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COMMUNICATION

Inheritance of Red Eyes in Ornamental Koi Carp

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Abstract
The purpose of this study was to investigate the inheritance of red eyes in ornamental koi, a variant of Common Carp *Cyprinus carpio*. The study included crossing fish with different eye color phenotypes and analyzing fish segregations in progenies. In progeny 1, obtained by crossing a red-eyed, yellow male with a black-eyed, white-red female, the segregation "red eyes : black eyes" in juveniles did not differ significantly from the 1:1 Mendelian ratio. In two progenies obtained by crosses of red-eyed fish from progeny 1, the segregations of larvae with light and dark lenses and the segregations "red eyes : black eyes" in juveniles not having black pigmentation on the body did not differ from the 3:1 Mendelian ratio. In two progenies obtained by crosses of black-eyed fish from progeny 1, larvae with only dark lenses and juveniles with only black eyes were observed. Based on these data, it was suggested that the appearance of red eyes in koi is controlled by a dominant mutation of one gene (*R*/*r*); koi with genotypes *RR* and *Rr* have red eyes, while koi with genotype *rr* do not possess this trait. Significant proportions of juveniles from fish of progeny 1 had black pigmentation on the body. In pigmented juveniles the ratio red eyes : black eyes was shifted towards black-eyed fish likely due to the influence of genes controlling the development of melanin on the body on the expression of mutation *R*. Based on the occurrence of fish with red eyes and black pigmentation on the body it was suggested that the appearance of red eyes in koi is caused not by an albino but by another demelanization mutation. In a progeny obtained by crossing a red-eyed female from progeny 1 with a Common Carp with wild-type color, all fish had black eyes while about 50% of the fish in this progeny had a light yellowish body. This shows that the mutation *R* causes the appearance of red eyes only in koi; in Common Carp with wild-type color this mutation causes only partial demelanization of the fish's body.

The Japanese ornamental koi, a variant of Common Carp *Cyprinus carpio*, is one of the most popular decorative fish in many countries all over the world, including the United States. Koi were developed approximately two centuries ago in Japan and are known by their large variability in colors and color patterns (Chapple 1999; Davies 1999). Scientific information on the inheritance of different color traits in koi is scarce (De Kock and Gomelsky 2015) and the inheritance of many color variants has never been investigated.

Albinism is the most frequent color modification observed in fish; albino individuals have been described in many aquarium fish, several aquaculture species, and among fish inhabiting natural waters. Albinism is the absence of black pigment, melanin, both in the skin and in the eyes. Albino fish have a yellowish body and red eyes; the eyes in albino organisms look red because the blood vessels inside the eyes become visible due to the absence of black pigment. There is a type of koi, called Akame Kigoi according to Japanese classification that literally means red-eyed, yellow fish; it is generally assumed that Akame Kigoi koi are albino (Chapple 1999). However, there are no data on inheritance of albinism in koi in the scientific literature. The initial purpose of this study was to investigate the inheritance of albinism in koi. However, during the study it became obvious that the appearance of red eyes in koi is a complex trait, the expression of which depends also on melanin pigmentation of the fish's body.

METHODS

The general scheme of experiments included crossing fish with different eye color phenotypes and an analysis of fish

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segregations in progenies with regard to eye color; variability of fish in progenies with regard to the presence or absence of black pigmentation on the body was also recorded. A total of 10 progenies were obtained and analyzed (see Tables 1, 2). Progeny 1 was obtained by crossing a red-eyed, yellow koi (Akame Kigoi) male without black pigmentation on the body with a black-eyed (normal eye color), white-red koi (Kohaku according to Japanese classification) female without black pigmentation on the body at Desert Rainbow Koi Farm, Tucson, Arizona, in spring 2013. This progeny was produced by natural spawning of the fish in a 1.1-m^3 tank; spawning ropes and mats were placed in the tank as the substrate. After spawning, the substrate with eggs was transferred to another 1.1-m^3 tank where hatching and swim-up of the larvae occurred. Later, fry in this tank were fed an artificial diet; fish also consumed the natural feed that developed in the tank. A sample of 40-d-old fry from the obtained progeny were shipped to the Aquaculture Research Center of Kentucky State University (KSU), Frankfort, and stocked for further raising in a 20-m^3 outdoor tank supplied with water from a reservoir. Later (at 2 months of age) we noticed that some of the fish from this progeny had red eyes and the ratio "red eyes : black eyes" was determined by analyzing 3-month-old juveniles.

