

Does divergence in female mate choice affect male size distributions in two cave fish populations?

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Sexual selection by female choice can maintain male traits that are counter selected by natural selection. Alteration of the potential for sexual selection can thus lead to shifts in the expression of male traits. We investigated female mate choice for large male body size in a fish (*Poecilia mexicana*) that, besides surface streams, also inhabits two caves. All four populations investigated, exhibited an ancestral visual preference for large males. However, only one of the cave populations also expressed this female preference in darkness. Hence, the lack of expression of female preference in darkness in the other cave population leads to relaxation of sexual selection for large male body size. While *P. mexicana* populations with size-specific female mate choice are characterized by a pronounced male size variation, the absence of female choice in one cave coincides with the absence of large bodied males in that population. Our results suggest that population differences in the potential for sexual selection may affect male trait variation.

Keywords: non-visual mate choice; sensory shift; sexual selection; size variation; *Poecilia mexicana* (Poeciliidae)

1. INTRODUCTION

Female mate choice can be a strong selective force in the evolution and maintenance of male secondary sexual traits (Andersson 1994), balancing the costs such traits may confer in terms of natural selection (Zuk & Kolluru 1998). Male secondary traits can disappear in populations both if costs of trait expression increase (Zuk *et al.* 2006) or if the potential for sexual selection by female choice declines; for example, through changes in environmental conditions that impair the use of sensory systems (Seehausen *et al.* 1997; Fisher *et al.* 2006). A drastic example is the colonization of cave habitats by previously surface-dwelling diurnal animals. In the absence of light, all visual female preferences become

obsolete, and accordingly male traits should vanish. Alternatively, male traits may remain under sexual selection if females evolve the capability of detecting male traits using another sensory system ('sensory shift', Plath *et al.* 2004).

We examined visual and non-visual female mate choice for large male body size in surface and cave populations of a small live-bearing fish (*Poecilia mexicana*). Female choice for large body size based on visual cues is widespread in poeciliid fishes (e.g. Reynolds & Gross 1992; Rosenthal & Evans 1998) and, in conjunction with natural counter-selection, seems to stabilize male size variation (Ryan *et al.* 1992). We compared female mating preferences and male size variation in two *P. mexicana* populations that independently colonized subterranean habitats (Tobler *et al.* submitted). Specifically, we tested whether the cave populations retained the ancestral poeciliid preference for large male size when exposed to light, and whether females evolved the ability to discriminate between different-sized males in darkness, indicating a sensory shift. Finally, we provide correlational evidence that non-visual female mate choice affects male size distributions in natural populations.

2. MATERIAL AND METHODS

(a) Populations

Cave-dwelling *P. mexicana* were collected in the Cueva del Azufre (CDA) and Cueva Luna Azufre (CLA) in Tabasco, Mexico. Surface-dwelling fish were collected from the nearby El Azufre creek and from the nearest bigger river, the Río Oxolotan (for details see Tobler *et al.* (2006, 2008)). Gene flow among the populations investigated is low (Tobler *et al.* submitted). All test fish were descendants of wild-caught individuals and were reared in large, randomly outbred populations at the Universities of Hamburg and Oklahoma. Mate choice data for two surface populations and the CDA cave form were reanalysed from Plath *et al.* (2004).

(b) Female mate choice

Mate choice experiments followed (Plath *et al.* 2004). Individual focal females (31.8 ± 1.6 mm standard length (SL), mean \pm s.e.) could choose between a large (33.0 ± 0.2 mm) and a small stimulus male (23.7 ± 0.4 mm) from the same population that were presented simultaneously on either side of a test tank. Males were confined to transparent Plexiglas cylinders in the visible light treatment or to wire mesh cylinders under infrared light to test for a derived non-visual preference. Unlike other cave fishes, cave-dwelling *P. mexicana* possess functional eyes, but none of the populations have IR-sensitive retinal photopigments (Körner *et al.* 2006). Association times during two subsequent 10 min observation periods (with interchanged stimuli) were used as a measure of preference. Owing to the absence of large males from the CLA laboratory stocks, CDA males were used as stimulus males in this case. To detect the female preferences for large male body size, association times near the two types of males were compared using paired *t*-tests. We also calculated the strength of preference ($SOP = \% \text{ time}_{\text{large male}} - \% \text{ time}_{\text{small male}}$). Arcsine-(square-root)-transformed SOPs were used as dependent variable in an ANOVA with 'treatment' (light or dark) and 'population' as independent variables.

