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XANTORIC VARIETY OF RAINBOW TROUT: STUDIES OF INHERITANCE AND BREEDING VALUE

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ABSTRACT. Studies of the inheritance of the atypical Xantoric coloration in rainbow trout reared at the Department of Salmonid Research (DSR) Rutki indicated that this trait is hereditary and controlled by the epistatic interaction of two genes. The dominant allele *A* responsible for Wild (W) coloration and the recessive allele *a* responsible for Xantoric (K) coloration occur at locus *A*. Allele *B* is responsible for Palomino (P) coloration, and allele *b*, in a homozygotic state, determines Albino (A) coloration. Locus *A* is epistatically dominant in relation to locus *B*. The alleles responsible for Palomino and Albino coloration have a negative pleiotropic impact on the growth and survival of trout. Through the application of gynogenesis, the females from which progeny inherit the Mottled (M) traits were identified for the first time. The data cited indicate that the described mutation is hereditary and related to the occurrence of Xantoric coloration.

Key words: ANDROGENESIS, GROWTH, GYNOGENESIS, HEREDITARY COLORATION, RAINBOW TROUT, TYPES OF COLORATION

1. INTRODUCTION

Coloration in fish results from the joint action of different hues that occur in chromatophores or pigment cells. Many different kinds of these cells occur in the skin of fish: melanophores control the expression of black pigment, xanthophores – yellow pigment, and erythrophores – red pigment. The coloration and its pattern on the body surface depends on the type, quantity, and positioning of the chromatophores. During the reproduction period, these cells are also regulated hormonally, which influences brighter fish coloration of higher contrast. Other types of pigment cells include iridocytes located in the epidermis that contain guanine crystals and influence the occurrence of variable white and blue colors.

In nature, mutated specimens occur in which pigment cells have either partially or totally disappeared. Specimens in which dark pigment cells (melanophores) have par-

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tially disappeared and the yellow pigment cells (xanthophores) have developed become golden colored. Fish with such coloration or those which are white are often albinistic and have red eyes that result from a lack of melanophores in the iris. The result of underdeveloped guanine crystals in fish skin is the occurrence of the color blue (alampia) (Kirpichnikov 1981).

Under natural conditions, individuals colored differently from that of the wild population are immediately noticed by predators and have a minimal chance of survival. As populations develop under controlled cultivation conditions, individuals with skin color that differs from that of wild specimens appear quite quickly. This results from the lack of natural predation and the consequential easy survival of these individuals.

The occurrence of varieties with atypical coloration has been confirmed in many species of cultivated fish. A high degree of heterogeneity has been confirmed in carp, *Cyprinus carpio* L., a fish that was domesticated thousands of years ago. The following types of coloration have been described in carp: golden (Moav and Wohlfarth 1968), blue (Wlodek 1963), red (Steffens 1958), and steel (Katasonow 1978). Currently, the most heterogeneous variety among colorful fish is the Koi carp. This name encompasses approximately 100 varieties of Japanese colorful carp which were created through crossbreeding initiated in Japan over a century ago that has incorporated various cultivated lines from the world over. Koi originates from domesticated carp and occupies the same systematic position. The colors that occur among Koi are red, white, pale pink, gold, yellow, orange, rarely blue or green, and a mixture of these colors. Individuals that are single colored or a mottle of two or more colors occur sporadically (Katasonov 1974, McDowall 1989, Wohlfarth and Rothbard 1991, Hilble and Langfeldt-Feldmann 2001).

The story of Crucian carp, which has been cultivated in China for more than a thousand years, is similar especially with regard to its ornamental form – the gold fish, *Carassius auratus auratus* (L.), of which there are many varieties with different colors and shapes (described in detail by Kirpichnikov 1981). In the 1990s cultivation techniques for many new species were developed, and color mutations have been noted in many of them. These species include, among others, rainbow trout (Clark 1970, Wright 1972, Bridges and Limbach 1972, Kincaid 1975, Kohlmann and Fredrich 1986, Dobosz et al. 1999, Goryczko and Dobosz 2004, Blanc et al. 2006), common dace, *Leuciscus leuciscus* (L.) (Witkowski et al. 1997), tench, *Tinca tinca* (L.) (Flajshans and

Kvasnicka 1997), channel catfish, *Ictalurus punctatus* (Rafinesque) (Bondari 1984), grass carp, *Ctenopharyngodon idella* (Valenciennes) (Tay et al. 1985), common whitefish, *Coregonus lavaretus* (L.), and grayling, *Thymallus thymallus* (L.) (personal observations).

Rainbow trout, a fish native to North America, was domesticated over a century ago. The ease with which the rainbow trout adapts to various environmental conditions has allowed for the rapid development and spread of its cultivation throughout the world. Rainbow trout belongs to the family Salmonidae, and until 1988 it was classified to the genus *Salmo* with the species name of *gairdneri*. Based on genetic research (Berg and Farris 1984, Thomas et al. 1986, Gyllensten and Wilson 1987), the American Fisheries Society in 1988 classified the rainbow trout to the genus *Oncorhynchus*, which led to the species name being changed to *mykiss* (Kendall 1988). Currently, the rainbow trout is cultivated as a consumer fish on all the continents using waters that do not exceed a temperature of 25°C (Goryczko 2005). The widespread cultivation of rainbow trout and the ease with which these fish can be observed in every stage of their development and growth permits noting the occurrence of individuals with coloration that differs from that of wild fish. Clark (1970) citing Beall (1963) described the history of the first cultivated trout that exhibited a non-wild (yellow) coloration in the state of Virginia. These trout initiated the so-called Golden line. These fish originated from a mottled female with wild-yellow coloration. Crossbreeding golden trout with those of wild coloration produced palomino fish (dark blond) (Wright 1972). Bridges and Limbach (1972) described a form of albinistic rainbow trout which lacked pigment in their irises and had white coloration in the juvenile stage and yellow at older stages. Kincaid (1975) first described trout with blue coloration that occurred in cultivated strains in the USA. Reports of mottled rainbow trout with wild-yellow coloration were made by Clark (1970), Maliszewski (1987), and Galbreath and Plemmons (2000). Over a period of about twenty years of rainbow trout cultivation at the Department of Salmonid Research in Rutki (DSR), mutant individuals were observed with palomino, albino, white, blue, cobalt (metallic) and mottled (wild-yellow and yellow-wild) colors (Goryczko and Dobosz 2004).

The immense phenotype plasticity of rainbow trout as well as the development of cultivation techniques resulted in the increased economic significance of and scientific interest in this species. Research into implementing new study methods and genetic

manipulation in rainbow trout has been and continues to be conducted. Many attempts have also been undertaken to evaluate the mechanisms of inheritance in mutant individuals (Wright 1972, Klupp and Kaufmann 1979, Kohlmann and Fredrich 1986, Dobosz et al. 1999, Galbreath and Plemmons 2000) as well as the relationship between coloration and the cultivation traits of rainbow trout (Clark 1970, Wright 1972, Kohlmann and Fredrich 1986, Dobosz et al. 2000, Siwicki et al. 2003). Yellow coloration in rainbow trout was used as a natural marker in genetic manipulations at the embryo stage (Chourrout 1982, Goryczko et al. 1991, Babiak et al. 2002) as well as a marker of the reference line, in order to estimate the impact of environmental factors between basins, during estimations of the growth of fish of various origins (Dobosz et al. 1995).

Due to the great popularity among anglers of golden and palomino trout, substantial numbers of these strains have been produced since the 1980s in the American states of Pennsylvania and Virginia with the goal of stocking open waters (Tave 1988). Golden trout are used at Rutki as natural markers when conducting various types of experiments. The sale of adult specimens of these colorations was begun in the early twenty-first century with the aim of making special sport fishing grounds (closed waters) more attractive to anglers.

The first results of crossbreeding as well as the increasing interest in golden trout at DSR Rutki prompted undertaking research aimed at determining the inheritance mechanisms of different colorations for subsequent generations. Simultaneously, it was determined whether the yellow coloration hereditary mutation had a pleiotropic impact on biological indices (growth rate, survival, age at sexual maturity), in comparison with those of trout with wild coloration.

TABLE 1

Names of coloration types as they appear in the present work and in the publications cited

Name	Abbreviation
Wild	W
Yellow	Y
Xantoric	K
Golden	G
Albino	A
Palomino	P
Mottled	M

The aim of this dissertation is to present a complex description of coloration inheritance and to evaluate the breeding value of rainbow trout with yellow and mottled coloration, in the case of the wild-yellow trout discussed, that have been cultivated at DSR Rutki since 1986.

2. MATERIAL

2.1. SOURCE OF STUDY MATERIAL

The DSR Rutki, which had been designed especially for conducting selective breeding projects, became operational in 1984. In the early 1990s, this facility acquired a system for tagging individuals with PIT (Passive Integrated Transponders) microprocessors. These tags are enclosed in glass capsules 12 mm long and 1 mm wide. This system permitted in 1991 initiating a long-term rainbow trout selective breeding program. The initial population was built from five different strains of rainbow trout with wild coloration that had been brought to Rutki by 1988; these were the Oleśnica, Jastarnia, Saitama (imported from Japan), A13, and Donaldson (imported from Finland, but originally from the USA). The initial population, a diallelic cross of the five introduced strains known at the outbred stock (Dobosz et al. 1992), provided the foundation the 100 families (half-sibs randomly chosen from two females and one male) of the first selection generation created in 1991 (Dobosz et al. 1995). The next generation of 100 selected families was created in the same way by crossing randomly (sets of 10×10) chosen fish (20 females and 10 males) from ten of the best families of the preceding generation. The choice of the best families as well as the best individuals in the families was conducted based on breeding traits such as growth rate, survival, fecundity, body shape, wild skin coloration, and the spring spawning period. The fifth selection generation was created in 2003. Simultaneously, the five strains introduced at Rutki were bred 'cleanly' for three generations as conservation stocks.

A few individuals with yellow coloration were noted among the progeny of the two indigenous strains (Oleśnica and Jastarnia). A few individuals with yellow and, rarely, single individuals with mottled coloration were also noted among the progeny of the outbred stock that was built. In subsequent selection generations, families in which yellow individuals occurred were eliminated. This permitted, beginning with the third generation, obtaining a trout population characterized solely by wild coloration, further

referred to as Wild (Wild coloration). In 1986, Ryszard Maliszewski delivered to the then Laboratory of Salmonid Research Rutki 10,000 eyed-egg stage yellow trout embryos that he had produced. These fish were the product of a mottled female (dark patches on a yellow background) and a male with brown coloration (Maliszewski 1987) and are further referred to as the Yellow strain. The first attempt to breed adult Yellow fish at Rutki indicated that this trait is inherited by the progeny.

Throughout further rearing different forms of Yellow coloration occurred. The coloration of these fish ranged from shades of yellow to dark blonde, and these fish are further referred to as Xantoric (Xantoric coloration). This name comes from the name of pigment cells responsible for the color yellow in fish skin. Further observations of the specially cultivated Xantoric trout of the Yellow strain that originated from eggs imported in 1986 and Xantoric fish chosen from among the first selection generation and the Jastarnia and Oleśnica strains, indicated that two distinct groups of Xantoric coloration occurred. One group was comprised of fish with coloration that ranged from pale yellow to orange with red irises. These fish were similar to those described in the literature as albino and are further referred to as Albino (Albino coloration). The second group was comprised of fish with coloration that ranged from light brown to dark blonde with dark colored irises. These fish were similar to those described in the literature as palomino and are further referred to as Palomino (Palomino coloration). In the initial stages of the research the interpretation of the inheritance of these traits in the trout at Rutki was controversial in comparison with that presented in the literature regarding albino, golden, and palomino coloration in trout. The coloration of single mottled specimens that occurred in cultivated populations was highly varied. Among adult fish there were individuals of Wild coloration with patches of Xantoric coloration and individuals of Xantoric coloration with patches of Wild coloration. There were also individuals with very tiny patches of Wild and Xantoric coloration of various shades. All of the fish with these colorations were referred to as Mottled (Mottled coloration).

Using the preliminary observations of fish with Xantoric and Mottled coloration (Wild-Xantoric), it was attempted to determine the reason for and the manner in which this coloration variety was inherited.

3. RESEARCH METHODS

3.1. CROSSBREEDING FISH WITHIN EXPERIMENTAL FAMILIES

In order to describe how Xantoric coloration is inherited and the type of inheritance occurring in rainbow trout, many attempts were made to cross trout with Xantoric, Mottled, and Wild coloration. Crossbreeding was based on mating two genetically different individuals with the goal of obtaining hybrids or progeny originating from the joining of two genetically different gametes.

The fish were crossbred according to a variety of methods and forms:

Crossbreeding methods:

- a) Outcrossing – crossing individuals originating from various strains of rainbow trout from the DSR that did not have common ancestors for at least 10 generations;
- b) Crossbreeding within strains – parent fish were of the same strain;
- c) Crossbreeding within families – crossbreeding method (F_1) that is applied to investigate the spread of traits in the second generation (F_2).

Form of crossbreeding:

- a) simple crossbreeding – a single crossbreeding of two chosen individuals;
- b) multiple crossbreeding – crossbreeding a cross again with an individual of known and desired coloration traits;
- c) reverse crossbreeding – crossbreeding specimens of various coloration but with an inverse sex combination.

3.2. GENOME MANIPULATION AND SEX CONTROL

Research into the engineering of fish genomes developed substantially in the last decade of the twentieth century, and was based primarily on manipulating the course of meiotic division as well as the first mitotic division in the embryo. Meiotic gynogenesis, mitotic gynogenesis, and androgenesis were applied in experiments designed to determine the mechanism of Xantoric coloration inheritance. These methods permitted obtaining either highly inbred progeny with genotypes from just one parent or those that were homozygotic at all genetic loci (Łuczynski et al. 2003).

Meiotic gynogenesis is induced by fertilizing fish eggs with semen in which the genetic material has been destroyed by irradiation (UV, gamma, X). The thus fertilized eggs are then subjected to either a physical or chemical shock that retains the second

polar body during the second meiotic division in the egg. The result is the creation of a diploid zygote in which the genetic material comes only from the mother. In fish species whose sex is determined by the chromosome system responsible for sex (i.e., XX-female, XY-male), the gametes of females are identical in the sex chromosomes they contain, thus all of the gynogenotes obtained will be females. Allendorf and Leary (1984) and Allendorf et al. (1986) reported that the mean inbred coefficient for progeny thus obtained was approximately 44% (for all loci); for given loci it can range from 0% to 100% (depending on the location of a given locus in relation to the centromere).

Mitotic gynogenesis is induced by applying an environmental shock to stop one of the first mitotic divisions of the newly created zygote with a haploid number of chromosomes. A haploid number of chromosomes results from fertilization with irradiated fish milt in which the genetic material in the sperm has been destroyed. Two haploid nuclei of the dividing zygote join together; this is how the missing set of chromosomes is built, which is a replica of the haploid set of chromosomes of the egg cell. This is how fully inbred individuals that have only maternal genetic material are obtained. All of the gynogenotes obtained this way, as is the case with meiotic gynogenesis, are females.