Fish from progeny 1 were then raised in indoor recirculating systems and earthen ponds at KSU Aquaculture Research Center for 2 years. In spring 2015, fish from progeny 1 reached sexual maturity and were crossed for the production of progenies for the present study; koi and Common Carp with wild-type color from the local KSU stock were also used in crosses. Progenies 2 and 3 were obtained by crossing red-eyed fish from progeny 1 (see Table 1). Both progenies 4 and 5 were produced by crossing red-eyed fish with black-eyed fish from progeny 1; progeny 4 was obtained by crossing a red-eyed female with a black-eyed male, while progeny 5 was produced using the reciprocal combination. Progeny 6 was produced by crossing a red-eyed female from progeny 1 with a black-eyed, white-red male without black pigmentation on the body from the local stock. Progenies 7 and 8 were obtained by crossing black-eyed fish from progeny 1 (see Table 1). Some fish parents from progeny 1 were used in more than one cross; the same red-eyed female was used for the production of progenies 2 and 4 while the same red-eyed male was used for the production of progenies 2 and 5; also, the same black-eyed female was used for production of progenies 5 and 8.

Progeny 9 was obtained by crossing a red-eyed female from progeny 1 with a Common Carp male with wild-type color from local stock, while progeny 10 was obtained by crossing the same wild-type color male with a black-eyed, white-red female (without black pigmentation on body) from local stock (see Table 2).

Progenies 2–10 were obtained by artificial spawning in a hatchery. The technique of artificial spawning was the same as that described by Gomelsky et al. (2015). To induce final oocyte maturation in females and spermiation in males, fish parents were injected with carp pituitary extract (Argent Chemical Laboratories, Redmond, Washington) at 3 mg/kg of body weight. Eggs were artificially fertilized in plastic bowls and were treated with a water–cow's milk mixture (volumetric ratio = 8:1) to remove adhesiveness. Embryos were incubated in McDonald jars; hatched larvae were collected in mesh hapas placed in flow-through raceway tanks. Swim-up larvae from progenies were stocked for rearing in separate, 20-m^3 outdoor tanks; from 3,400 to 4,300 swim-up larvae (quantities were evaluated by volumetric method) were stocked in each tank. Tanks were supplied with water from a 0.2-ha reservoir. Fish in tanks were fed an artificial diet; fish also consumed zooplankton and benthic organisms that developed in the tanks or flowed in with the reservoir water.

Segregations of fish in progenies 2–10 with regard to eye color were determined twice: at larval and juvenile stages. Some larvae in the obtained progenies had unusual light-colored eye lenses. Segregations of "light eye lens : dark eye lens" were determined by observing randomly taken samples of larvae under a dissecting microscope. Segregations of "red eyes : black eyes" were determined by analyzing randomly taken samples of 5–6-month-old juveniles raised in outdoor tanks. Some of the fish in progenies 2–8 raised in outdoor tanks acquired areas of black pigmentation on their bodies at the age of 1.5–2 months. Therefore, along with recording eye color in 5–6-month-old juveniles, the presence or absence of black pigment on fish bodies was also recorded.

In progenies 9 and 10 segregations of juveniles with dark, wild-type, Common Carp body color and light body color (see below) were recorded. To investigate the presence of black pigment melanin in the skin of juveniles with light body color, several fish with this color type were examined under a dissecting microscope.

Observed segregations of fish in progenies were compared with theoretical ratios using a chi-square test (Zar 1999).

