Genetic segregation for male body coloration and female mate preference in the guppy

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Research note

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Abstract

Objective The purpose of this study was to segregate the genetic lines responsible for the orange area of coloration in males and the response to orange coloration exhibited by females in the guppy (Poecilia reticulata) through artificial selection. This study is part of a project that uses QTL-seq to search for candidate genes involved in male orange coloration and female response to male coloration. We created two lines: high-selected lines of males having large areas of orange spots and of females with high response to male orange coloration; and low-selected lines of males having small areas of orange spots and of females with low response to male orange coloration.

Results The male orange area and the female response became significantly different between high- and low-selected lines after three generations of artificial selection. This indicates that the differences in the frequencies of alleles at loci affecting the orange area and the female response between the lines increased over the generations through selection.

Introduction

The guppy, *Poecilia reticulata,* is a model fish for studies on sexual selection [1]. Female guppies generally prefer mating with males that possess larger and more colorful orange spots [2,3]. However, substantial variations in both male coloration and female preference are maintained between and within populations [4–6]. A better understanding of the mechanism underlying this diversity would be gained from identifying the associated genes. To date, however, no sequence variant affecting male sexual traits and female mate preference have been determined, although variation in the expression of opsin genes is known to affect the female response to male coloration [7].

In the guppy, artificial selection experiments for increased and decreased male orange area have shown high and low selection lines with a strong divergence [8]. In addition, as a result of artificial selection for high and low lines of male orange area, females from high lines tended to show stronger preference for male orange coloration than did females from low lines [9]. These results indicate that both male orange area and female preference are based on genetic variations and a genetic correlation between male orange coloration and female preference for orange coloration has been demonstrated [9]. It has also been shown that there is a high heritability for orange area in introduced populations of guppies [10]. Quantitative trait locus (QTL) studies for a set of variable male traits including size and color pattern have been performed using linkage data of 790 single-nucleotide polymorphism (SNP) markers [11], with results suggesting that several loci located at different linkage groups affect the orange area, although the exact SNP could not be determined.

Recently, genome-wide association analysis has been used to identify the regions affecting the phenotypes [12–14]. Usually, a large number of individuals are required to detect significant SNPs. QTL-seq is another genome-wide method which uses phenotypically different breeding lines for identifying QTL loci [15]. We set up a project that uses QTL-seq to search for candidate genes involved in male orange coloration and female preference to orange coloration. In preparation, we artificially selected for male orange coloration and female preference to orange coloration, and created lines with opposite traits: males with large and small orange area and females with high and low preference to orange coloration. In this paper, we report on the results of the artificial selection.

Methods

Fish

Feral guppies were collected in March 2012 from 5 sites on Okinawa Island, Japan: Isa River, 2661N, 12800E; a water channel in Gabusoka, 2662N, 12800E; a spring pond in Inoha, 2666N, 12791E; Hiji River, 2672N, 12818E; and Okuma River, 2673N, 12817E. The caught guppies were transported to the University of Tohoku in Sendai, Japan, and kept in plastic tanks. After one month, once the fish had become accustomed to laboratory conditions, females were isolated in plastic tanks (2 L) and allowed to give birth. Thirty-one females produced broods (1 Isa, 1 Inoha, 2 Gabusoka, 11 Hiji, 16 Okuma). In addition, two females from a laboratory strain of feral guppy from Okinawa Island were isolated and allowed to give birth. In total, 85 male (P male) and 82 female (P female) offspring were collected from the 33 broods. These offspring were reared in plastic tanks (7 L) with males and females kept separately. After 6 months, male orange coloration and female mate preferences were measured.

All fish were maintained under constant conditions (251 °C, aerated and filtered water, 12:12 h light:dark cycle), and fed once daily with newly hatched brine shrimp (*Artemia salina)* nauplii and commercial flake food (Tetramin, Tetra Werke).

Male coloration

Male orange area was measured as the ratio of the total area of orange spots to the total area of the body and caudal fin following the procedure in Additional File 1. The left and right sides of anesthetized males were photographed using a digital camera (D3300, Nikon, Japan), and the images analyzed using Photoshop CS4 (Adobe, San Jose).

Female preference

A detailed description of the procedure can be found in Additional File 1. Briefly, female preference to male orange coloration was estimated as the response to digitally modified video images of a male. A focal female was introduced into the test aquarium (18 30 17 cm) next to which was placed a color liquid crystal display monitor (FlexScan L 367, Eizo) at 2 cm from the side. After 5 min acclimation, the images of two males were displayed on the screen. One was of a male with large/colorful orange spots (high-orange, HO), the other was of a male with small/drab orange spots (low-orange, LO). The length of time that the focal female was facing the images and swimming within 5 cm of the digital image of each male was measured over 5 min. The test was then repeated with the positions of the images of the two males reversed. The proportion of time that the female spent viewing the HO male image in relation to the total time that the female spent on both the HO and LO male images was taken as the female preference to HO.