Androgenesis is induced by applying an environmental shock to stop one of the first divisions of the newly created zygote with a haploid number of chromosomes from the father. This requires destroying the genetic material in the egg cells with gamma- or X-radiation. As with mitotic gynogenesis, the diploid individuals obtained are fully inbred, although the genetic materials in the nucleus come only from the father. Since trout sex depends on the presence of the sex chromosomes *X* or *Y* in the male haploid chromosome set (contained in the sperm), the androgenetic individuals obtained were either females or males at the same sex ratio of 1:1. In this case, the sex genotype of all the males is *YY*.

In the studies described in the present work, ultraviolet (UV) irradiation was applied according Goryczko et al. (1991) to destroy, or deactivate, the genomes in the sperm used to induce gynogenesis. This method requires diluting milt forty-fold and mixing it with a magnetic mixing stick and then irradiating it with a $2075 \mu\text{W cm}^{-2}$ dose of UV. Egg inactivation for androgenesis was done with a cobalt bomb; the eggs were radiated with a 35 kilorads of gamma rays (Babiak et al. 2002). Until the end of the 1990s, meiotic gynogenesis was induced with thermal shock; this method of retaining the second polar body was performed by submerging eggs in 28°C water for 10 mins. The eggs were fertilized and held at a constant temperature of 10°C until

the application of the thermal shock 40 mins later (Goryczko et al. 1991). In 2000 the DSR acquired a device for performing pressure shocks that are more effective than thermal shocks. This procedure is performed by placing the eggs and water in a special chamber and then subjecting them to pressure of approximately 7000 psi for about 4 mins (Chourrout 1984). Thanks to this device, in addition to meiotic gynogenesis, it is also possible to perform mitotic gynogenesis and androgenesis. Applying the pressure method described above in the 350 mins following fertilization is most effective for these methods of genetic manipulation at the embryonic stage (Ihssen et al. 1990).

Changes in sex phenotype were achieved by subjecting XX female genotype fry to hormonal treatment with methyltestosterone delivered in the feed in a quantity of 3 mg kg⁻¹ for 60 days (Bieniarz et al. 1991); this was done in order to obtain phenotypic male gynogenotes.

3.3. REARING FISH AND DETERMINING BIOLOGICAL TRAITS

The experimental fish were subjected to artificial spawning. Incubation was performed in flow-through incubators with a capacity of 5000 eggs, or in a California-type incubators with chambers that can accommodate from 400 to 1000 eggs, depending on study requirements. Incubation was conducted in spring water with a temperature of 7 to 8°C. The alevins, fry, and older experimental fish were held in plastic basins with a surface area of 1 m² or 2 m² and a capacity of 300 or 1000 l water or in concrete basins with a surface area of 3 m² and a capacity of 2500 l water, depending on the number, weight, and age of the fish. The basins were supplied with surface waters from the Radunia River that ranged in temperature from 0 to 21°C (depending on the season of the year).

The experimental fish were fed pellet rainbow trout feed. The optimal daily feed ration (From and Rasmussen 1984) was calculated with the computer program Djournal Production Program v.3. Depending on necessity, the fingerlings were tagged with individual PITs (Passive Integrated Transponder, FishEagle). These tags are injected to the abdominal cavity of the fish with a syringe and have a neutral impact on their development and survival (Dębowski et al. 1998a, Buzby and Deegan 1999). The experimental fish were inspected annually in either spring or fall to determine the number of fish in the various coloration groups and to determine the mass, sex, and degree of sexual maturity.

Immunological tests were done to determine the nonspecific cellular immune response potential based on the metabolism and phagocytic activity of macrophages as well as on lymphocyte activity evaluated by their proliferation response following mitogen stimulation. The cells used in the immunological studies were isolated from the kidney head or the spleen following centrifugation in Gradisol (Polfa) solution.

The metabolic activity of macrophages was determined on the degree of respiratory burst activity (RBA) after the cells were stimulated with phorbol myristate acetate (PMA- Sigma) according to the method described by Secombes (1990). The phagocytic activity of the macrophages was evaluated by determining the potential killing activity (PKA) using the microcolorimetric method described in Siwicki et al. (1996). Lymphatic activity was determined based on the lymphocyte proliferative response to mitogen stimulation with Con A (concanavaline A, Sigma) and LPS (Lipopolisaccharide, Sigma) according to the method described by Mosmann (1983).

3.4. ANALYZING THE IMAGE OF MOTTLED FISH AND THE STATISTICAL ANALYSIS OF BIOLOGICAL TRAITS

Images of the Mottled coloration in gynogenotes were analyzed using Multiscan v 4.2 by Computer Scanning Systems Ltd. (Warsaw, Poland). Comparisons were made by analyzing images obtained by scanning photographs (Dębowski et al. 1998b). They were made using a dorsal segment from each fish that comprised a square as wide as the width between the eyes and that extended to the line that determined width at the base of the dorsal fin. Since the fish differed in size, the measurements were standardized. In order to eliminate changes in the natural coloration that could have occurred when making the pictures, the images were analyzed on a 255 degree grayscale. The results obtained were processed into histograms of each image and used to determine the pigment saturation within five ranges of the grayscale: 0-50; 51-100; 101-150; 151-200; 201-255. The uniformity of the Mottled coloration pattern of the gynogenetic fish examined was analyzed based on the similarity (correlation) between the shades of gray in the histograms of individuals.

Statistica (StatSoftTM, Tulsa, USA) was used for statistical calculations. The uniformity between the assumed coloration inheritance hypothesis and the number of fish in the various coloration groups was analyzed with the chi-squares test (χ^2). Analysis of variance (ANOVA) was applied to perform the statistical analysis of the mean weight

data of fish in various coloration groups, and significant differences between mean weights were compared with either Student's t-test or Duncan's multiple range test. Two levels of significance (0.05 – significant difference; 0.01 – highly significant difference) were designated to verify the hypothesis.

4. RESULTS AND ELEMENTS OF THEIR DISCUSSION

4.1. XANTORIC VARIETY

4.1.1. INHERITANCE OF XANTORIC COLORATION TRAITS

The first attempts to crossbreed adult individuals within the Yellow strain (specially imported Maliszewski strain) resulted in all of the progeny having Xantoric coloration traits. After fish from the Jastarnia and Oleśnica strains that exhibited Xantoric coloration attained sexual maturity, a series of crossbreeding experiments were conducted within the three strains. The progeny obtained from these crossbreedings were all of Xantoric coloration. Subsequently, attempts were made to crossbreed individuals of Xantoric and Wild coloration within and between strains (Table 2).

TABLE 2

Crossbreeding of Wild and Yellow individuals from strains in which Yellow coloration occurred and the coloration of their progeny

Strain/coloration Female	Strain/coloration Male	Coloration of the progeny
Yellow/Xantoric	Yellow/Xantoric	Xantoric
Jastarnia/Xantoric	Jastarnia/Xantoric	Xantoric
Oleśnica/Xantoric	Oleśnica/Xantoric	Xantoric
Jastarnia/Xantoric	Yellow/Xantoric	Xantoric
Oleśnica/Xantoric	Yellow/Xantoric	Xantoric
Yellow/Xantoric	Jastarnia/Wild	Wild/Xantoric
Yellow/Xantoric	Oleśnica/Wild	Wild
Jastarnia/Wild	Yellow/Xantoric	Wild
Jastarnia/Wild	Oleśnica/Xantoric	Wild
Jastarnia/Wild	Oleśnica/Wild	Wild/Xantoric
Jastarnia/Wild	Jastarnia/Wild	Wild/Xantoric
Oleśnica/Wild	Jastarnia/Wild	Wild

At this stage of the experiment the number of progeny in the various coloration groups was not counted. Based on the results of the coloration of progeny, it was hypothesized that Xantoric coloration is an inherited mutation of the gene that controls Wild coloration in rainbow trout. Simultaneously, these results provide evidence that it is a mutation of the same allele in all the strains in which the occurrence of Xantoric fish was confirmed. The allele responsible for Wild coloration is denoted by the symbol A , while that responsible for Xantoric coloration, which based on the results presented in Table 2, should be considered to be recessive in relation to A , is denoted by the symbol a .

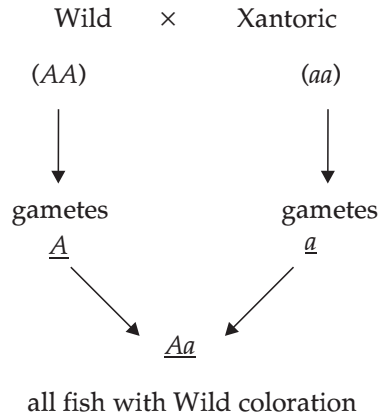
According to this assumption, fish with Wild coloration have an AA genotype, while Xantoric fish have an aa genotype. Fish with the heterozygotic genotype Aa have Wild coloration and pass on the allele for Xantoric coloration to the next generation.

Simultaneously, it was observed that at the eyed egg stage, Xantoric fish have no eye pigment, while the Wild embryos have visible, dark eyes. This trait clearly differentiates Xantoric from Wild fish as early as at this stage of development (Fig. 1).



Fig. 1. Eggs at the eyed stage. On the right, visible eye pigmentation (future Wild coloration fish embryos), on the left, a lack of eye pigment (future Xantoric coloration fish embryos).

The lack of eye pigment in Xantoric fish during embryonic development was exploited to conduct a series of crossbreedings in order to gain an understanding of Xantoric coloration inheritance. The progeny chosen for the experiment were fully Wild in coloration and originated from the cross of a Wild female from the Jastarnia strain with a Xantoric male from the Yellow strain (Table 2). Their progeny should have a coloration genotype of Aa (schematic illustration below).



After these fish had attained sexual maturity, a series of crossbreedings (males and females) were performed with Xantoric individuals of both sexes from the Yellow strain with a known coloration genotype of aa . During the eyed-egg stage the number of specimens with or without dark eye pigmentation were counted. The hypothetical ratio of Wild to Xantoric embryos of 1:1 was evaluated with the χ^2 test (Table 3).

TABLE 3

Number of eyed eggs with or without visible eye pigment that resulted from crossbreedings of various individuals from families with the Wild coloration phenotype and a hypothetical genotype of Aa with individuals from the Yellow strain with a hypothetical genotype of aa

Female - coloration	Male - coloration	Number of eggs		Value χ^2
		With eye pigment	Without eye pigment	
Wild (Aa)	Xantoric (aa)	111	98	0.809
Wild (Aa)	Xantoric (aa)	139	122	1.107
Wild (Aa)	Xantoric (aa)	146	130	0.928
Xantoric (aa)	Wild (Aa)	66	74	0.457
Xantoric (aa)	Wild (Aa)	88	92	0.089
Xantoric (aa)	Wild (Aa)	152	139	0.581

The series of crossbreedings of various individuals from the family of Wild coloration and a heterozygotic genotype (Aa) with individuals from the Yellow strain with Xantoric coloration and a homozygotic genotype (aa) indicated at the eyed-egg stage that all of the progeny groups had individuals with Wild and Xantoric coloration. The numerical ratio of Wild or Xantoric individuals was confirmed with the χ^2 test based on the number of embryos with pigmented eyes (individuals with Wild coloration) and embryos with a lack of eye pigmentation (individuals with Xantoric coloration) in all samples was 1:1 (Table 3). This confirmed the proposed hypothesis regarding the recessiveness of the allele responsible for Xantoric coloration.

The results of the experiment also confirmed that Wild and Xantoric coloration is determined by one gene (locus) and that this trait is passed on faithfully from generation to generation. Two alleles occur in this gene: A – that determines Wild coloration and is dominant with regard to allele a that determines Xantoric coloration. The inheritance and distribution scheme of the Wild and Xantoric coloration traits in the subsequent generations, in accordance with Mendel's first and second laws, is presented in Fig. 2.

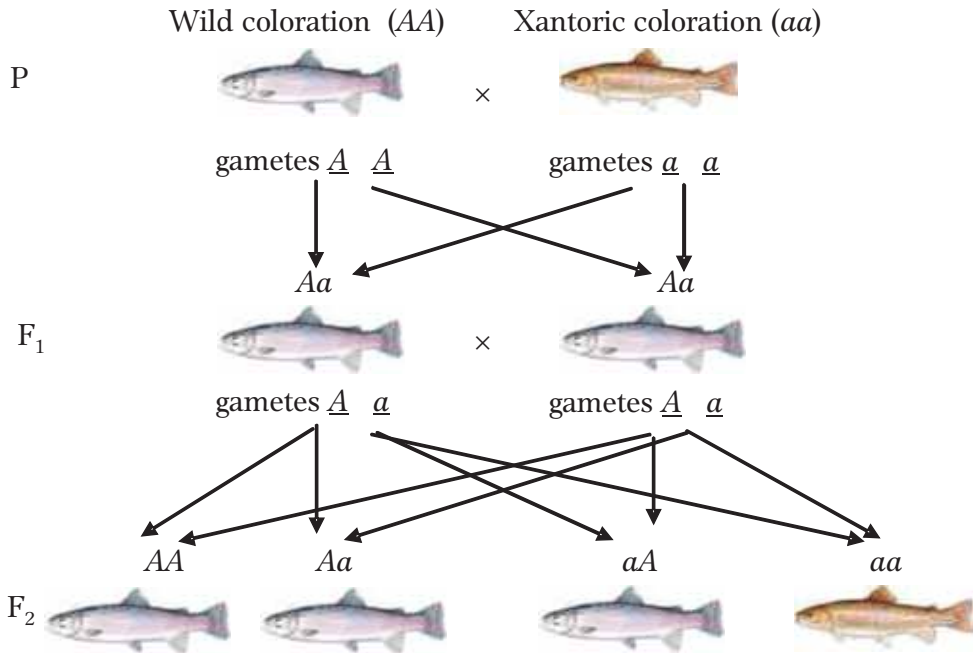


Fig. 2. Inheritance scheme for Wild and Xantoric coloration in progeny in subsequent generations by crossbreeding fish with homozygotic genotypes that determine a given coloration: in the second generation (F_2) the Wild:Xantoric coloration traits segregate as 3:1.

4.1.2. XANTORIC FISH IN EXPERIMENTS CONDUCTED AT DSR RUTKI

Knowing the inheritance mechanism for genes that determine the Xantoric coloration allowed this trait to be used as a marker in genetic experiments conducted on rainbow trout at the DSR Rutki. For the first time, Xantoric females from the Yellow strain were used to optimize the parameters essential for the effective induction of meiotic gynogenesis. A series of experiments were conducted to determine optimal degree of milt dilution and UV irradiation dose (exposure time) needed to eliminate the sperm genome (Goryczko et al. 1991). The eggs from these females were fertilized with milt that had been inactivated with UV irradiation. The genome of the inactivated sperm came from males of the Donaldson strain, in which the occurrence of Xantoric individuals had never been noted. Simultaneously, when eggs from females of the Yellow strain were fertilized with the milt from males of the Donaldson strain, all of the progeny had Wild coloration. Thus, in order to verify the effectiveness of the gynogenesis method, the gynogenetic progeny should have Xantoric coloration. These experiments served to determine the optimal degree of milt dilution and the conditions and exposure time of radiation (Goryczko et al. 1991).

