Color Inheritance in Ornamental (Koi) Carp (Cyprinus Carpio L.)

Prepared by

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Abstract

Koi are an ornamental variety developed from the common carp which was cultivated domestically for food. While Koi and common carp belong to the same species, they differ significantly in both qualitative and quantitative traits. There are many classes of Koi that are differentiated by color, color combinations, and patterns, finnage (standard or long fin), scalation (full scale, mirror, linear, or leather), reflective scale types (ginrin types or absence thereof), and body sheen (metallic or non-metallic). Most of the color variants have emerged from Japan, beginning in the early 1900's in Niigata, and after World War II, refinement and popularity has increased worldwide. Koi are classified and judged in Koi shows based on their phenotype (expressed) characteristics rather than on their genotype. Many of the color variants have been developed using crossbreeding and artificial segregation to refine these varieties.

Koi have high fecundity and there are few published studies on Koi genotypes for color inheritance. Koi breeding historically was highly competitive, with breeding secrets being closely guarded within families, severe culling, and inbreeding. Many of these variants appear to be the result of a few genes (both recessive and dominant) but with high variability.

This paper describes various studies conducted on color phenotypes in Koi and colored common carp.

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1 Introduction

Koi are an ornamental strain of common carp (Cyprinus Carpio) and differ widely in color and hues, color pattern types and orientation, scale types and fin length. All of these traits are independently inherited and contribute to the visible appearance and varying degrees of attractiveness of the Koi. Koi that are entered in shows are judged holistically based on several factors including body conformation, color, pattern, and scalation based on their variety. The expression of a trait is known as the phenotype. Although the development of Koi began several hundred years ago by breeders in Niigata, Japan, there have been few studies that document the underlying genotypes of these fish. These different color studies on common and ornamental carp have been conducted in various countries including Russia, Israel, Poland, China, Germany, Hungary, Japan, Indonesia and the United States. These studies describe the Koi parents and offspring colors often independent of Japanese naming conventions (i.e., Kohaku, Showa, Sanke, Asagi, Magoi), with generic descriptions and often without photographs. Typically, studies done on colored common carp were performed to understand the inheritance of certain colors, many which were deemed unfavorable/undesirable. The pigmentation either was not attractive enough to be sold as ornamental fish, customers preferred certain colors for consumption, or the fish exhibited slower growth (weight and size) and/or survival rates. Fish farmers used the results of these studies to eliminate the colored individuals from breeding stocks.

Color, a qualitative morphological trait, is inherited according to Mendel's principles and is determined by a relatively low number of genes.[1] It is suggested that the environment (i.e., temperature, PH, hardness, water quality, nutrition, stress, sunlight, etc) can influence or enhance how well these colors are expressed[2] and that coloration in some particular varieties of Koi stabilize at different ages of maturation. Nutrients are required to produce certain proteins and for ornamental fish kept in artificial ponds, some plant (spirulina) and other carotenoids are supplemented in fish feed to enhance body coloration.

Koi are available in at least 15 colors[3] including black, blue, red, yellow, orange, white and green color markings as solid colors as well as color combinations and patterns. Color inheritance has

[1] Reference 7 (Gomelsky) p. 37
[2] Reference 8 (Gustiano) p. 87
[3] Reference 7 (Gomelsky) p. 47

high variability in Koi since most varieties were established by crossbreeding. Progenies of the same color parental Koi contain many different color types and established color type varieties.[4] Although Koi have high fecundity, low percentages of offspring exhibit all the characteristics necessary to become valuable, show champions.

2 Principles of Color Development and Inheritance

2.1 Koi Karyotype, Chromosomes, Loci, and Alleles

Chromosomes are found within the nucleus of every cell and are composed of linear deoxyribonucleic acid (DNA) molecules.[5] DNA is comprised of extensive chains of base pairs supported by sugars and phosphorous and is the molecule storing genetic information in fish.[6] Within the DNA molecule are hereditary complex elements called genes. There are only four compounds (adenine, cytosine, guanine, and thymine) found in the sequence of base pairs.[7]These compounds occur only in paired states and these states can only pair with only one other. The physical location of genetic chemical instructions for the production of a protein occurs at the locus of the chromosome[8] as depicted in Figure 1. These alternate forms of the same gene, known as alleles, produce different forms of that gene's protein.[9]

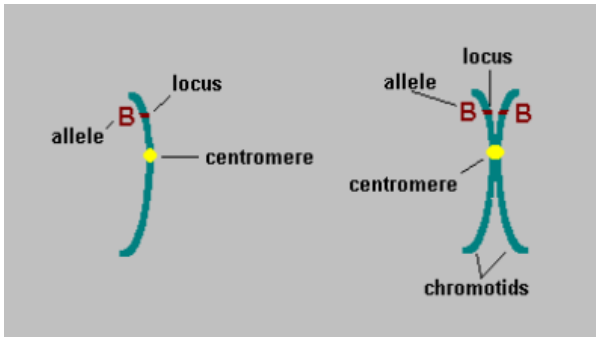


Figure 1 Chromosome Illustration[10]

[4] Reference 12 (Lutz), p. 20
[5] Reference 10 (Klug), p. 6
[6] Reference 12 (Lutz), p. 5
[7] Ibid, p. 5
[8] Reference 16 (FAO)
[9] Ibid (FAO internet article)
[10] Reference 19 (KSU internet article)

A diploid individual can possess only one or two alleles at a given locus. When both sets of instructions are the same for a particular locus, the fish is homozygous for that allele at that locus. An allele can be either dominant or recessive homozygous. If there are two distinct alleles present on the two chromosomes including the locus (one on the chromosome and another on the homolog), the fish is heterozygous. In this case, one or both allele may be expressed.[11]A trait such as color is easily distinguished as an observable characteristic of a heterozygote with two distinct alleles. In some cases, the phenotype does not distinguish the underlying genotype as homozygote or heterozygote. In the case where the observable condition does not distinguish between homozygous and heterozygous, the allele is said to be dominant since it masks the presence of the allele in the phenotype. [12]

