RESEARCH ARTICLE

Establishment and maintenance of sexual preferences that cause a reproductive isolation between medaka strains in close association

Mayuka Ikawa*, Emi Ohya*, Hiroka Shimada, Makiko Kamijo and Shoji Fukamachi‡

ABSTRACT
Animals choose reproductive partners based on their sexual preferences which are established at a certain time point before, during, or after sexual maturation. The preferences are often divergent within a species, which suppresses gene flow between populations and may promote speciation. There are two strains of medaka (Oryzias latipes) that differ by a single transgene and mate assortatively depending on skin color. Here, we demonstrate that symmetrically biased (mutually exclusive) sexual preferences are (1) gradually established during growth depending on skin color and the color of surrounding fish, (2) strong enough to minimize gene flow between the strains at a population level, and (3) inflexibly retained after sexual maturation, even after weeks of daily mating with partners of the other strain. Thus, these laboratory strains of medaka are under premating isolation with the simplest genomic structure. They provide an empirical platform for assessing the complex and hypothetical mechanisms of speciation by mate choice.

KEY WORDS: Mate choice, Medaka, Somatolactin alpha, Skin color, Sympatric speciation

INTRODUCTION
Mate choice or selecting reproductive partners is one of the most active fields of research in animal biology, and many studies have been conducted using various species for decades (Jones and Ratterman, 2009; Scordato et al., 2014). Secondary sexual characteristics, such as color, ornaments, dances, songs, and pheromones, often appear in either sex to attract the other and are divergent among species, demonstrating that different animals choose mates based on different criteria. Generally, males are more exaggerated and less choosy than females, and research tends to focus on mate choice by females (female choice); however, there is increasing evidence for male choice (Avigiano et al., 2016; Bloch et al., 2016; Utagawa et al., 2016) showing the importance of sexual preferences which are established at a certain time point before, during, or after sexual maturation. The preferences are often divergent within a species, which suppresses gene flow between populations and may promote speciation. There are two strains of medaka (Oryzias latipes) that differ by a single transgene and mate assortatively depending on skin color. Here, we demonstrate that symmetrically biased (mutually exclusive) sexual preferences are (1) gradually established during growth depending on skin color and the color of surrounding fish, (2) strong enough to minimize gene flow between the strains at a population level, and (3) inflexibly retained after sexual maturation, even after weeks of daily mating with partners of the other strain. Thus, these laboratory strains of medaka are under premating isolation with the simplest genomic structure. They provide an empirical platform for assessing the complex and hypothetical mechanisms of speciation by mate choice.

*These authors contributed equally to this work
‡Author for correspondence (fukamachi@fc.jwu.ac.jp)

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

Received 10 October 2016; Accepted 22 December 2016
perception of traits, and decision of mating), which may provide a hint in understanding the complex sexual selection that occurs in the field. Based on our previous study (Utagawa et al., 2016), we further characterized the symmetrically biased sexual preferences of ci and Actb-SLα:GFP males, focusing on their stability (Experiment I), strength (Experiment II), and establishment (Experiment III).

RESULTS

Experiment I: the stability test

We placed matured males of ci and Actb-SLα:GFP (n=8 for each strain), which had preferred females of the same strain (Subjects 0 in Fig. 2), in either of the following conditions for 12 weeks: (1) the mixed condition where one ci male, one Actb-SLα:GFP male, one ci female, and one Actb-SLα:GFP female were kept in a tank (four sets), or (2) the different-sex-different-strain (DSDS) condition in which two ci males and two Actb-SLα:GFP females, or two Actb-SLα:GFP males and two ci females, were kept in a tank (two sets each; see Fig. 1). During breeding, we tested sexual preferences of each male once a week. This means that we measured sexual preferences of four males from each strain and condition every week.

Medaka spawn every morning, and mating between ci and Actb-SLα:GFP did occur under the DSDD conditions (see Experiment IIb) although they are mutually unattractive (Fukamachi et al., 2009a; Utagawa et al., 2016). That is, males in the DSDD condition mated daily with females of the different strain for 12 weeks. However, the males in either of the mixed or DSDD conditions never preferred females of the different strain (Fig. 2). Even alleviation of the biased preferences seemed not to occur.