The growth rates and survival of fish with Xantoric and Wild coloration were compared in a second experiment (Dobosz et al. 1995). Two independent evaluations were performed on progeny groups of different origin. In one case these were gynogenetic strains: Yellow and Saitama of Xantoric and Wild coloration, respectively. In the second case, they were the progeny of five females from the Jastarnia strain with Wild coloration and the coloration genotype *Aa* and three males from the Yellow strain with Xantoric coloration and the coloration genotype *aa*. The eggs from the females of the second group were fertilized with the mixed milt of the males. The individuals obtained from this group were of the same origin and the Wild to Xantoric coloration ratio was 1:1. During the first fry rearing period, all of the experimental groups were held in quantities of about 2000 individuals in separate plastic basins with a volume of 1 m³. During the second fingerling rearing period, 300 gynogenetic fish individuals from both coloration groups were chosen at random and stocked into the same types of basins in three replicates and randomly chosen fish originating from crossbreeding (175 individuals from both coloration groups) were stocked into a single basin. The mean fish weight for the initially reared fry in the experimental groups was determined by the overall weight of randomly chosen individuals, while during the next period

weight was determined based on the individual weight of specimens that survived. The fish were fed formulated feed throughout the growth period.

During the first fry rearing period (the first 30 days following the beginning of feeding) the gynogenetic Xantoric fish (Yellow strain) had the lowest growth rate obtaining a mean weight of 0.51 g. This was lower by 27.1% in relation to the other three groups which attained a mean weight of 0.81 g. During this period, no fish deaths were noted in the three experimental groups.

During the second fingerling rearing period (92 days), the gynogenetic Xantoric fish had a significantly lower mean weight (11.9%) and a highly significantly lower survival rate in comparison to the gynogenetic fish with Wild coloration (Saitama strain). Xantoric fish originating from the crossbreeding of two strains (Jastarnia, Yellow) exhibited during the fingerling stage an 8.1% significantly higher mean weight at identical survival rates in comparison to fish with Wild coloration and originating from the same crossbreeding (Table 4).

TABLE 4

Mean initial and final body weight, and survival of gynogenetic Xantoric fish from the Yellow strain and gynogenetic Wild fish from the Saitama strain and Xantoric and Wild fish from crossbreeding the Yellow and Jastarnia strains in second fingerling rearing period

Origin of fish	Initial body weight (g)	Final body weight (g)	Survival (%)
Gynogenetic Xantoric	0.59	25.9 ± 9.52*	22.0**
Gynogenetic Wild	0.81	29.4 ± 12.54*	71.5**
Xantoric	0.81	40.0 ± 9.85*	82.9
Wild	0.81	37.0 ± 9.43*	82.3

* - significant ($P < 0.05$) and ** - highly significant ($P < 0.01$) differences in survival attained by mean weight, estimated separately within the gynogenetic and crossbred fish

Only gynogenetic individuals of various coloration were subjected to further evaluation of growth rate and survival. After 120 gynogenetic Xantoric fish from the Yellow strain and gynogenetic Wild fish from the Saitama strain had been tagged with PITs, they were placed in a concrete basin with a water volume of 2.5 m³. After six months of rearing when the fish had attained individual market weights ranging from 300-500 g, the weight of each tagged fish was evaluated individually. The growth rate of the gynogenetic individuals with Xantoric coloration was highly significantly lower in comparison to that of the gynogenetic individuals with Wild coloration. During tagging,

the mean weight of the Wild gynogenetic fish was significantly higher by 12.5% than the mean weight of the gynogenetic Xantoric fish, and following six months of rearing this difference was highly significant having risen to 28.4%. The survival of the tagged fish during the rearing period was 52.5% and 60.5% for the Wild and Xantoric individuals, respectively, and was significantly higher in the latter.

4.2. FORMS OF ALBINO AND PALOMINO COLORATION AMONG XANTORIC FISH

Two distinct types of coloration were noted among the Xantoric fish in the first year of life during the fingerling stage (fish weight of at least 20 g). The first type was characterized by the lack of eye pigment (red) and skin coloration ranging from light to dark yellow. The second type had dark eye color and skin color that ranged from dark orange to light brown. Among Xantoric fish, individuals of the first type were described as Albino fish (Albino coloration), while the second were described as Palomino fish (Palomino coloration).

Observations conducted over a two-year period of fish with Xantoric coloration during embryonic development and the fry stage indicated that in the eyed-egg stage these fish lack melanophores in their irises. Xantoric trout alevins also initially exhibited the red eye color that is typical of the Albino form; however, iris pigmentation began to appear in a portion of the Xantoric alevins during the period from the end of yolk sac resorption and the beginning of exogenous feeding (Fig. 3).

4.2.1. INHERITANCE OF PALOMINO AND ALBINO COLORATION TRAITS

At the alevin stage the progeny of females from the Jastarnia strain with Wild coloration and the males from the Yellow strain with Xantoric coloration described previously had Wild and Xantoric coloration at a ratio of 1:1 (Table 3) (Dobosz et al. 1995). In the fingerling stage all of the Xantoric fish were classified as Palomino fish (black eyes and light brown skin color), while the fathers of this progeny had Xantoric coloration from the Albino group (red eye color and light yellow skin color). The lack of Albino fish among the progeny prompted undertaking further crossbreedings aimed at determining the way in which Palomino and Albino coloration are inherited. After reaching sexual maturity, the progeny were crossbred to obtain generation F₂. Six females and six males were chosen, and six families were created by crossbreeding

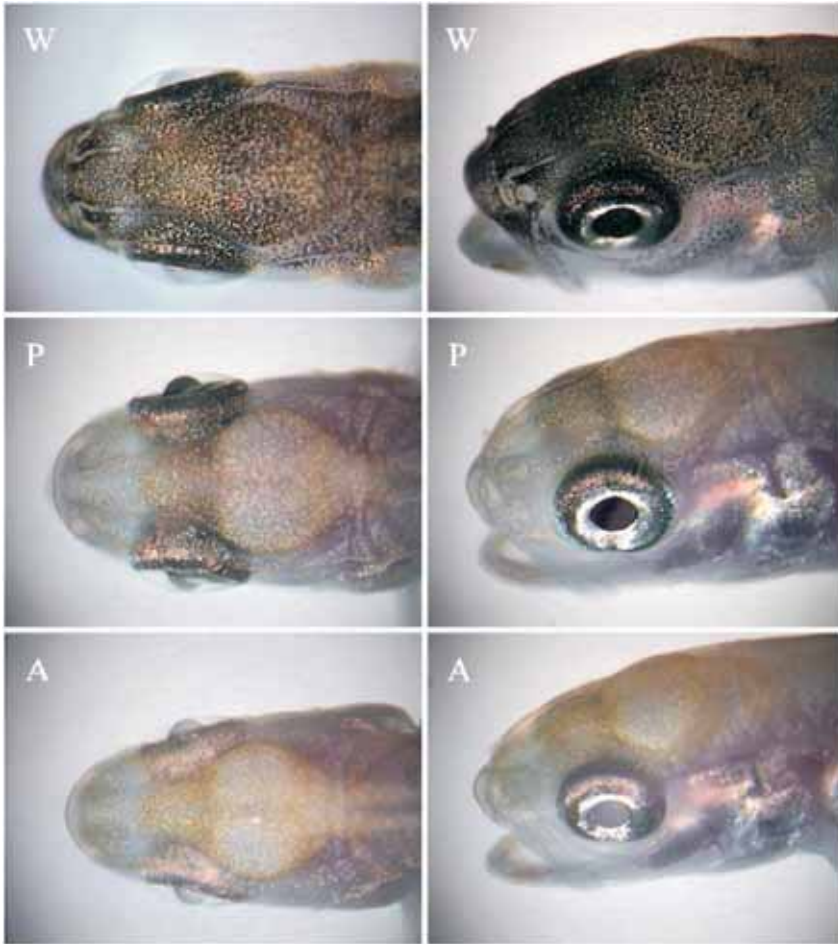


Fig. 3. Forms of skin and eye color in rainbow trout alevins: W – Wild skin coloration and black eye pigment (Wild coloration), P – Xantoric skin coloration and black eye pigmentation (Palomino coloration), A – Xantoric skin coloration and red eye pigment (Albino coloration) (Photo M. Korwin Kossakowski) (after Dobosz et al. 1999).

them. Three of these originated from females with Wild coloration and males with Palomino coloration, while the other three families originated from females with Palomino coloration and males with Wild coloration. During the fingerling stage, the occurrence of Wild, Palomino, and Albino fish among the progeny (F₂) was noted in all the families. The number of fish with Wild, Palomino, and Albino coloration within the families was similar, at a ratio of 4:3:1, respectively. The number of fish of various colorations in

the individual families was subjected to the χ^2 test. In four cases, the χ^2 test confirmed the anticipated 4:3:1 ratio of fish with Wild, Palomino, and Albino coloration. The deviation from the hypothetical number in one family was significant, while in the second it was highly significant (Table 5). These deviations might have been caused by the different survival rates of the fish of different coloration in these families since the fish were evaluated following four months of rearing and until this time a significant number of fish deaths was noted.

TABLE 5

Number of fish with Wild, Palomino, and Albino coloration in the experimental families and the values of the χ^2 test applied to compare the comparability of the number, respectively, with the anticipated ratio of 4:3:1 (after Dobosz et al. 1999)

Parental coloration (Female × Male)	Number of progeny of various colorations (number anticipated)			Value χ^2
	Wild	Palomino	Albino	
1. Palomino × Wild	131 (121)	73 (90)	37 (30)	5.74
2. Palomino × Wild	50 (52)	32 (39)	22 (13)	7.56*
3. Palomino × Wild	201 (183)	127 (137)	38 (46)	3.85
4. Wild × Palomino	104 (106)	78 (79)	30 (27)	0.53
5. Wild × Palomino	136 (106)	58 (79)	18 (27)	17.03**
6. Wild × Palomino	155 (145)	96 (109)	39 (36)	2.39

* $P < 0.05$, ** $P < 0.01$

Assuming that the 4:3:1 ratio of Wild, Palomino, and Albino fish among the progeny of the six families is correct, it can be concluded that skin coloration in rainbow trout is determined by two genetic loci. Allele *A* (Wild coloration), which is dominant with respect to allele *a* (Xantoric coloration), is segregated in one locus while the second locus segregates allele *B* (Palomino coloration), in relation to allele *b* (Albino coloration); however, locus *A* dominates epistatically over locus *B* (Fig. 4).

Since in two of the six cases the hypothesis of the segregation ratio in Wild, Palomino, and Albino of 4:3:1 was disproved (Table 5), more crossbreeding was performed at this stage of the experiment to further verify the hypothetical inheritance model of these three coloration varieties (Fig. 4). Additionally, the appearance of eye pigmentation in Palomino fish during the alevin stage was exploited (Fig. 3). This trait allowed differentiating the Palomino and Albino fish according to phenotype in the early swimming alevin stage, and not, as was the case in the previous experiment, at the fingerling

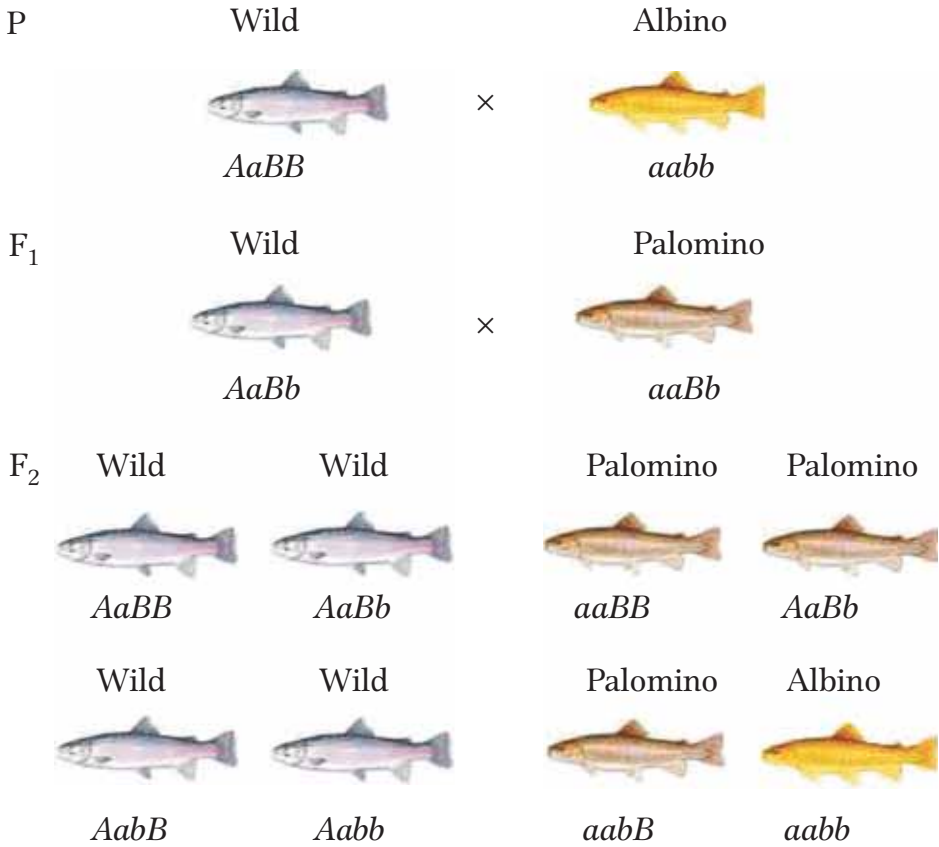


Fig. 4. Schematic illustration of hypothetical phenotypes, genotypes, and allelic segregation in subsequent generations (P, F₁, F₂) from crossbreeding fish of different coloration.

stage. Eggs were obtained from eight gynogenetic females with Albino coloration and a hypothetical genotype of *aabb*. After mixing them, the eggs were divided into nine portions, each of which was fertilized with milt from nine different males (Wild, Palomino, Albino coloration), that were full sibs and originated from Albino females with a hypothetical genotype of *aabb* and from males with Wild coloration and a hypothetical genotype of *AaBb*. Portions 1 to 5 were fertilized with semen from males with Wild coloration (hypothetical genotype *AaBb* or *Aabb*), portions 6, 7, and 8 were fertilized with semen from males with Palomino coloration (hypothetical genotype *aaBb*), portion 9 was fertilized with semen from a male of Albino coloration (hypothetical genotype *aabb*). During the eyed-egg stage the unfertilized eggs and live embryos were counted

in each portion. Among the living embryos, the number with and without eye pigment were counted. All of the embryos fertilized by the Wild males (portions 1-5) either had or did not have eye pigment in the eyed-egg stage. The embryos from all of the Palomino (portions 6-8) and Albino (portion 9) males did not exhibit any eye pigment. These results permit the conclusion that all of the embryos without eye pigment will be of Xantoric coloration. The χ^2 test confirmed agreement between the quantity of embryos with red eyes and the Xantoric coloration inheritance principle (Table 6).

