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INHERITANCE OF THE WHITE-RED *(KOHAKU)* COLOR COMPLEX IN ORNAMENTAL (KOI) CARP *(CYPRINUS CARPIO* L.)

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Abstract

Color variability was investigated in three normal (amphimictic) and three meiotic gynogenetic progenies obtained from white-red (*kohaku*, according to Japanese classification) koi parents. All the amphimictic and gynogenetic progenies consisted of three color types – white, white-red and red. The ratio of colors depended on the relative coverage by red patches in the *kohaku* parents. The lowest percentage of red fish and highest proportion of white fish were recorded in progeny obtained from *kohaku* parents with relatively weak development of the red color. The percent coverage by red patches was measured in a sample of thirty or sixty white-red fish from each amphimictic progeny and the distribution of color classes within the sample was determined. The distribution of the sample, together with the white:white-red:red ratio in the total progeny, was used to estimate the color class distribution in the entire progeny (including the solid white and solid red individuals). In two of the amphimictic progenies, the fish clearly divided into two groups: non-red (solid white and white-red) and solid red fish. Appearance of these groups might be explained by the existence of some major color-determining gene(s), which determine(s) the background color (either white or red) of the individual fish. It is also suggested that the development of red patches in fish with a white body background is controlled by many genes with alleles that either maintain the white color or induce the appearance of red patches.

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Introduction

White-red (*kohaku*, according to Japanese classification) is one of the most popular color types of koi (Kuroki, 1981; Tamadachi, 1990). The mode of inheritance of the white-red color complex, as well as other multicolor traits in koi, is not yet resolved and few studies have addressed this topic. Iwahashi and Tomita (1980) investigated color segregation in progenies obtained from white-red parents, as well as from solid white and solid red fish originated from *kohaku* during three generations. Based on their data, they suggested that the white-red color complex is controlled by numerous genes of white and red colors (Iwahashi and Tomita, 1980). Taniguchi et al. (1986) compared color variability in diploid, triploid and gynogenetic progenies produced from koi and suggested that the genes for red and white colors are non-allelic.

Based on color variability in normal and gynogenetic progenies, Gomelsky et al. (1996) also concluded that the white-red color complex in koi is polygenic. They assumed that the color ratio (white:white-red:red) in each progeny depends on the rate of red patch development in its parents, so that weak or strong development of the red color in the parents results in corresponding development of the red color in their offspring. The present study was conducted to verify this assumption. To describe more precisely the rate of red coloration in offspring, the relative area covered by red patches in samples of white-red fish was determined in some progenies.

Materials and Methods

Normal (amphimictic) and meiotic gynogenetic progenies were produced at the Fish and Aquaculture Research Station in Dor, Israel, from *kohaku* (white fish with red patches) koi parents with different rates of red color coverage.

Three amphimictic and three gynogenetic progenies were produced and analyzed (Table 1). The amphimictic progenies were designated F1xM1, F4xM3 and F5xM4 according to their female (F) and male (M) parents. The gynogenetic progenies were designated G-F1, G-F2 and G-F3 according to the female parent only. The relative portion of the body covered with red patches in the parents is given in Table 1. The amphimictic progenies F1xM1 and F4xM3 were obtained from *kohaku* with relatively strong development of red patches (30-45% body coverage), while progeny F5xM4 was obtained from *kohaku* with relatively weak development of red patches (1-3% body coverage). Photographs of two of the koi parents are presented in Fig. 1.

Relative red body coverage in white-red fish was determined by the following method. The fish was anesthetized with ethyl-amino-benzoate (benzocaine) and placed in a transparent plastic bag of corresponding size. The outline of the body from the tip of the snout to the end of caudal peduncle and the red patches were traced onto the covering plastic film with a permanent-ink pen. The fish was turned over and the procedure was repeated for the opposite side. The plastic film with the outlines of the body and the red patches was photocopied onto white paper. The paper was cut along the outline of the body and weighed to the nearest 0.01 g, followed by a similar weighing of the cut-out outlined red patches. The ratio of the two weights gave the relative body coverage by red patches. To estimate the error of this method, three fish were measured three times each, resulting in a mean error of about 2.0%.

Methods used to obtain normal and meiotic gynogenetic progenies were described by Gomelsky et al. (1996). After transition to active feeding, the larvae were reared in 110 l tanks and fed *Artemia* nauplii for 7-10 days. Subsequently, each group of fry was separately stocked in 0.04 ha earthen ponds for further rearing.

The color segregation in the progenies was evaluated after 3-4 months of pond-rearing when the fish attained a mean length of 15-25 cm. The color type of each fish in each progeny was recorded. The ratios of the color types (white, white-red and red) in the progenies were compared using the Chi-square test; differences were considered significant at $p<0.05$.

