



## Characterization of assortative mating in medaka: Mate discrimination cues and factors that bias sexual preference



Umi Utagawa<sup>a</sup>, Shoichi Higashi<sup>b</sup>, Yasuhiro Kamei<sup>b</sup>, Shoji Fukamachi<sup>a,\*</sup>

<sup>a</sup> Laboratory of Evolutionary Genetics, Department of Chemical and Biological Sciences, Japan Women's University, Mejirodai 2-8-1, Bunkyo-ku, Tokyo 112-8681, Japan

<sup>b</sup> Spectrography and Bioimaging Facility, Core Research Facilities, National Institute for Basic Biology, Okazaki-shi, Aichi 444-8585, Japan

### ARTICLE INFO

#### Article history:

Received 25 May 2014

Revised 24 May 2016

Accepted 29 May 2016

Available online 31 May 2016

#### Keywords:

Medaka

Mate choice

Monochromatic light

Skin color

Somatolactin alpha

Spectral reflectance

### ABSTRACT

Somatolactin alpha (SL $\alpha$ ) is a peptide hormone that regulates skin color, and SL $\alpha$ -deficient and SL $\alpha$ -excess strains have been established in medaka (*Oryzias latipes*). Their skin colors differ conspicuously and males prefer to mate with females from the same strain. Pre-mating sexual isolation in this model vertebrate provides an ideal platform for investigating the molecular mechanisms of mate choice. Thus, we studied the sensory cues utilized by these fish to discriminate the same and different strains. When males were given a choice under monochromatic light, where the skin colors differed only in terms of brightness but not in hue, mating occurred but it was not assortative. This suggests that: (1) a visual cue is essential for mate discrimination rather than odor or acoustic cues; (2) the visual cue is color and not shape, size, or motion; and (3) the color cue needs to be perceived as the relative balance of brightness at multiple wavelengths rather than the brightness at a specific wavelength. In addition, we introduced another skin-color mutation into the SL $\alpha$ -excess strain and found that this new strain and the original SL $\alpha$ -excess strain, which also overexpressed SL $\alpha$  but exhibited distinct skin colors, preferred different colors. This demonstrates that SL $\alpha$  is not a primary determinant of sexual preference. The symmetrically biased sexual preferences of the SL $\alpha$ -deficient and SL $\alpha$ -excess strains may be acquired postnatally depending on their individual skin color or that of tank mates.

© 2016 Elsevier Inc. All rights reserved.

### Introduction

Many animals select reproductive partners but males are often less choosy than females. Thus, behavioral mate choice experiments are often conducted by presenting two (or more) males to one female in order to assess the sexual preference of the female (female choice). Female choice has been investigated most extensively in fish. Using various species under various conditions, researchers have shown that females choose males according to their skin color (Barlow and Siri, 1987), popularity among other females (Dugatkin and Godin, 1992), boldness against predators (Godin and Dugatkin, 1996), body size (Rosenthal and Evans, 1998), fin size (Kunzler and Bakker, 2000), genotype of the *major histocompatibility complex* locus (Landry et al., 2001), nest size (Takahashi and Kohda, 2002), genitalia size (Langerhans et al., 2005), olfactory cues (Plenderleith et al., 2005), foster mother (Verzijden and ten Cate, 2007), electric discharge (Feulner et al., 2009), bower shape (Young et al., 2010), familiarity (Mariette et al., 2010), age (Kanuga et al., 2011), acoustic cues (Maruska et al., 2012), or eye color (Novales Flamarique et al., 2013). In addition, studies of male choice (or mutual choice) in fish (e.g., pipefish (Sandvik et al.,

2000), goby (Amundsen and Forsgren, 2001), blue-eye (Wong and Jennions, 2003), molly (Plath et al., 2008), mosquitofish (Deaton, 2009), stickleback (Candolin and Salesto, 2009), cichlids (Baldauf et al., 2010, 2009), and mangrove killifish (Ellison et al., 2013), have demonstrated that males do select their mating partners during reproduction.