TABLE 6

Survival of embryos in the eyed-egg stage and the number of embryos exhibiting differences in eye pigmentation in the experimental progeny groups (after Dobosz et al. 1999)

Male coloration (hypothetical genotype)	Number of eyed eggs (indiv.)	Embryo survival (%)	Number of embryos with dark eyes (indiv.)	Number of embryos with red eyes (indiv.)	Value χ^2
1. Wild (<i>Aa?b</i>)	1084	84.2	535	549	0.18
2. Wild (<i>Aa?b</i>)	577	45.3	288	289	0.00
3. Wild (<i>Aa?b</i>)	355	28.0	187	168	1.02
4. Wild (<i>Aa?b</i>)	948	79.9	464	484	0.42
5. Wild (<i>Aa?b</i>)	921	78.0	445	476	1.04
6. Palomino (<i>aaBb</i>)	1051	83.4	0	1051	-
7. Palomino (<i>aaBb</i>)	955	77.8	0	955	-
8. Palomino (<i>aaBb</i>)	1018	81.4	0	1018	-
9. Albino (<i>aabb</i>)	439	34.1	0	439	-

*agreement of the number of embryos with dark eyes with those with red eyes at a ratio of 1:1 tested,
 $\chi^2 P_{0.05} = 3.84$

In order to identify the fish of Palomino and Albino coloration among those of Xantoric coloration, the occurrence of eye pigment at the swimming up alevin phase when exogenous feeding began was evaluated. In each group of progeny the fish of Wild and Xantoric coloration were counted; additionally, the fish with dark or red eye color among the Xantoric individuals were also counted.

In progeny groups 1, 2, 3, and 5 a similar number of alevins with Wild coloration and dark eyes and with Xantoric coloration and red eyes (Albino) were confirmed by the χ^2 test at a ratio of 1:1, which indicates that the progeny originated from males with Wild coloration and an *Aabb* genotype. Among the alevins from group 4, individuals were confirmed with Wild coloration and dark eyes, individuals with Xantoric

coloration and dark eyes (Palomino), and individuals with Xantoric coloration and red eyes (Albino) in quantities that had been confirmed by the χ^2 test and in agreement respectively with the ratio 2:1:1. This proves that males with Wild coloration had an *AaBb* genotype. In progeny groups 6-8 that originated from males with Palomino coloration and a hypothetical *aaBb* genotype, nearly equal numbers of alevins occurred with Xantoric coloration and dark eyes (Palomino) and red eyes (Albino) (this was confirmed with the χ^2 test). In group 9, eggs fertilized with milt from a male with Albino coloration and a hypothetical *aabb* genotype had Xantoric coloration and red eyes (Albino) (Table 7).

TABLE 7

Survival from the eyed-egg stage to the swimming up alevin stage and body and eye color in rainbow trout alevins divided into groups originating from a female with Albino coloration and from males of various skin coloration (after Dobosz et al. 1999)

Male coloration (genotype)	Number of alevins (indiv.)	Alevin survival (%)	Number of alevins with Wild coloration (indiv.)	Number of alevins with Xantoric colorations and dark eyes (indiv.)	Number of alevins with Xantoric colorations and red eyes (indiv.)	Value χ^2
1. Wild (<i>Aabb</i>)	924	85.2	462	0	462	0.00
2. Wild (<i>Aabb</i>)	518	89.8	262	0	256	0.07
3. Wild (<i>Aabb</i>)	309	87.0	167	0	142	2.02
4. Wild (<i>AaBb</i>)	840	88.6	412	219	208	0.56
5. Wild (<i>Aabb</i>)	781	84.8	390	0	391	0.01
6. Palomino (<i>aaBb</i>)	948	90.2	0	455	493	1.52
7. Palomino (<i>aaBb</i>)	843	88.3	0	427	416	0.14
8. Palomino (<i>aaBb</i>)	915	89.9	0	450	465	0.25
9. Albino (<i>aabb</i>)	408	92.9	0	0	408	

$\chi^2 P_{0.05} = 3.84; 5.99$ (for either 1 or 2 degrees of freedom, respectively)

The results obtained confirmed the correctness of the inheritance model for Palomino and Albino coloration in rainbow trout (Fig. 4). Allele *A*, which is responsible for Wild coloration, dominated over allele *a* that is responsible for Xantoric coloration (Table 7, groups 1-5). Allele *B* is responsible for Palomino coloration, and its expression depends on the presence of the recessive homozygotes *aa* (Table 7, groups 6-8). Albino coloration is the result of the occurrence of the double recessive homozygotes *aabb* (Table 7, group 9).

4.2.2. COMPARISON OF GROWTH AND SURVIVAL OF FISH WITH PALOMINO, ALBINO, AND WILD COLORATION

Three groups of fish that were half sibs and the progeny of parents with known genotypes were chosen for the experiment (Table 7, groups 1, 4, 7). These families originated from eight females with Albino coloration and the known genotype *aabb*, whose eggs were mixed and then fertilized with milt from different males. The following names were assigned to the families in this experiment:

- WA family (Table 7, group 1) originated from a male with Wild coloration and the known genotype *Aabb* and 50% of the fish had Wild (W) coloration and the known genotype *Aabb* while 50% had Albino (A) coloration with the genotype *aabb*;
- WPA family (Table 7, group 4) originated from a male with Wild coloration and the known genotype *AaBb* and 50% of the fish had Wild (W) coloration and the known genotypes *AaBb* or *Aabb*, 25% of the fish had Palomino (P) coloration and the genotype *aaBb*, and 25% of the fish had Albino (A) coloration and the genotype *aabb*;
- PA family (Table 7, group 7) originated from a male with Palomino coloration and the known genotype *aaBb*; 50% of the fish had Palomino (P) coloration and the genotype *aaBb* while 50% of the fish had Albino (A) coloration and the genotype *aabb*.

The alevins from each family were stocked into separate plastic basins with a water volume of 0.3 m³ where the fish were fed *ad libitum*. After a month of rearing, the fish in each family were divided and counted according to phenotype coloration and simultaneously weighed as a group to determine their mean weight (Table 8).

TABLE 8

Survival from alevins and mean body weight in families and within different phenotype coloration groups of progeny, in three analyzed families following the first month of rearing (after Dobosz et al. 2000)

Families	Fish of different coloration							
	All fish		Wild		Palomino		Albino	
	Survival (%)	Mean body weight (g)	Survival (%)	Mean body weight (g)	Survival (%)	Mean body weight (g)	Survival (%)	Mean body weight (g)
WA	92.9	0.62	94.6	0.65	-	-	91.1	0.59
WPA	89.7	0.54	93.9	0.55	88.6	0.53	82.7	0.52
PA	91.0	0.54	-	-	91.6	0.56	90.4	0.52

“-” no fish in this coloration group

In the first month of rearing, the fish in the Albino group exhibited the lowest mean weight and survival of all the families in relation to all the other coloration groups. Fish with Palomino coloration exhibited a higher mean weight and survival in the PA family as well as median weight and survival in relation to fish with Wild and Albino coloration in the WPA family. The growth rate of fish in coloration groups in the different families was not analyzed statistically, but survival was analyzed with the χ^2 test, which indicated that these differences were not significant.

The fish families were next rejoined and stocked into separate concrete basins with a water volume of 2.5 m³. During this period of the evaluation, the fish were fed with 12-hour automatic feeders. The daily ration was calculated with the DJournal program. Fish losses in the basins were noted. Following the subsequent four and eight months of rearing, the amount of fish in the analyzed families was evaluated and individual weight was determined in phenotype coloration groups.

TABLE 9

Analysis (ANOVA) of the relation between fish skin coloration (Wild – W, Palomino – P, Albino – A) and body weight with the three families (WA, WPA, PA) after 5 and 9 months of rearing (after Dobosz et al. 2000)

Family – fish coloration	Evaluation period (month)	Degree of freedom (Df)	MC impact	Df – error	MC – error	F value
WA	5	1	65146**	580	183.4	355.2
	9	1	231501**	563	797.3	290.4
WPA	5	2	14324**	591	195.3	73.3
	9	2	50575**	538	845.6	59.8
PA	5	1	10816**	535	147,3	73.4
	9	1	38943**	508	734.7	53.0

** – highly significant ($P < 0.001$) relation between coloration and body weight

The individual evaluation of fish after 5 and 9 months of rearing from the beginning of exogenous feeding indicated a highly significant relation between coloration and fish growth in the studied families (Table 9). The estimation of the F value following 5 and 9 months of fish growth in all the families indicated that this relationship weakens in older fish. The difference in the mean weight attained by fish of different coloration, at both five and nine months of rearing, was highly significant in all the families (Table 10).

TABLE 10

Survival, mean weight, and coefficient of variation of fish with different body coloration in three families during evaluation after 5 and 9 months of rearing from alevin to fingerling stage (after Dobosz et al. 2000)

Family	Month	Fish coloration								
		Wild			Palomino			Albino		
		Survival (%)	Mean body weight (g)	CV (%)	Survival (%)	Mean body weight (g)	CV (%)	Survival (%)	Mean body weight (g)	CV (%)
WA	5	74.7**	44.5±15.6	35.2	-	-	-	51.3**	23.0±9.6	42.1
	9	71.6**	88.7±31.9	36.0	-	-	-	50.6**	47.8±22.0	46.3
WPA	5	80.8**	43.5±16.2	47.0	67.8**	24.0±11.3	47.0	54.3**	17.5±9.2	54.3
	9	73.3**	73.0±31.4	43.0	61.2**	53.6±26.1	48.8	50.5**	39.1±25.4	65.0
PA	5	-	-	-	69.6*	32.9±12.8	39.0	57.7*	23.8±11.2	47.2
	9	-	-	-	64.6	67.5±27.7	41.0	56.3	50.0±26.4	52.9

*significant ($P < 0.05$) and ** highly significant ($P < 0.01$) differences in survival of various coloration phenotypes among families

Differences between mean fish weight and different phenotype colorations among the studied families were highly significant

CV - coefficient of variation

“-” no fish in a given coloration group

Fish with Albino coloration exhibited the lowest growth rates in all of the families. Those with Palomino coloration grew faster than those with Albino coloration in the PA family and medial with fish of Wild and Albino coloration in the WPA family. The slowest growth rate among Albino fish was correlated with the highest coefficient of variance (CV) for body weight. The growth of fish with Palomino coloration was medially linked to the body weight coefficient of variance. Fish with Wild coloration were the fastest growing and had the lowest coefficient of variance for body weight (Table 9). After five months of evaluation, differences in survival among the fish phenotype coloration groups were highly significant ($P < 0.01$) within the families WA and WPA and significant ($P < 0.05$) in the PA family. After nine months of evaluation the differences remained highly significant in the WA and WPA families, while in the PA family the differences in the survival of fish with Palomino and Albino coloration became insignificant. Fish with Albino coloration exhibited the lowest survival rates in all of the families following both five and nine months of rearing. Fish with Palomino coloration exhibited higher survival than those of Albino coloration in the PA family and medial between fish with Wild and Albino coloration in the WPA family (Table 9). The estimated feed coefficient (weight of feed consumed/weight of fish growth) in all the fami-

lies following nine months of evaluation was similar at 0.79 in the WPA and WA families and 0.80 in the PA family.

4.2.3. NONSPECIFIC IMMUNITY IN FISH OF VARIOUS COLORATION

The fish used in this experiment came from the WPA family. They were in their second year of rearing and weighed between 180 to 200 g. Blood samples were drawn from ten individuals from each coloration group (Wild – genotype *AaBb* or *Aabb*; Palomino – genotype *aaBb*; Albino – genotype *aabb*). The samples were used to evaluate the link between coloration and the activity of blood macrophages and lymphocytes (Siwicki et al. 2003). The results obtained indicated a statistically significantly ($P < 0.05$) higher value for all the studied traits in fish with Wild coloration in comparison with fish with Palomino and Albino coloration. Fish with Albino coloration had the lowest values, but they were statistically insignificant in comparison with the fish with Palomino coloration (Table 11). These studies confirm that there is a link between Wild and Xantoric coloration and intracellular immunity. Fish with Wild coloration are characterized by higher unspecific immunity.

TABLE 11

Respiratory burst activity (RBA) and potential killing activity (PKA) of blood phagocytes and the proliferative response (LP) of blood lymphocytes stimulated by mitogens ConA, and LPS in Wild, Palomino, and Albino colored rainbow trout (after Siwicki et al. 2003)

Immunological parameters	Phenotype coloration (genotype)		
	Wild (<i>AaBb.Aabb</i>)	Palomino (<i>aaBb</i>)	Albino (<i>aabb</i>)
RBA	0.48 ± 0.04	0.40 ± 0.03*	0.37 ± 0.02*
PKA	0.41 ± 0.03	0.34 ± 0.04*	0.30 ± 0.02*
LP stimulated with ConA	0.60 ± 0.03	0.52 ± 0.04*	0.48 ± 0.04*
LP stimulated with LPS	0.59 ± 0.04	0.50 ± 0.03*	0.46 ± 0.02*

mean value ± standard deviation

* statistical significance in relation to fish with Wild coloration verified with the Student's *t*-test ($P < 0.05$)

4.2.4. FISH WITH PALOMINO AND ALBINO COLORATION IN EXPERIMENTS CONDUCTED AT DSR RUTKI

Males of Xantoric (Palomino and Albino) coloration from the WPA family were used to improve the androgenesis method. A Palomino male (*aaBb*) was used in one of the experiments to determine the dosage of gamma irradiation required to eliminate the genome in the eggs of females with Wild coloration (*AA*) and the timing of the pres-

sure shock that stops the first mitotic division (Babiak et al. 2002). All of the androgenetic diploid progeny in the eyed-egg stage exhibited Xantoric coloration, while, in the control group (eggs not subjected to irradiation) all of the progeny had Wild coloration. The recessive Xantoric coloration of the androgenetic progeny confirmed that the nuclear genome in these fish came only from the father. In the fry stage (three weeks after feeding began), the ratio of Palomino and Albino fish among the 905 androgenetic Xantoric fish individuals was close to 1:1. During the first six months of rearing, significant fish deaths occurred and after eight months at the end of the first season 205 fish were left – 114 androgenetic homozygotic individuals with Palomino coloration (*aaBB*) and 91 with Albino coloration (*aabb*). All of the fish were males with a homogametic set of *YY* sex chromosomes.

