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Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish

Martin Plenderleith, Cock van Oosterhout, Rosanna L. Robinson and George F. Turner*

Department of Biological Sciences, University of Hull,
Hull HU6 7RX, UK
**Author for correspondence (g.f.turner@hull.ac.uk)*

Research on reproductive isolation in African cichlid fishes has largely focused on the role of nuptial colours, but other sensory modes may play an important role in mate choice. Here, we compare the relative importance of visual and olfactory cues in mate recognition by females of a Lake Malawi cichlid species. Female *Pseudotropheus emmiltos* were given a choice of spawning next to a conspecific male or a male of the closely-related sympatric *Pseudotropheus fainzilberi*. Significant preference for conspecific males only occurred when olfactory cues were present. This suggests that divergence of olfactory signals may have been an important influence on the explosive radiation of the East African species flock.

Keywords: olfaction; Cichlidae; mate choice; reproductive isolation; *Pseudotropheus*; sexual selection

1. INTRODUCTION

It has been proposed that sexual selection may be a major driving force behind the speciation events leading to the spectacular diversity of cichlids found within the large African lakes (Seehausen *et al.* 1997; Knight & Turner 2004). Studies on cichlid fish have also inspired a number of models which have demonstrated that under certain conditions sexual selection might cause sympatric speciation (Arnegard & Kondrashov 2004)

Three sympatric species from Lake Malawi mate assortatively in the laboratory indicating that direct mate-preference may prevent hybridization between recently diverged species (Knight *et al.* 1998). Partial assortative mating between allopatric colour variants of *Pseudotropheus zebra* suggests incipient allopatric speciation by divergent sexual selection (Knight & Turner 2004).

Several studies have focused on the role of colour in cichlid mate choice (Seehausen & van Alphen 1998; Maan *et al.* 2004; Pauers *et al.* 2004), but other cues, such as sound, may also be important (Amorim *et al.* 2004). Olfactory cues are increasingly recognized to play an important role in mate recognition (Blows & Allan 1998; Ortiz-Barrientos *et al.* 2004), as well as in the assessment of mate quality and compatibility (Penn 2002), in many taxa, including fish, such as swordtails (McLennan & Ryan 1997) and sticklebacks (McLennan 2004).

Here, we compare the relative importance of visual versus olfactory cues in mate recognition by females of a Lake Malawi cichlid species. Female *Pseudotropheus emmiltos* were given a choice of spawning next to a pair of males: a conspecific or a heterospecific male of a closely related sympatric species, *Pseudotropheus fainzilberi*. We tested female preference using a partial partition design (Turner *et al.* 2001) which allowed free contact and a full range of sensory cues. Then, females were allowed to spawn next to males visible through a transparent partition, either with or without the olfactory cues from the males.

2. MATERIAL AND METHODS

(a) *Experimental animals*

Pseudotropheus emmiltos and *P. fainzilberi* are members of the species-rich *P. zebra* complex of the mbuna group of rocky shore cichlid fishes endemic to Lake Malawi. The species are closely related (Allender *et al.* 2003) and occur sympatrically, but population genetic studies using microsatellites have indicated that they are reproductively isolated (M. E. Knight, personal communication). The species occur sympatrically in the northwest of Lake Malawi. In this study, fish from Mphanga Rocks (10.45 °S, 34.67 °E) were used. The water was kept at approximately 22 °C using an external thermostatic heater and filtered through a trickle filter and UV sterilizer. The fishes were kept on a 12-hour day using timed fluorescent lights. Fishes were fed on a mixture of fish flakes and small goldfish pellets twice daily.

(b) *Experimental procedure*

Mate choice experiments were run in a single (180×45×33 cm) tank that was split into three equal-sized compartments, with two male compartments flanking a central female compartment. Twenty female *P. emmiltos* were placed into the central compartment. Individual females spawn about once every 3–4 months and were unlikely to have spawned more than once in any experiment; the low frequency of spawning meant that females would not interfere with each other, while keeping them in a large group helped them acclimatize and minimized intragroup aggression. A single male *P. emmiltos* and a male *P. fainzilberi* were placed into opposite flanking compartments. In all experimental designs, the males were size-matched for weight (±5 g) and standard length (±5 mm). As soon as practical after each spawning, the males were swapped to minimize the effects of individual (within population) variation in male attractiveness. Also the side allocated to the male of each species was swapped to control for directional bias. A total of 12 males of each species were used in these experiments. Where more than one spawning occurred within a replicate, the male that received the most spawnings was taken to be the preferred male.