Typical fish karyotypes have between 42 and 60 chromosomes, and most of the fish in the Cyprinidae family have between 48 and 50 chromosomes with the exception of common carp and goldfish with about 100 chromosomes.[13]The specific number of chromosomes is referred to as the diploid number (2n) and these occur in pairs. One chromosome set is maternal, the other set is paternal. The number of different types of chromosomes in a diploid species is half the diploid number, called the haploid number (n). [14]

The genes that exist in primary gametocytes are important for reproduction purposes since these cells develop into the gametes (sperm and eggs) via a process called meiosis that carry the genes of future offspring. Replication of chromosomes occurs early in meiosis as the paternal and maternal homologues replicate and then come together to form a pair.[15] Although mutations are rare during the replication process, because Koi have high fecundity (a female has hundreds of thousands of eggs, a male can produce even higher numbers of sperm), each parent is almost assured of producing a few gametes that contain one or more mutations which increases genetic variability.[16]Reduction of the chromosome number from the diploid state to the haploid state in primary oocytes and primary spermatocytes prevents doubling of chromosomes each generation

[11] Reference 16 (FAO)
[12] Reference 20 (FAO)
[13] Reference 7 (Gomelsky) p. 10
[14] Reference 16 (FAO)
[15] ibid
[16] Ibid

since the diploid state is restored when the egg is fertilized with the sperm occurs in Meiosis I and in Meiosis II.[17]The chromatids are separated as depicted in Figure 2.

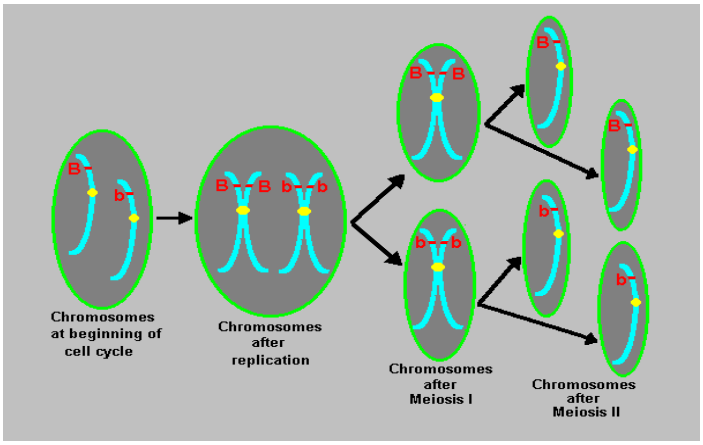


Figure 2 Meiosis Steps and Products[18]

Since the number of Koi chromosomes is double the number of other members of the Cyprinidae family, DNA content is higher and a little more than half the enzymes are expressed in duplicates[19], so common carp and goldfish are considered to be tetraploids (4n), having undergone this genome duplication between 16 and 58 million years ago.[20] The genome is thought to have undergone allo-tetraploidization based on the studies of Ohno et al., in 1967.[21]DNA markers, such as microsatellites and Amplified Fragment Length Polymorphism (AFLP), are being developed to study cyprinus carpio L and map the genome.[22]

2.2 Types of Chromatophores

Fish skin color is determined by the combination of color pigment-containing cells called chromatophores.[23] There are 6 different types of pigment cells[24], five of which can be found in Koi. Melanophores contain melanin (black pigment), xanthophores contain yellow pigment (carotenoid which is a pteridine), erythrophores (similar to xanthophores) contain predominantly

[17] Reference 16 (FAO)
[18] Reference 19 (KSU)
[19] Reference 3 (David) p. 8
[20] Ibid, p.9
[21] Ibid, p.9
[22] Ibid, p.7
[23] Reference 7 (Gomelsky) p. 37
[24] Reference 21 (Kelsh)

red pigment, leucophores contain white pigment and iridophores contain crystals of colorless pigment guanine/purine which reflects and refracts light giving Koi their metallic appearance.[25]The sixth type of cell pigment is called cyanophores (electric blue pigments) which are revealed in very few fish species.[26]The blue and green colors exhibited in Koi are the result of chromatic interactions between melanophores, xanthophores, and reflective iridophores.[27]

Today's colorful Koi are descendants of the wild-type common carp, a dark blackish/brown colored carp. The melanophores and erythrophores combined to give Koi cryptic coloration which allowed them to be less visible to predators in the wild. Lighter color Koi were less likely to pass on their genes to offspring because they are more attractive to predators.[28]Mutations of genes which control the synthesis of pigments or the structure and distribution of pigment cells are responsible for the hereditary variability in Koi. Breeding these mutations in captivity to other Koi and cross breeding with other oddities has produced many color morphs Koi enthusiasts have come to appreciate.

2.3 Gene Interaction Types and Color Expression

Qualitative phenotypes are divided into two categories, autosomal and sex-linked.[29] Autosomal genes are inherited and are expressed equally for males and females. Sex-linked genes are also inherited but are expressed differently in males and females. Genes that are linked to sex chromosomes are common in ornamental fish (e.g., the platy, guppy) but none yet have been found in Koi. Most qualitative phenotypes that have been discovered in cultured food fish are autosomal.[30]

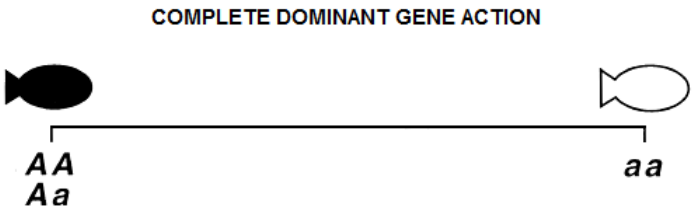
There are several interactions of genes that result in color variability and its inheritance such as complementary gene action, recessive epitasis, dominant epitasis, and the action of duplicate genes in the case of melanin inheritance.[31]