In 2000, a second generation of androgenetic fish (AF_2) was bred. Six clonal families were created: three from Palomino males (androgenetic fish with a known homozygotic genotype (*aaBB*) and three from Albino males (*aabb*). Only individuals from the family of the androgenetic Palomino males, which were theoretically genetic copies of their fathers, survived to the age of two years. In 2002, three androgenetic males with Palomino coloration from various clonal AF_2 families were reproduced to obtain in the third generation (AF_3) three different families which were theoretically copies of the AF_2 generation families and of grandfathers in the AF_1 generation (Fig. 5a). In the same year, three neomales were reproduced androgenetically (genetic females with *XX* sex genotype changed into physiological males through the application of synthetic methyltestosterone analogue hormone; Bieniarz et al. 1991). The neomales were meiotic gynogenetic individuals from six Albino females from the Yellow strain. These fish were described as the GF_1AF_2 generation. The pedigree and schematic illustration of the families obtained with Palomino and Albino coloration through embryonic manipulation are presented in Figures 5a and b.

The aim of this experiment was to produce homozygotic fish and then a clonal family through embryonic genetic manipulation. Using Palomino fish permitted evaluating the similarity of progeny of clonal fish in the families of generation AF_3 as well as to the fathers of generation AF_2 . It was determined through visual evaluation that there was substantial similarity in coloration and body shape within the families. Low differentiation with regard to Palomino coloration among the families was also confirmed (Fig. 6). In the case of androgenetic fish originating from neomales with Albino coloration, there

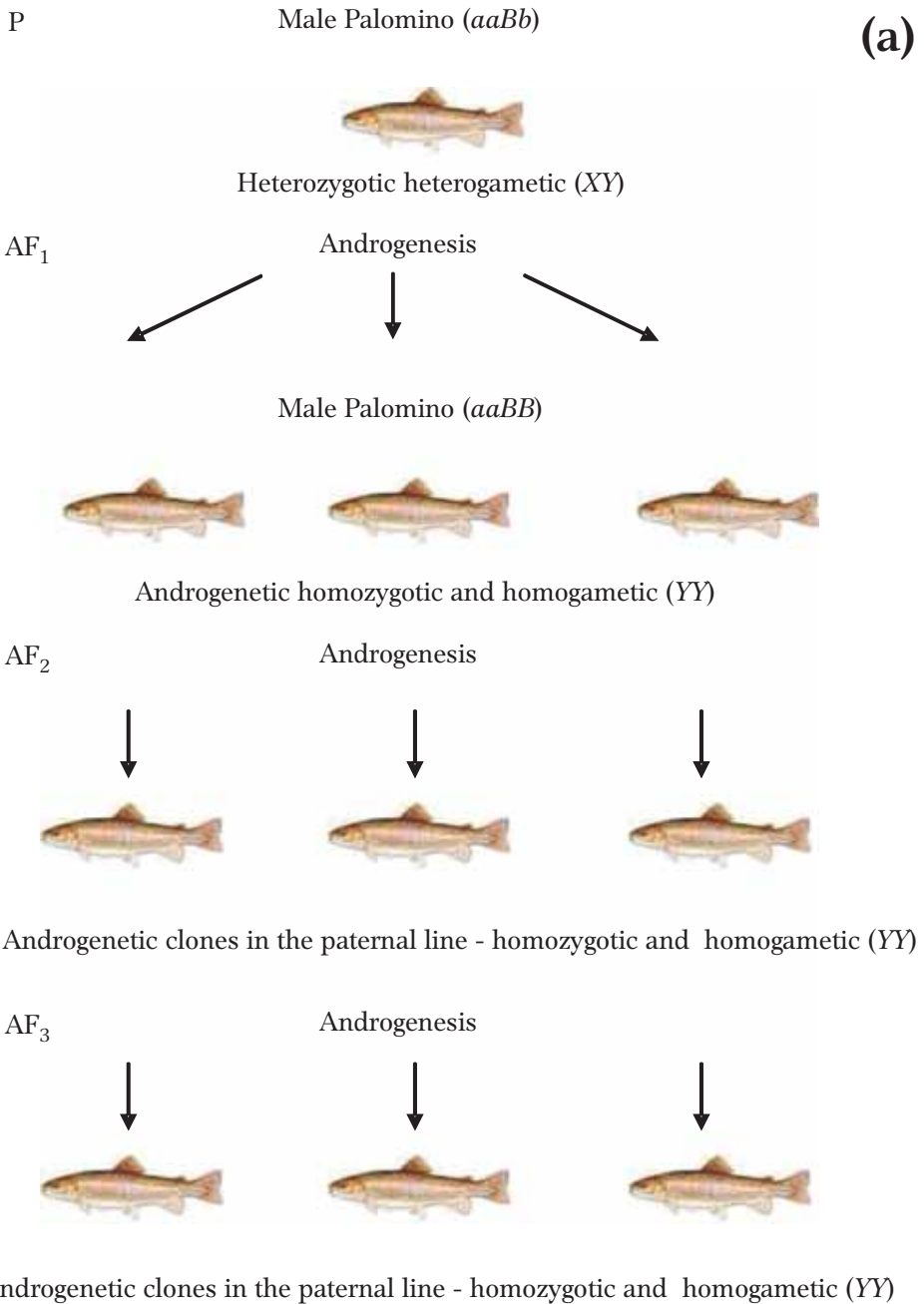


Fig. 5. Schematic illustration of the three androgenetic homozygotic families with Palomino coloration AF₃ (a), and three homozygotic families with Albino coloration GF₁AF₂ (b).

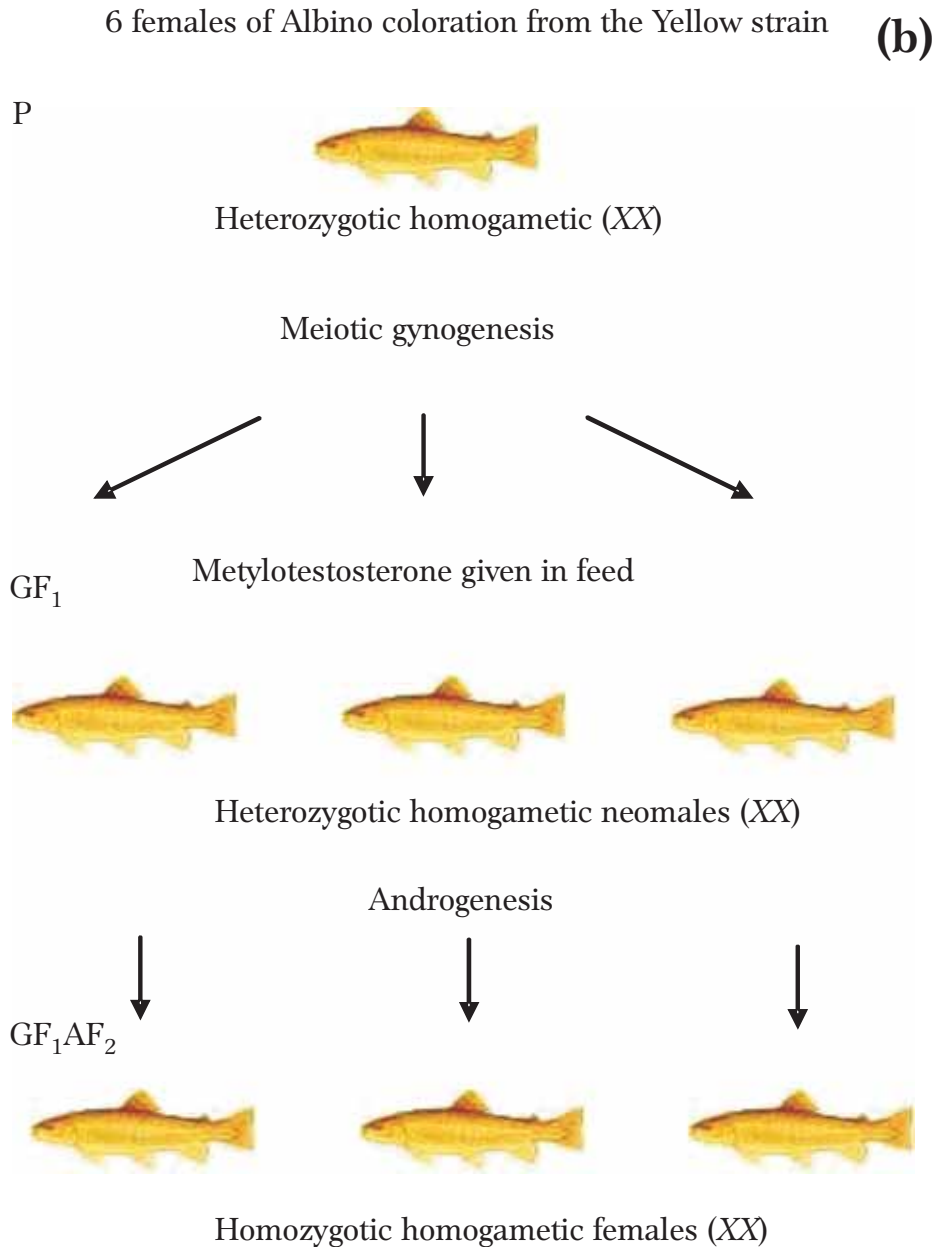


Fig. 5. Schematic illustration of the three androgenetic homozygotic families with Palomino coloration AF₃ (a), and three homozygotic families with Albino coloration GF₁AF₂ (b).



Fig. 6. Individuals from three different androgenetic families with Palomino coloration and the known genotype *aaBB* of the generation AF₃ (Fig. 5 a).

was also substantial similarity within families and slight coloration differences among families (Fig. 7).

Worthy of note is the dark shade of Palomino coloration in the fish of clonal families with homozygotic genotype coloration *aaBB* (Fig. 6) with the substantially lighter shade of Palomino coloration in fish from the family of known heterozygotic Palomino coloration *aaBb* originating from the PA and WPA families (Table 7, groups 7 and 4. Fig. 8).

4.3. MOTTLED COLORATION VARIETY

The Yellow strain fish were the progeny of females with Mottled coloration (Maliszewski 1987). In the subsequent generations of this Yellow strain with Xantoric coloration, no individuals with Mottled coloration were noted. In the early 1990s, single individuals with Mottled (M) coloration were observed in the population of fish selected for fast growth at DSR Rutki that originated, from among others, from the Jastarnia and Oleśnica strains. These fish were reared to sexual maturity with the aim



Fig. 7. Individuals of three different androgenetic families originating from neomales of Albino coloration of the generation GF_1AF_2 (Fig. 5 b).



Fig. 8. Individuals originating from the two different WPA and PA families and of known heterozygotic genotype Palomino coloration ($aaBb$).



Fig. 9. First Mottled coloration female reared at DSR Rutki.

of reproducing them and determining the inheritance pattern of the Mottled coloration form. The first identified female had only a few patches of Albino coloration (Fig. 9).

The eggs obtained from this female were subjected to meiotic gynogenesis with thermal shock. At the fingerling stage her progeny was comprised of seven individuals with Albino coloration and 83 with Wild coloration. In two subsequent gynogenetic breeding attempts made with other females with Mottled coloration, distinctly more Palomino (over) than Wild coloration was observed, and in the second the opposite was noted; all of the progeny had background color.

4.3.1. FIRST INDIVIDUALS WITH MOTTLED COLORATION OBTAINED AMONG THE GYNOGENETIC PROGENY OF THE MOTTLED FEMALE M

In 2000, two adult individuals of Mottled coloration from each sex were chosen from the production fish stock. These individuals were crossbred within and with fish of other coloration. Their sexual products were subjected to meiotic gynogenesis, mitotic gynogenesis, and androgenesis. Pressure shock was applied to duplicate the genetic material.

4.3.2. PROGENY OF THE FIRST GENERATION (F₁) OF FISH WITH MOTTLED COLORATION

The androgenetic progeny of the Mottled male (Fig. 10b) and that obtained by crossbreeding this male with females with Wild coloration and the Mottled female M (Fig. 10a) all exhibited Wild coloration.

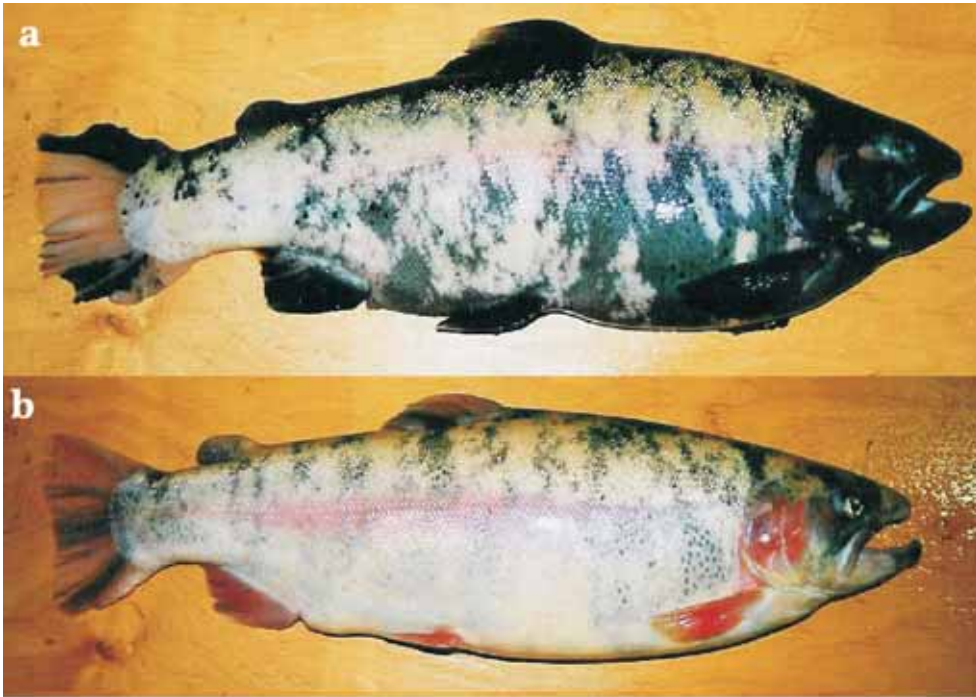


Fig. 10. Adult individuals with Mottled coloration: female M (a), male (b).

The gynogenetic meiotic and mitotic progeny of Mottled female M in the eyed-egg stage partially exhibited Xantoric coloration (red eyes) and partially Wild coloration (black eyes). At the alevin and reared fry stages, most of the individuals among the Xantoric fish were identified as Palomino, and there were a few Albino individuals.

The progeny of the Mottled female M and males with Wild coloration all had Wild coloration. In the eyed-egg stage, the progeny of this female and the Palomino male were either of Wild or Xantoric coloration, while in the alevin and fry stages their coloration was either Wild or Palomino. The crossbreeding of the Mottled female M with the Albino male did not produce viable progeny.

The precise number of fish in the families and in progeny coloration groups is presented in Table 12.