Artificial selection

Fifteen males with the largest orange area and 15 males with the smallest orange area were selected from the P males as the high- and low-selected lines, respectively. Fifteen females with the highest preference (from the female preference test) and 15 females with the lowest preference were selected from the P females for high- and low-selected lines, respectively. Details of the individuals selected are given in Additional File 1.

A male and a female were selected from each line and paired such that males and females from the same broods did not mate. One female was placed into a plastic tank (2 L) with her paired male and they were maintained until the female produced a brood (F1). To ensure that all offspring were reared at the same density, four male and four female offspring were randomly selected after sex discrimination and reared under the same conditions as the P individuals. Once these offspring had reached 6 months of age, the male coloration and the female preference were measured. Next, males and females were selected from the F1 generation and paired in the same way as for the P generation. The process and measurements were repeated until generation F3.

Statistical analysis

The male orange area and the female preference between selected lines were compared using *t*-test on independent samples in each generation. The *t*-test with assumed heteroscedasticity was used because variances of male orange area between selected lines were not homogeneous (Levene's test; $F > 10.78$, $P < 0.01$).

Realized heritability (h^2) was calculated separately for the low- and high-selected lines for male orange area and female preference as:

$$h^2 = 2\left(\frac{R}{S}\right)$$

where R represents the cumulative response (the sum of means in generation i minus means in generation i–1) and S represents the cumulative selection differential (the sum of selected individuals' means in generation i minus all individuals' means in generation i) [16]. The slope of the regression of R

on S was estimated, with the multiplier of 2, and was used as selection for each trait for the separate sexes. Statistical analyzes were performed using SPSS version 25.0.

Results

In the high-selected lines, the orange area for 53, 54, 45 males and mate preference for 50, 55, 45 females were measured at F1, F2, F3, respectively (Table S1 in Additional File 2). In the low-selected lines, the orange area for 47, 51, 45 males and mate preference for 48, 53, 47 females were measured at F1, F2, F3, respectively (Table S1). After the three generations of artificial selection, the mean male orange area of the high-selected line increased 110.8% and that of the low-selected line decreased 15.6%, compared to those of P males (Fig. 1). The male orange area differed significantly between high- and low-selected lines in all generations (F1: $T_{64.710} = 5.045$, $P < 0.001$; F2: $T_{82.897} = 9.970$, $P < 0.001$; F3: $T_{64.072} = 10.025$, $P < 0.001$). The realized heritability of male orange area estimated from the high-selected line was significantly larger than zero, whereas that from the low-selected line was not (Table 1, h^2 for each generation is shown in Table S2 of Additional File 2).

After the three generations of artificial selection, the mean female response to HO in high- and low-selected lines were 4.8% and 24.6% lower, respectively, than that of P females (Fig. 1). The female response to HO in high- and low-selected lines did not differ in F1 ($T_{83.483} = 1.286$, $P = 0.202$), but differed significantly in F2 and F3 (F2: $T_{99.049} = 3.394$, $P = 0.001$; F3: $T_{77.320} = 3.842$, $P < 0.001$). The realized heritabilities of female response to HO estimated both from the high- and low-selected lines were not significantly larger than zero (Table 1, Table S2).

Discussion

In this study, the area of male orange coloration became significantly different between high- and low-selected lines after the three generations of artificial selection. This indicates that the differences in the frequencies of alleles at loci affecting the orange area between the lines increased over the generations through selection. The realized heritability estimated from male orange area in high- and low-selected lines was 1.47 and 0.32, respectively, consistent with values in a previous study (0.20–1.50) [8]. Since the response to artificial selection and the heritability estimates would depend largely on initial genetic variation, the similar estimates of heritability between the present and previous studies indicates that similar levels of genetic variation for male orange area might be maintained in the populations in Okinawa and Trinidad. The heritability estimated from the low-selected line was not significantly larger than zero, indicating that the frequencies of alleles affecting smaller orange area might be larger in the initial populations of the artificial selection.

The female responses to HO also became significantly different between high- and low-selected lines after three generations of artificial selection. This indicates that differences in female response to orange coloration were partly due to allelic differences between selected lines. However, although females that responded to HO males were selected in the high-selected lines, the female response to HO males did not increase over generations (Fig. 1), and the estimated heritability from the high-selected line was not significantly larger than zero. On the other hand, in low-selected lines, the female response decreased over generations and the heritability was marginally significant (0.595, $P = 0.057$). This might be because the initial population included allelic variations for decreasing response, but did not include allelic variations for increasing female response.