[25] Reference 8 (Gustiano) p. 88
[26] Reference 21 (Kelsh)
[27] Reference 23 (Svensson) p. 300
[28] Reference 22 (Fletcher) p. 25
[29] Reference 16 (FAO)
[30] ibid
[31] Reference 7 (Gomelsky) p. 38

2.3.1 Color Inheritance Controlled by Single Autosomal Genes

2.3.1.1 Complete Dominant Gene Action

Complete dominant gene action, as depicted in Figure 3, the allele is so strong, that the phenotype color is reflective of the dominant gene. In the case of common carp, most of the color genes are recessive mutations and are expressed in the phenotype when they are recessive homozygous.[32]



Phenotype	Genotype
Dominant	Homozygous dominant
Dominant	Heterozygous
Recessive	Homozygous recessive

Species	Recessive phenotype	Dominant phenotype
Common carp	blue	normal pigmentation
	gold	normal pigmentation
	grey	normal pigmentation
	normal pigmentation	light yellow band on dorsal fin; yellow on head

Figure 3 Complete Dominant Gene Action[33]

2.3.1.2 Incomplete Dominant Gene Action

Incomplete dominance is the result of a dominant gene which is not strong enough to completely suppress the recessive allele in the heterozygous state, so that each of the 3 genotypes has a corresponding phenotype.[34] The dominant gene is only expressed when the fish inherits the dominant gene from each parent as depicted in Figure 4.[35]Only one observance of incomplete dominant gene action has been revealed in carp, the light colored gene (L), which is briefly discussed in section 3.1.7.

[32] Reference 16 (FAO)
[33] Ibid
[34] ibid
[35] ibid

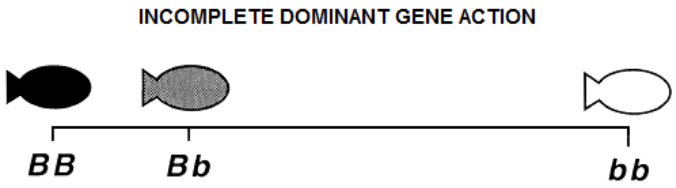


Figure 4 Incomplete Dominant Gene Action[36]

2.3.1.3 Additive Gene Action

Additive gene action differs from incomplete dominant gene action in that the resulting phenotype is intermediate between the dominant homozygote and the recessive homozygote and has not been revealed to date in carp.[37]This interaction between genes has been revealed in only rainbow trout. The mutant gene allele (G') induces a reduction of melanin producing a variant body color midway between the wild type dominant color and the recessive golden color (albino trout but with black eye pigment).[38]

2.2.2 Color Inheritance Control by Two Autosomal Genes - Albinism

Albinism, which is the absence of black pigment both in the skin and eyes, is due to an autosomal recessive mutation and is simply inherited.[39] The black pigment melanin has a photo protective effect because it absorbs UV irradiation and protects skin, eyes and body from damage. Albinos are more sensitive to visible light since they lack this pigmentation.[40]

The synthesis of melanin from the amino acid tyrosine is a complex metabolic process and melanin production is under control of several genes.[41]Mutation of any of these genes can lead to the termination of melanin synthesis and appearance of albino animals could be the mutation of one gene or several genes. The albino mutant gene can interact with other color modifying genes and in this case, color is determined according to the rules of the dihybrid cross.[42]

Katasonov (1978) studied melanin in wild type carp and determined it is controlled by 2 dominant duplicate genes B_1 and B_2.[43]When wild-type color carp were crossed with carp with

[36] Ibid (FAO)
[37] Ibid (FAO)
[38] Reference (Gomelsky) p. 41-42
[39] Reference 12 (Lutz) p. 25
[40] Reference 7 (Gomelsky), p. 41
[41] Ibid, p. 40
[42] Ibid. p. 40
[43] Ibid, p. 47

color, the F1 progenies were all of the wild type color. When the F1 offspring were crossed with each other, the F2 progeny had a ratio of 15:1 (wild:color).[44]The akame (red-eyed) Kigoi is the result of a double recessive mutation with a matte, non-metallic lemon yellow body color.

3 Color Inheritance Studies in Common Carp and Koi

The inheritance of color in Koi is still under investigation, and many Koi hobbyists have relied on the genealogy of the colored Koi lineage assembled by Mr. Masanaga Kataoka for the best insight into the bloodlines of the modern day varieties (reference Figure 5). The history of Nishikigoi is full of stories, myths and folklore and establishing a factual history of true crosses may never be accomplished.