At the eyed-egg and alevin stages in the progeny groups from the mitotic gynogenesis of the Mottled female M and the progeny obtained by crossbreeding her

TABLE 12

Number of progeny coloration groups in different stages of development and originating from a Mottled female M and/or Mottled male Xantoric – K, Wild – W, Palomino – P, Albino – A, Mottled – M

Families Origin of progeny	Development stage – coloration		
	Eyed-eggs – W/K	Alevins – W/P/A	Fry – W/P/A
♀ Mottled meiotic gynogenesis	207/61	166/94/3	60/28/0
♀ Mottled mitotic gynogenesis	130/145	110/117/14	37/29/4
♀ Mottled × ♂ Wild (MW)	Wild	Wild	Wild
♀ Mottled × ♂ Palomino (MP)	183/182	181/180/0	161/158/0
♀ Mottled × ♂ Albino (MA)	0	0	0
♀ Mottled × ♂ Mottled (MM)	Wild	Wild	Wild
♂ Mottled androgenesis	Wild	Wild	Wild
♂ Mottled × ♀ ♀ Wild	Wild	Wild	Wild

with the Palomino male the ratio of embryos with Wild to Xantoric coloration was 1:1 as confirmed by the χ^2 test. Among the fish progeny of meiotic gynogenesis 36.7% were of Xantoric coloration as alevins while at the eyed-egg stage these individuals comprised just 23% of all the embryos. Since the number of all the specimens in both stages of development was similar, this indicated that a portion of the individuals with black eyes which were classified to the groups of Wild individuals at the eyed-egg stage, were identified as Palomino at the alevin stage (Table 12).

While red-eyed individuals occurred at the eyed-egg stage in groups of progeny originating from the Mottled female M, at the alevin stage very few individuals with Albino coloration were confirmed only in the groups of gynogenetic fish. Three Albino (1.14%) individuals were confirmed in the meiotic gynogenesis progeny group while in the mitotic gynogenesis progeny group 14 Albino (5.81%) individuals were confirmed (Table 12).

When the fingerlings of gynogenetic fish attained a weight of about 20 g the appearance of the first Mottled individuals was confirmed. The number of these individuals increased as the fish grew. The evaluation of the number of Mottled fish was first performed in the early fall at a mean fish weight of 75.8 g in the mitotic gynogenetic group and of 98.7 g in the meiotic gynogenetic group. The quantitative division of gynogenetic

fish into groups according to coloration and mean weight attained in these groups is presented in Table 13.

Two groups of Mottled coloration were distinguished within the Mottled group. The first was within the Xantoric coloration and described as Mottled fish from the Palomino-Albino group (P-A), while the second was within the Wild and Xantoric coloration and was described as Mottled fish of the Wild-Xantoric (W-K) group.

Table 13

Mottled female M gynogenetic progeny – number and mean body weight (\pm standard deviation) within coloration groups at the fall fingerling stage

Progeny coloration groups	Mitotic gynogenesis		Meiotic gynogenesis	
	Number (indiv.)	Mean body weight (g)	Number (indiv.)	Mean body weight (g)
Wild	11	93.4 \pm 37.1	32	100.1 \pm 41.2
Palomino	7	78.1 \pm 24.5	1	152
Mottled Palomino-Albino (P-A)	3	50.3 \pm 33.8	-	-
Mottled Wild-Xantoric (W-K); advantage of Wild coloration	13	67.8 \pm 36.4	19	92.6 \pm 22.6
Mottled Wild-Xantoric (W-K); advantage of Xantoric coloration	8	71.9 \pm 34.4	3	105.0 \pm 26.2
Total	42	75.8\pm35.0	55	98.7\pm35.2

“-” no fish in the given coloration group

During the evaluation of the fall fingerlings in the remaining progeny groups originating from the Mottled female M and/or the Mottled male, all of the fish exhibited Wild coloration, with the exception of the fry from the family obtained through crossbreeding the Mottled female M with the Palomino male, in which 50% of the fish had Wild coloration and 50% had Palomino coloration. Another evaluation of the gynogenetic fish progeny of the Mottled female M was conducted at the age of 2 years with additional photographic documentation of the Mottled group, W-K different Xantoric hues in each individual were observed. It was also impossible to divide the Xantoric patches into Palomino or Albino coloration groups due to the indistinct Wild patch image and the black eyes in all the Mottled fish (Table 14).

Four Mottled female progeny from meiotic gynogenesis achieved sexual maturity at the age of 2 years. Consequently, gynogenetic reproduction was conducted, but no viable embryos were obtained. The groups originating from the Mottled female M were left for further rearing – all of the daughters obtained through gynogenesis and 60 individu-

TABLE 14

Number and mean body weight (\pm standard deviation) within coloration groups at 2 years of age

Progeny coloration groups	Mitotic gynogenesis		Meiotic gynogenesis	
	Number (indiv.)	Mean body weight (g)	Number (indiv.)	Mean body weight (g)
Wild	5	1787 \pm 269	15	1774 \pm 609
Palomino	5	1189 \pm 384	1	2025
Mottled Palomino-Albino (P-A)	3	784 \pm 246	-	-
Mottled Wild-Xantoric (W-K); advantage of Wild coloration	4	896 \pm 432	20	862 \pm 446
Mottled Wild-Xantoric (W-K); advantage of Xantoric coloration	3	726 \pm 196	2	978 \pm 342
Total	20	1150 \pm 507	38	1278 \pm 652

“-” no fish in the given coloration group

als chosen at random from each family obtained through crossbreedings with males of various coloration (generation F₁).

All of the fish left were tagged individually with PITs.

4.3.3. COLORATION OF SECOND GENERATION (F₂) FISH PROGENY ORIGINATING FROM MOTTLED FEMALE M

In 2003 adult individuals (generation F₁) of the gynogenetic progeny of Mottled female M as well as its crossbreedings with a Wild male were reproduced. Successful reproduction was achieved with two daughters (obtained through meiotic gynogenesis) with Mottled coloration (M1 and M2, Fig. 11) and their half-sibs, six females from the F₁ generation with Wild coloration (W1 - W6) that originated from the Mottled female M and the male with Wild coloration (family MW, Table 12). The eggs of eight chosen females were divided into three or four portions depending on the quantity of eggs obtained. Two portions of eggs from each female were subjected to gynogenesis in the stages of meiosis and mitosis, while the remaining portions from individual females were fertilized individually with the milt from six different males from the F₁ generation. Three of them originated from the same MW family as the Wild females with Wild coloration (W2, W3, W4) and from three originating from the MP family (Table 12), one with Wild (W1) and two with Palomino (P1, P2) coloration. Each egg portion was incubated separately.



Fig. 11. Mottled daughters of the Mottled female M obtained through meiotic gynogenesis. 3-year-old fish just prior to egg collection; (a) M1, (b) M2.

It was not possible to divide the embryos into groups with dark and red eyes during the eyed-egg stage. This was due to the wide variety of eye color that ranged from red to russet, brown, and then to black. The results of the alevin coloration determined to be Wild (initial skin and eye pigmentation) and Palomino (lack of skin pigmentation and dark eyes) of the F_2 generation (taking into consideration origin) are presented in Table 15.

Viable F_2 progeny alevins were obtained in all the meiotic gynogenetic crossbreedings, with the exception of the Wild female W6. However, in the groups of progeny obtained through mitotic gynogenesis, viable fry was produced by just four (M2, W1, W3, W5) of eight females. Since the all the progeny of female W1 exhibited Wild coloration, they were excluded from further rearing. The occurrence of Albino coloration was not noted in any of the families. In all the remaining progeny groups from the F_2 generation individuals with Wild and Palomino coloration were obtained at the fry stage as a result of meiotic gynogenesis and crossbreeding. In groups of progeny obtained through mitotic gynogenesis, it was confirmed that females W3 and W5

TABLE 15

Origin and number of progeny with different coloration (F₂ generation) of Mottled female M;
in the alevin and fry stages (mean weight of approximately 0.5 g)

Families Origin of progeny	Fish coloration			
	Alevins		Fry	
	Wild	Palomino	Wild	Palomino
♀ Mottled M1 meiotic gynogenesis	3	25	3	6
♀ Mottled M1 mitotic gynogenesis	-	-	-	-
♀ Mottled M1 × ♂ Palomino P1 (F ₁ -MP)	56	45	51	34
♀ Mottled M2 meiotic gynogenesis	3	53	1	32
♀ Mottled M2 mitotic gynogenesis	0	8	0	6
♀ Mottled M2 × ♂ Wild W1 (F ₁ -MP)	51	18	41	15
♀ Wild W1 meiotic gynogenesis	80	0	0*	0*
♀ Wild W1 mitotic gynogenesis	10	0	0*	0*
♀ Wild W1 × ♂ Wild W2 (F ₁ -MW)	138	0	0*	0*
♀ Wild W2 meiotic gynogenesis	58	34	54	33
♀ Wild W2 mitotic gynogenesis	-	-	-	-
♀ Wild W2 × ♂ Wild W3 (F ₁ -MW)	70	23	0**	0**
♀ Wild W3 meiotic gynogenesis	17	25	14	20
♀ Wild W3 mitotic gynogenesis a	10	20	6	5
♀ Wild W3 × ♂ Wild W1 (F ₁ -MP)	82	23	0**	0**
♀ Wild W4 meiotic gynogenesis	49	35	22	26
♀ Wild W4 mitotic gynogenesis	-	-	-	-
♀ Wild W4 × ♂ Palomino P2 (F ₁ -MP)	147	145	75	84
♀ Wild W5 meiotic gynogenesis	89	69	30	16
♀ Wild W5 mitotic gynogenesis	26	22	23	18
♀ Wild W5 × ♂ Wild W2 (F ₁ -MW)	71	23	71	23
♀ Wild W5 × ♂ Palomino P1 (F ₁ -MP)	84	89	73	73
♀ Wild W6 meiotic gynogenesis	-	-	-	-
♀ Wild W6 mitotic gynogenesis	-	-	-	-
♀ Wild W6 × ♂ Wild W4 (F ₁ -MW)	194	62	112	48

* excluded from further rearing due to all progeny having Wild coloring

** excluded from further rearing due to the hatch being mixed in the hatching apparatus

“-” no fish in the given coloration group

of Wild coloration produced individuals with Wild and Palomino coloration, while the Mottled M2 female produced only individuals of Palomino coloration (Table 15).

Among the meiotic gynogenetic progeny (F₂) from the Wild females of the F₁ generation only in the case of female W3 did the individuals with Palomino coloration (60%)

prevail. In the remaining three groups of meiotic gynogenetic progeny (F_2) from females W2, W4, and W5 individuals of Wild coloration were more numerous than those of Palomino coloration and comprised 63, 58, and 56% of the families, respectively. Assuming that these fish had the heterozygotic genotype Aa at locus A , then with gynogenetic reproduction (with solely the maternal genome) and the assumption of a lack of the impact of the crossing over phenomenon at a single locus (reproduction in the meiosis phase), the coloration ration of Wild and Xantoric among progeny should be 1:1. Analysis with the χ^2 test of the abundance of these two coloration groups among the meiotic gynogenetic progeny exhibited a significant difference only in the case of the progeny of female W2 (calculated value $\chi^2=6.26$, where the boundary value exceeded with probability of 0.05 at 1 degree of freedom is 3.84).

In the second meiotic gynogenetic generation of the Mottled females M1 and M2 distinctly more (89% and 95%) individuals with Palomino coloration than those with Wild coloration were observed. This was opposite to the case of the first generation of meiotic gynogenetic progeny that was obtained from their Mottled M mother (36% Palomino individuals, Table 12).

In the case of the progeny of generation F_2 of the Mottled female M, the crossbred half or full sib families (originating from generation F_1 parents with Wild coloration) were comprised 100% of individuals with Wild coloration or of individuals with Wild and Xantoric coloration at a ratio close to 3:1. When females with Wild coloration were crossed with males of Palomino coloration this ratio was close to 1:1 (in all cases deviation tested with the χ^2 test was insignificant) (Table 15). This proves that the Mottled female M that gave rise to the subsequent generation had a heterozygotic genotype of Aa at locus A and passed her progeny either allele A responsible for Wild coloration or allele a responsible for Xantoric coloration in accordance with the schematic illustration presented in Figure 4. Her gynogenetic daughters (M1 and M2, Fig. 11) also have the heterozygotic genotype Aa at locus A . Confirmation of this is the coloration of the fry obtained from crossbreeding the female M2 with the male W1 of Wild coloration and the known heterozygotic genotype Aa in locus A , as it originated from a male of Palomino coloration and the homozygotic genotype aa in locus A . The observed number of fish in the Wild and Xantoric coloration groups corresponded to the hypothetical ratio of 3:1 (as confirmed by the χ^2 test). With the crossbreeding of the M1 female with the P1 male of Palomino coloring the ratio of Wild to Xantoric individuals in the progeny

was 56 to 45 individuals, respectively. In comparison to the remaining groups of siblings in generation F₂, the number of Wild to Xantoric individuals deviated most from the ratio of 1:1. However, the value calculated for this group with the χ^2 test was 1.198 (below the probability of the greater value 3.84 for P = 0.05), which allowed identifying this deviation as accidental (Table 15).

All of the groups of progeny from the F₂ generation, with the exception of the group excluded due to the occurrence only of individuals of Wild coloration and that which became mixed in the incubator (Table 15), were left in separate basins for further rearing. The first individuals with Mottled coloration appeared in some of the groups at the fall fingerling stage. Another precise analysis of progeny coloration along with photographic documentation of the Mottled individuals was conducted following one year of rearing the fish to a weight of 40 to 60 g. The results are presented in Table 16 as well as in Figures 12a-j.

TABLE 16

Number of generation F₂ progeny of the Mottled female M during the evaluation at age 1+ taking into consideration the division into various coloration groups

Families Parent origin and coloration	Fingerling coloration			
	Wild	Palomino	Mottled W-K	Mottled P-A
♀ Mottled M1 meiotic gynogenesis	-	-	2	-
♀ Mottled M1 × ♂ Palomino P1 (F ₁ -MP)	23	13	13	14
♀ Mottled M2 meiotic gynogenesis	1	-	12	1
♀ Mottled M2 mitotic gynogenesis	-	-	-	-
♀ Mottled M2 × ♂ Wild W1 (F ₁ -MP)	37	-	-	-
♀ Wild W2 meiotic gynogenesis	41	-	16	-
♀ Wild W3 meiotic gynogenesis	7	-	15	-
♀ Wild W3 mitotic gynogenesis	6	-	2	-
♀ Wild W4 meiotic gynogenesis	17	-	8	-
♀ Wild W4 × ♂ Palomino P2 (F ₁ -MP)	48	59	-	-
♀ Wild W5 meiotic gynogenesis	27	-	13	1
♀ Wild W5 mitotic gynogenesis	5	5	8	1
♀ Wild W5 × ♂ Wild W2 - (F ₁ -MW)	35	10	-	-
♀ Wild W5 × ♂ Palomino P1 (F ₁ -MP)	48	31	9	7
♀ Wild W6 × Wild W4 (F ₁ -MW)	52	34	-	-

“-” no fish in the given coloration group

Fig. 12. Progeny groups with Mottled coloration (generation F₂, Table 16) Mottled female (Fig. 10a).