Experiment IIa: the strength test (two-male-one-female trials)

We quantified the degree of sexual isolation between ci and Actb-SLα:GFP in two ways (Experiments IIa and IIb). First, we questioned why ci and Actb-SLα:GFP males seldom court females of different strains (Fig. 2) (Fukamachi et al., 2009a; Utagawa et al., 2016). To address this question, we observed mating behaviors under the condition in which a pair of ci and Actb-SLα:GFP males was presented with one ci or one Actb-SLα:GFP female. Body lengths of the competing males were not identical; among the male pairs tested (n=4), ci was larger than Actb-SLα:GFP in three of the four pairs.

We presented to each male pair a total of eight females (four ci and four Actb-SLα:GFP) on four consecutive days (one ci and one Actb-SLα:GFP female per day). In all the 32 trials (four male pairs x eight females), one of the males extensively courted a female, whereas the other male seldom or never did. It was apparent that this was not because of a hierarchical relationship between the males because courting males changed when the females presented were changed. All males extensively courted only when a female of the same strain was presented (Fig. 3). Thus, the males seemed to have little interest in females of the different strain.

Fig. 1. Brief overview of the fish breeding and mate-choice trials. During larval/juvenile stages, we kept fish under separated or mixed conditions (not shown in this figure). During adolescent/adult stages (left), we put four or more fish into a tank and kept them under either separated, mixed, or different-sex-different-strain (DSDD) conditions. On the day before mate-choice trials (middle), we prepared four tanks using 16 fish (eight males and eight females) by putting one ci male, one Actb-SLα:GFP male, one ci female, and one Actb-SLα:GFP female into each tank, and kept different sexes in different compartments using a separator (dotted line). On the day of the mate-choice trials (right), we took one male out from each tank and performed the first trials. Then, we exchanged the males and performed the second trials. In Experiment III, we repeated the trials four times on four consecutive days by rotationally putting the males into different tanks.
Experiment IIb: the strength test (paternity identification after mass breeding)

Next, we hypothesized that the symmetrical, strong, and long-lasting sexual preferences (or sexual indifferences) of ci and Actb-SLα:GFP (Figs 2 and 3) may suppress gene flow between these conspecific and post-zygotically compatible strains. To test this, we put six males and six females in a tank, collected eggs every morning, and identified the paternity of each embryo by GFP fluorescence.

We first put six ci females and six Actb-SLα:GFP males in a tank for five days. Four to six females spawned daily, but the spawning often started late in morning (about 10:00 h); for instance, one female spawned in the afternoon on the first day. We obtained a total of 101 fertilized eggs and all the embryos were, needless to say, GFP-positive (Trial I in Table 1). We then replaced the six Actb-SLα:GFP males with three ci males and three Actb-SLα:GFP males and continued collecting eggs for another six days. Similar to Trial I, four to six females spawned daily, but all spawning was usually completed by 09:00 h. We obtained a total of 217 fertilized eggs, 21 of which were GFP-positive, demonstrating that the ci females produced 9.3-fold more offspring with the ci males than they did with the Actb-SLα:GFP males (Trial II in Table 1).

We repeated Trial II using different individuals of males and females. Because the new ci females spawned a fewer number of...
females. A gene flow from Actb-SLα:GFP males to ci females is strongly (Trial II) or completely (Trial III) suppressed, even when the strains were in close contact in a small tank. We assume that this is largely because of the sexual indifference of Actb-SLα:GFP males to ci females (Fig. 3), but do not exclude a significant contribution from female choice, i.e., rejection of spawning by the females.

A gene flow in the other direction, from ci males to Actb-SLα:GFP females, cannot be assessed by this method because all eggs that Actb-SLα:GFP females spawn will become GFP-positive, irrespective of paternity; however, we suspect that it would also be suppressed considering the symmetrical characteristics of sexual preferences between ci and Actb-SLα:GFP medaka (Figs 2 and 3).