(c) Male size variation

To assess the size variation, we collected *P. mexicana* from the four natural habitats in spring 2007. Male and female SLs were measured using callipers to the closest millimetre. For data analysis, males and females were assigned to three size classes: SL < 25 mm, 25–29 mm and > 29 mm (see Morris *et al.* 1996). The relative frequencies of the different size classes were compared using chi-squared tests.

3. RESULTS

(a) Female mate choice

Females from all populations showed a highly significant visual preference for the larger of the two stimulus males (figure 1a). In the absence of visible light,

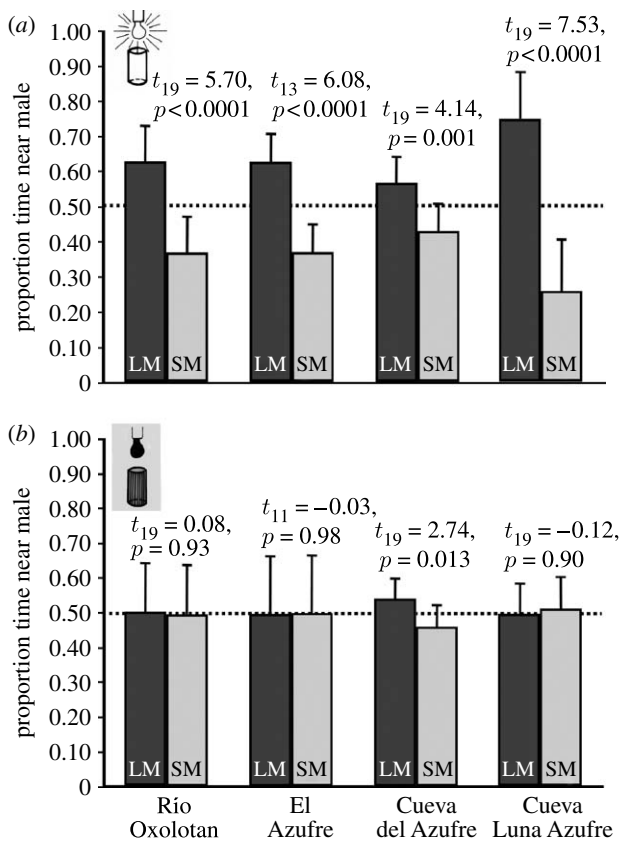


Figure 1. The mean (\pm s.d.) time *P. mexicana* females spent associating with a large (black bars, LM) and a small stimulus male (grey bars, SM). Males were presented in (a) transparent Plexiglas cylinders under visible light or (b) wire mesh cylinders under infrared conditions. Association times were compared using paired *t*-tests.

only the CDA females showed a significant preference (figure 1b). Comparing the strength of preference across treatments and populations revealed a significant interaction effect ($F_{3,138} = 6.72$, $p < 0.001$) indicating that populations responded differently in the two experimental treatments. Both the factor population ($F_{3,138} = 3.35$, $p = 0.021$) and treatment ($F_{1,138} = 49.37$, $p < 0.001$) were also significant.

(b) Male size variation

Male and female size distributions differed significantly among populations (figure 2). Large males were absent in the CLA population ($n = 38$, mean = 21 mm (range: 18–29 mm)), but common in the others (Río Oxolotan: $n = 131$, mean = 31 mm (19–56 mm)); El Azufre: $n = 53$, mean = 30 mm (24–39 mm); CDA: $n = 203$, mean = 29 mm (21–45 mm)). Although large females were present in the CLA (figure 2b), females were, on average, smaller ($n = 38$, mean = 29 mm (20–49 mm)) than those from other populations (Río Oxolotan: $n = 263$, mean = 37 mm (22–62 mm)); El Azufre: $n = 117$, mean = 35 mm (23–55 mm); CDA: $n = 303$, mean = 35 mm (20–52 mm)).