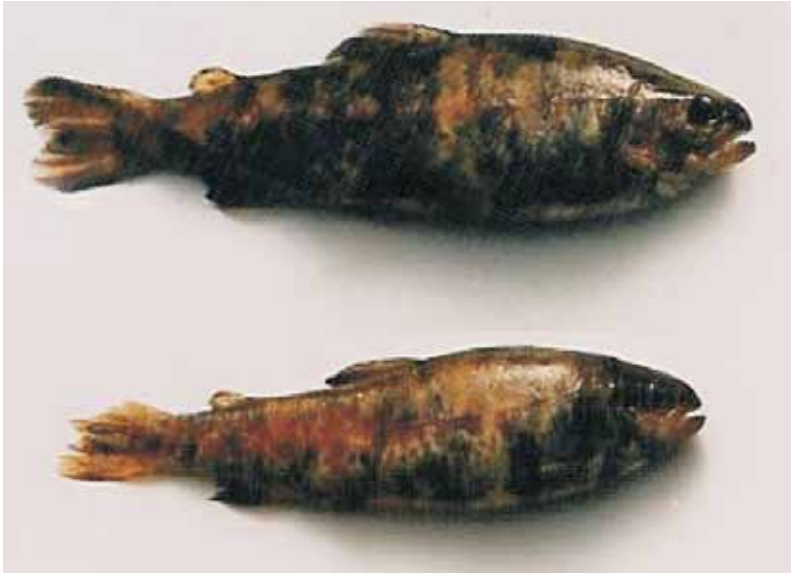


Fig. 12a. Meiotic gynogenotes ; ♀Mottled M1 (F₁ – meiotic gynogenote ♀ Mottled M).

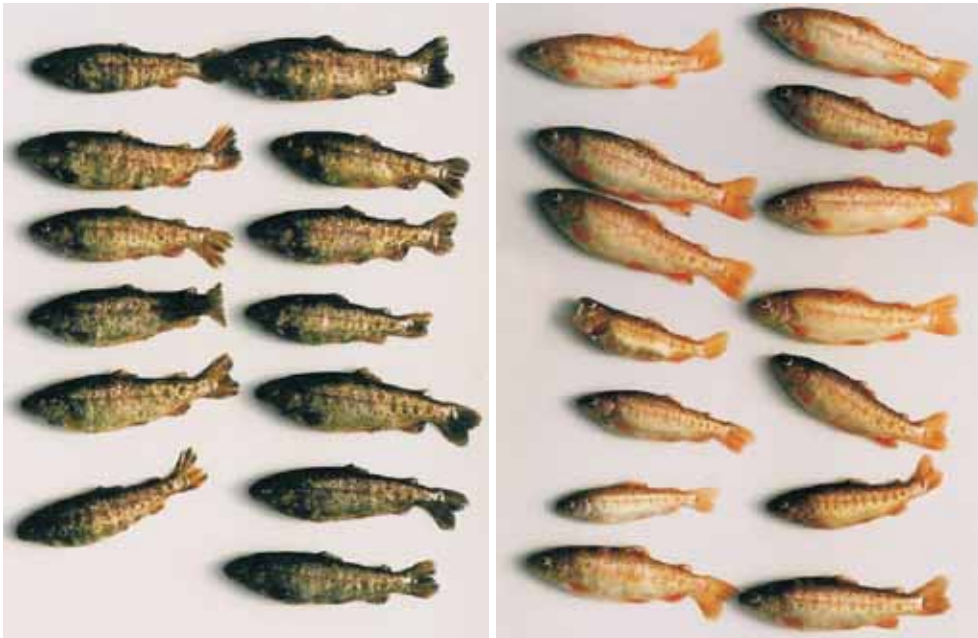


Fig. 12b. Progeny; ♀Mottled M1 (F₁ – meiotic gynogenote ♀Mottled M) × ♂ Palomino P1 (F₁ – ♀ Mottled M × ♂ Palomino).



Fig. 12c. Meiotic gynogenotes ; ♀Mottled M2 (F_1 – meiotic gynogenote ♀Mottled M).



Fig. 12d. Meiotic gynogenotes ; ♀Wild W2 (F_1 – ♀Mottled M \times ♂ Wild).

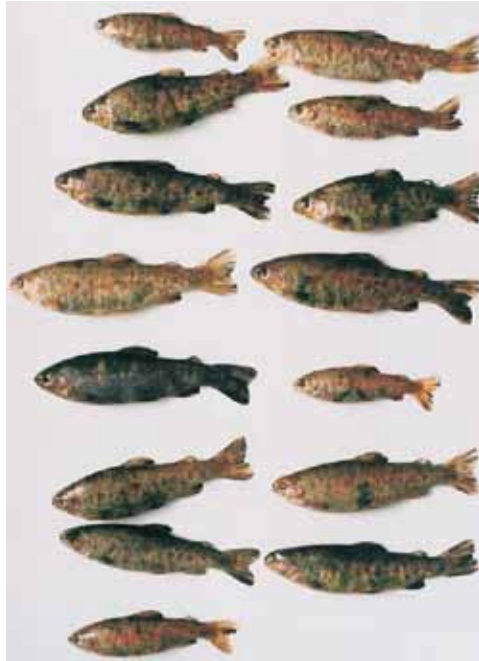


Fig. 12e. Meiotic gynogenotes ; ♀Wild W3 (F_1 – ♀Mottled M × ♂ Wild).



Fig. 12f. Mitotic gynogenotes ; ♀Wild W3 (F_1 – ♀Mottled M × ♂ Wild).



Fig. 12g. Meiotic gynogenotes ; ♀Wild W4 (F_1 – ♀Mottled M \times ♂ Wild).



Fig. 12h. Meiotic gynogenotes ; ♀Wild W5 (F_1 – ♀Mottled M \times ♂ Wild).



Fig. 12i. Mitotic gynogenotes ; ♀Wild W5 ($F_1 - \text{♀Mottled M} \times \text{♂ Wild}$).

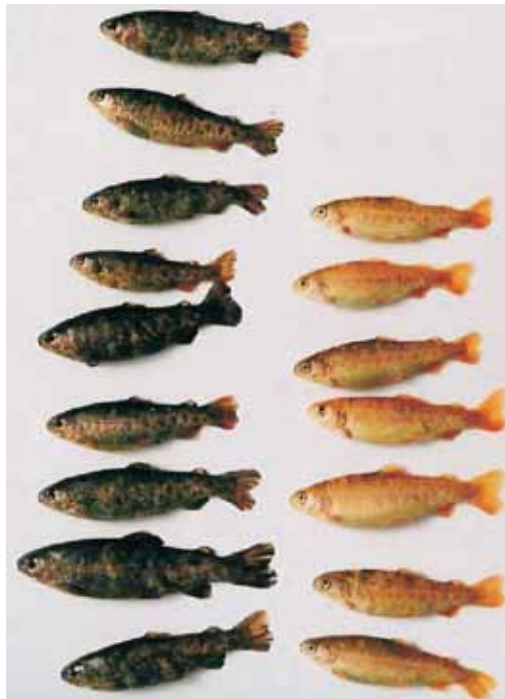


Fig. 12j. Progeny; ♀Wild W5 ($F_1 - \text{♀Mottled M} \times \text{♂ Wild}$) \times ♂ Palomino P1 ($F_1 - \text{♀ Mottled M} \times \text{♂ Palomino}$).

In all of the groups of gynogenetic progeny from generation F_2 reared further, individuals of Mottled coloration appeared at the fingerling stage. Of five groups of crossbred progeny, the occurrence of Mottled individuals was confirmed only in two families originating from the Palomino male P1 and the females M1 and W5. In two other families originating from the Wild female W4 and the Palomino male P2 and the Wild female W5 and the Wild male W4 only individuals with Wild and Palomino coloration were confirmed. In the family that originated from the Mottled female M2 and the Wild male W1 only individuals with Wild coloration survived to the fry stage, while the individuals of Palomino coloration observed at the fry stage died in the first two months of rearing (Table 16). These results indicate that not all the progeny of Mottled females that have in the genotype allele *a* responsible for Xantoric coloration received the Mottled trait. In both families originating from the Palomino P1 male, two equally numerous coloration groups described as Wild-Xantoric (W-K) and Palomino-Albino (P-A) occurred within the Mottled individuals (Fig. 12b and j). Single Mottled individuals from the Palomino-Albino (P-A) coloration groups were also confirmed in the groups of gynogenetic progeny of females M2 and W5 (Table 16).

The survival of the F_2 generation both in families as well as in coloration groups during the growth period from alevin to fingerling was highly varied and difficult to interpret.

4.3.4. GROWTH OF GENERATION F_2 FISH WITH MOTTLED COLORATION

In the second year of life of the F_2 generation originating from Mottled female M another evaluation of individual fish coloration and weight was done, except families without mottled fish. The results are presented in Table 17.

Analyzing the survival of Wild and Mottled fish between the first and second years of life and treating all the groups of progeny as a whole, it was determined that fish with Mottled coloration have a slightly higher survival rate than those with Wild coloration (80.9 and 74.0%, respectively). Attention should be drawn, however, to the progeny groups with Mottled coloration (W-K) that originated from the Palomino male P1 and the Mottled female M1 and Wild female W5 as, paradoxically, in the second year there were two and three more Mottled fish, respectively, than there had been in the previous year (Tables 16 and 17). It is possible that in the first year of life Mottled coloration does not manifest in all individuals. It can be assumed that individuals described as Mottled

TABLE 17

Mean weight (g) \pm standard deviation and number (in parentheses) of individuals (age 2+) of coloration groups of families from the F₂ generation of Mottled female M

Families Parent origin and coloration	Coloration and mean weight \pm standard deviation (number)			
	Wild	Palomino	Mottled W-K	Mottled P-A
♀ Mottled M1 \times ♂ Palomino P1	572 \pm 155 (20) ^{C,d}	495 \pm 167 (12) ^c	363 \pm 139 (15) ^{A,b}	410 \pm 147 (11) ^a
♀ Mottled M2 meiotic gynogenesis	-	-	707 \pm 365 (7)	-
♀ Wild W2 meiotic gynogenesis	766 \pm 103 (27) ^C	-	226 \pm 102 (8) ^A	-
♀ Wild W3 meiotic gynogenesis	880 \pm 620 (3)	-	568 \pm 406 (14)	-
♀ Wild W4 meiotic gynogenesis	823 \pm 297 (15)	-	564 \pm 252 (6)	-
♀ Wild W5 meiotic gynogenesis	729 \pm 247 (16) ^C	-	410 \pm 98 (7) ^A	440 (1)
♀ Wild W5 mitotic gynogenesis	950 \pm 143 (5) ^C	400 (1)	649 \pm 167 (7) ^A	-
♀ Wild W5 \times ♂ Palomino P1	447 \pm 104 (39)	393 \pm 113 (28)	411 \pm 162 (12)	335 \pm 110 (5)

Letters a, b, c, d – (small letters) significant differences, (capital letters) highly significant between mean weights attained by fish in coloration groups in particular families

“-” no fish in the given coloration group

at the age of 2 years but are characterized by a prevalence of Wild coloration (80-90%) could have been recognized as Wild at the age of 1 year. A similar situation was confirmed in the case of meiotic and mitotic gynogenetic progeny of generation F₁ of the Mottled female M, in which, along with growth, there were distinctly fewer fish with Wild coloration in relation to the remaining coloration groups (Tables 13 and 14).

In all the groups of gynogenetic progeny in which at age 2+ only specimens with Wild and Mottled coloration occurred it was confirmed that the mean weight of Wild fish was distinctly higher. Among the gynogenetic progeny of females W2 and W5 these differences were highly significant, while among the gynogenetic progeny of females W3 and W4 these differences were insignificant; this could have been caused by the low numbers of individuals and high variation in individual fish weight within these coloration groups.

In the progeny groups of females M1 and W5 and the male P1, individuals with Wild, Palomino, and Mottled coloration occurred. The individuals with Mottled coloration were divided into two groups – Wild-Xantoric (W-K) and Palomino-Albino (P-A).

Analysis of variance indicted that coloration within the progeny of the female M1 had a highly significant influence on the different mean weights attained by the fish in these coloration groups. The application of Duncan's multiple range test permitted

concluding that fish with Wild coloration had the highest mean weight and exceeded highly significantly the mean weight of Mottled fish with W-K coloration, which had the lowest mean weight, and significantly exceeded the mean weight of Mottled fish with P-A coloration. The mean weight of fish with Palomino coloration fell between the mean weight of fish with Wild and Mottled coloration and significantly exceeded that only of fish with Mottled W-K coloration.

In the family originating from the Wild female W5 and the Palomino male P1, fish coloration was not found to influence the mean weight attained by the progeny of this family. Nevertheless, the highest mean weight in this family was attained by fish with Wild coloration, followed by Mottled fish from the W-K group, followed by Palomino fish, while the lowest mean weight was attained by fish with Mottled coloration from the P-A group (Table 17).

4.3.5. ATTEMPT TO EVALUATE THE INHERITANCE OF MOTTLED COLORATION TRAITS USING THE GYNOGENESIS METHOD

The subject of the tests was the Mottled female M2 from meiotic gynogenetic generation F₁ (Fig. 11b) and ten of her meiotic gynogenetic generation F₂ progeny that had survived to the age of 1+ and that all had Mottled coloration of the Wild-Xantoric pattern (Fig. 12 c). The coloration was tested with regard to the passing on of the Mottled trait pattern to the next generation. Based on pictures of a segment of the dorsal section (Fig. 13), the distribution of patches and the intensity of particular pigments were analyzed on the body of each fish examined.

The results of the analysis of segments of the dorsal area were compared between the female and her progeny and among the progeny. The body coloration of the female and her gynogenetic progeny was, in all instances, Mottled (Fig. 14a and b). The comparison of pigment intensity with variance analysis did not reveal significant differences among the fish ($P > 0.05$). Variation in the distribution of particular patches, their surface area, and pigment intensity was, however, apparent in all of the fish examined. Taking into consideration pigment intensity and the relation of dark to light spots, it was confirmed that there are two coloration forms that differ from one and other. Four individuals (1, 2, 6, 8) were assigned to the group with a predominance of dark spots with bright pigmentation, while seven fish (3, 4, 5, 7, 9, 10) as well as the female had lighter colors (Fig. 14a and b). A significant statistical correlation of the share of back-

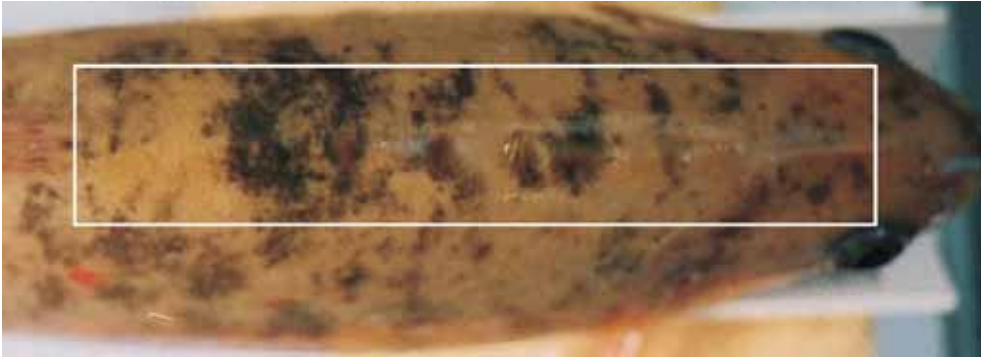


Fig. 13. Segment of the dorsal image of the fish used for the comparative analysis of body coloration in Mottled fish.

ground pigment and patches among the female and her progeny was confirmed in individuals 3, 4, 7, 9, and 10 (Table 18, Fig. 14 a and b). Reciprocal concurrence of color among progeny was estimated to be 30.2%.

