Effect of the first mating experience on mating behaviors and brain gene expression in male medaka

Masahiro Daimon

Okayama University

Takafumi Katsumura

Kitasato University

Hiroataka Sakamoto

Okayama University

Satoshi Ansai

Tohoku University

Hideaki Takeuchi (✉ hideaki.takeuchi.a8@tohoku.ac.jp)

Tohoku University

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Abstract

The first mating (sexual) experience leads to the maturation of male mating behavior across species (insects, fish, rodents). Here, we investigated whether the first mating experience leads to maturation of male mating behavior in medaka using repeated mating tests. In “naïve” (sexually inexperienced) males after the first mating experience, the latency to mate with the same female partner was significantly decreased, whereas when the partner was swapped, the latency to mate was not affected. These findings suggest that repeated matings (3 times) enhanced male mating activity for the familiarized female, but not for an unfamiliarized female. In “experienced” (> 7 matings) males, repeated matings (3 times) with the same partner did not influence the latency to mate, suggesting that multiple matings (> 7 times) abolish the mate preference of naïve males. Furthermore, we identified 10 highly and differentially expressed genes after the mating experience in the brains of the post-naïve males, and revealed 3 genes that are required for a functional cascade of the thyroid hormone system. These findings together suggest that the first mating experience abolishes the preference to mate with a familiarized female via neural maturation triggered by thyroid hormone activation in the medaka brain.

Introduction

The first mating experience leads to the maturation of male mating behavior across species. For example, in fruit flies (*Drosophila melanogaster*), the first mating experience in males shortens the latency to the first courtship with a female¹. Furthermore, in a triangle relationship (naïve male, experienced male, and receptive female), experienced males more frequently exhibit abdomen bends (attempted copulation) than naïve males. In rodents, the first male mating experience decreases the latency to the first intromission (mounting behavior with penis insertion) in mice^{2,3} and rats⁴. In fish species, however, only one published report indicates that the emergence of mate preference depends on the mating experience. Vega-Trejo et al. quantified mate preference in mosquitofish (*Gambusia holbrooki*) using a 3-chamber test, and demonstrated that the amount of time spent with a novel female is significantly increased by the mating experience, but not by visual and olfactory familiarization⁵.

In rodents, the behavioral maturation triggered by the first mating experience is associated with brain neural maturation. The number of neurons in the olfactory bulbs and the density of mushroom spines in the medial preoptic area (mPOA) are increased by the first mating experience in mice^{2,6}. In contrast, the mushroom spine density in the mPOA decreases and the expression of *vgf*, which encodes the neuropeptide precursor VGF in the mPOA, affects behavioral maturation (shortening mating latency) following the mating and ejaculation experience in male rats^{4,7}. We recently reported that the expression of gastrin-releasing peptide and oxytocin receptors is increased in the spinal ejaculation generator in the lumbosacral cord after the first mating experience with an ejaculation in male rats⁸. Furthermore, the first mating experience also decreases neuronal activity in the center part of the mPOA, suggesting that the first mating experience reconstructs the neural network associated with the male mating behavior⁹. No

studies to date have revealed whether the neural/molecular mechanisms underlying behavioral changes dependent on the first mating are conserved among vertebrates.

To address this question, we used medaka fish (*Oryzias latipes*) in the present study. There are many advantages to using medaka fish for studies of mating behavior. First, medaka mating behavior comprises several steps (approach, courtship display, wrapping, and spawning), which allow for quantification of male mating activity under laboratory conditions¹⁰. Second, as the female reproductive cycle is 24 h, the same female ready to spawn can be used for mating tests every morning¹¹. Third, medaka is a model animal for molecular genetics and state-of-the-art molecular genetic techniques are available. Here, we show that the first male mating experience abolishes the mating preference for familiarized females in medaka fish. Furthermore, transcriptome studies suggested the possible involvement of the thyroid system in the behavioral changes dependent on the male mating experience in medaka fish.

Methods

Ethics statement

All the methods in this study were carried out in accordance with relevant guidelines and regulations. The work in this paper was conducted using protocols specifically approved by the Animal Care and Use Committee of Okayama University (permit number: OKU-2015467). All surgery was performed under deep anesthesia using ice, and all efforts were made to minimize suffering following the NIH Guide for the Care and Use of Laboratory Animals Fish and breeding conditions. The study was carried out in compliance with the ARRIVE guidelines (<https://arriveguidelines.org/arrive-guidelines>).