(c) *Assaying mate preference*

Female *P. emmiltos* are mouthbrooders, depositing their eggs singly or in batches of 2–3 on the substrate and quickly collecting them in their mouths (Konings 2001). Although clutches were generally of 20–40 eggs laid in a single day, females may exercise the option of leaving a male after a single batch has been fertilized. In the present study, male compartments were floored with plastic grating of 15 mm squares ('egg crating'), preventing the females from collecting their eggs and allowing us to assign mate preference based on where eggs were laid (Kidd *et al.* in press). As these fish prefer to spawn in shelter, artificial caves were provided in the form of plastic boxes opaque on the top and one side and with two sides open.

(d) *Experiment 1: full contact mate choice*

Two grids separated the tank into three compartments. The mesh-size of the grid was sufficiently large to allow free movement of the focal female, but small enough to prevent the males from escaping their respective areas. The floor of each male compartment was covered with a layer of egg crating.

(e) *Experiment 2: mate choice with visual but not olfactory cues*

Solid, 5 mm thick, acrylic partitions separated the tank into three compartments. Each compartment had its own water inflow and drain. This prevented any chemical communication. A tray covered over with egg crating was placed on the floor of the tank at either

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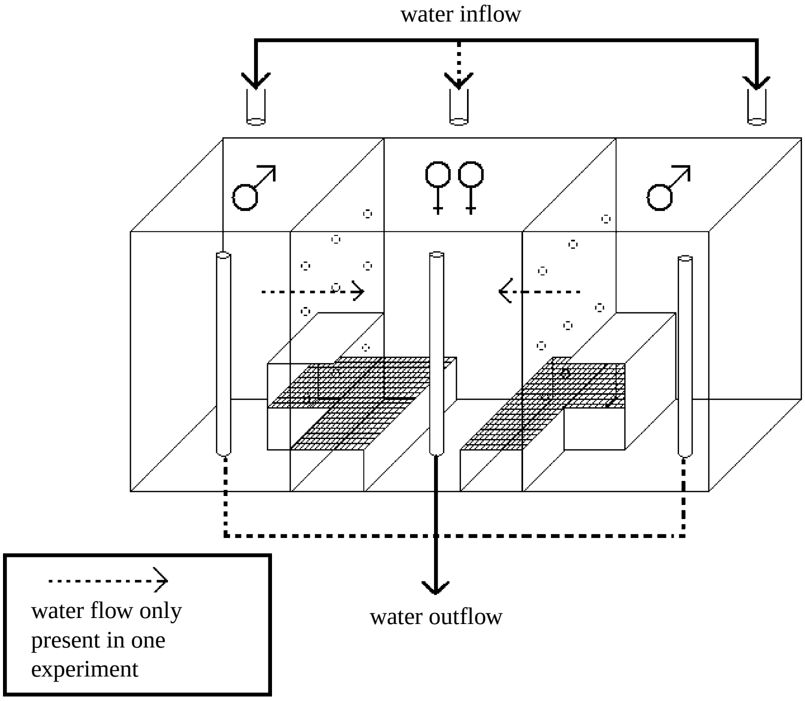

Figure 1. Design for experiments 2 and 3. In experiment 2, there were no perforations in the acrylic partition. In experiment 3, the central inflow was turned off and the central drain pipe cut down so that no water flowed out the drains in the two outer compartments. Thus, water flowed from the male sections to the female compartment through the holes in the partitions.

side of the female's compartment. Males were given a small artificial cave in their compartment, adjacent to the female's compartment (figure 1). Spawnings occurred on the trays in the female's compartment, facing the courting male's cave.

(f) *Experiment 3: mate choice with olfactory and visual cues*

The design of this experiment was identical to that of experiment 2, except that each acrylic divider was perforated with small holes, allowing unidirectional water flow from each male's compartment (with water inflow) to the females' compartment (with drain; figure 1).

(g) *Statistical analysis*

We tested the null hypothesis that the hetero- and conspecific males are equally attractive to females using a binomial test. A chi-squared test was used to examine whether spawning frequencies differed between experiments.

3. RESULTS

In all 12 replicates in experiment 1 (full contact) spawning occurred exclusively with the conspecific *P. emmiltos* males (figure 2; binomial, $p=0.0002$). In experiment 2 (visual cues only), females showed no significant preference, showing preference for each male type in six of the twelve replicates (binomial, $p=0.612$). In 11 out of 12 replicates in experiment 3 (visual and olfactory contact), females preferred conspecific males (binomial, $p=0.003$). Female preferences in experiment 2 differed significantly from those in experiments 1 (chi-squared test, $\chi_1^2=8.00$, $p=0.005$) and 3 ($\chi_1^2=5.04$, $p=0.025$).

4. DISCUSSION

Our study demonstrated that the strong preference by female *P. emmiltos* for conspecific males over those of the closely related sympatric *P. fainzilberi* (experiment 1) broke down when visual cues alone were presented to females (experiment 2). When chemical communication between the compartments was restored, females again showed a significant preference for conspecific males (experiment 3). This indicates that non-visual cues are paramount in the selection of conspecific males in this species.