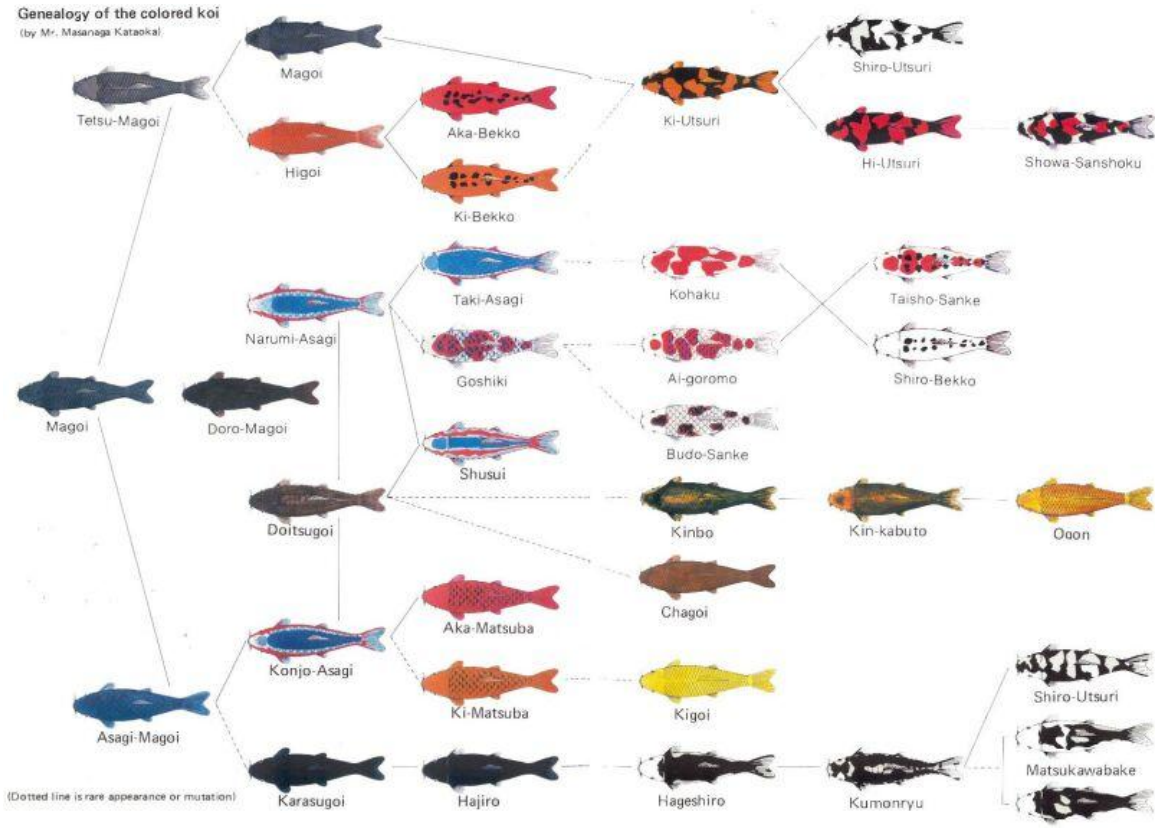


Figure 5 Genealogy of Colored Koi[45]

[44] Ibid, p. 47, 50
[45] Reference 24 (AKA)

3.1 Color Pigmentation and Pattern Inheritance Studies

Pigment cell studies were performed on red, yellow, white, green and blue color morphs of common carp by Gustiano et al., in Indonesia believed to have been introduced from China and green carp introduced from Europe.[46]Other studies of pigmentation and color patterns have been performed on carp and Koi in various countries. As a result of pigmentation studies carried out by Bagnara and Hadley (1973), color polymorphisms are a result of various combinations of several types of pigments to produce the colorful carp phenotypes.[47]Chemical analyses indicated xanthophores (yellow) and erythrophores (red) in carp have the same pigment content called carotenoid.

3.1.1 Orange Color

Bialowqs (2004) performed a study on the inheritance of orange pigmentation in common carp in Poland and determined that two pairs of alleles at two gene loci determined fish pigmentation and that carp with orange pigmentation were double recessive homozygotes.[48]

Katasonov (1974, 1978) performed studies on the orange Japanese carp and concluded that crossing parents heterozygous in the genes B_1 and B_2 ($B_1b_1B_2b_2$) resulted in only one-sixteenth of the progeny with orange pigmentation.[49, 50]At larval stage, the double homozygotes were transparent and did not contain black pigment cells but later isolated pigmented regions appeared on their bodies resulting in carp becoming spotted, black and orange ("spotty").[51]The two dominant duplicate[52] $B_1$and B_2 genes (which generate the common green body background) are epistatic to R_1 (red) and R_2 (yellow) genes.[53]

Results of orange colored (Koi) carp in Israel studied by Wohlfart/Rothbard (1991) and Cherfas, et al., (1992) matched the genetic inheritance model proposed by Katosonov.[54]

[46] Reference 8 (Gustiano) p. 86
[47] Ibid, p. 88
[48] Reference 1 (Bialowqs) p. 147
[49] Reference 9 (Kirpichnikov) p. 62
[50] Reference 7 (Gomelsky) p. 47
[51] Reference 9 (Kirpichnikov) p. 62
[52] Reference 7 (Gomelsky) p. 50
[53] Reference 8 (Gustiano) p. 89
[54] Ibid, p. 89

3.1.2 Blue Color

Studies were conducted in Poland, Israel and German on blue carp. Results by Wlodek (1963) on Polish carp indicated the F2 generation inheritance was 3:1 (nonblue:blue) with approximately 20.3-23.8% exhibiting the blue coloration. Studies on Israeli carp (bl gene) were similar to the results conducted on Polish carp. For German blue carp the second generation ratio was near the expected 3:1 and 1:1 for Fb (backcross). These studies indicated that the blue gene was an autosomal recessive mutation in all 3 cases.[55]Blue color carp has absence of red (erythrophores) and yellow pigmentation (xanthophores).[56]

3.1.3 Steel Color

Results from studies conducted by Katasonov on Japanese Koi revealed the steel coloration was due to a recessive mutation (gene r) and these colored carp were characterized by a reduced amount of red and yellow pigment cells (xanthophores and erythrophores).[57]

3.1.4 Grey Color

Studies performed on Israeli carp by Wohlfart (1968) revealed the grey pigmentation depends on the presence of one autosomal recessive gene (gr) and was not linked to gold (g gene) or blue (bl gene).[58]

3.1.5 White Color

Katosonov (1978) suggested that white color may be a due to the recessive b_1 and b_2 genes together with the recessive gene (r) of blue color to yield a white colored Koi ($b_1b_1b_2b_2rr$)[59] White carp appeared to be missing orange/yellow (xanthophores) and black pigmentation (melanophores).[60]

3.1.6 Green Color

Indonesian common green colored carp exhibited B_1 and B_2 genes (wild-type alleles). The green color morph had four types of pigment cells.[61]

[55] Reference 18 (FAO)
[56] Reference 8 (Gustiano) p.88
[57] Reference 9 (Kirpichnikov)
[58] ibid
[59] Reference (Gustiano) p. 89
[60] Reference 18 (Kirpichnikov)
[61] Reference (Gustiano) p. 89