**Experiment III: the establishment test**

The only difference between genomes of ci and Actb-SLα:GFP medaka is the SLα transgene (Fukamachi et al., 2009b). Hence, this transgene must be the cause of their symmetrically biased sexual preferences. However, this does not necessarily mean that the preferences are genetic traits because the fish used in the previous and present studies had been reared under separated conditions; different strains had experienced different breeding conditions prior to the mate-choice trials.

Thus, there seemed to be three possible, not necessarily alternative, mechanisms in which the SLα transgene biases sexual preference: (1) SLα acts directly on the brain and its low or high expression makes medaka prefer ci or Actb-SLα:GFP, respectively (i.e. preference is a genetic trait); (2) SLα controls only the skin color and medaka prefer fish with the same color (i.e. preference is a genetic but acquired trait); and (3) SLα controls only the skin color and medaka prefer the color of fish that they were reared with (i.e. preference is an acquired trait).

To test these hypotheses, we reared ci and Actb-SLα:GFP medaka in various conditions and assessed their preferences. First, we transferred two-month-old males (spawning typically starts at three months after hatching) from the separated condition to the mixed condition and let them mature. This breeding provided males the opportunity to associate with females of the different strain prior to mate-choice trials. However, the males strongly preferred females of the same strain (Fig. 4C). Taken together, associating or mating experiences during either the larval/juvenile or adolescent/adult stages are insufficient in making males interested in females of the different strain.

When males had been reared under the mixed condition during both the larval/juvenile and adolescent/adult stages, however, interest in females of the different strain significantly increased (Fig. 4D; see also Fig. 5), demonstrating that sexual preference could be affected by tank mates. This result supports the third of the above hypotheses. Nevertheless, it simultaneously supports the first and/or second hypotheses, because preferences of the males were still symmetrically biased toward females of the same strain in spite of the identical (i.e. mixed) conditions that the males had experienced.

Consistent results could be obtained when we transferred males from the mixed condition to the DSDS condition; males’ interest in females of the different strain was significantly increased (Fig. 4E,F; see also Fig. 5). Interestingly, preference of the ci males became not at all biased or even oppositely biased, although such phenomena could not be detected in the Actb-SLα:GFP males. The cause of these exceptionally asymmetrical results between ci and Actb-SLα:GFP males is discussed below.

**DISCUSSION**

**Genetic factors that influence sexual preference**

The ci and Actb-SLα:GFP males, which had consistently been bred in mixed conditions, still preferred females of the same strain (Fig. 4D). This result forecasts the existence of an innate factor that makes males prefer females of the same strain, the SLα transgene (Fukamachi et al., 2009b). However, the transgene does not always make males prefer Actb-SLα:GFP females; for instance, Actb-SLα:GFP males with the r mutation, which reduces xanthophores, preferred ci females instead of Actb-SLα:GFP females (Utagawa et al., 2016). Therefore, there seems to be an indirect mechanism in which the SLα transgene biases sexual preference; medaka seem to perceive and prefer mates with skin color the same as their own.

In this indirect mechanism, any skin-color gene can potentially influence sexual preference, and the r gene has actually been shown to be one of them (Utagawa et al., 2016). However, the slc45a2 gene that controls melanophores or the GFP or DsRed transgene that artificially stains the trunk green or red had little effect on sexual preference; i.e. the mutant or transgenic males, which had been bred in separated conditions, did not necessarily prefer females of the same strain (Fukamachi et al., 2009a; Nakao and Kitagawa, 2015; Utagawa et al., 2016). Thus, there seem to be specific colors that medaka perceive as preferable when selecting reproductive partners.

Speciation theories sometimes suppose pleiotropic effects of a gene that controls both sexual trait and sexual preference.
(Wolf et al., 2010). Although such genes (for instance, a gene that causes exaggerated ornaments to develop and makes neural circuits to prefer the ornaments) sound somewhat unrealistic, our study, probably for the first time by genetic engineering, clearly demonstrated that such a harmoniously pleiotropic gene could exist. The \textit{ci} and Actb-SL\alpha:GFP medaka, which are one mutation (transgene) away from each other and assortatively mate in complete sympatry (Table 1), might represent the initial stage of speciation by mate choice (further discussed below).