RESULTS

Data on segregations of fish with regard to eye color are presented in Tables 1 and 2. In progeny 1, obtained by crossing a black-eyed, white-red female and red-eyed, yellow male, the segregation "red eyes : black eyes" in juveniles did not differ significantly from the 1:1 Mendelian ratio ($P > 0.05$) (Table 1). No fish from progeny 1 had any black (melanin) pigmentation on the body. As an illustration, a photograph of a red-eyed fish from progeny 1 is shown in Figure 1. We observed that red eyes in koi were darker than the eyes of typical albino fish in other fish species. In this case the red color of the eyes was clearly visible only at certain light angles.

In progenies 2 and 3, obtained by crossing red-eyed fish from progeny 1, the ratios of larvae with light and normal dark

TABLE 1. Characteristics of koi parents and segregation of fish in progenies with regard to eye color and black pigmentation of the body.

Progeny number	Phenotype of parents with regard to eye color		Number of larvae analyzed	Segregation of larvae (%)		Number of juveniles analyzed	% of pigmented juveniles	Segregation in nonpigmented juveniles (%)		Segregation in pigmented juveniles (%)	
	Female[a]	Male[b]		Light eye lens	Dark eye lens			Red eyes	Black eyes	Red eyes	Black eyes
1	Black-eyed	Red-eyed				64	0	48.4[c]	51.6[c]	0	0
2	Red-eyed	Red-eyed	102	75.5[d]	24.5[d]	358	52.0	74.4[d]	25.6[d]	40.3[f]	59.7[f]
3	Red-eyed	Red-eyed	115	75.6[d]	24.4[d]	491	28.9	78.8[d]	21.2[d]	26.8[f]	73.2[f]
4	Red-eyed	Black-eyed	69	40.6[c]	59.4[c]	447	62.0	55.9[c]	44.1[c]	15.5[g]	84.5[g]
5	Black-eyed	Red-eyed	90	56.7[c]	43.3[c]	309	52.1	58.1[e]	41.9[e]	16.1[g]	83.9[g]
6	Red-eyed	Black-eyed	78	53.8[c]	46.2[c]	268	34.3	56.3[c]	43.7[c]	19.6[g]	80.4[g]
7	Black-eyed	Black-eyed	103	0	100	285	62.5	0	100	0	100
8	Black-eyed	Black-eyed	132	0	100	364	70.9	0	100	0	100

[a] Females used in crosses for production of progenies 2–8 are fish from progeny 1 without black pigmentation on the body.

[b] Males used in crosses for production of progenies 2–5 and 7–8 are fish from progeny 1 without black pigmentation on the body; for production of progeny 6 a koi male from local stock without black pigmentation on the body was used.

[c] Not significantly different from the 1:1 theoretical ratio at $P > 0.05$.

[d] Not significantly different from the 3:1 theoretical ratio at $P > 0.05$.

[e] Not significantly different from the 1:1 theoretical ratio at $P = 0.0485$.

[f] Significantly different from the 3:1 theoretical ratio at $P < 0.0001$.

[g] Significantly different from the 1:1 theoretical ratio at $P < 0.0001$.

TABLE 2. Results of crosses of red-eyed and black-eyed koi females with Common Carp male with wild-type color.

Progeny number	Phenotype of parents with regard to eye color and body pigmentation: Female	Phenotype of parents with regard to eye color and body pigmentation: Male	Number of larvae analyzed	Segregation of larvae with regard to body pigmentation and eye lens color (%): Unpigmented body and light eye lens	Segregation of larvae with regard to body pigmentation and eye lens color (%): Pigmented body and dark eye lens	Number of juveniles analyzed	Segregation of juveniles with regard to eye color (%): Red eyes	Segregation of juveniles with regard to eye color (%): Black eyes	Segregation of juveniles with regard to body color (%): Light color	Segregation of juveniles with regard to body color (%): Wild-type body color
9	Red-eyed koi without black pigmentation on the body[a]	Black-eyed Common Carp with wild-type pigmented body[b]	117	53.0[c]	47.0[c]	203	0	100	52.7[c]	47.3[c]
10	Black-eyed koi without black pigmentation on the body[b]	Black-eyed Common Carp with wild-type pigmented body[b]	86	0	100	215	0	100	0	100

[a] Fish from progeny 1.