3.1.7 Light Colored Carp (L)

Due to a dominant mutation, carp, homozygous with respect to the mutant gene (LL), die either at the larvae stage or early development period, and no living fish with this genotype are found.[62]Fish that are heterozygous (Ll) to this gene survive but have lower viability to the recessive homozygous (ll) and are a lighter colored carp.[63] The gene L in a heterozygous state has a pronounced pleiotrophic action resulting in carp with other morphological differences including longer pectoral fins, and larger head dimensions.[64]

3.1.8 Patterned (D for Design)

A dominant mutation "Domas" is associated with a light-yellow pattern (a stripe on the back and ornamental pattern on the head).[65] The autosomal D and d gene pair was responsible for presence and absence of specific pattern on the body of the fish (Katosonov 1973). The gene D is pleiotropic similar to the gene L and carp with such a pattern, both homozygous and heterozygous (DD and Dd) are reasonably viable but exhibit morphological differences such as wild type body shape but with larger heads.[66]Crossing the heterozygote yields a 3:1 (pattern: no pattern) with respect to the trait.[67]

3.2 Study of Color Inheritance of Oujiang Color Common Carp-China

These pigmentation tests were performed on colored common carp which were "red, red with large black spots, red with small black spots, white, and white with big black spots" as shown in Figure 6 from China. Fifteen breeding pairs were selected of the same description and the F1 offspring were examined once they obtained 15-20 cm and had stable pigmentation.[68]These

[62] Reference 9 (Kirpichnikov) p. 63
[63] ibid
[64] ibid
[65] Reference (Gustiano) p. 87
[66] ibid
[67] ibid
[68] Reference 11 (Li) p. 59

Figure 6 Oujiang China Colored Common Carp[69]

Results (reference Figure 7) indicated that the F1 progenies produced by crossing like pairs of solid colored parents (red x red, white x white) were nearly 100% aligned with the parents and the same colored patterns produced almost a 3:1 ratio of parent pattern: no pattern. Only the pairing of the red color with small black spot parents (mating #2) resulted in a progeny of a patterned carp which appeared to produce the black spotted carp with different black coverage (small and big), which would support the suggestion of Gomelsky that the black pattern (Bekko) is highly variable. There were no crosses performed with non-like phenotypes or F1 crosses performed. The results of the whole red parents are similar to studies performed on Koi by David and Gomelsky. The results of the white parental cross did not appear to match those of studies on Japanese transparent/white Koi yielding almost a 100% white versus ratios typical 1:1 (white: non-white) and most were homozygous to the dominant black pattern (less than 1% in one mating).

[69] Ibid p. 57

Color phenotype in F1 generations from various pigmentation types parents in Oujiang color common carp.

Parents color	Color pattern in F1(%)				
	Offspring color	In mating combination 1	In mating combination 2	In mating combination 3	Mean rate
Whole red	Whole red	99.11	99.76	Occasionally died	99.44
	Red color with big black spots	0.89	0.24		0.56
Red color with big black spots	Whole red	4.45	23.53	37.28	21.75
	Red color with big black spots	95.55	76.47	62.72	78.25
Red color with small black spots	Whole red	24.52	11.22	10.46	15.40
	Red color with big black spots	0	27.55	11.72	13.09
	Red color with small black spots	75.48	61.23	77.82	71.51
Whole white	Whole white	99.21	100	100	99.74
	White color with big black spots	0.79	0	0	0.26
White color with big black spots	Whole white	26.41	4.26	20.98	17.22
	White color with big black spots	73.59	95.74	80.12	82.78

Naga, The ICLARM Quarterly (Vol. 24, Nos. 3 & 4) July-December 2001

Figure 7 Color Phenotype in F1 Progenies of Oujiang Colored Common Carp[70]

3.3 Inheritance of White-Red (Kohaku) Color Combination

Koi hobbyists classify the kohaku as a non-metallic white based fish with a red pattern. It is also one of the earliest hybridized ornamental Koi dating back to 1888 as a cross between a red-headed Magoi with a red-cheeked light Asagi Magoi yielded a sarasa Koi.[71]Several genetic studies have been performed to study the inheritance of the red-white color combination in ornamental Koi.

Early studies (Iwahashi and Tomita, 1980) suggested that the Kohaku is a heterozygote as well as its white offspring and that red progeny were pure breeds.[72] The amount of red in the pattern of the progenies seemed to be proportional to the amount of red exhibited by the parents. Studies by Gomelsky et al., (1996) concluded the white-red complex is polygenic and that the amount of

[70] Ibid p. 59
[71] Reference 25 (AKCA- Jordan)
[72] Reference 2 (David) p. 130

red coverage in offspring is based upon the amount of parental red patches.[73] A follow-up study by Gomelsky and Cherfas et al., (2003) concluded that the inheritance of red areas in Kohaku are controlled by many genes with alleles that either maintain the white color, or allow the appearance of red patches.[74]Novelo and Gomelsky (2009) re-examined the appearance of red patches in Kohaku by analyzing digital photographs using two different software image analysis programs to study the quantitative amount of red in Kohaku progenies obtained from the parents and concluded the red area coverage was highly variable.[75]In 2004, David et al., studied the inheritance of the red color on Israeli carp whose ancestors were imported from Japan or Europe within the past twenty years.[76]This study involved multiple (13) crosses involving white Koi (transparent), all red Koi, and Kohaku. In addition, the amount of red patches was also studied with Kohaku parents selected with body coverage of 1/3, 2/3 and 3/3 as shown in Figure 8 and with parents with red patches assessed at either less than 50% (low) or greater than 50% (high) for the crossings.[77]