Fig. 4. Results of Experiment III, the establishment test. In each figure, presented females are shown on top (Actb-SL\alpha:GFP) and bottom (\textit{ci}), and each male is represented by a dotted vertical line (n=4 per strain per condition). Four mate-choice trials were performed using each male, and the ratios of approaches between the presented females are shown as four circles on each line (white, \textit{ci}; black, Actb-SL\alpha:GFP). Missing circles indicate invalid data (e.g. the right-most Actb-SL\alpha:GFP male in A approached females less than 10 times in all the four trials, and therefore there is no circle on the dotted line). A box with a horizontal line in the middle indicates a mean and s.e.m. (A) Separated conditions for the first two months and then mixed conditions afterwards. (B) Separated conditions for the first two months and then different-sex-different-strain (DSDS) condition afterwards. (C) Mixed conditions for the first two months and then separated condition afterwards. (D) Mixed conditions. (E) Mixed conditions for the first three months (high fish densities suppressed the speed of growth and maturation), and then DSDS condition afterwards (until five months old). (F) Mixed condition for the first two months, and then DSDS condition afterwards.
Environmental factors that influence sexual preference

The genetic factors discussed above, however, bias sexual preferences only weakly (see Fig. 4D), as is the case in other animals (Schiele et al., 2010). Significant effects apparently come from environmental factors such as surrounding fish (Fig. 5).

Although the sample size in Experiment III is not very large (n=8 per breeding condition) and the results may fluctuate, the present study seems to indicate that the sexual preferences of medaka are established gradually during growth and maturation. As shown in Fig. 4D–F, males have increased interest in females of the different strain only when rich opportunities for association/mating with the different strain were provided. Association/mating opportunities during either the larval/juvenile or adolescent/adult stages did not increase interest in the different strain (Fig. 4A–C). These results are similar to those demonstrated over 50 years ago using doves, whereby “not only the early experience during an optimum period but also continued experience throughout the bird’s life has an effect on adult behavior” (Klinghammer and Hess, 1964).

Our study provided an additional insight into the adult behavior. Once established, sexual preferences can hardly be altered by sexual experiences (Fig. 2). This finding in a model fish is implicative. If the strongly biased preferences of ci and Actb-SLα:GFP medaka can secondarily be relieved or reversed somehow, the methods might be applicable to sexual or reproductive problems in other vertebrates. Our result already indicates that forced association or mating has little effect in this regard (Fig. 2). Further studies on the brain or sensory organs of ci and Actb-SLα:GFP medaka would reveal how this continual maintenance could be achieved, for instance, by gene expression, neural circuits, or neural activities.

The asymmetrical preferences between ci and Actb-SLα:GFP medaka (Fig. 4E,F) need further investigation. The increased interest by ci males may indicate learned preferences by repeated mating with Actb-SLα:GFP females (i.e. a rewarding system) (Tenk et al., 2009); however, such effects were not observed in the Actb-SLα:GFP males. Among many considerable reasons, it is most likely that phenotypes of the presented females might be different in addition to the skin-color difference. That is, the ci and Actb-SLα:GFP males, which had established weak skin-color preferences because of the rich opportunities to associate/mate with the different strain (Fig. 4D), might choose females based on other criteria such as body size or susceptibility to spawning.

Indeed, a similar result could be obtained using males that had been bred in a consistently mixed condition. Both the ci and Actb-SLα:GFP males, which had not experienced the DSDS condition, significantly preferred larger Actb-SLα:GFP females (body length, 29.3±0.4 mm; body height, 4.4±0.2 mm) to smaller ci females (body length, 26.3±0.4 mm; body height, 3.5±0.1 mm) (data not shown). Thus, mating experiences seemed to have only negligible effects on, not only maintenance (Fig. 2), but also the establishment of sexual preferences.

Sympatric speciation by mate choice alone?