Taking the female response to orange coloration in high and low-selected lines as a whole, the heritabilities of female preference estimated in this study were lower than those estimated in a previous studies in the guppy (0.3) [9], and in a study in the three-spined stickleback (*Gasterosteus aculeatus)* (0.43, s.e. = 0.37) [17]. Beside the initial genetic variation for female preference, female responses estimated from exposure to digital images of a male might be different from female preference when presented with real males. Orange spots on real male guppies are more chromatic, and female guppies prefer males based on the combination of chroma, luminance and color pattern [7]. It has been showed that variations in female response to HO males is positively correlated with the expression of multiple opsin genes which are affected by both the light environment and by an allelic difference in the LWS−1 gene [7]. Thus, it is possible that environmental variation of opsin expression might have caused the lower genetic variation seen in this study.

The present experiments support previous studies showing that variations in male orange area and female preference are partly due to genetic variation, and are reflected in this study’s success in obtaining genetically different lines of males with large and small orange areas. Males in the high- and low-selected lines could be used for QTL-seq for determining candidate genomic regions affecting male orange area.

Limitations

Feral guppy populations from Okinawa, Japan were used in this study, but the artificial selection experiment involving male orange area has already been tested using Trinidadian guppies. In addition, we did not set up replicates or controls for the artificial selection experiment.

Declarations

Authors’ contributions

AS involved in measuring male orange area and female mate preference, breeding for artificial selection, statistical analyzes and writing the manuscript. MK designed and supported the study, and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and material

All data generated or analyzed during this study are included in this published article and in Additional File 3.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All of animal care and breeding were performed according to the guidelines of the animal care and use committee of Tohoku University. The protocol was approved by the committee (permit number: 2017LsA–025).

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Tables

Table 1 Realized heritability of male orange area and female preference. *P* indicates probability from one-sample t-test for difference to zero.

		h^2± SEM	P
Male orange area	High line	1.472±0.243	0.026
	Low line	0.315±0.111	0.105
Female preference	High line	-0.122±0.120	0.416
	Low line	0.595±0.148	0.057

Figures

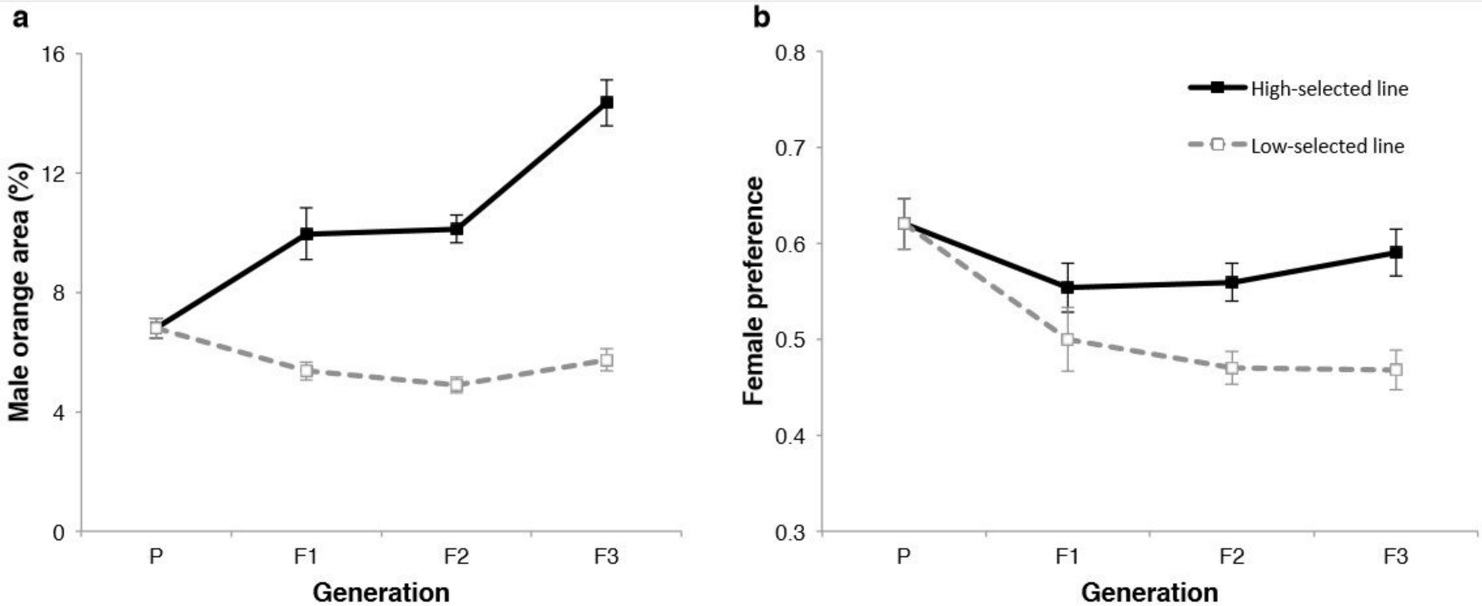

Figure 1

Responses of male orange area (a) and female preference (b) to artificial selection. Plots are mean SEM.

Supplementary Files

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