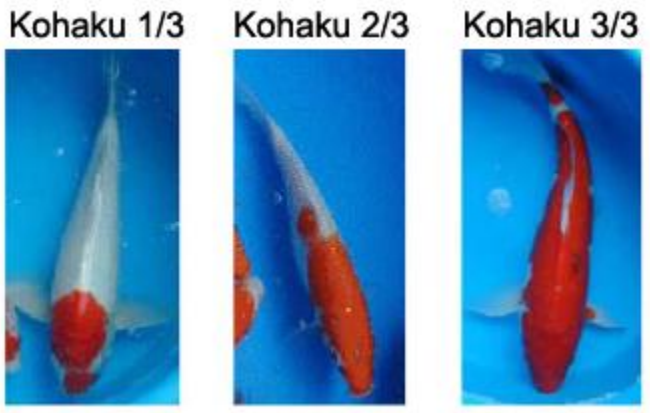


Figure 8 Kohaku parent with red patches used in Study by David 2004

David concluded that the resultant progenies were highly variable among the crosses but that all progenies resulted in only the three phenotypes (transparent (tp)/white, Kohaku, red). Crosses between Kohaku parents yielded some percentage of Kohaku offspring, crosses between solid red Koi yielded all red offspring and crosses between all transparent (white) parents had some offspring with red.[78]These

[73] Reference 26 (Gomelsky) p. 219
[74] Reference 6 (Gomelsky) p. 147
[75] Reference 5 (Novelo/Gomelsky) p. 113
[76] Reference 2 (David) p. 131
[77] Ibid p. 131-132
[78] Reference 2 (David) p. 135

results are shown in Figure 9[79] where n is the number of crosses. In addition, David concluded that the proportion of red offspring increased logarithmically along with the increase of red color in the parents and the proportion of transparent/white Koi fry varied inversely as the square root of the amount of red exhibited by the parents.[80] Although none of the three progeny phenotypes correlated to the degree of red coverage in the parents, there was some parental effects which could be detected, namely the female with two-thirds red coverage produced the highest quantity of Kohaku offspring independent of the male parent and that the male parent with one-third coverage gave the same results when paired with females with one-third and three-thirds red patches in females.[81]Females with low red coverage produced more offspring with transparent/white Koi when compared to the female with high red coverage. David's conclusion was that the red color traits are controlled by a minimum of 3 genes with "inter-locus and intra-locus interactions"[82] and suggested additional studies include crossing F1 transparent Koi obtained from crosses of Kohaku parents to generate F2 progenies were needed.[83]

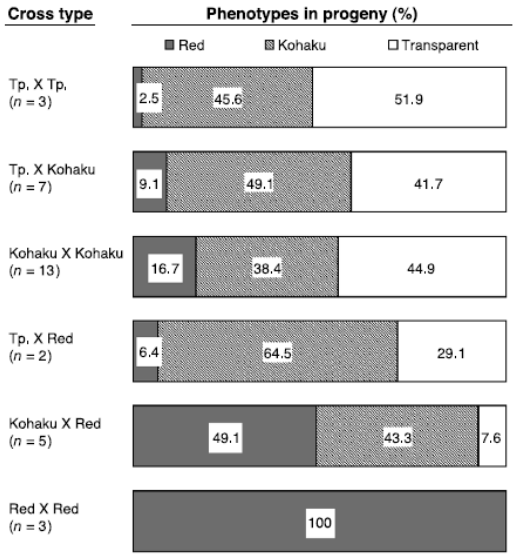


Figure 9 Average Proportions of Red, Kohaku, Transparent Offspring

[79] Ibid p. 136
[80] Reference 3 (David) p. 23
[81] Ibid p. 24
[82] Ibid p. 29
[83] Ibid p. 30

Gomelsky concluded that Kohaku homozygous (blbl) to the dominant gene (Bl) do not have black patches and may be white, red or white-red.[84]

3.4 Inheritance Studies of the Black (Bekko) Pattern

Koi hobbyists view the Bekko pattern as patterns of small black spots arranged attractively on a non-black-based fish. In show quality specimens, this pattern is not located on the head and is typically located on the dorsal half of the body above the lateral line[85] as shown in Figure 10.

Figure 10 Shiro Bekko[86]

A study by Gomelsky in 1998 suggested that the Bekko pattern was the result of a gene (Bl) that has a "dominant effect of the black pattern". [87]Koi larvae with the mutant gene Bl are not born as black fry but instead begin producing melanin at 7-10 days (14 days post hatching) after these larvae transitioned to active feeding.[88,89]

David in 2004 performed a study on offspring of parental Koi with a Bekko pattern (Shiro Bekko, Sanke, and Aka-Sanke). Based on the ratios of offspring (48.7% Bekko offspring with one Bekko parent and 75.9% offspring had a Bekko pattern when both parents had the Bekko pattern),[90]David suggested "that the black Bekko pattern is controlled by a single gene Bl with

[84] Reference 7 (Gomelsky) p.50
[85] Reference 27 (AKCA.org)
[86] Ibid
[87] Reference 7 (Gomelsky) p. 50
[88] Reference 17 (Gomelsky) p. 135
[89] Reference 3 (David) p. 32
[90] Reference 2 (David) p. 137

dominance of the black pattern allele over the non-Bekko allele".[91] If one were to assume the Bekko parents were heterozygote (Blbl) and parents without the Bekko pattern were blbl genotype, then the ratios 3:1 and 1:1 (Bekko:non-Bekko) are consistent with the results of Gomelsky.[92]

Gomelsky concluded the major gene Bl/bl determines the presence or absence of black patches. The interesting observation that most of the fish used in the studies were not homozygous for this gene may be the result of severe culling (fish are unattractive and hence are not selected), or there is some mechanism which has not yet been discovered that prevents reproduction of this trait.[93]When heterozygote Blbl white-red-black Koi (Sanke) are crossed, all 6 color combinations were present in progeny and the ratio of Koi with the Bekko pattern to Koi without the black Bekko pattern was 3:1 suggesting the coverage and amount of black patches is likely under the control of many genes and the same is true for red patches.[94]