The medaka is a small freshwater teleost, which is a native of the Far East (Wittbrodt et al., 2002), that has been established as a model vertebrate for genetic and developmental studies (Takeda and Shimada, 2010). Its natural history has been investigated for a century and is well described (Kinoshita et al., 2009). Mate choice had been less well characterized in the medaka, but it was recently shown that visual familiarization enhances the female's preference for males and the neurons responsible for this characteristic were identified (Okuyama et al., 2014). In fact, the medaka may be one of the best models for studying mate choice because mature adults spawn every day and their behavior in the morning should definitely reflect their mating preference (instead of their association or shoaling preferences; see Kidd et al. (2013) for more on this problem in cichlids).

Male choice is also found in medaka. Previously, we showed that one of four types of pigment cells in the skin, i.e., orange xanthophores, plays a crucial role in mate attraction, where females with decreased xanthophores were courted less frequently by males (Fukamachi

\* Corresponding author.

E-mail address: [fukamachi@fc.jwu.ac.jp](mailto:fukamachi@fc.jwu.ac.jp) (S. Fukamachi).

et al., 2009a). This result appears to be consistent with many other reports of a role for carotenoids as secondary sexual characteristics in fish and other vertebrates. In addition, we found that this xanthophore-decreased mutant medaka (*color interfere*, *ci*; Fukamachi et al., 2004) and a xanthophore-increased transgenic medaka (Actb-SL $\alpha$ :GFP; Fukamachi et al., 2009b) strongly prefer to mate within the strain; i.e., these conspecific color variants have mutually opposite sexual preferences and they mate assortatively.

The only difference between the genomes of *ci* and Actb-SL $\alpha$ :GFP is in terms of the absence or presence of a transgene that constitutively expresses a hormone called somatolactin alpha (SL $\alpha$ ). The transgene also expresses green fluorescent protein (GFP) as a reporter, but its effect on assortative mating must be subtle (if not absent) because: (1) the fluorescence is faint and not detectable under white light by humans, or in terms of spectral reflectance (see Results); and (2) much stronger GFP fluorescence in the OIMA1-GFP medaka, the trunk of which is apparently green even under white light, did not decrease or increase sexual attraction (Fukamachi et al., 2009a). Thus, the difference in the expression of SL $\alpha$  alone appears to be sufficient to harmoniously change the skin color and sexual preference to establish a reproductive barrier within the species.

Pre-mating sexual isolation (assortative mating) is a hot topic in evolutionary biology because it may drive sympatric speciation. However, although many studies have reported assortative mating by fish in the field and in laboratories, few have investigated its mechanism using straightforward methods such as genetic engineering or cell/tissue manipulation. This may be because the experimental techniques that are applicable to these wild (non-model) species are limited. Thus, the *ci* and Actb-SL $\alpha$ :GFP medaka may be helpful tools for linking the evolutionary concept and its molecular/cellular mechanism, i.e., by identifying the molecules and neural circuits that are responsible for perceiving sexual traits, being attracted, and making a decision about mating.

In the present study, as the first step toward this goal, we investigated two fundamental questions using this experimental platform: (1) the cues used by *ci* and Actb-SL $\alpha$ :GFP to discriminate the same and different strains; and (2) whether the expression of SL $\alpha$  alone might be the primary determinant of their symmetrically biased sexual preferences.

## Materials and methods

### *Fish and breeding conditions*

All of the fish were born and raised in the laboratory, where the water temperature was kept at 27 °C and light was provided by ordinary fluorescent lamps for 14 h per day. The breeding water was circulated using a central filtration system. Fish were fed live brine shrimps and commercial flake foods five times per day. Different strains were bred in separate tanks, although there may have been visual contact between neighboring tanks through the translucent tank walls.

