

FEMALE PREFERENCES FOR NATURALLY-OCCURRING NOVEL MALE TRAITS

by

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Summary

We report two independent cases of female preferences for novel male traits in two species of poeciliid fish, *Poecilia latipinna* and *Poecilia mexicana*. In both cases the preference predates the occurrence of the trait, lending strong support to the pre-existing bias hypotheses. This support is independent of the assumptions associated with phylogenetic inference. Unlike the two sexual species, the unisexual hybrid *P. formosa* had no detectable preference for the novel male traits.

Keywords: pre-existing bias, novel ornament, sexual selection, tumor, *Poecilia latipinna*, *Poecilia mexicana*, *Poecilia formosa*.

Introduction

The temporal sequence of the evolution of preferences and traits is almost always unknown. Both trait and preference can evolve simultaneously or either trait or preference can evolve first and then come under sexual

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selection. The latter idea, known as pre-existing bias theory (Endler & Basolo, 1998) or more specifically sensory exploitation (Ryan, 1997, 1998; Endler & Basolo, 1998) has recently been supported empirically.

Studies in sexual selection have shown that females can have preferences for traits that are not exhibited by conspecific males (Ryan, 1997, 1998). Such studies usually demonstrate female preferences for a conspecific male whose phenotype has been manipulated with the addition of a trait (*e.g.* sword, call component) over the typical, unmanipulated male phenotype (see Shaw, 1995; Ryan, 1998 for examples).

In these studies the argument that a pre-existing bias influenced character evolution explicitly depends on knowledge of the phylogeny of the particular group studied and thus on assumptions of phylogenetic reconstruction (Hillis *et al.*, 1996). Although phylogenetic inference has greatly contributed to our understanding of sexual selection in particular and behavioural ecology in general, it is often based on a parsimonious interpretation of the character state distribution. Ryan (1996) cautioned that phylogenetic support for pre-existing biases often relies on the difference between two phylogenetic hypotheses that differ in only a single step. He also illustrated, using the argument for pre-existing biases in tungara frogs, how changing phylogenetic hypothesis can complicate interpretations of a pre-existing bias.

Another source of support for the pre-existing bias theory comes from studies that use artificial traits and test female responses to them. For example Burley *et al.* (1982) found that female zebra finches (*Taeniopygia guttata*) are more attracted by males wearing red leg bands. A similar finding was later reported for American goldfinches (*Carduelis tristis*) (Johnson *et al.*, 1993). In another study Burley & Simanski (1998) found female preferences for artificially added white crests in two species of grassfinches. Witte & Curio (1999) studied female preferences for a novel red feather in Javanese Mannikin (*Lonchura leucogastroides*) and noticed that previously unattractive males will gain attractiveness, whereas previously attractive males will lose attractiveness. Studying leg bands in bluethroats (*Luscinia s. svecica*) Fiske & Amundsen (1997) found that in addition to colour, symmetry may influence preferences for novel, artificial ornaments. Although these studies show that females have preferences for traits not exhibited by conspecifics, more direct evidence for the pre-existing bias hypothesis would show such preferences for naturally occurring novel male traits. The difficulty of doing this is clear.

Here we report of two cases of naturally occurring novel ornaments and the associated female preferences in two species of the genus *Poecilia*. The species we used are *P. latipinna*, *P. mexicana* and their natural hybrid, *P. formosa*. All are livebearing fish, with internal fertilisation that are widespread in North and Central America (Page & Burr, 1991). The natural unisexual hybrid, the Amazon molly, is essentially a clonal fish (for review see Schlupp *et al.*, 1998). It is gynogenetic (Hubbs & Hubbs, 1932), thus it needs sperm to trigger embryogenesis. This sperm is provided in nature by males of *P. latipinna* or *P. mexicana*. Theoretically, Amazon mollies do not need sexual preferences other than those required for species recognition (Marler *et al.*, 1997; Körner *et al.*, 1999; Landmann *et al.*, in press), as any male molly that provides sperm should be sufficient for them to reproduce (Hubbs & Hubbs, 1946a, b). Nonetheless, *P. formosa* has sexual preferences, such as one for large male body size (Marler & Ryan, 1997).