Animal maintenance

All fish (*Oryzias latipes*; d-rR strain) were bred in our laboratory. Fish larvae were fed Paramecium or small pellet foods (Medaka no Mai Next, Kyorin, Japan), juveniles were fed small pellet foods (Medaka no Mai Next), and adult medaka were fed pellet foods (Otohime B2, Nisshin-marubeni, Japan) a few times a day. Juvenile and adult fish were fed brine shrimp once a day. Medaka were maintained in groups in plastic aquariums (13 cm x 19 cm x 12 cm height) or polypropylene containers (48 cm x 36 cm x 20 cm height). The water temperature was maintained at 24–28°C with white LED lights (Eco-slim, OHM ELECTRIC INC, Japan) for 14 h per day (08:00–22:00). At another laboratory (Okayama University, Japan), all medaka were maintained in plastic aquariums (13 cm x 19 cm x 12 cm height); the larvae were fed Paramecium, juveniles were fed small pellet foods (Hikari lab., Meito system, Japan), and adult medaka were fed flake foods (TetraMin, Tetra, Germany) a few times a day and brine shrimp once a day.

Animal preparation for mating tests

Adult male (>5 months of age) and female (>3 months of age) fish were used for this experiment. To prepare “naïve males”, we separated sexually immature males from females 1–2 months after hatching.

We determined their sexes based on the body color difference¹² and fin shape. We used sexually matured females that had spawned fertilized eggs continuously for at least 3 days as sexually-matured ready-to-spawn eggs. In the present study, we defined “naïve” males as sexually inexperienced and “experienced males” as adult males that had mated with females more than 7 times.

Mating test using fixed pairs

The mating test was performed as previously described¹³. To separate the male from the female, a plastic cup (CE-300, Kenis, Japan, 37 mm [radius] x 90 mm [height]) with white opaque paper was used from the night to the next morning (16:00–10:00). To habituate the fish to this experimental condition before starting the mating test, males were placed in the cup overnight at least 3 times. The day before the mating test, the opaque plastic cup with the naïve or experienced male was placed into a tank containing a female. The next morning, a male was released toward the tank containing the female, which allowed them to begin their mating behavior (9:30–10:30). We recorded their mating behavior for 15 min using a Web camera (BSW200MKB, Buffalo, Japan). We repeated the mating tests for 7 days (times) using the same pairs. If a female did not spawn at least once over the 7 days, we excluded all data from the analysis. We manually measured the timing of the courtship display (male quick-circle dance), wrapping (crossing each body), wrapping rejection (wrapping with no spawning), and spawning by viewing the video, and calculated the latency to mate (period from releasing the male to the wrapping with spawning), the latency to the first courtship display, and the latency to mate from the first courtship. Experienced males who had mated with females more than 7 times were used for the same experiments as a control.

Mating test using swapped pairs

To determine whether the mating experience with the same partner was essential for changing the mating behavior, we performed the mating test using fixed and swapped pairs using naïve males continuously for 3 days (times). The procedure was the same as for the mating test described above.

Statistical analysis

Statistical analysis was run by R (version 4.0.5) with generalized linear mixed models (GLMMs) by the “glmer” function in the package lme4 (version 1.1-27) to reveal whether the number of matings

(experience) affected medaka mating behavior. The gamma distribution (latency to mate, latency to the first courtship display, and latency to mate from the first courtship) and Poisson distribution (number of courtship displays, wrappings, wrapping rejections, and courtship from courtship [Cou → Cou], wrapping from courtship [Cou → Wra], courtship from wrapping rejection [W.R → Cou], and wrapping rejection from wrapping [Wra → W.R]) with a log link function were used for each statistical analysis. Individual male and female (only in swapped pairs) identification numbers were included as random intercepts. We constructed both full models including mating times as an explanatory variable and null models with no explanatory variable, and then compared the models using the likelihood ratio test. To select the model with the best predictability, we compared the Akaike Information Criterion (AIC) between the 2 models. When the likelihood ratio test indicated a significant effect of the mating times ($P < 0.05$), adjusted P values calculated using the emmeans package (version 1.6.1) with the Tukey method are shown for post hoc test.