The strongly or completely suppressed gene flow between ci and Actb-SLα:GFP medaka in perfect sympatry (Table 1) is intriguing; if this suppression continues for generations, the strains could speciate. As we have shown, their symmetrical, strong, and long-lasting preferences can be established even when they have temporal opportunities for association/mating during growth and maturation (Fig. 4A–C). That is, a shoaling preference (Engeszer et al., 2007) or background preference (Bault et al., 2015) may be sufficient for establishing a reproductive barrier. However, such conditions are not sympatric specifically, even when the medaka strains are living in close physical contact. Thus, as some theoretical studies have suggested (Arens and Kondrashov, 2004; Kirkpatrick and Nuismer, 2004; van Doorn et al., 2004), our results may provide an empirical example supporting the theory that, in perfect sympatry (Fig. 4D), speciation by mate choice alone is not very likely.

MATERIALS AND METHODS

Fish

All experiments were conducted using two medaka strains, ci (Fukamachi et al., 2004) and Actb-SLα:GFP (Fukamachi et al., 2009b). All fish hatched,
Petri dishes for embryonic development. Several days. Spawned eggs were collected every morning and incubated in fine-ground TetraMin (Tetra) and live Paramecium. Juveniles and adults were fed with live Artemia. All the following experiments have been approved by the Animal Experiment Committees of Japan Women’s University.

**Experiment I: the stability test**

We prepared mature fish of the ci and Actb-SLα:GFP strains, which had been reared separately since hatching. These fish were then kept in either of the following conditions for 12 weeks: (1) the mixed condition where one ci male, one Actb-SLα:GFP male, one ci female, and one Actb-SLα:GFP female were kept in the same tank, or (2) the different-sex-different-strain (DSDS) condition where two ci males and two Actb-SLα:GFP females (or two Actb-SLα:GFP males and two ci females) were kept in the same tank. The tank size was 20×13 cm² with a water level of ~5 cm. During breeding, we tested sexual preferences of the males by mate-choice trials once a week as follows (see also Fig. 1).

A day before the trials, we prepared four tanks, in each of which one ci male, one ci female, one Actb-SLα:GFP male, and one Actb-SLα:GFP female were placed. The males and females were kept in different compartments using a translucent separator with slits in order to prevent spawning until the next morning.

The following morning, the four tanks were set under a commercial video camera. One of the two males was taken out of each tank, the separators were removed, and the mating behaviors were recorded for 30 min. Then, we replaced the males and continued the recording for another 30 min. Males removed in the first trials were changed weekly. After these eight mate-choice trials (four tanks×two sets), we put all the males back into the tanks and continued mixed or DSDS breeding.

Females often spawned eggs during the first trials, but we left the eggs attached to the cloaca as in previous studies (Fukamachi et al., 2009a; Utagawa et al., 2016). This is because males of ci and Actb-SLα:GFP seemed to prefer females of the same strain irrespective of spawning stages, for example, they approached spawned females of the same strain much more frequently than yet-to-spawn females of the different strain. This characteristic of males is not always fruitless because females sometimes spawn multiple times a day.

The recorded behaviors were manually analyzed by counting the number of approaches (jerking motions) of a male toward each female (see Fukamachi et al., 2009a for rationales) and the ratio was regarded as male sexual preference in the trial. We ignored all trials in which fewer than 10 approaches could be counted because the ratios could be biased or unbiased by accident. These ratios were used to calculate the mean and the standard error of the mean (s.e.m.) for each strain, condition, and week. We calculated percentages as bias if the s.e.m. (which were nearly equal to 95% confidence intervals; data not shown) did not include 50-50.

**Experiment II: the strength tests**

The protocol for Experiment IIa (two-male-one-female trials) is almost identical to that of the one-male-two-female trials described above. The only differences were that we took one female (instead of male) out of each tank for the first and second trials and repeated the trials on four consecutive days by rotationally changing female individuals [i.e. a total of eight females (two females per day) were presented to each competing pair of males]. Video-recorded behaviors were manually analyzed by counting the number of approaches from each male in a trial, which were used to calculate a ratio between the male pair. The mean and s.e.m. of the ratios were also calculated for each male pair and presented strain of female.

