

Variation of Female Preference for Male Coloration in the Eastern Mosquitofish *Gambusia holbrooki*

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The preference for melanistic males was studied in two populations of eastern mosquitofish (*Gambusia holbrooki*, Pisces: Poeciliidae), one from Florida and one from northern Italy. Melanism in the eastern mosquitofish is a Y-linked character, expressed in males only. Melanistic males have black spots varying in size and number. In the Florida population, melanistic males are common, whereas in the Italian population they have never been observed. Females were male-deprived for at least 2 months before being tested in a dichotomous choice chamber. Italian females showed a significant preference for unpigmented males from their own population, whereas Florida females preferred melanistic males. When given the choice between males with few (<10% of the body surface) and males with many (>50%) black spots, Italian females preferred males with few black spots and Florida females those with many black spots. The preference of the Italian females for unpigmented males was confirmed in females reared from birth to maturity in the presence of only melanistic males. The preference of Florida females for melanistic males was also confirmed in females reared from birth to maturity in the presence of only unpigmented males. Altogether, these results demonstrate that in the eastern mosquitofish there is polymorphism in female preference and that this preference does not have an environmental basis.

KEY WORDS: Female choice; sexual selection; melanism; ornaments; poeciliids; rare phenotype; *Poecilia reticulata*.

INTRODUCTION

It is now generally accepted that secondary sexual traits can evolve through mate choice. The evolution of sexually selected characters requires that individuals of one sex (usually the females) are choosy, and that both selected characters and female mate preference are inherited traits. Mate choice has been demonstrated in a variety of animals (Andersson, 1994).

There is also good evidence that sexually selected characters have substantial heritability, as large as that of other morphological or behavioral traits (see Andersson, 1994, and references therein). On the contrary, the genetic basis for female mate preference has been demonstrated in a limited number of cases (for a review see Bakker and Pomiankowski, 1995). The most compelling evidence for genetic control of female preference comes from insects. Information on vertebrates, despite being preferred subjects for studies of sexual selection (Andersson, 1994), is scant. Among the few exceptions, the genetic basis of female preference for carotenoid coloration has been convincingly demonstrated in two fish, the three-spine stickleback *Gasterosteus aculeatus* (Bakker, 1993) and the guppy *Poecilia reticulata* (Houde and Endler, 1990; Houde, 1994).

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The latter species belongs to the family Poeciliidae, in which several species exhibit sex-limited, polymorphic color patterns (Liley, 1966; Farr, 1984; Angus, 1989). However, there is limited information on female mate choice based on male color in other poeciliid species. One of the cases that has been studied is the eastern mosquitofish *Gambusia holbrooki*. In this species, males, like females, are usually gray and unpigmented, but black-spotted males have been observed in variable proportions in several populations (Regan, 1961). Melanistic spotting is inherited as a Y chromosome-linked trait (Angus, 1989) and is expressed only in males. The presence of these pigmented males raises the question of why melanism is maintained when there is presumably natural selection for crypsis. Available information is contradictory. Although some studies have suggested that females prefer melanistic males (Karplus and Algom, 1996; Gould *et al.*, 1999), the majority of studies have failed to observe such a preference or found a mild preference for unpigmented males (Martin, 1977; Nelson and Planes, 1993; Taylor *et al.*, 1996). In agreement with the latter studies, females from an Italian feral population show a significant preference for unpigmented males (Bisazza *et al.*, 2000).

These results make the problem of the maintenance of male melanism in the eastern mosquitofish even more intriguing, since black spotted males are apparently at a disadvantage by both sexual and natural selection. One possible explanation for the conflicting results found in different studies on female preference may be that, in this species, polymorphism exists not only for male coloration, but also for female preference. The association between melanistic pigmentation and female choice in poeciliids has been studied in detail in the guppy, and there is no general agreement on its function in this species (Houde, 1997). The aim of the present study is to understand the role of female choice in maintaining male coloration in the eastern mosquitofish.

In this study we compare the mate preference of females from two populations, an American population with a high frequency of melanistic males and an Italian population in which this phenotype is absent. In the first experiment, we examined the mating preferences of female from both populations for melanistic males and Italian wild-type males. In order to control for the possibility that females were selecting males on the basis of traits other than coloration, we repeated the first experiments allowing females to choose between American males differing in the degree of melanism. The females of the two populations tested

in the two previous experiments had been raised in the presence of only one male phenotype (American males were in 90% of the cases melanistic, whereas this phenotype is absent in the Italian population). In order to rule out the possible environmental influence on female preference variation, we performed a sort of cross-fostering experiment. We tested female preference for male coloration in females from the Italian population that were raised from birth to maturity in the presence of only melanistic males and females from the American population that were raised in the presence of males only from the Italian population.