However, David's studies of multiple crosses led him to suggest that the Bekko pattern inheritance may be more complicated.[95] David's studies involved 23 crosses which had 17 parental Koi with the Bekko pattern. Examination of the ratios of progenies from these crosses revealed that none of the parents were homozygous (BlBl). David's observation was an "under-representation of the dominant homozygotes in a mature population".[96] Secondly, the amount of offspring exhibiting the Bekko pattern was higher for crosses where both parents had the Bekko pattern versus those with the only one Bekko parent. Because of this observation, it was suggested by David that in some crosses of the Blbl parental Koi, 25% of the Bekko offspring were missing. In the study by Gomelsky, only 1 parent out of 17 had the BlBl homozygote (F1 male parent #6 did not differentiate from the other males exhibited as heterozygote with respect to the black patches).[97]Since at least one parent in the Bekko studies was homozygote, this implies the trait is not lethal but that some selection has occurred to remove a substantial portion

[91] Reference 3 (David) p. 30
[92] ibid
[93] B. Gomelsky email correspondence, November 23, 2011.
[94] Reference 7 (Gomelsky) p. 50, 51
[95] Reference 3 (David) p. 30
[96] Ibid p. 30
[97] Reference 17 (Gomelsky) p.134,138

of these Koi from the genetic pool.[98]All offspring of this male determined to be homozygote (BlBl) with a homozygous (Kohaku) female#2 (blbl) yielded all black pigmented Koi.[99]

Crosses between all transparent/white parents yielded some Bekko F1offspring. This led David to suggest that the Tp gene which may inhibit the red color expression may also inhibit the expression of the Bekko pattern.[100]This theory would suggest that white/transparent Koi may have the dominant Bl allele but not the black color, which could be the result of duplicate gene action similar to studies on zebra fish because it is an ancient tetraploid.[101]Further studies show that Kohaku are recessive homozygotes to one dominate gene for black patches (blbl).[102]David's study confirmed this when no Bekko pattern was expressed in crosses with fish with the red color which confirmed Gomelsky's findings that the red color and Bekko pattern were controlled by different and unlinked loci.[103]

David further suggested that the "Tp locus may be epistatic to both the genes" for the red color and the Bekko pattern.[104] The Shiro Bekko phenotype suggests these Koi do not have "red color loci (R^a and R^b) and not due to the dominant Tp allele."[105] This suggestion could be proven if the transparent F1 offspring of Kohaku parents were crossed. If "there are two separate inhibitor loci, one epistatic to genes controlling the red color (R^a and R^b) and the other to the Bl gene that controls the Bekko pattern, then a study of crossing Shiro Bekko parents would yield red colored offspring."[106]

Further studies using parental Koi with different amounts of the Bekko pattern and studies of those suggested by David should show that inheritance of the gene controlling the black Bekko pattern is independent of the genes controlling other pigments and would confirm Katosonov's

[98] Reference 3 (David) p. 31
[99] Reference 17 (Gomelsky) p.136
[100] Reference 3 (David) p. 31
[101] Reference 2 (David) p. 143, 144
[102] Reference 7 (Gomelsky) p. 50
[103] Reference 3 (David) p. 31
[104] Ibid p. 29, 31
[105] Ibid p.31
[106] ibid p. 29

(1978) conclusions that the black pattern does not appear to be connected with the inheritance of orange and white pigments.[107]

3.5 Inheritance Studies of the Black (Utsuri) Pattern

Koi hobbyists classify the Utsurimono variety as either a white, red or yellow solid color Koi with a wrapping black pattern attractively arranged in a checkerboard pattern from the back of the Koi extending below the lateral line.[108]This Koi must also have black on the head and can have black in the fins[109] and are depicted in Figure 11.[110]High quality Utsurimono should have few scales of black (freckles) to detract from the otherwise beauty of the contrasting base color and wrapping pattern.

Figure 11 Shiro Utsuri[111]

To date, Gomelsky has not studied Koi exhibiting the Utsuri pattern.[112] David performed nine crosses of parents both with the Utsuri pattern and determined that hatched fry that later develop the Utsuri pattern already contain black/dark pigmentation; and are found in low proportion (from 0% to 16%) and in lower quantities than other fry exhibiting black pigmentation at

[107] Reference 8 (Gustiano) p. 89
[108] Reference 28 (AKCA.org)
[109] ibid
[110] Reference 29 (MKPS.org)
[111] Reference 28
[112] Email correspondence with Dr. Gomelsky on 23 November 2011

hatching.[113] David concluded that the genes controlling the Utsuri (wrapping) pattern are different from the genes controlling the Bekko pattern.[114]More studies are needed to study the inheritance of this pattern.

4 Discussion

Ornamental (Koi) carp is available in many colors and patterns. Koi have evolved over a relatively short time period (~200 years) with the domestication of carp in Japan through segregation and cross-breeding, with new phenotypes appearing with higher frequency after the end of World War II and with the increasing worldwide popularity and demand from hobbyists. Koi have high fecundity and many successive generations of careful breeding and culling of offspring with undesirable characteristics in the phenotype are removed (culled) from breeding stock using segregation. Even with artificial segregation, the offspring obtained by crossing highly attractive and valuable Koi still results in high variability. Cyprinus Carpio is thought to have undergone a genome duplication event based on the number of chromosomes somewhere between 11 and 21 million years ago[115]so it is possible that the complex color inheritance may result from duplicate loci that control those traits, specifically those related to the multiple genes that control the amount of red coverage studied in Kohaku crosses and the inheritance of the Utsuri wrapping pattern. Studies performed by Katasonov indicated as few as 5 genes (b1, b2 (wild type/melanin or orange pigment), g (gold), r (blue/white), and gr (grey) were responsible for most of the base pigmentation colors. The absence/presence of the Bekko pattern has been revealed in several studies as the result of a single gene, bl (black). David's hypothesis based on his studies on Koi in Israeli populations is that there is a linkage/mechanism of some type between the genes that control melanin production and genes which control other pigments, specifically with regard to the Tp allele. Other researchers have shown that melanin production is inherited independently. The gene (or genes) for the black Utsuri pattern is different from the gene that controls the Bekko pattern as revealed in the pigmentation studies in Koi larvae. Segregation by breeders can occur as early as 14 days (based on fry pigmentation and morphological abnormalities) and severe culling occurs many times during the first year to reduce the amount of undesirable offspring to eliminate problems resulting from overstocking

[113] Reference 2 (David), p. 139, 143
[114] Reference 2 (David) p. 129
[115] Reference 3 (David) p. 83-84

(i.e., cannibalistic individuals) and water quality issues (i.e., the reduced growth rate, disease). Some complex color patterns do not stabilize until after the Koi has reached sexual maturation (for example, Asagi net pattern matures late compared to other color pigmentation) and some Koi fry, either based on weak color inheritance, environmental conditions, or metabolism will lose coloration as it ages.