4. DISCUSSION

Female *P. mexicana* from all populations exhibited a visual preference for large males, suggesting the persistence of the ancestral character state in both cave populations even though visual preferences are not

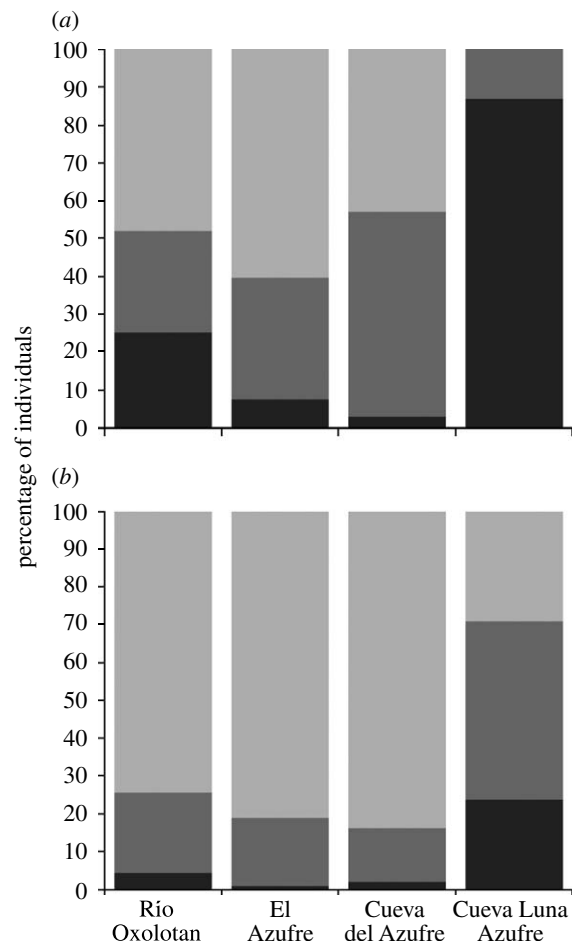


Figure 2. (a) Male ($\chi^2 = 178.4$, $p < 0.001$) and (b) female ($\chi^2 = 79.7$, $p < 0.001$) size distributions differed significantly among populations. The bars depict the relative frequency of small (<25 mm SL; black), medium (25–29 mm; grey) and large individuals (>29 mm; light grey) in the different populations.

currently under selection. However, the potential for sexual selection through female mate choice appears to differ among the two cave populations. CDA females also discriminated among different-sized males in darkness, indicating a sensory shift, whereby both mechano-sensory and chemical cues may play a role (Plath et al. 2007a). By contrast, CLA females, just like surface populations, did not exhibit a preference in darkness. Conceivably, CLA females might not have responded to non-visual cues emanating from CDA males; however, females readily responded in visual choice tests, and tests with CLA males were not feasible due to the absence of large males in this population.

Despite their spatial proximity, both caves were independently colonized by *P. mexicana*; the two populations represent morphologically and genetically distinct lineages (Tobler et al. 2008, submitted). The inability of the CLA population to evolve non-visual mate choice could be a consequence of lower evolutionary potential due to small population size (see Willi et al. 2006) or a more recent colonization as compared to the CDA population.

Female mate choice for large-sized males is thought to maintain male size variation in natural populations (Ryan et al. 1992). While large males are favoured due to sexual selection, they face a cost by natural selection

as they take longer to mature (Morris & Ryan 1990), are more conspicuous in ornamentation and behavioural displays (Ptacek & Travis 1996) and, thus, are preferred targets of predators (Trexler *et al.* 1994; Johansson *et al.* 2004; Tobler *et al.* 2007). Lack of non-visual mate choice was accompanied by the absence of large-sized males in the CLA population. Although our sample size is limited due to the small population size in this cave, more than 80% of males were smaller than 25 mm. The relaxation of sexual selection thus could have directly favoured the early maturity, resulting in the loss of large-sized males; although, unlike in the CDA (Tobler *et al.* 2007), predators seem to be absent in this cave (M. Tobler *et al.* 2006, 2007, unpublished data).

The low average body size in CLA females also suggests a possible role of natural selection on body size reduction. For example, low energy availability could select for slow growth rates and smaller size at maturity (Reznick 1990; Arendt & Reznick 2005). But, although the CLA is indeed a resource-poor habitat (Tobler *in press*), energy limitation is unlikely to solely account for the loss of large-sized males. *P. mexicana* from the El Azufre and CDA population exhibit equally low body conditions (Tobler *in press*), probably owing to energetically costly adaptations necessary to cope with the toxic hydrogen sulphide present in the latter two habitats (Plath *et al.* 2007b); still large males are frequent in these populations.

The experiments reported in this paper comply with the current legislation of the European Union and the USA.

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