Table 1. Characteristics of *kohaku* (white-red) koi parents used in crosses and proportions of color types in their amphimictic and gynogenetic progenies.

Progeny	*Coverage with red patches in parents (%)*		*Number of analyzed fish*	*Proportion of color types (%)*		
	Female	*Male*		*White*	*White-Red*	*Red*
Amphimictic progenies						
F1xM1	44.8	29.5	575	3.7^{x}	73.6^{xy}	22.8^{x}
F4xM3	42.0	30.4	215	5.1^{x}	66.5^{x}	28.4^{x}
F5xM4	1.1	3.0	1407	10.5^{y}	76.8^{y}	12.7^{y}
Gynogenetic progenies						
G-F1	44.8	-	244	1.6^{x}	88.9^{x}	9.4^{xy}
G-F2	14.4	-	389	7.5^{y}	87.1^{x}	5.4^{x}
G-F3	36.6	-	215	1.9^{x}	83.3^{x}	14.9^{y}

Values within a column with different superscripts are significantly different (p<0.05). Significance of difference was evaluated separately for amphimictic and gynogenetic progenies.

Samples of thirty white-red fish from progeny F1xM1 and sixty fish from progenies F4xM3 and F5xM4 were taken to determine the relative body coverage by red patches using the previously described method. Resulting data were used to determine the distribution of the sampled fish into classes of red body coverage. The distribution of the sample, together with the overall color ratio of the progeny, was used to estimate the color class distribution of the total progeny (including solid white and solid red individuals). The distributions were compared to normal and other common unimodal distributions by the Chi-square Goodness-of-Fit Test (Rosner, 1995) using Crystal Ball™ software.

Results

Data on the numbers of analyzed fish and segregation into color types are given in Table 1. All the progenies contained three color types – white, white-red and red, with white-red always being most numerous. The proportions of color types in progenies F1xM1 and F4xM3, obtained from parents with relatively strong development of red patches, did not significantly differ from each other. In progeny F5xM4, obtained from parents with relatively weak development of red patches, the proportion of white fish was significantly higher than in the other two amphimictic progenies, while the proportion of red fish was significantly lower.

The same tendency was observed in the gynogenetic progenies. In progeny G-F2, obtained from a female with relatively lower red body coverage, the proportion of white fish was significantly higher than in the other two gynogenetic progenies (G-F1 and G-F3) obtained from females with stronger development of red patches. On the contrary, the proportion of red fish in progeny G-F2 was lower than in the other two gynogenetic progenies. The proportion of white-red fish was higher in

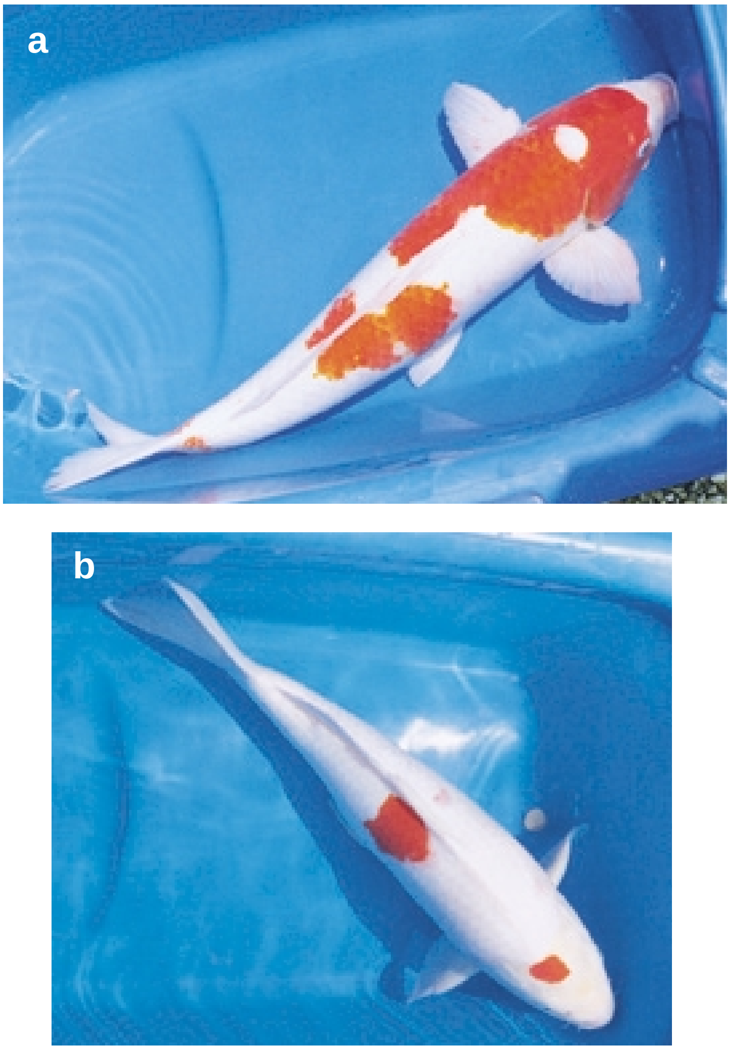

Fig. 1. Two of the *kohaku* (white-red) koi used to produce progeny in this study: (a) male 3 (relative red body coverage 30.4%) and (b) male 4 (relative red body coverage 3.0%).