In this paper we present two case-studies of naturally occurring novel traits: one studying a *P. latipinna* male was conducted in Austin, Texas, the second one using a *P. mexicana* male was conducted in Hamburg, Germany. In both cases we tested whether conspecific females had a preference for the novel trait and additionally tested whether sympatric gynogenetic Amazon mollies had a comparable preference.

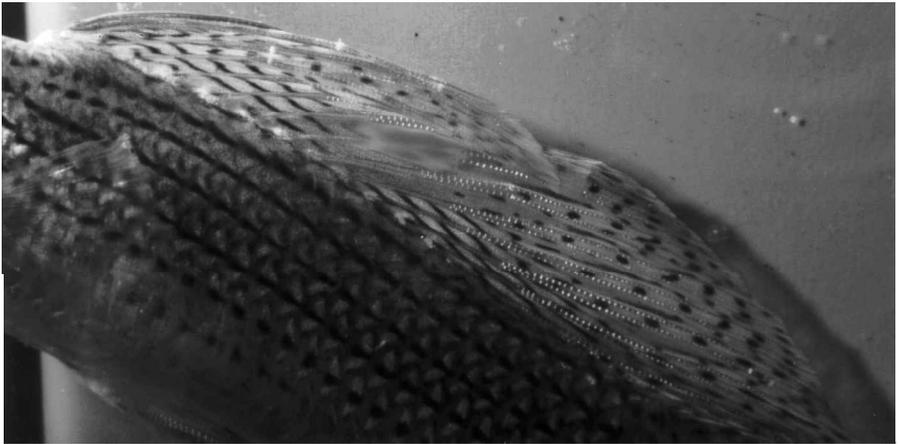
Materials and methods

P. latipinna

The *P. latipinna* and *P. formosa* were collected from the San Marcos River near Martindale, TX, where we have studied these species intensively over the last six years (Schlupp *et al.*, 1994; Schlupp & Ryan, 1996, 1997). We collected a single male bearing a conspicuous orange tumor in the dorsal fin. The tumor was a nodular benign pigmented lesion consisting mainly of carotene containing pigment cells interspersed with melanophores and leucophores (Schartl, pers. comm.). The spindle shaped tumor had an area of a few cm² and was growing three-dimensional between the 7th and 9th fin ray (Fig. 1a). During the last six years we have collected more than 150 males from this site. Only the male described here was bearing an orange tumor. Furthermore *P. latipinna* is widely used as a model organism (see Meffe & Snelson, 1989), and such tumors have never been reported.

The male appeared healthy otherwise and sired more than 100 offspring in the laboratory. These offspring were used to establish a new stock for future research.

For a choice test a female was selected randomly from a stock tank, that contained only females. The species were also kept separate, but as all fish were collected in the field as adults, they probably have met both males and females of *P. latipinna* and females of *P. formosa*. The males were kept separate from each other but had access to conspecific



A



B

Fig. 1. (a) The orange tumor in the dorsal fin of a *P. latipinna* male; (b) The orange spot in the dorsal fin of a *P. mexicana* male.

females. As we were testing a unique and novel trait, a bright orange tumor, we could only use the one male bearing this trait. In that sense the data are not independent.

The standard length of the tumor bearing male was 51 mm. We used two other males of the same body size ± 2 mm (51 and 53 mm) and otherwise comparable coloration to match the tumor-bearing male.

The choice tank was a $124 \times 34 \times 55$ cm ($l \times w \times h$) aquarium divided into five sections. The two end sections were separated with clear Plexiglas dividers and contained the stimulus males. The middle zone was a neutral zone, the two sections adjacent to the side compartments were defined as preference zones. The dividers allowed visual communication but effectively disrupted chemical communication.