### *Mate choice experiments under white light*

We performed mate choice experiments at Japan Women's University (JWU) in Tokyo and the National Institute for Basic Biology (NIBB) in Aichi. For the experiments in NIBB, fish raised at JWU were transported to NIBB 10 days before starting the mate choice trials for acclimation. The dimensions of the tanks (water volumes) used for the mate choice trials (and also for breeding) were L 210 × W 135 × H 70 mm<sup>3</sup> at JWU and L 150 × W 100 × H 120 mm<sup>3</sup> at NIBB (the body length of a mature medaka is about 3 cm).

Before starting the mate choice experiments, we prepared four or five tanks, each of which held four adults (a male and a female from one strain, and a male and a female from another strain). The tank could be partitioned using a translucent separator with slits and we

kept different strains in separate compartments (a pair from a strain on one side and a pair from the other strain on the other side). After the females started spawning eggs regularly each morning, we started a series of mate choice trials, as follows.

One day before the trial, we changed the combination of fish in the compartments to prevent spawning, with two males from different strains in one compartment and two females from different strains in the other compartment. We paired females of similar size, but the body length was not precisely equal in the mate choice experiments, except for those using Actb-SL $\alpha$ :GFP-rr (Fig. 4), where we verified that the body length was not significantly different between the females.

On the day of a trial, we took one male out of each tank, removed the separators, and let the other male mate freely with the two females. Free interaction was also allowed between the females as well as between females and the male, but females were basically passive and they appeared indifferent to each other (we did not note any competing, attracting, guarding, or other behaviors in females that might have affected male choice). The behaviors were recorded from a bird's-eye view using a commercial video camera at JWU, and charge-coupled device (CCD) video cameras and PowerDirector software (CyberLink) at NIBB. After 30 min, we separated the male and females using the separator, replaced the male with the one removed previously, and repeated the trial. After the second trial, all of the males were placed back into different tanks, so we could perform trials the next morning by presenting different females to the males. We repeated these procedures for four or five consecutive days (i.e., each male met all females in the four or five tanks). Light was provided solely by ordinary fluorescent lamps on the ceiling.

### *Mate choice experiments under monochromatic light*

We used the Okazaki Large Spectrograph (OLS) at NIBB as a source of monochromatic light (see Results for details). The procedures followed in the mate choice trials were basically the same as those described above. We prepared five tanks, each of which held four adults (a *ci* male, a *ci* female, an Actb-SL $\alpha$ :GFP male, and an Actb-SL $\alpha$ :GFP female), and placed them on a horseshoe-shaped table in the OLS room so the tanks could be irradiated with monochromatic lights at different wavelengths. The OLS irradiated monochromatic light in the horizontal direction. Thus, we interrupted its direct irradiation of the tanks using black boards, set stand mirrors above the tanks at an angle of 45°, and irradiated the monochromatic light in the direction of gravity (see Fig. 2B). The center of a tank was irradiated with monochromatic light at the intended wavelength (e.g., 500 nm), and thus the actual light irradiated each tank at a specific wavelength  $\pm$  5 nm (e.g., 495–505 nm).

The trials were repeated for five consecutive days using the same sets of fish (i.e., unlike the experiments under white light, we did not rotate the combinations of males and females in the tanks) but under different wavelengths on different mornings. Thus, each male was presented to the same females under five types of monochromatic light over five days. The set of wavelengths comprised either 400, 500, 600, 700, and 800 nm, or 450, 550, 650, 750, and 840 nm. The inside of the tank (bottom and sides) was painted white to avoid reflections of internal images on the tank wall in the dark OLS room (see Fig. 2B) and to provide sufficient light to induce mating, which occurred rarely in black-painted tanks (data not shown).

### *Evaluation of mating preferences and statistics*

The video-recorded behaviors of the males were analyzed manually, as described previously (Fukamachi et al., 2009a). Briefly, a series of medaka mating behaviors was initiated by male's swimming under a female ("following"; Kinoshita et al., 2009) and we counted the male's approaching behaviors toward each female (not necessarily following because the females sometimes swam fast as if escaping approaching

Download English Version:

<https://daneshyari.com/en/article/323026>

Download Persian Version:

<https://daneshyari.com/article/323026>

[Daneshyari.com](https://daneshyari.com)