RNA-Seq and Data Analysis

Whole brains with the pituitary were collected from naïve males with no mating experience and post-naïve males (after 2 mating experiences) in the morning just before the third mating. The dissected brains were stabilized with RNA-later (Thermo Fisher Scientific, USA) until the extraction steps. Total RNAs were extracted using TRI Reagent (Cosmo Bio, Japan) and then purified using an RNeasy plus mini kit (Qiagen, Germany). RNA extraction solutions (same concentrations) from 3 individuals were included as 1 sample for RNA-seq (each sample size was 2). All library preparations and sequencing were outsourced to a company (GENEWIZ, Japan). The cDNA libraries were prepared using a NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, USA). The libraries were multiplexed and loaded on an Illumina HiSeq X Ten (2 x 150 bp, Illumina, USA). Adapter sequences were trimmed using Cutadapt¹⁴ (v1.9.1). Reference genome sequences and gene model annotation files of medaka (Annotation NO: ASM223467v1) were used. Second, HISAT2¹⁵ was used to index the reference genome sequence. Finally, clean data were aligned to the reference genome via software HISAT2 (v2.0.1). We used edgeR¹⁶ (v3.4.6) for differential expression analysis. After normalization with the TMM method, differential expressed genes were identified with an FDR adjusted p value < 0.01 and $|\text{Fold change}| > 2$.

Results

The first mating experience in males decreased the latency to mate

To investigate how the first mating experience in males could influence mating behavior in medaka fish, we performed mating tests using naïve males and experienced males who had mated with females more than 7 times. To prepare naïve males, we separated juvenile males into groups and bred them without any females until performing the mating tests. We also prepared sexually mature females (> 3 months after hatching) for the mating test. The mating tests were carried out using 11 fixed pairs for 7 days (7

times) (Fig. 1A, 1B). The latency to mate was defined as the interval from “releasing the male” to “crossing with spawning”. To evaluate the effect of the first mating experience on the latency to mate, we used a GLMM, which revealed that the latency of naïve males significantly decreased after the first mating experience and biased the distribution for 5 days, but not for 7 days (Fig. 1C, Table S1, GLMM; day1 vs day3 estimate $\pm$ s.e. = 0.5094 ± 0.192 , z.ratio = 2.646, $P = 0.0406$, day1 vs day5 estimate $\pm$ s.e. = 0.8494 ± 0.189 , z.ratio = 4.488, $P < 0.0001$, day1 vs day7 estimate $\pm$ s.e. = 0.4231 ± 0.219 , z.ratio = 1.934, $P = 0.2137$, day3 vs day5 estimate $\pm$ s.e. = 0.3401 ± 0.191 , z.ratio = 1.781, $P = 0.2825$, day3 vs day7 estimate $\pm$ s.e. = -0.0862 ± 0.217 , z.ratio = 0.398, $P = 0.978$, day5 vs day7 estimate $\pm$ s.e. = -0.4263 ± 0.218 , z.ratio = -1.951 , $P = 0.2067$). In experienced males, on the other hand, the mating experience had no significant effect on the latency to mate (Fig. 1C, Table S1). Together, these findings indicated that the male mating experience influenced medaka mating behavior only in naïve males. We confirmed that these tendencies were reproduced in experiments performed at a different laboratory (Fig. S1, Table S2, S3). Therefore, we concluded that the first mating experience in naïve males decreased the latency to mate.