MATERIALS AND METHODS

We studied female preference for melanistic males using fish deriving from two distinct populations. The first one is a feral population living in a stream near Padova (Idrovia Padova-Venezia, Camin), Italy. The eastern mosquitofish was introduced into Spain at the beginning of this century and is now widespread and well established in most shallow waters of southern Europe. In the Italian populations melanistic males have not been reported so far (Zulian *et al.*, 1995). The other population was sampled in the drainage ditches near the Disney Epcot Center, Orlando, Florida, and maintained in our laboratory. In this population melanistic males are common (M. Schadle, personal communication). After the experiments, fish from the Italian population were released back to the site from which they were originally captured.

The two populations were maintained in distinct, heterosexual groups of 20–25 individuals in 150-L aquaria (temperature, 24–26°C; photoperiod, 0500–2100 h) and fed with brine shrimp and dry food for tropical fish. Females used in the choice test were deprived of males for at least 2 months before being tested. In a previous study (Bisazza *et al.*, 2000), we showed that females deprived of males for a long time show the same tendency to approach males as postpartum females, but we used deprived females for practical reasons.

Choice tests were carried out as by Bisazza *et al.* (2000). Briefly, we used a classical three-chamber choice apparatus. Female mosquitofish are gregarious. In order to distinguish sexual from social motivation three companion females were always visible to the test female, in the rear of the test compartment (Fig. 1). Once introduced into the choice apparatus, the test female rapidly resumed normal swimming behavior. Two groups of three stimulus males (matched in size)

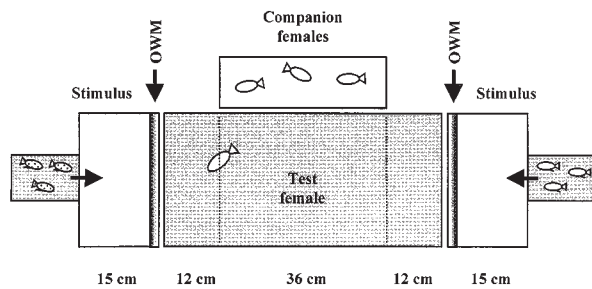


Fig. 1. Choice apparatus used for testing female preference for male coloration (from above). Companion females and stimulus compartments were illuminated by a neon light suspended about 25 cm above the water surface; the others (shaded) received only indirect light laterally from the nearby illuminated compartments. Fish in the shaded compartments were able to see the fish in the well-illuminated ones, but not the other way around (OWM, one-way mirror). The central compartment was divided into three virtual sectors by means of two black adhesive tape stripes (dotted lines) stuck on the front glass.

were simultaneously presented to the test female on the two sides of the test compartment, making them enter the stimulus compartment through a door opened from a distance by the observer. By means of one-way mirrors and differential illumination, stimulus males were prevented from seeing test and companion females. Before the choice tests, the stimulus males were kept for some days in aquaria with a one-way mirror on one side, in order to get them accustomed to their reflected image. Test females were introduced into the test compartment 60 min before being tested. Males were then introduced into lateral compartments and the test female was consecutively observed for the following 30 min. The position of the test female was recorded every 15 s, and mate preference was expressed as the proportion of time she spent in the 12-cm sector near one male compartment over the total time spent in the 12-cm sectors near male compartments.

Fish from the two populations were housed in isolation from one another. Therefore females from each population had never seen a male from the other population prior to the behavior trials. Three experiments were carried out. In the first choice experiment, Florida and Italian females were given the opportunity to choose between three melanistic and three wild-type males which were matched for size. Stimulus males differed in color, but also belonged to two distinct populations. Therefore, any preference may be due to other morphological or behavioral male characteristics. In order to ascertain whether females exerted their choice on male color, we ran a second experiment in which all males were from the Florida population but differed in

their amount of black. For this purpose, males were photographed and the amount of black pigmentation was estimated by means of an image analysis program (Scion Image 1.62). Females were given the choice of three melanistic males with black spots covering more than 50% of the total body surface (range, 55–78%) and three males with few black spots (range, 0–7%).

Because females from the Italian population had never experienced a melanistic male prior to the tests, any difference between female preference among the populations may be due to an environmental effect. To control for this possibility, we reared approximately 20 newborn Florida females from birth to maturity in the presence of 3 adult Italian males. We also reared a similar number of Italian females in the presence of three melanistic males. The sex ratio and population density were similar to those at which we maintained the females in the first experiment. The preference for wild-type and melanistic males was then tested as in the first experiment.