all the gynogenetic progenies than in the amphimictic progenies.

The color class distributions of the samples of white-red fish from each amphimictic progeny are presented in Table 2. These distributions, together with the white:white-red:red ratios, were used to estimate the overall color class distributions in the amphimictic progeny (including solid white and solid red individuals; Table 2). Application of the Chi-square Goodness-of-Fit test showed that, in all the amphimictic progenies, the overall distribution did not fit any common unimodal distribution (Table 3). In progenies F1xM1 and F4xM3, the frequency of solid red fish was much higher than the frequency of adjacent

Table 2. Frequency (%) of red body coverage in fish of amphimictic progenies.

Classes (% red body coverage)	*F1xM1*		*F4xM3*		*F5xM4*	
	Among red-white fish	*Among all fish*	*Among red-white fish*	*Among all fish*	*Among red-white fish*	*Among all fish*
0 (solid white)	-	3.7	-	5.1	-	10.5
>0.0-10.0	20.0	14.8	13.3	8.8	26.7	20.5
10.1-20.0	16.7	12.3	15.0	10.0	10.0	7.7
20.1-30.0	20.0	14.8	13.3	8.8	5.0	3.8
30.1-40.0	6.7	4.9	18.3	12.2	15.0	11.6
40.1-50.0	3.3	2.4	6.7	4.5	5.0	3.8
50.1-60.0	6.7	4.9	8.3	5.5	6.7	5.1
60.1-70.0	0	0	5.0	3.3	6.7	5.1
70.1-80.0	10.0	7.3	8.3	5.5	6.7	5.1
80.1-90.0	10.0	7.3	6.7	4.5	3.3	2.5
90.1-<100.0	6.7	4.9	5.0	3.3	15.0	11.6
100 (solid red)	-	22.8	-	28.4	-	12.7

Table 3. Observed probability distributions of red body coverage in koi, compared to common unimodal distributions.

	Progeny		
	F1 x M1	*F4xM3*	*F5xM4*
All frequency data	No fit[a]	No fit[a]	No fit[a]
All frequency data excluding solid red fish (100% red color coverage)	Beta distribution $\alpha = 0.43$; $\beta = 0.71$ χ^2 p-value = 0.13[b]	Beta distribution $\alpha = 0.66$; $\beta = 1.03$ χ^2 p-value = 0.04[b]	No fit[a]

[a] Based on comparison with all common unimodal distributions.
[b] Observed distribution corresponds to theoretical distribution if χ^2 p-value>0.01.