The first mating experience shortened the latency to a first courtship display

We further examined which behavioral component could influence the latency to mate in naïve males in repeated mating tests. First, we counted the number of courtship displays and measured the latency to the first courtship display, revealing that the latency to the first courtship display was significantly decreased only in naïve males, and not in experienced males (Fig. 2A, Tables S1, GLMM; day1 vs day3 estimate $\pm$ s.e. = 0.0973 ± 0.189 , z.ratio = 0.515, $P = 0.9555$, day1 vs day5 estimate $\pm$ s.e. = 0.6015 ± 0.186 , z.ratio = 3.229, $P = 0.0068$, day1 vs day7 estimate $\pm$ s.e. = 0.7165 ± 0.201 , z.ratio = 3.562, $P = 0.0021$, day3 vs day5 estimate $\pm$ s.e. = 0.5043 ± 0.171 , z.ratio = 2.951, $P = 0.0167$, day3 vs day7 estimate $\pm$ s.e. = 0.6192 ± 0.198 , z.ratio = 3.135, $P = 0.0093$, day5 vs day7 estimate $\pm$ s.e. = 0.115 ± 0.196 , z.ratio = 0.586, $P = 0.963$). On the other hand, the number of courtship displays did not significantly change in either naïve or experienced males (Fig. 2B, Table S1). Next, we compared the latency to mate from the first courtship display, which negatively correlated with the degree of female receptivity^{12–14}. The latency was significantly decreased only in naïve males (Fig. 2C, Table 1, GLMM; day1 vs day3 estimate $\pm$ s.e. = 1.4486 ± 0.331 , z.ratio = 4.379, $P = 0.0001$, day1 vs day5 estimate $\pm$ s.e. = 1.5358 ± 0.346 , z.ratio = 4.44, $P = 0.0001$, day1 vs day7 estimate $\pm$ s.e. = 0.3013 ± 0.4 , z.ratio = 0.754, $P = 0.875$, day3 vs day5 estimate $\pm$ s.e. = 0.872 ± 0.326 , z.ratio = 0.268, $P = 0.9933$, day3 vs day7 estimate $\pm$ s.e. = -1.1472 ± 0.377 , z.ratio = 3.041, $P = 0.0126$, day5 vs day7 estimate $\pm$ s.e. = -1.2344 ± 0.385 , z.ratio = -3.207 , $P = 0.0073$), and strongly suggested that the female mating experience with the naïve males enhanced female receptivity. Furthermore, we analyzed other behavioral elements (wrappings and wrapping rejections), and found no significant change between the number of the 2 behavioral elements and the number of mating in either naïve or experienced males (Fig. S3, Table S1). Next, we analyzed behavioral transitions in the mating test, and found that the number of courtship displays significantly decreased between day1 vs day3 and day1 vs day5 in experienced males (Fig. S5, Tables S1, GLMM; day1 vs day3 estimate $\pm$ s.e. = 0.636 ± 0.234 , z.ratio = 2.717, $P = 0.0333$, day1 vs day5 estimate $\pm$ s.e. = 0.820 ± 0.251 , z.ratio = 3.26, $P = 0.0061$,

day1 vs day7 estimate $\pm$ s.e. = 0.42 ± 0.223 , z.ratio = 1.881, $P = 0.2361$, day3 vs day5 estimate $\pm$ s.e. = 0.184 ± 0.283 , z.ratio = 0.649, $P = 0.9158$, day3 vs day7 estimate $\pm$ s.e. = -0.216 ± 0.258 , z.ratio = -0.838 , $P = 0.8365$, day5 vs day7 estimate $\pm$ s.e. = -0.400 ± 0.273 , z.ratio = -1.464 , $P = 0.4592$). In addition, we confirmed that these tendencies were reproduced in experiments performed at a different laboratory (Fig. S4, S6, Tables S2, S3). Accordingly, we revealed that the first mating experience shortened the latency to the first courtship display only in naïve males.

Table 1
RNA-seq results. List of differentially expressed genes (DEGs) that were more highly expressed in post-naïve samples (2 matings) than in naïve samples. Genes are ordered from high to low expression using FPKM values as an index.

	gene	$\log_2 \text{FC} $	FDR	FPKM (naïve)	FPKM (post-naïve)
1	<i>fkbp5</i>	1.89	1.010E-18	23.68	87.57
2	<i>tshba</i>	2.72	3.320E-39	6.99	45.93
3	<i>hapln2</i>	1.12	4.760E-05	19.22	41.74
4	LOC101155558	1.40	3.284E-03	11.44	30.26
5	<i>macrosialin</i>	1.04	1.990E-05	14.57	29.91
6	<i>stat1a</i>	1.13	5.290E-07	10.18	22.34
7	<i>lgals17</i>	1.76	1.730E-06	4.97	16.87
8	<i>dio2</i>	1.05	9.331E-04	7.30	15.10
9	<i>lgals3bp</i>	1.39	5.390E-07	5.77	15.10
10	<i>klf9</i>	1.10	4.730E-08	6.24	13.36

The behavioral change in naïve males occurred only in fixed pairs

The first male mating experience mainly influenced the latency to mate and the latency to the first courtship display in fixed pairs in 7 continuous mating tests. Here we examined whether this effect was specific for fixed pairs. To compare mating behavior between fixed pairs and swapped pairs, we performed mating tests for 3 days (Fig. 3A, each $n = 8$). The latency to mate and the latency to the first courtship display were significantly decreased in the fixed group (GLMM; day1 vs day2 estimate $\pm$ s.e. = 0.205 ± 0.00818 , z.ratio = 2.5, $P = 0.0332$, day1 vs day3 estimate $\pm$ s.e. = 0.345 ± 0.00779 , z.ratio = 4.432, $P < 0.0001$, day2 vs day3 estimate $\pm$ s.e. = 0.141 ± 0.0785 , z.ratio = 1.791, $P = 0.1725$), but did not change in swapped pairs (Fig. 3B, 3C, Table S4). The latency to mate from the first courtship display (Fig. 3D, Table S4), numbers of other events (Fig. S7, Table S4), and behavioral transitions (Fig. S8, Table S4) did not significantly change in either fixed or swapped pairs. The latency to mate and the latency to the first

courtship display significantly decreased in repeated behavioral experiments (3 times). These findings strongly suggest that the naïve males recognized the first mating partner and thus the latency to mate and the latency to the first courtship display were decreased according to the mating experience.