To initiate a trial, the female was placed in the middle compartment of a choice tank and allowed to acclimate in a clear Plexiglas cylinder (15 cm diameter, 6 mm wall thickness). After five minutes the cylinder was gently removed and the time the female spent near the two males was measured for five minutes. The observer sat quietly about 1.0 m in front of the tank. After the first trial, the males were switched and the above procedure was repeated to detect side biases. We defined *a priori* that a side bias had occurred when a female spent more than 80% of her choosing time on one side of the tank. Such trials were excluded from the data analysis. We used non-parametric statistics to analyse the data.

P. mexicana

The *P. mexicana* and *P. formosa* were collected from the Rio Purificacion, Nuevo Leon, Mexico, brought to the laboratory in Hamburg and maintained as random outbred stocks. We collected a single male with an orange spot in the anterior part of the dorsal fin (Fig. 1b), in position and size comparable to the tumor found in the *P. latipinna* male. The male was healthy and sired over 30 offspring, which were used to establish a new stock for future research. The collecting site has been studied intensively by us for four years and by others between 1970 and 1978 (Balsano *et al.*, 1989). During this time this phenotype has never been reported and is also unreported for the species.

Due to limitations in the availability of females we used two populations of *P. mexicana*: one was the population from which the novel male was collected, the second one was collected near Tampico, Tamaulipas, Mexico. Both populations were maintained in mixed sex groups and were naive in respect to the novel phenotype. The fish used here were first or second generation offspring of wild fish.

We tested 17 *P. mexicana* females with the novel male *versus* one of six males of equal size (± 2 mm) and nine *P. formosa* females (Rio Purificacion) with the novel male *versus* one other male of equal size (± 2 mm).

The choice tests were conducted in a round Plexiglas aquarium (83 cm diameter, 30 cm high) to avoid corner and edge effects (Timmann & Schlupp, unpubl.). The water level was maintained at 10 cm and the temperature was 25°C. The tank was divided into four equal sectors (I-IV) and surrounded by a light blue cardboard of 50 cm height. Each test was filmed from above and scored on a video-monitor to avoid any disturbance by the observer. The tank was indirectly illuminated by a 1000 W light source of 6200 K light temperature. The stimuli were presented in two clear Plexiglas cylinders (10 cm diameter) randomly in opposite sectors of the tank. The other two sectors contained empty cylinders.

A trial was started by placing the two males in the cylinders. The test female was placed in the middle of the tank and the time she spent in the two sectors was recorded for ten minutes after she crossed three sectors or ten minutes. After this the stimuli were switched to avoid sector-biases. We used non-parametric statistics to analyse the data.

Results

P. latipinna

Females of *P. latipinna* had a significant preference for the tumor-bearing male (Wilcoxon test, $z = -2.7$, $p = 0.007$, $N = 14$, Fig. 2).

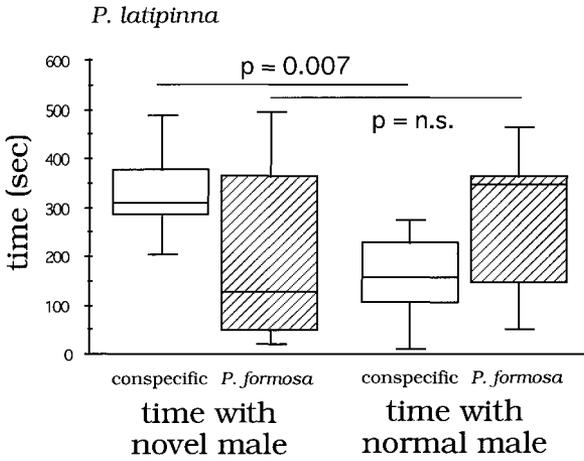


Fig. 2. Box plots of the female preferences of *P. latipinna* and *P. formosa* from Texas.