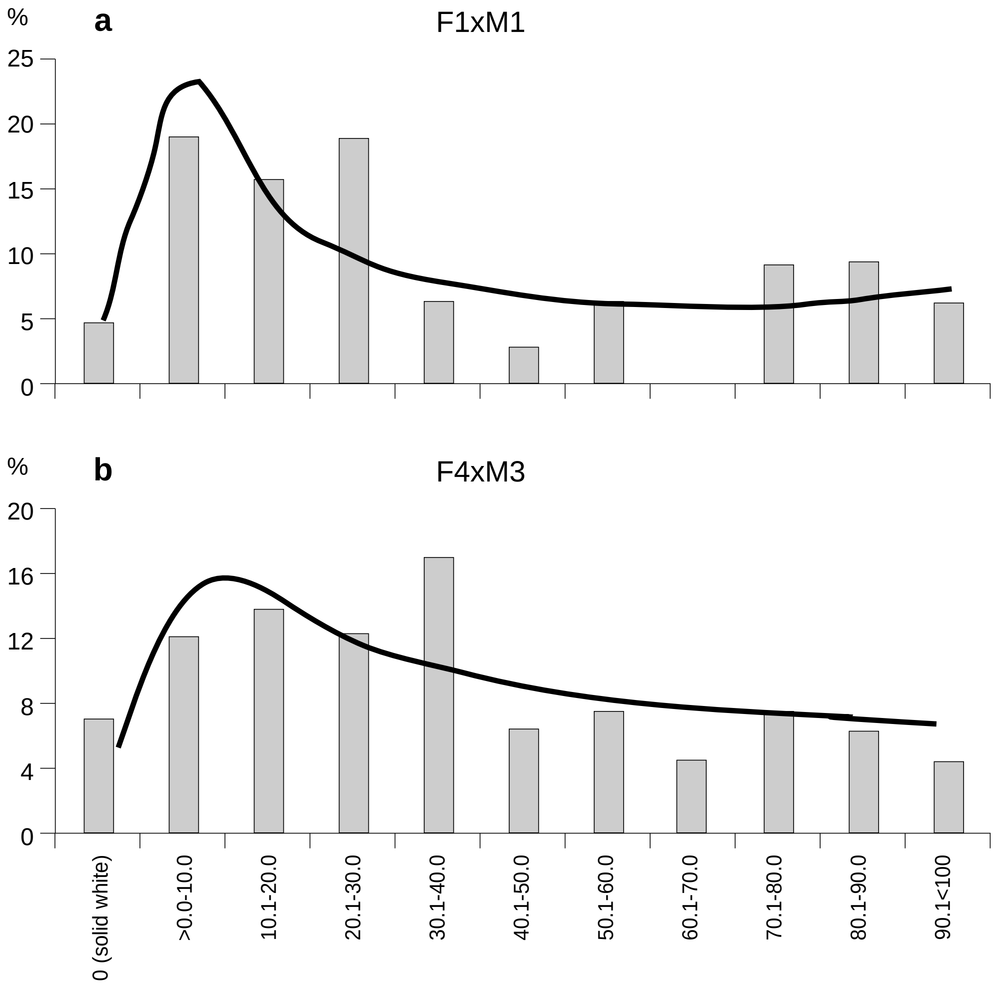

Fig. 2. Frequency of color classes (excluding solid red fish) in amphimictic progenies (a) F1xM1 and (b) F4xM3 compared to curves of fitted Beta distributions.

classes of white-red fish. Application of the Chi-square Goodness-of-Fit test to progenies F1xM1 and F4xM3 showed that the color class distributions of the solid white and white-red fish (i.e., excluding solid red individuals) fit a Beta distribution (Table 3, Fig. 2). In both progenies, the distribution was positively skewed towards a lower percentage of red coverage.

In progeny F5xM4, the color class distribution, excluding the solid red fish only or together with the adjacent class of white-red fish (90.1-<100.0), did not fit any unimodal distribution (Table 3).

Discussion

The present study reveals the dependence of the color ratio in a progeny on the relative coverage by red patches in its white-red (*kohaku*) parents, as suggested earlier by Gomelsky et al. (1996). Among the amphimictic progenies, the lowest percentage of red fish and highest proportion of white fish were recorded when white-red parents with very weak development of red color (1-3%, progeny F5xM4) were crossed. Similarly, among the gynogenetic progenies, the largest proportion of white fish and lowest proportion of red fish was observed in progeny G-F2, obtained from female F2 with the lowest red body coverage (14.4%). As in our previous study (Gomelsky et al., 1996), the proportion of white-red fish in meiotic gynogenetic progenies was higher than in amphimictic progenies.

The observed variability of the fish with regard to the development of red color supported suggestions made by Iwahashi and Tomita (1980) and Gomelsky et al. (1996) that the white-red color complex is a quantitative trait. However, in progenies F1xM1 and F4xM3, solid red fish had the highest frequency and this end of the distribution of classes did not correspond to the tail end of unimodal distributions typical for quantitative traits (Strickberger, 1985). The fish in these two progenies clearly divided into two groups with regard to background body color: non-red (solid white and white-red) and solid red fish. Taking into account that the white-red color complex is a polygenic trait, the appearance of these groups might be explained by the existence of some major color-determining gene(s), which determine(s) the background color (either white or red) of individual fish in a progeny.

The distribution of red coloration in fish with a white background (i.e., solid white and white-red fish) in progenies F1xM1 and F4xM3 was unimodal. Hence, we suggest that the development of red patches in fish with a white background is controlled by many genes with alleles that either maintain the white color or induce the appearance of red patches. The skewness of distributions towards higher frequencies of fish with weaker development of red coverage may be explained by a dominance relationship between the alleles. The effect of dominance skews the distribution of quantitative traits towards the dominant direction (Strickberger, 1985). Other factors, such as interaction between major gene(s) and polygenes, also may cause an asymmetrical distribution.

The method of measuring relative red body coverage used in this study provides an opportunity to investigate the development of red color in progenies obtained from *kohaku* parents as a trait with continuous variability and provides further information for understanding the mechanisms of white-red color complex inheritance in koi. Suggestions made in this study will be checked in further experiments.

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