The first mating experience changed gene expression in the male brain

To evaluate the effect of the first mating experience on the brain gene expression patterns, we compared gene expression profiles using whole brains between the naïve and post-naïve male medaka that had 2 mating experiences (Fig. S9). In the present study, we performed RNA-seq analysis using the whole brain with the pituitary, because in some fish species such as zebrafish and cichlid, social status has significant effects on gene expression at the whole brain level^{17,18}. We identified 10 differentially expressed genes (DEGs) that were upregulated by the mating experience and had a greater than 2-fold change, suggesting that the first mating experience could influence brain gene expression in male medaka. We listed the DEGs in descending order of the expression level (FPKM) (Table 1). Interestingly, we found that 3 genes (*tshba*, *dio2*, *klf9*) of the top 10 DEGs were associated with functional expression of the thyroid hormone system (Table 1). *tshba* encodes a thyroid stimulating hormone that promotes the synthesis and secretion of inactivated thyroid hormone (T4). *dio2* encodes type II iodothyronine deiodinase, which converts inactivated thyroid hormone to activated thyroid hormone (T3). *klf9* encodes a transcription factor, Krüppel-like factor 9, which is induced by thyroid hormone (T3)¹⁹. These findings indicate that the first mating experience changed the gene expression patterns and upregulated thyroid hormone-related genes. In addition, early-life stress exposure increases *fkbp5* expression in the brain and FKBP5 regulates glucocorticoid receptor activity²⁰. High *fkbp5* expression and early-life stress interact to increase anxiety-like behavior mediated by AKT signaling in association with hippocampal synaptic plasticity²¹. Hapln2 (also called Bral1) is essential for formation of the functional extracellular matrix and neuronal conductivity in mice²².

Discussion

The findings of the present study revealed, in medaka fish, that naïve males altered their mating behavior according to their first mating experience. Interestingly, the pattern of behavioral alterations after the first mating in medaka fish was quite different from those of other species such as fruit flies^{1,23}, mice^{2,3} and rats⁴, in which the mating experience increases male sexual motivation, but does not influence mate preference. In medaka fish, repeated matings (3 times) with the same partners decreased the latency to mate in naïve males, but not in experienced males. This finding suggests that naïve medaka males have a mate preference for familiar females, while experienced medaka males do not. In other words, the first mating experience might abolish familiarized mate preference in male medaka. Most animals change their social strategy from the juvenile stage to the adult stage. During the juvenile stage, the social strategy mainly promotes growth and survival, while in the adult stage, the social strategy includes reproductive behaviors to produce more progeny. For example, juvenile guppies tend to form kin groups to achieve an effective transfer and protect against predators²⁴, while adult guppy shoals do not maintain

kin groups to prevent inbreeding²⁵. In other fish species, such as humbug damselfish²⁶ and three-spot dascyllus²⁷, juveniles tend to exhibit a social preference for familiar individuals. In cichlid, juvenile fish kept in a kin group grow faster²⁸ and approach a predator for surveillance more often than solitary individuals that do not live in a group²⁹. At the adult stage, mosquito fish exhibit a mate preference for unfamiliar mates, which emerges after the first mating experience. To our knowledge, these findings provide the first evidence indicating that the first mating experience abolishes mate preference in any species. Further studies are needed to elucidate why naïve medaka males have a mate preference based on familiarization and why the multiple (> 7 times) mating experience abolishes the preference. It is possible that the first mating experience contributes to changes in social strategy during life.