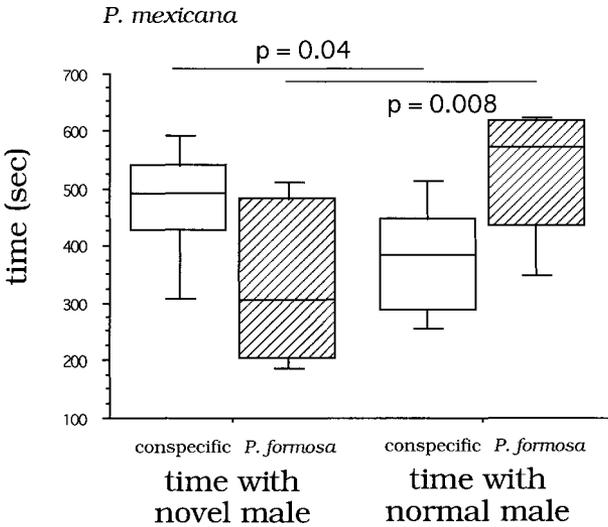


Fig. 3. Box plots of the female preferences of *P. mexicana* and *P. formosa* from Mexico.

In another experiment using the same males as in the above experiment but females of the gynogenetic *P. formosa*, we found no preference for the tumor bearing male (Wilcoxon test, $z = -0.66$, $p = 0.51$, $N = 10$, Fig. 2).

P. mexicana

Females of *P. mexicana* had a significant preference for the male with the orange spot (Wilcoxon Test, $z = -2.01$, $p = 0.044$, $N = 17$, Fig. 3). Females of *P. formosa* significantly preferred the male without a spot (Wilcoxon test, $z = -2.67$, $p = 0.008$, $N = 9$, Fig. 3).

Discussion

We report of two independent but similar cases in which a female sexual preference predates the evolution of a respective trait in males. To our knowledge this report is the first to document pre-existing female preferences for naturally-occurring novel traits. Our data provides the first evidence that is completely independent of phylogenetic inference, clearly supporting the pre-existing bias hypothesis.

As we were only able to use one individual each, it is possible, however, that the preference was for traits correlated with the orange spots, although no such trait was obvious to us. Future research will use artificial ornaments mimicking the described natural ones, to further investigate the issue. Interestingly, the sexual preference in *P. mexicana* is much weaker as in *P. latipinna*, a pattern that has been reported consistently from several studies (Schlupp *et al.*, 1991; Ryan *et al.*, 1996). In both cases we found a preference for the novel trait in the sexual species, but not in the sperm dependant asexual, *P. formosa*. It is not clear to us why the preference for the novel ornament would be present in the sexual species and not in the gynogenetic species. The absence of the trait in *P. formosa* and simultaneous presence in the host species could have resulted from several patterns of trait evolution. *P. formosa* might have lost the preference during or after the hybridization. One potential explanation would be assuming that the novel phenotypes also attract predators. Thus, the preference for the novel phenotypes may entail a higher risk of predation for the female, too. While this risk may be compensated in the sexual species, because *e.g.* the male offspring also have the attractive phenotype, in the asexual species this cost cannot be compensated for, because no genetic material is transmitted. In other words, for *P. formosa* it might be advantageous to avoid novel phenotypes to avoid predation, whereas for the sexual species it might pay to show their preference despite the risk of predation.

Females of the three species differ slightly in their visual pigments (Körner & Loew, pers. comm.) and thus colour perception potentially leading to different preferences.

Apparently the preferences were present before the novel traits arose as they appear in closely related taxa as well. Male guppies (*P. reticulata*) are well known for their array of bright colours, including orange, and females typically prefer males with more orange (Houde, 1997), as do some male sailfin mollies when red markings are artificially applied (Ptacek & Travis 1997). We suggest that the female preference for such general markings, might be a characteristic of a number of poeciliid species which in one case manifests itself as a preference for a tumorous male.

Our study of the tumor bearing *P. latipinna* male also provides an example how tumors, if they come under sexual selection may spread in a population. In this case, even if the measured female preference is not due to the tumor, this male would have had considerable reproductive success.

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