Although cichlids produce sound during courtship (Amorim *et al.* 2004), the low frequency pulsed sounds produced by courting males are audible through the thin acrylic partitions that we used, so females should have been able to discriminate conspecific and heterospecific males on the basis of auditory cues in all three experiments. Thus, our results suggest that female preference for conspecific males is based largely on olfactory cues.

Previous studies on sexual selection in cichlids have focused largely on female preference for male nuptial colours as a driving force for speciation (Seehausen *et al.* 1997; Seehausen & van Alphen 1998; Couldridge & Alexander 2002; Maan *et al.* 2004). A major role for sexual selection in speciation is consistent with the breeding system of these fishes, where females care for the offspring and males compete to attract females. This leads to a male-biased operational sex-ratio and potential for strong sexual selection. The role of preference for visual traits is consistent with the striking differences in male breeding dress among many closely-related sympatric species (Seehausen *et al.* 1997) and with the smaller levels of colour differentiation among assortatively mating allopatric races (Knight & Turner 2004). However, species and geographic races may have diverged in signal traits other than colour. Empirical evidence that colour *per se* is paramount in cichlid assortative mating is largely based on a study by Seehausen & van Alphen (1998), investigating a pair of sympatric Lake Victoria species that differ in

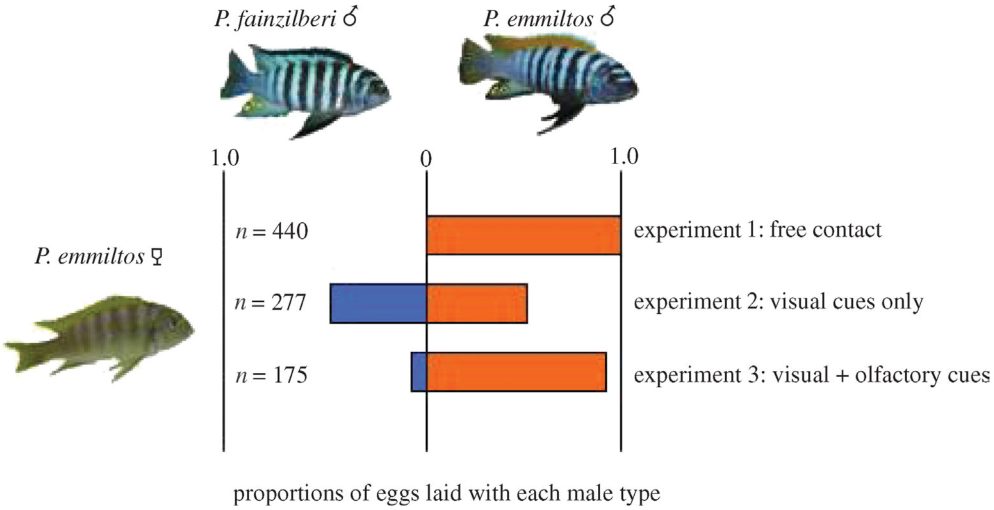

Figure 2. Proportions of eggs laid by female *P. emmiltos* with conspecific and *P. fainzilberi* males for each experiment. Total numbers of eggs laid in the experiment (*n*) are indicated.

male breeding dress. They found that females responded more to conspecific males under a broad light spectrum, but not under monochromatic orange light.

However, Jordan *et al.* (2003) found that females of three Lake Malawi cichlids associated more with conspecific males behind solid partitions which prevented olfactory contact, even under monochromatic light. This suggests that shape, pattern and behaviour may have been more important than colour in species discrimination in this group. Females which could not see males did not show behavioural responses to odour from male conspecifics. This suggests that multiple cues are required to elicit a positive courtship response (e.g. Candolin 2003), rather than ruling out the importance of olfaction.

Fishes show tremendous variation in olfactory receptor genes (Niimura & Nei 2005). Genetic changes in mate preferences associated with olfactory cues are implicated in speciation of taxa including *Drosophila* (Ortiz-Barrientos *et al.* 2004) and Mexican pupfish (Strecker & Kodric-Brown 1999). Previous studies have linked olfactory-related mate preference to variation at the major histocompatibility complex and female preference for genetically dissimilar males (Penn 2002). However, this mechanism may have the potential to generate the variability needed to provide the basis for divergence of female preferences between incipient species.

Our results suggest that divergence of male breeding colour has occurred in this species pair without associated divergence in female preference for visual traits. Recently, it has been proposed that divergence of male nuptial colour may be driven by male competition, since males of an uncommon colour may stimulate reduced territorial aggression and so may more readily establish a territory than males of the ancestral colour form (Seehausen & Schluter 2004).

In conclusion, our study suggests that divergence in olfactory signals and preferences may play a greater role than previously thought in the spectacular rapid speciation of this species flock.

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