Experiment IIb (paternity identification after mass breeding) proceeded as follows. We prepared mature males of ci and Actb-SLα:GFP and verified by mate-choice trials (see Experiment III) that each male preferred females of the same strain. Then, six of the males (either six Actb-SLα:GFP or three ci plus three Actb-SLα:GFP) were put into a tank (150×100 cm² with a water level of 8 cm) with six ci females and were left to mate freely for several days. Spawned eggs were collected every morning and incubated in Petri dishes for embryonic development.

The transgene in Actb-SLα:GFP weakly expresses the reporter GFP (Fukamachi et al., 2009b). Therefore, the embryos become GFP-positive or GFP-negative, when a ci female is mated with an Actb-SLα:GFP or ci male, respectively. Thus, by observing the presence or absence of GFP fluorescence in each embryo under a SZX16 fluorescence stereomicroscope (Olympus), we identified their paternity.

For statistics, we performed a chi-square test using a null hypothesis that the ci females randomly mated with the ci and Actb-SLα:GFP males reproducing equal numbers of GFP-positive and GFP-negative embryos.

**Experiment III: the establishment test**

Before the sex of the medaka became apparent through secondary sexual characteristics in the dorsal and anal fins (called the larval/juvenile stages in this manuscript), we tested two conditions of breeding. The separated condition, in which different strains were kept in different tanks in a similar density, and the mixed condition, in which different strains were kept in the same tank in similar numbers.

After the sex became apparent at approximately two months after hatching (called the adolescent/adult stages in this manuscript), we kept them in one of the following three conditions: the separated condition, the mixed condition, or the DSDS condition (see Experiment I for the latter two conditions). All the four or more fish in each tank freely associated and mated with tank mates until being used in mate-choice trials.

We conducted mate-choice trials using these fish as described in Experiment I, except that we repeated the trials on four consecutive days by rotationally changing combinations of males and females (Fig. 1). Preference of a male was calculated as an average ratio of valid trials (i.e. with 10 or more approaches). These average preferences were then used to calculate a mean and s.e.m. for each strain and breeding condition. Statistical differences among the conditions were analyzed using an ANOVA and a Tukey’s HSD post hoc test.

**Acknowledgements**

We thank Shin-ichi Chisada of the National Institute for Basic Biology for providing us with Paramecium and breeding advice, and Haruka Kajimura and Kaoru Naito of JWU for their support in the mate-choice experiments.

**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**

M.I. and E.O. performed most of the experiments and analyzed the data. H.S. and M.K. performed part of the experiments and data analyses, including the double-blind check of the recorded behaviors. S.F. designed the experiments, reanalyzed the data, and wrote the manuscript.

**Funding**

This research was supported by research funds from Jungwon University (JWU) and the Bio-Imaging Center in JWU to S.F. and Grant-in-Aid for Research Activity Start-up (#15H06652) from Japan Society for the Promotion of Science (JSPS) to M.K.

**References**

Aida, T. (1921). On the inheritance of color in a fresh-water fish, APLOCHIELLUS LATIPES Temnick and Schiegel, with special reference to sex-linked inheritance. Genetics 6, 554-573.

Ansai, S. and Kinoshita, M. (2014). Targeted mutagenesis using CRISPR/Cas system in medaka. Biol. Open 3, 362-371.

Arnegard, M. E. and Kondrashov, A. S. (2004). Sympatric speciation by sexual selection alone is unlikely. Evolution 58, 222-237.

Avigliano, E., Scardamaglia, R. C., Gabelli, F. M. and Pompilio, L. (2016). Males choose to keep their heads: preference for lower risk females in a praying mantid. Behav. Processes 129, 80-85.

Bault, Z. A., Peterson, S. M. and Freeman, J. L. (2015). Directional and color preference in adult zebrafish: implications in behavioral and learning assays in neurotoxicology studies. J. Appl. Toxicol. 35, 1502-1510.

Bloch, A. N., Estela, V. J., Leese, J. M. and Itzkowitz, M. (2016). Male mate preference and size-assortative mating in convict cichlids: a role for female aggression? Behav. Processes 130, 81-85.