5 Conclusion

Genetic studies performed (to date) reveal that in ornamental (Koi) carp (cyprinid carpio L) color is inherited due to autosomal mutations of a few genes resulting in highly complex variations. Most of the different color phenotypes indicate that they are inherited according to Mendelian principles. The inheritance of the black Bekko pattern is the result of a single dominant mutation of a single gene but the amount of spots is highly variable and may be under the control of several genes. The red dorsal pattern in Koi (Kohaku) is also highly variable and appears to be under the control of several genes, proportional to the amount of body coverage in the parents. The black Utsuri pattern is the result of a different gene (or genes) than the gene linked to the small spot (Bekko) pattern.

All of these studies used normal scaled Koi for pairings as some doitsu cross combinations are lethal. One dominant lethal mutation (homozygous LL) for light-colored Koi was discovered by Katasonov but this gene L has not been cited by other researchers for linkage in mortality rates for offspring in other field research. Albino Koi (akame kigoi) are viable and a result of a recessive mutation where this type of Koi is absent black pigment.

Further studies are needed to assess the inheritance of the genes associated with the formation of lateral patterns (e.g., Shusui, Kumonryu, Kikisui), dorsal/step patterns of different colors (besides red and black) as well as the inheritance of acromelanism (temperature dependent pattern) which gives the Kumonyru a dynamic unstable appearance.

6 Glossary

"Adenine (A): A nitrogenous base, one member of the *base pair* A- T (adenine- thymine).

Alleles: Alternative forms of a genetic *locus*; a single allele for each locus is inherited separately from each parent (e.g., at a locus for eye color the allele might result in blue or brown eyes).

Base pair (bp): Two nitrogenous bases (adenine and thymine or guanine and cytosine) held together by weak bonds. Two strands of DNA are held together in the shape of a double helix by the bonds between base pairs.

Chromosomes: The self- replicating genetic structures of cells containing the cellular DNA that bears in its nucleotide sequence the linear array of genes. Eukaryotic genomes consist of a number of chromosomes whose DNA is associated with different kinds of proteins.

Complementary sequences: Nucleic acid base sequences that can form a double- stranded structure by matching base pairs; the complementary sequence to G- T- A- C is C- A- T- G.

Cytosine (C): A nitrogenous base, one member of the *base pair* G- C (guanine and cytosine).

Diploid: A full set of genetic material, consisting of paired chromosomes one chromosome from each parental set. Most animal cells except the gametes have a diploid set of chromosomes

DNA (deoxyribonucleic acid): The molecule that encodes genetic information. DNA is a double- stranded molecule held together by weak bonds between base pairs of nucleotides. The four nucleotides in DNA contain the bases: adenine (A), guanine (G), cytosine (C), and thymine (T). In nature, base pairs form only between A and T and between G and C; thus the base sequence of each single strand can be deduced from that of its partner.

Eukaryote: Cell or organism with membrane- bound, structurally discrete nucleus and other well- developed sub-cellular compartments. Eukaryotes include all organisms except viruses, bacteria, and blue-green algae.

Gene: The fundamental physical and functional unit of heredity. A gene is an ordered sequence of nucleotides located in a particular position on a particular chromosome that encodes a specific functional product (i.e., a protein or *RNA* molecule).

Gene expression: The process by which a gene's coded information is converted into the structures present and operating in the cell. Expressed genes include those that are transcribed into *mRNA* and then translated into protein and those that are transcribed into *RNA* but not translated into protein (e.g., transfer and ribosomal RNAs).

Guanine (G): A nitrogenous base, one member of the base pair G- C (guanine and cytosine).

Haploid: A single set of chromosomes (half the full set of genetic material), present in the egg and sperm cells of animals and in the egg and pollen cells of plants.

Heterozygosity: The presence of different alleles at one or more loci on homologous chromosomes.

Homologous chromosomes: A pair of chromosomes containing the same linear gene sequences, each derived from one parent.

Karyotype: A photomicrograph of an individual's chromosomes arranged in a standard format showing the number, size, and shape of each chromosome type; used in low- resolution physical mapping to correlate gross chromosomal abnormalities with the characteristics of specific diseases.

Locus (pl. loci): The position on a chromosome of a gene or other chromosome marker; also, the DNA at that position. The use of locus is sometimes restricted to mean regions of DNA that are expressed.

Mutation: Any heritable change in DNA sequence.

Nitrogenous base: A nitrogen- containing molecule having the chemical properties of a base.

Nucleic acid: A large molecule composed of nucleotide subunits.

Nucleotide: A subunit of DNA or RNA consisting of a nitrogenous base (adenine, guanine, thymine, or cytosine in DNA; adenine, guanine, uracil, or cytosine in RNA), a phosphate molecule, and a sugar molecule (deoxyribose in DNA and ribose in RNA). Thousands of nucleotides are linked to form a DNA or RNA molecule.

Nucleus: The cellular organelle in eukaryotes that contains the genetic material.

Somatic cells: Any cell in the body except gametes and their precursors.

Thymine (T): A nitrogenous base, one member of the base pair A- T (adenine- thymine)."[116]

[116] Reference 30 – all definitions are verbatim

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