[b] Fish from local stock.

[c] Not significantly different from the 1:1 theoretical ratio at $P > 0.05$.

lenses did not differ significantly ($P > 0.05$) from the 3:1 Mendelian ratio (Table 1). In progenies 4–6, which were obtained by crossing red-eyed koi from progeny 1 with black-eyed koi (from either progeny 1 or local stock), ratios of larvae with light and dark lenses did not differ significantly ($P > 0.05$) from the 1:1 Mendelian ratio. Koi larvae with both light and dark lenses had a light, unpigmented, body color, which is typical for koi. As an illustration, koi larvae with light and dark lenses from progeny 6 are shown in Figure 2. As seen in the photograph, the retinal epithelium (area of eye around lens) was pigmented in larvae with both light and dark lenses. Analysis of juveniles from progenies 2–6 showed that segregations "red eyes : black eyes" were very different for fish with and without black pigmentation on the body. In progenies 2 and 3 segregations "red eyes : black eyes" in unpigmented juveniles did not differ from the 3:1 Mendelian ratio ($P > 0.05$), while in pigmented juveniles segregations were drastically shifted to the prevalence of black-eyed fish resulting in a significant difference from the 3:1 ratio ($P < 0.0001$); proportions of red-eyed fish among juveniles with pigmented bodies in progenies 2 and 3 were 40.3% and 26.8%, respectively. In progenies 4, 5, and 6 segregations "red eyes : black eyes" in unpigmented juveniles did not differ from the 1:1 Mendelian ratio ($P > 0.05$ in progenies 4 and 6 and $P = 0.0485$ in progeny 5). In these three progenies (4–6) segregations "red eyes : black eyes" in pigmented juveniles were drastically shifted towards the prevalence of fish with black eyes, resulting in a significant different from the 1:1 ratio ($P < 0.0001$); proportions of red-eyed fish among pigmented juveniles in these progenies varied from 15.5% (progeny 4) to 19.6% (progeny 6) (Table 1). In progenies 2–6 juveniles with black pigmentation on their bodies had darker red eyes than did unpigmented juveniles from the same progenies.

FIGURE 1. Red-eyed koi female from progeny 1.

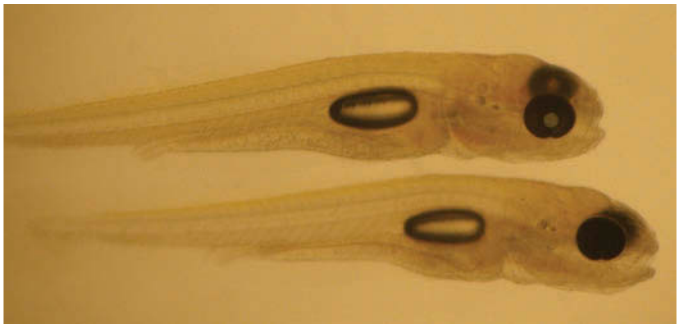

FIGURE 2. Koi larvae from progeny 6 with light (upper larva) and dark (lower larva) eye lens. Note the pigmented retinal epithelium (area of eye around lens) in larva with light lens.

FIGURE 3. Juvenile Common Cap from progeny 9 with dark wild-type (upper juvenile) and light (lower juvenile) body color.

In progenies 7 and 8, which were obtained by crossing black-eyed fish from progeny 1, all analyzed larvae had dark eye lenses and all analyzed juveniles had black eyes (Table 1). Black pigmentation of the bodies was observed in 62.5% and 70.9% of juveniles in progenies 7 and 8, respectively.