Furthermore, after the mating experience, we observed the upregulation of 3 genes (*tshba*, *dio2*, *klf9*) related to functional expression of the thyroid hormone. Thyroid hormone is required for brain maturation and development^{30,31}. Interestingly, in Japanese quail, thyroid hormone is a trigger hormone for the release of gonadotropin-releasing hormone in the brain to mature gonads for reproduction³². A relationship between seasonal reproduction and thyroid hormone in the brain is reported across species, including mice³³ and fish³⁴, among seasonally reproductive animals. In this study, sexually mature males were used for the mating test and there was no change of seasonal information (i.e., water temperature and day length remained the same) in the laboratory, but medaka do show seasonal reproductivity³⁵, suggesting that the thyroid hormone system is activated in the brain by the first mating experience and not a seasonal change. To our knowledge, there are no reports of thyroid hormone activation triggered by the first mating experience in any species. In addition, *klf9*, which is a transcription factor dependent on the thyroid hormone¹⁹, contributes to dendritic spine maturation in the mouse hippocampus³⁶. The first mating experience leads to maturation of mating behavior and shapes male sexual motivation with neural maturation in the mPOA, which is the center of the male mating behavior in rodents^{37,38}. Therefore, the first mating experience may mature male mating social behavior with neural maturation according to thyroid hormone levels in male medaka (Fig. 4). Behavioral maturation by the first mating experience in male rodents might also be related to the thyroid hormone system. The center of the mPOA, including the sexually dimorphic nucleus of the preoptic area, is more activated in naïve male rats than in experienced male rats⁹. In addition, knockdown of *vgf* expression erases the behavioral maturation dependent on the first mating experience⁴. Interestingly, thyroid hormone regulates *vgf* expression in hamsters³⁹, implying that behavioral maturation by the first mating experience in male rodents is also related to the thyroid hormone system. These findings, however, are fragmented and further studies are necessary to elucidate whether the molecular mechanisms associated with male mating maturation dependent on the first mating experience are conserved among vertebrates.

Declarations

Data availability

All sequence read data are available from DDBJ (DRA013480) and the data that support the findings of this study are available from the corresponding author, [HT], upon reasonable request.

Competing interests (mandatory)

The authors declare no competing interests.

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Author contributions

M. D., H. S. and H. T. designed research; M. D. performed research; M. D., T. K. and S. A. analyzed data; M. D. and H. T. wrote the paper; All authors reviewed manuscript.

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Figures

Figure 1

Design and results of the mating test using naïve males. (A) (B) Outline of the behavioral experiment. (A) 10 (naïve) and 11 (experienced) males were used for the study. Male and female pairs were fixed during the experiment and the experiment was carried out for 7 consecutive days. (B) Experimental procedure. The male was separated a day before the experiment and released to the female the next morning. Their mating behavior was recorded for more than 10 min and analyzed. (C) Behavioral test results. Left and right panels show the transition of the latency to mate in naïve and experienced males, respectively. The latency significantly decreased in naïve males depending on the number of matings for 5 days, but not in experienced males. Each dot represents the results of each individual. The boxplot indicates median (the center line), minimum, maximum, first quartile, third quartile, and outlier points. *P*-value shows the results of the post hoc test with Tukey's adjustment method in a generalized linear mixed model (gamma distribution, log link function).

Figure 2

Change in the courtship behavior, latency to the first courtship display (A), and the number of courtship displays (B) and the latency to mate from the first courtship display (C). Each dot represents the results of each individual. The boxplot indicates median (the center line), minimum, maximum, first quartile, third quartile and outlier points. *P*-value shows the results of the post hoc test with Tukey's adjustment method in a generalized linear mixed model (gamma distribution (A and C) and Poisson distribution (B), log link function).

Figure 3

Effect of the familiarization (repeated matings with the same partner) on mating behavior of naïve males. (A) Outline of the behavioral experiment. Each group contained 8 males in this study (fixed and swapped groups). (B) Latency to mate (left: fixed group, right: swapped group). The latency to mate significantly decreased only in the fixed group, and not in the swapped group. (C) Latency to the first courtship display (left: fixed group, right: swapped group). The latency significantly decreased only in the fixed group, and not in the swapped group. (D) Latency to mate from the first courtship. Each dot represents the results of each individual. The boxplot indicates median (the center line), minimum, maximum, first quartile, third quartile and outlier points. *P*-value shows the results of the post hoc test with Tukey's adjustment method in a generalized linear mixed model (gamma distribution, log link function).

Figure 4

Possible model of the effect of the first mating experience in male medaka. In this study, we found that 3 genes (*tshba*, *dio2*, *klf9*) were upregulated after the first mating experience. *tshba* encodes thyroid stimulating hormone, which activates inactivated thyroid hormone (T4). DIO2, which is an enzyme that converts T4 to activated thyroid hormone T3. KLF9 is expressed depending on the T3 level and contributes to spine maturation. The first mating experience may lead to maturation of male mating behavior via neural maturation depending on the thyroid hormone system in the male medaka brain.

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