Engeser, R. E., Da Barbiano, L. A., Ryan, M. J. and Parichy, D. M. (2007). Timing and plasticity of shoaling behaviour in the zebrafish, Danio rerio. Anim. Behav. 74, 1269-1275.
Fukamachi, S., Sugimoto, M., Mitani, H. and Shima, A. (2004). Somatolactin selectively regulates proliferation and morphogenesis of neural-crest derived pigment cells in medaka. Proc. Natl. Acad. Sci. USA 101, 10661-10666.

Fukamachi, S., Kinoshita, M., Aizawa, K., Oda, S., Meyer, A. and Mitani, H. (2009a). Dual control by a single gene of secondary sexual characters and mating preferences in medaka. BMC Biol. 7, 64.

Fukamachi, S., Yada, T., Meyer, A. and Kinoshita, M. (2009b). Effects of constitutive expression of somatolactin alpha on skin pigmentation in medaka. Gene 442, 81-87.

Ijiri, K. (1995). Fish mating experiment in space – what it aimed at and how it was prepared. Biol. Sci. Space 9, 3-16.

Kasahara, M., Naruse, K., Sasaki, S., Nakatani, Y., Qu, W., Ahsan, B., Yamada, T., Nagayasu, Y., Doi, K., Kasai, Y. et al. (2007). The medaka draft genome and insights into vertebrate genome evolution. Nature 447, 714-719.

Klinghammer, E. and Hess, E. H. (1964). Imprinting in an Altricial Bird: the blond ring dove (Streptopelia Risoria). Science 146, 265-266.

Nakao, R. and Kitagawa, T. (2015). Differences in the behavior and ecology of wild type medaka (Oryzias latipes complex) and an orange commercial variety (himedaka). J. Exp. Zool. A Ecol. Genet. Physiol. 323, 349-358.

Okuyama, T., Yokoi, S., Abe, H., Isoe, Y., Suehiro, Y., Imada, H., Tanaka, M., Kawasaki, T., Yuba, S., Taniguchi, Y. et al. (2014). A neural mechanism underlying mating preferences for familiar individuals in medaka fish. Science 343, 91-94.

Ortiz-Barrientos, D. and Noor, M. A. F. (2005). Evidence for a one-allele assortative mating locus. Science 310, 1467.

Schielzeth, H., Bolund, E. and Forstmeier, W. (2010). Heritability of and early environment effects on variation in mating preferences. Evolution 64, 998-1006.

Scordato, E. S. C., Symes, L. B., Mendelson, T. C. and Safran, R. J. (2014). The role of ecology in speciation by sexual selection: a systematic empirical review. J. Hered. 105 Suppl. 1, 782-794.

Tenk, C. M., Wilson, H., Zhang, Q., Pitchers, K. K. and Coolen, L. M. (2009). Sexual reward in male rats: effects of sexual experience on conditioned place preferences associated with ejaculation and intromissions. Horm. Behav. 55, 93-97.

Utagawa, U., Higashi, S., Kamei, Y. and Fukamachi, S. (2016). Characterization of assortative mating in medaka: mate discrimination cues and factors that bias sexual preference. Horm. Behav. 84, 9-17.

van Doorn, G. S., Dieckmann, U. and Weissing, F. J. (2004). Sympatric speciation by sexual selection: a critical reevaluation. Am. Nat. 163, 709-725.

Williams, J. R., Catania, K. C. and Carter, C. S. (1992). Development of partner preferences in female prairie voles (Microtus ochrogaster): the role of social and sexual experience. Horm. Behav. 26, 339-349.

Wolf, J. B. W., Lindell, J. and Backstrom, N. (2010). Speciation genetics: current status and evolving approaches. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365, 1717-1733.

Yamazaki, K., Beauchamp, G. K., Kupniewski, D., Bard, J., Thomas, L. and Boyse, E. A. (1988). Familial imprinting determines H-2 selective mating preferences. Science 240, 1331-1332.

Yang, H., Norris, M., Winn, R. and Tiersch, T. R. (2010). Evaluation of cryoprotectant and cooling rate for sperm cryopreservation in the euryhaline fish medaka Oryzias latipes. Cryobiology 61, 211-219.