In progeny 9, which was obtained by crossing a red-eyed female from progeny 1 with Common Carp with wild-type color, segregation of larvae with light and dark eye lenses did not differ significantly from the 1:1 Mendelian ratio ($P > 0.05$) (Table 2). Larvae with light lenses in this progeny also had light body color, while larvae with black lenses had dark pigmented body color, which is typical for larvae of Common Carp with wild-type color. All juveniles in this progeny had black eyes, and segregation with regard to fish body color was observed. Approximately 53% of juveniles had a light (yellowish) body color while about 47% had the dark body color typical for Common Carp wild-type color; segregation of juveniles with light and dark body color did not differ significantly from the 1:1 Mendelian ratio ($P > 0.05$). As an illustration, photographs of juveniles with light and dark body colors from progeny 9 are presented in Figure 3. Observation of juveniles with light body color under a dissecting microscope showed that these fish had few, mostly contracted melanophores. In progeny 10, which was obtained by crossing black-eyed koi with the same (as in cross for production of progeny 9) wild-type color Common Carp male, all larvae had dark eye lens and all juveniles had black eyes (Table 2); both larvae and juveniles in progeny 10 had the dark body color typical for wild-type Common Carp.

DISCUSSION

The appearance of red eyes in fish (as well as in other animals) is usually connected with the action of albino mutations (Bridges and von Limbach 1972; Rothbard and Wohlfarth 1993). The inheritance of albinism has been analyzed earlier in many aquarium fishes and several aquaculture species including Rainbow Trout *Oncorhynchus mykiss* (Bridges and von Limbach 1972; Nakamura et al. 2001), Channel Catfish *Ictalurus punctatus* (Bondari 1984), and Grass Carp *Ctenopharyngodon idella* (Rothbard and Wohlfarth 1993). In most investigated cases the albinism was controlled by an autosomal recessive mutation; however, both recessive and dominant albino mutations were described in Rainbow Trout (Bridges and von Limbach 1972; Nakamura et al. 2001). Also, in several fish including Rainbow Trout (Dobosz et al. 1999), Goldfish *Carassius auratus* (Yamamoto 1973; Kajishima 1977), and Japanese Medaka *Oryzias latipes* (Yamamoto 1969) an interaction of albino mutations with other color-determining genes was described. As mentioned above, the initial purpose of the present study was to investigate the inheritance of albinism in koi.

In progeny 1, which was obtained by crossing red-eyed and black-eyed koi, the segregation “red eyes : black eyes” did not differ significantly from the 1:1 theoretical ratio. This Mendelian ratio is typical for a monohybrid cross (when one gene is involved) when a heterozygous individual is crossed with a homozygote for the recessive allele. To determine which allele (controlling either red eyes or normal black eyes) is dominant and which is recessive, the results of crossing fish from progeny 1 with the same phenotype (i.e., red-eyed × red-eyed and black-eyed × black-eyed) are decisive. Parental genotypes in both crosses can be determined based on which ratios appear in the resulting progenies. Crossing two heterozygotes will result in the 3:1 Mendelian ratio in the progeny while crossing two homozygotes for the recessive allele will produce only fish with the parental phenotype. As shown above, in progenies 2 and 3, obtained by crossing red-eyed fish from progeny 1, the segregations of larvae with light and dark lenses and of nonpigmented juveniles with red and black eyes did not differ from the 3:1 ratio, while the cross of black-eyed fish from progeny 1 gave only larvae with dark lenses and juveniles with black eyes in progenies 7 and 8. Based on these data and the considerations of Mendelian principles of inheritance presented above, it was suggested that the appearance of red eyes in koi is controlled by a dominant mutation of one gene. Usually the gene for an albino mutation with two alleles is designated as *A/a*; however, as shown below, it is very probable that appearance of red eyes in koi is caused by the action of not albino but another demelanization mutation. Therefore, the gene under investigation was designated as *R*/*r*; koi with genotypes *RR* and *Rr* have red eyes, while koi with genotype *rr* do not possess this trait. In this case, progenies 1 and 4–6, which demonstrated the 1:1 theoretical ratio, resulted from crossing heterozygous (*Rr*) red-eyed fish with black-eyed fish homozygous for the recessive allele (*rr*).

