observed female preference represent true mate choice behaviour? Gabor (1999) found Sailfin molly (*P. latipinna*) females to discriminate even between large and small females. The author concludes that female preferences for large body size may be derived from a non-sexual context and the discrimination between large and small males may be a by-product of such already-existing preference. We assume that female preferences for large male body size are true mating preferences in the Cave molly, because there is no alternative explanation for what adaptive significance the preference for large males should have in the Cave molly – apart from finding a “high quality mate”. Shoaling and aggressive behaviour are reduced (Parzefall 1974; 1979; 1993) and can be ruled out as explanation for the evolution of this non-visual preference in the Cave molly.

Fig. 2 - The time (A) Cave molly females from mixed-sex stock tanks, (B) females after parturition and (C) sexually inexperienced (virgin) females spent near a large male (LM) or a small male (SM) when allowed to choose between them. I. tests in light, II. tests in darkness. Boxplots, showing the median (middle line), the interquartile range (box) and the 5th and 95th percentiles (whiskers).
**Sexual experience and female choice**

Females that were taken from stock tanks significantly preferred to associate with the large male in light as well as in darkness. This finding is in accordance with previous studies (Körner 1999; Parzefall 2001; Plath et al 2004). In females that had been tested after parturition, we had evidence that all focal females were sexually experienced. These females did not significantly discriminate during the tests in light, but clearly discriminated when the experiment was carried out in the dark. In darkness, discrimination behaviour was more pronounced than in females from the stock tanks. Sample sizes are small in our experiments, therefore the negative result must be discussed carefully due to low statistical power. The fact that females that had given birth before the tests did show a strong preference in darkness indicates that they were generally clearly motivated to choose.

Females that had been raised without males (virgin females) had had no prior sexual experience in our experiments. Nevertheless, virgin females readily discriminated between males of different sizes in light and in darkness. Like females from the stock tanks, they preferred to associate with the large stimulus male. If females acquired this mating preferences during male-female interactions, choice behaviour should have been absent in virgin females. Hence, the mating preference for large male body size appears not to be acquired during sexual encounters of young females. The mating preference for large males in probably innate in Cave molly females.

In summary, we propose that the mating preference for large male body size in Cave molly females is innate rather than acquired by sexual interactions with males. This study provides further evidence that Cave molly females prefer to associate with large males in light as well as in darkness, indicating that sexual selection by female mate choice occurs even in cave dwelling animals.

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**REFERENCES**