All data sets were normally distributed (Kolmogorov–Smirnov test with Lilliefors correction for small sample size, $p > 0.05$). We therefore used a parametric test (one-sample Student t test) to verify whether the observed proportion of time spent near the melanistic male (or the male with greater pigmented area in the case of the second experiment) was significantly different from 0.5. All probabilities are two-tailed. Statistical analysis was performed using SPSS 8.0.

RESULTS

Females from the Italian feral population spent significantly more time near wild-type males (one-sample t test, $t = 4.04$, $p = 0.002$; $n = 12$), whereas the opposite tendency was found in females from Florida ($t = 2.95$, $p = 0.013$; $n = 12$) (Fig. 2a). When given the choice between heavily and lightly pigmented males from the American population, Italian females preferred males with fewer black spots ($t = 2.76$, $p = 0.022$; $n = 10$), whereas Florida females preferred the more pigmented males ($t = 2.50$, $p = 0.034$; $n = 10$) (Fig. 2b). Sexually mature Italian females that were reared from birth in the presence of melanistic males showed the same preference for wild-type males as the females collected from the wild ($t = 6.39$, $p < 0.001$; $n = 12$). Similarly, Florida females, reared in the presence of only wild-type males, consistently preferred melanistic males ($t = 3.18$, $p = 0.013$; $n = 9$) (Fig. 2c). On average, females spent $33.9 \pm 1.89\%$ (SE) of their time in

the compartment near the males with the preferred phenotype (versus $16.5 \pm 0.47\%$ on average in the rest of the aquarium, corrected for differing sizes of the compartments), which is significantly more than expected by chance ($p < 0.05$ for all the experiments).

DISCUSSION

The results of our first experiment showed that there were differences among females in the preference for male coloration: Italian mosquitofish females preferred wild-type males, whereas Florida females preferred males from their own population. The preference of Italian females for wild-type males indicates that they did not base their preference on the "rare phenotype" (Partridge, 1983), since the Italian females we tested had never seen a melanistic male before this experiment. The second experiment demonstrates that females base their preference on male coloration, and not on other behavioral or morphological characteristics possibly differentiating the males of the two populations, since Italian females preferred males with a few black spots, whereas Florida females preferred heavily spotted males. In another study (Bisazza *et al.*, 2000, unpublished results) we found that male behavior in the stimulus compartment did not differ among the two populations tested here. This, of course, cannot exclude the possibility that females base their choice on criteria other than male coloration. However, the existence of a preference for melanism per se has been demonstrated by Gould *et al.* (1999) using mosquitofish dummies with different degrees of melanism. This suggests that male melanism is an important cue used by females during mate choice.

The third experiment indicates that these preferences do not have an environmental basis: Italian females continued to prefer wild-type males even if they were raised in the presence of only melanistic males, and Florida females preferred melanistic males even if they grew up in the presence of only wild-type males. The female preference we observed in the first experiment was therefore not due to environmental influence (e.g., imprinting or imitation) determined by the sexual and social conditions experienced by the females before the experiment. As far as we know, this is one of the few cases, among fish, showing that female preference is polymorphic and has a genetic basis, the other two species being the sticklebacks and the guppy (for a review see Bakker and Pomiankowski, 1995). However, in contrast to the latter two species, female choice in the