In progenies 2–5 and 7–8, which were produced by crossing fish from progeny 1 without black pigmentation on the body, a large proportion of fish (from 29% to 71%) had black pigmentation on the body; also 34% of pigmented fish were observed in progeny 6, which was produced by crossing red-eyed female fish from progeny 1 with black-eyed, white-red male fish without black pigmentation on the body from local stock. These results were quite unexpected. Up to now, two types of black pigmentation have been described in koi. "Bekko" type of black pigmentation is developed approximately at age 10–14 d and is controlled by a dominant mutation of one gene (*Bl*/*bl*) (Gomelsky et al. 1998; David et al. 2004). "Utsuri" type of black pigmentation develops in the embryo, and hatched larvae are already pigmented (the same as hatched larvae with wild-type color); mechanisms of inheritance of the "Utsuri" type of black pigmentation in koi is still unknown (David et al. 2004; De Kock and Gomelsky 2015). The type of black pigmentation in koi that was observed in the present study differed from both of these known types of pigmentation. As mentioned above, fish started acquiring this type of pigmentation at age 1.5–2 months, much later than the typical timing of appearance of Bekko and Utsuri types of pigmentation. Also, the pigmented fish found in progenies 2–8 were obtained by crosses of unpigmented koi. This is not typical for the types of black pigmentation previously described in koi. It can be suggested that the genotype of the yellow male with red eyes, which was used for production of progeny 1, contained recessive mutations for black pigmentation. These mutations were not expressed in fish of progeny 1 but caused the appearance of black pigmentation in fish in the next generation, in progenies obtained by crossing the fish from progeny 1. Further studies are needed for a more detailed description and analysis of inheritance of this newly described type of black pigmentation in koi.

In progenies 2–6 the segregations of red-eyed and black-eyed nonpigmented juveniles did not differ significantly from corresponding Mendelian ratios (3:1 or 1:1) and coincided with the segregations of larvae with light and dark lenses recorded in these progenies. However, segregations of eye color in pigmented juveniles from these progenies were drastically shifted towards the prevalence of fish with black eyes. This shows that the presence of genes, which caused black pigmentation in the fish body, influenced expression of the investigated mutation *R*. The action of these genes resulted in the appearance of black pigmentation not only in the body but also in the eyes, and so many fish in these progenies acquired the black eye color. As mentioned above, pigmented juveniles had darker red eyes than nonpigmented fish. Apparently some amount of melanin accumulated in the eyes of these fish, but the eyes still looked dark red (not black).

Analysis of variability of eye and body pigmentation of fish in progeny 9, which was obtained by crossing a red-eyed female from progeny 1 with a Common Carp having wild-type color, provided the possibility of investigating the influence of genes controlling wild-type color pigmentation on the expression of the investigated mutation *R*. It is known that wild-type pigmentation in Common Carp is controlled by dominant alleles of two duplicated genes (B_1/b_1 and B_2/b_2); the presence of one dominant allele at any gene in the fish genotype results in the appearance of wild-type color (Katasonov 1978; De Kock and Gomelsky 2015). Koi do not have this type of pigmentation since they are homozygous for the recessive alleles at both genes (genotype $b_1b_1b_2b_2$). All fish in progeny 10, which was obtained by crossing a wild-type Common Carp male with a black-eyed unpigmented koi female, had wild-type color. This shows that this male was homozygous for the dominant allele at least at one gene (genotype B_1B_1– or –B_2B_2). When this male (genotype *rrBB*, only one of the duplicated genes is shown for simplicity) was crossed with the red-eyed koi female (genotype *Rrbb*), the resulting progeny 9 would be expected to consist of fish with genotypes *rrBb* and *RrBb* with the 1:1 theoretical ratio. As described above, about 50% of the larvae in this progeny had dark bodies and dark eye lenses, and about 50% of the juveniles had black eyes and wild-type dark color; it is obvious that fish of this group had genotype *rrBb*. The other 50% of fish in this progeny with genotype *RrBb* had unpigmented bodies and light eye lenses at the larval stage and black eyes and light yellowish color at the juvenile stage. This shows that the presence of dominant alleles of genes controlling the wild-type color in Common Carp genotype significantly influences the expression of the investigated mutation *R*. At the larval stage mutation *R* causes the appearance of larvae with unpigmented bodies and light eye lenses. However, genes controlling wild-type color in Common Carp induce the production of some amount of melanin in body skin and eyes. As a result, juveniles with genotype *RrBb* have a light body color because of the reduced amount of melanin in the skin and black eyes. This shows that the investigated mutation *R* causes the appearance of red eyes only in koi; in Common Carp with wild-type color, this mutation causes only partial demelanization of the fish's body.