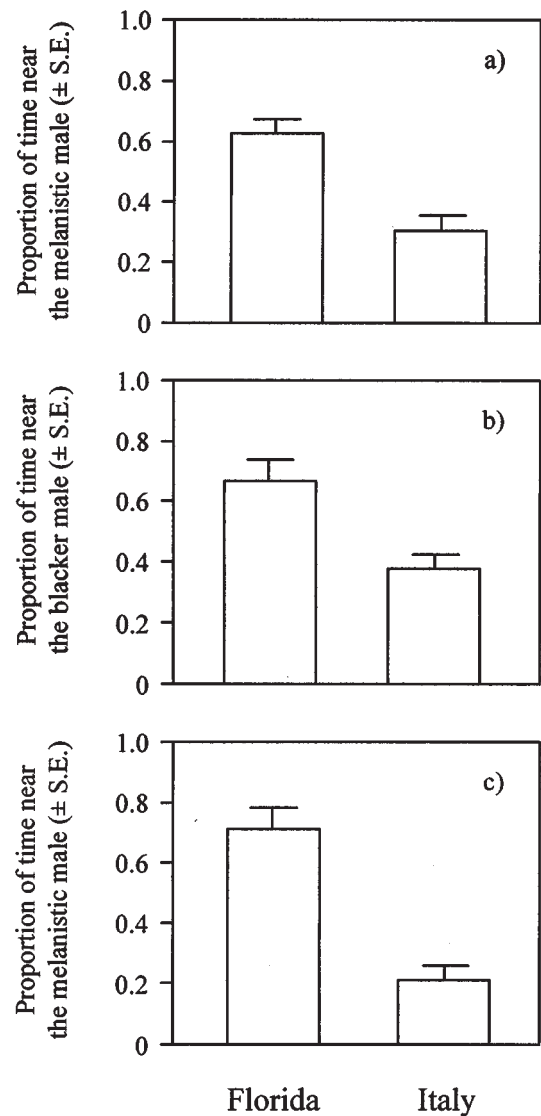


Fig. 2. Mate choice between Florida melanistic males and Italian wild-type males of eastern mosquitofish females according to the population of origin. Female preference is expressed as the proportion of the time (means and SE) spent by the test female within the virtual sector near the black—spotted males (a) or the males with more black spots (b) over the total time spent within the two side sectors. In the third experiment (c) Florida females were reared from birth in the presence of Italian wild—type males, and Italian females were reared in the presence of Florida melanistic males.

eastern mosquitofish selects for a melanin-based color, whereas mate choice in sticklebacks and guppies is based on the extent and intensity of carotenoid pigmentation (Bakker, 1993; Houde, 1997). Melanistic spots, which are present in the guppy, are thought to work as a signal amplifier for carotenoid spots (Brooks, 1996).

Previous studies on female preference for melanistic males in the eastern mosquitofish have not conclu-

sively demonstrated that the polymorphism in male coloration in the eastern mosquitofish is maintained through female choice. It has been shown that, in the wild, melanistic males are found swimming in association with a female significantly more often than expected on the basis of random pairing according to the frequency of melanistic males in the population (Karplus and Algom, 1996). This observation has been considered as evidence for female preference for melanistic males. However, it may also be the result of a greater sexual activity or competitive ability of melanistic males, since they are usually more aggressive and interfere more often with the sexual activity of other males than unpigmented males (Martin, 1977; Karplus and Algom, 1996). Dichotomous choice tests under laboratory conditions which controlled for male behavior failed to show any evidence of female preference at all (Nelson and Planes, 1993) or even demonstrated a preference for unpigmented males (Taylor *et al.*, 1996). However, as mentioned above, Gould *et al.* (1999) found that females prefer male models with black spots or black fins or totally black males over wild-type male models. Such strikingly contrasting results may be explained by a variation of female preference with respect to male pigmentation, as observed in the two populations we studied. Our results therefore suggest that male melanistic coloration may be maintained through female preference in some populations but not in others. Although our results reconcile the conclusions reached by different authors about female preference for melanism in the eastern mosquitofish, they do not support the idea of a preexisting sensory bias as a mechanism by which females choose melanistic males (e.g., see Gould *et al.*, 1999). If preexisting bias were driving the preference for melanistic males in mosquitofish, the females from the Italian populations should have shown a preference for melanistic males rather than native unpigmented ones as shown here.

A wider survey of mosquitofish populations varying in the proportion of melanistic males is necessary to confirm that preference for melanistic males and their proportion in the population covary, similarly to what has been found in the guppy for carotenoid coloration (Houde and Endler, 1990). The reason for the observed polymorphism in female preference remains unclear. One possibility is that ecological conditions, such as predation pressure, differ among the two populations thus favouring crypsis in some populations and conspicuousness in others. For example, female guppies are more susceptible to predation when they are courted

by brightly colored males, and thus can also pay a cost for male ornamentation (Pocklington and Dill, 1995). Guppy males from high-predation populations are generally less colored than those from low-predation populations (Endler, 1980) and female preference for bright males shows a corresponding pattern (Stoner and Breder, 1988; Endler and Houde, 1995). If this also applies to the eastern mosquitofish, then a higher predation rate should reduce both the proportion of melanistic males and the female preference for them. Recent results suggest that Italian females avoid melanistic males rather than prefer wild-type ones (A. Bisazza, unpublished results), which is more in agreement with the hypothesis that black ornamentation is costly than with the lack of preference for melanistic males. However, to test whether the proportion of melanistic males, female preference, and predation intensity covary, it is necessary to investigate other populations.

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