As previously mentioned, interaction of albino mutations with other color-determining genes has been described in several fish species. In all these cases, some color morphs with an intermediate (between the wild-type color and albino) level of melanin development appeared. Dominant epistasis was described for the interaction of albino genes with other pigmentation-causing genes in Goldfish (Yamamoto 1973; Kajishima 1977) and Rainbow Trout (Dobosz et al. 1999), while recessive epistasis was described for gene interaction in Japanese Medaka (Yamamoto 1969). In F_2 progenies obtained by crossing fish heterozygous for two interacting genes, the 12:3:1 or 9:3:4 ratios (wild-type color : intermediate type : albino) were observed in cases of dominant or recessive epistasis, respectively, which are modifications of the 9:3:3:1 classical Mendelian ratio for a dihybrid cross (i.e., when two genes are involved). In the present study, fish with intermediate development of melanin between wild-type color and red-eyed fish without black pigment on the body were also observed. However, results of the present study and examples of interactions of albino mutations with other genes described previously differ in large extent with regard to inheritance and expression of mutations. Expression of mutation *R* in koi was influenced not by one gene but by different genes (by both genes of black pigmentation on the koi body and genes controlling wild-type color). Also, interactions between albino mutations and other genes are observed only in cases of some specific allelic variability of interacted genes; in other cases in the same species albinism is determined by one gene only (as a monohybrid trait) with wild-type and albino alleles. In contrast, results of the present study show that mutation *R* is not allelic to genes B_1/b_1 and B_2/b_2 that determine wild-type color. Finally, in the present study red-eyed fish with black pigmentation on the body have been observed; this type of fish has not been described before in studies on the interaction of albino mutations with other genes in other species.

Based on the occurrence of fish with red eyes and black pigmentation on the body it can be suggested that the appearance of red eyes in koi is caused not by an albino but by another demelanization mutation, which decreases the quantity of melanin to some extent in both the skin and eyes but not completely. This type of mutation has been described in fish before. Shimada et al. (2002) described about 20 mutations that decreased melanin content in the eyes and skin of Japanese Medaka; usually there was a correlation between the reduction of melanin in the skin and eyes, but the degree of reduction varied from mutation to mutation. Streisinger et al. (1986) described the mutation *gol-2* (golden) in Zebrafish *Danio rerio* (also known as the Zebra Danio). The retina in larvae having this mutation were pigmented (brown) in contrast to the completely unpigmented eyes in albino (mutation *alb-1*) larvae; adult Zebrafish with mutation *gol-2* had deep ruby-red eyes in contrast to light red eyes in albino fish. The mutation causing red eyes in koi investigated in the present study has some similarity in its expression with mutation *gol-2* in Zebrafish. As mentioned above, red eyes in koi were darker than red eyes of albino fish in other species. Also, koi larvae with light eye lenses had dark, pigmented, retinal epithelium.

Further studies will be aimed at a more detailed investigation of inheritance and expression of the mutation controlling the appearance of red eyes in koi.

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