Female - dorsal section

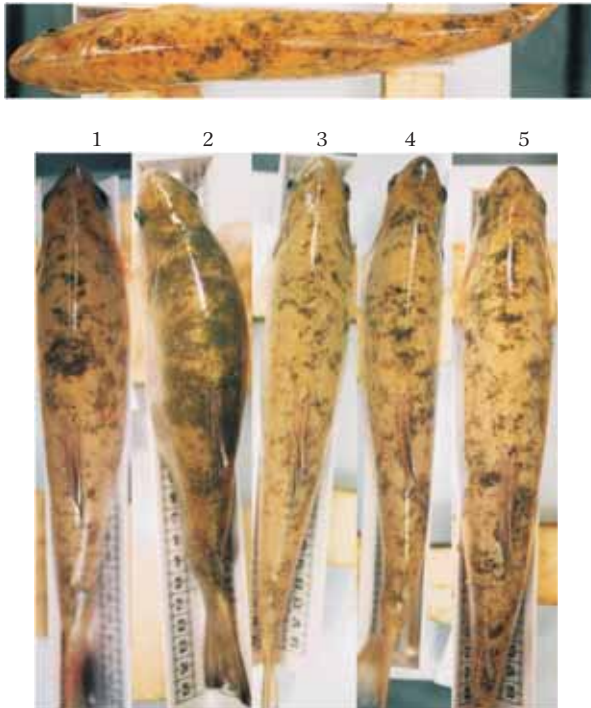


Fig. 14a. Female Mottled M2 coloration and her gynogenetic progeny (below).

Female - dorsal section



Fig. 14b. Female Mottled M2 coloration and her gynogenetic progeny (below).

TABLE 18

Correlation coefficients for body coloration distribution in Mottled fish ($P < 0.05$)

	Fish 1											
Fish 1	-	Fish 2										
Fish 2	0.86	-	Fish 3									
Fish 3	0.01	0.48	-	Fish 4								
Fish 4	0.34	0.75	0.93	-	Fish 5							
Fish 5	0.08	0.33	0.91	0.77	-	Fish 6						
Fish 6	0.99	0.92	0.15	0.48	0.03	-	Fish 7					
Fish 7	0.52	0.86	0.79	0.96	0.55	0.65	-	Fish 8				
Fish 8	0.91	0.82	0.17	0.47	0.02	0.93	0.65	-	Fish 9			
Fish 9	0.54	0.86	0.78	0.95	0.57	0.66	1.00	0.67	-	Fish 10		
Fish 10	0.28	0.68	0.88	0.96	0.62	0.42	0.96	0.47	0.96	-	Female	
Female	0.27	0.69	0.93	0.99	0.71	0.41	0.96	0.44	0.95	0.99	-	

Boldface type indicates a statistically significant dependence

5. DISCUSSION

5.1. OCURRENCE OF XANTORIC COLORATION IN RAINBOW TROUT

Xantoric body color in rainbow trout at the DSR Rutki is hereditary and is controlled by the epistatic interaction between two loci. The occurrence of the following alleles at locus *A* was confirmed: *A* – dominant and responsible for Wild coloration, *a* – recessive and responsible for Xantoric coloration and the lack of eye pigment in embryos in the eyed-egg stage. The following alleles were confirmed to occur at locus *B*: *B* – responsible for Palomino coloration and the appearance of eye pigment in the early fry stage, *b* – responsible for Albino coloration and the lack of eye pigment throughout the individual's life. Locus *A* dominates epistatically in relation to *B*; this means that the impact of the allele from locus *B* appears when the locus *A* is recessive homozygotic (*aa*). This mechanism for Xantoric coloration inheritance in rainbow trout was first documented by Dobosz et al. (1999).

Another mechanism for the inheritance of Xantoric coloration is described in the literature. The occurrence of the first rainbow trout with Xantoric body coloration was confirmed in the 1950s in the United States (Clark 1970). However, the inheritance mechanism for Xantoric coloration described for these fish was decidedly different from that described above. Clark (1970) identified two coloration groups and called them Golden and Palomino. Golden trout have golden coloration with a distinct red band extending along the lateral line. Palomino trout are distinctly darker, nearly dark blond, and have a less distinct red band that runs along the lateral line. The irises of both Golden and Palomino fish are dark. The inheritance of these forms of coloration is controlled by a single autosomal locus (gene) called *G* with the additional participation of two alleles. Allele *G* is responsible for Wild (normal) coloration in trout, while allele *G'* is responsible for a reduction in the quantity of melanophores. Fish of the *GG* genotype have Wild coloration, while the homozygotic genotype *G'G'* significantly lowers the quantity of melanophores occurring in the skin which causes the coloration phenotype of the so-called Golden trout. The heterozygotic genotype *GG'* also causes a lowering of melanophore quantity to a lesser extent and the effect is something between Wild and Golden – the so-called Palomino coloration (Wright 1972; in Tave 1988, Purdom 1993).

Bridges and Limbach (1972) as well as Klupp and Kaufmann (1979) described the occurrence of Xantoric trout that is a shade of bright yellow with red eyes – the so-called Albino. The mutation responsible for the occurrence of Albino trout is hereditary, and this coloration is determined by a single autosomal gene. Two alleles occur at this locus called *A*; the dominant allele *A* is responsible for Wild coloration and the recessive allele *a* is responsible for Albino coloration. Since allele *A* is completely dominant with regard to allele *a*, the fish of the genotypes *AA* and *Aa* have phenotypically Wild coloration, while the phenotype of fish with the genotype *aa* is Albino.

The Albino phenotype described in these publications corresponds to the Albino coloration presented in the current work. It can be assumed that the same mutation and gene are responsible for the inheritance of both of the Albino forms described. If it is assumed that in the Xantoric population from Rutki the allele *B* does not occur at locus *B*, which is responsible for Palomino coloration, then the only phenotype colorations that would occur would be Wild and Albino. Locus *A* alone is responsible for their occurrence since locus *B* always occurred as the homozygotic recessive *bb*. Similarly to fish populations described by Bridges and Limbach (1972) and Klupp and Kaufmann (1979), individuals with the genotype *AA* or *Aa* would have Wild coloration (analogously to the fish with *AAbb* and *Aabb* genotypes in Rutki), while fish with an *aa* genotype would be Albino (analogously to the fish with a *aabb* genotype in Rutki).

The trout described by Chourrout (1982) in experiments with gynogenesis using milt from Yellow trout with a dominant gene responsible for this coloration are certainly an example of another hereditary form of Xantoric coloration. Nakamura et al. (2001) also identified, described, and determined the location of the gene responsible for the occurrence of the Yellow albino variety of trout coloration that is fully dominant in relation to Wild coloration. Presumably, in all instances the occurrence of the mutation responsible for Xantoric coloration is related to the blocking the development of melanophores and the synthesis of melanin that is responsible for black coloration.

The experiments performed to evaluate the growth and survival of Xantoric fish from Rutki indicated that the genes responsible for coloration have a negative, pleiotropic influence on the growth, survival, and vitality of the fish (Tables 8, 9, 10, 11, Dobosz et al. 2000, Siwicki et al. 2003). This effect appeared more distinctly in Albino (*aabb*) fish, and while Palomino (*aaBb*) fish exhibited faster growth and higher survival rates than did the Albino fish, the levels of these indicators were lower than those

achieved by fish with Wild coloration. All of the fish of various colorations compared were half-sibs that were reared in one basin. However, similar results were not always obtained in earlier studies. Gynogenetic fish with Albino coloration from the Yellow strain exhibited a slightly lower growth rate in relation to that of the gynogenetic fish with Wild coloration that originated from the Saitama strain imported from Japan in 1985 at distinctly lower survival rate in their first year of growth (Table 4). In this case, the fish of various colorations were held in separate basins. In the second year of life, the fish were reared; n one pond and at the end of the growth period the gynogenetic fish with Wild coloration exhibited a significantly higher weight at an insignificantly lower survival rates.

Another experiment was done to analyze the growth of two equally numbered groups of full sibs with Palomino and Wild coloration that were reared in one pond. In the fry stage at a similar survival rate, the individuals with Palomino coloration exhibited a higher mean mass than their sibs with Wild coloration did (Table 4).

Observations confirming lower growth and survival rates in Albino fish in comparison to those of fish with Wild coloration and held in one pond were published by Kohlmann and Fredrich (1986). These differences became smaller in older fish at the ages of two and three years.

Okumus et al. (2001) reported that fish of the same origin with Albino and Wild colorations grew identically when held in separate basins, but when held in one basin, the individuals with Wild coloration attained a significantly higher mean weight in comparison to the Albino fish. The evaluation was performed following 122 days of growth from the fry stage until the fish had attained commercial weight. Based on breeder observations, Clark (1970) reported that trout with Golden coloration were less active, more sensitive to light, and grew to smaller sizes than Wild coloration trout. The author's own experiments and data available in the literature confirm these observations; the Xantoric variety of trout is less vital, which is apparent in its lower growth and higher mortality rates. To date, the author has not been able to find data in the literature pertaining to the growth and survival of trout with dominant genes responsible for Xantoric coloration.

5.2. OCCURRENCE OF MOTTLED COLORATION IN TROUT

Reports of single individuals of Mottled trout were made at the same time as the appearance of the first trout with Xantoric coloration (Wright 1972, Maliszewski 1987). Single Mottled individuals were also noted in Rutki during the first years of trout rearing (1986-88) (Fig. 9). The first attempts to reproduce Mottled fish by crossbreeding individuals with Wild and Xantoric colorations and by meiotic gynogenesis with Mottled females did not produce progeny with Mottled coloration.

Galbreath and Plemmons (2000) presented the first attempts to evaluate how Mottled coloration is inherited in rainbow trout. The eggs of Mottled females were divided into five portions; three were fertilized individually by males of different colorations (Wild, Golden, Albino) and two were subjected to gynogenesis during the meiotic phase which produced progeny that came only from the mother. In the group of progeny fathered by the Golden trout, two Mottled individuals were obtained, but they did not appear until the fish had been reared for six months. The remaining fish in this group were of Palomino coloration. The progeny of the Mottled female and the male with Wild coloration were exclusively of Wild coloration. In the progeny group of the Mottled female and the Albino male, 8% of the hatchlings were of Wild coloration, while the remainder were Albino. In the two gynogenetic groups that originated from the Mottled female a total of five individuals with Wild coloration (12%) were noted, while the remaining fish were of Albino coloration. These results provide evidence that the female was a mosaic that had been created in an early stage of zygote development. When Mottled coloration originates in this way, it is not inheritable, and the coloration of the trout progeny is either Wild or Albino.

Yamazaki et al. (2006) performed genetic examinations of 12 individuals of cherry salmon, *Oncorhynchus masou ishikawe* (Brevoort) with Mottled coloration. They determined the DNA content in the cells of their erythrocytes using flow-cytometry. The studies showed that three were diploid, eight were haploid-diploid mosaic, and one was diploid-triploid mosaic. Since these individuals were found within the group of Albino fish, it was assumed that Mottled coloration was connected to the occurrence of the recessive gene *a* responsible for Albinism. Nine Mottled fish that had attained sexual maturity were crossbred with different individuals with Albino coloration and the known recessive homozygotic genotype *aa* that is responsible for this coloration.

The groups of progeny that were obtained from the crossbreeding of Mottled and Albino fish were as follows:

- individuals with Albino and Wild coloration at a ratio of 1:1, they were the progeny of one Mottled individual that was identified as diploid or three identified as haploid-diploid, which suggests that the genotypes of their Mottled parents were mosaics of Aa/Aa or a/Aa , respectively;
- individuals of only Wild coloration that were the progeny of three Mottled individuals that had been identified as haploid-diploid, which suggests that the genotype of these Mottled fish was the mosaic of a/AA ;
- individuals of only Albino coloration that were the progeny of a Mottled individual that was identified as a diploid-triploid, which suggests the genotype mosaics aa/AAA , aa/AAa or aa/Aaa ;
- individuals that were mostly of Albino coloration with a few of Wild coloration that were the progeny of a Mottled individual identified as diploid, which suggests the genotype mosaic Aa/aa .

Similar results that confirmed the mosaic origins of Mottled coloration were obtained when the first Mottled female was spawned at Rutki (Fig. 9); in comparison with the individuals of Albino coloration, the gynogenetic progeny was comprised primarily of individuals with Wild coloration. In the androgenetic progeny of a Mottled male (Fig. 10b) and that of the same male crossbred with a three-year old Mottled female (marked with the index 'M'; Fig. 10a) produced only individuals with Wild coloration (Table 12).

Decidedly different results were obtained with the gynogenetic reproduction of Mottled female M (Fig. 10a). Among her gynogenetic progeny (both meiotic and mitotic), a substantial quantity of fingerlings were confirmed to have Mottled coloration; during the evaluation of the fall fingerling stage, it was determined that in the mitotic gynogenetic progeny group they comprised 57%, while in the group of meiotic gynogenesis they comprised 40% of all the fish in a given group (Table 13). There were no Mottled individuals in the progeny groups of the F_1 generation originating from the Mottled female M and various males of various coloration (Wild, Palomino, Mottled). In the Mottled female M generation F_2 , mottled fish were observed in some groups of the gynogenetic progeny of her daughter and in two families of the six produced by full sibs of the F_2 generation (Table 16; Fig. 12). Based on these results, it can be concluded

that the Mottled coloration trait was confirmed in Mottled female M and is inherited by the subsequent generation, but not by all individuals. Among the Mottled fish of generation F₂ that were noted, the occurrence of Wild-Xantoric (W-K) and Palomino-Albino (P-A) were confirmed. The coloration patterns and the surface areas of certain colorations on the fish skin differed in different fish.

The analysis of the skin coloration of the progeny of generation F₁, Mottled M female crossbred with a Palomino male (Table 12) and the generation F₂ family after crossbreeding the Mottled female M1 and Wild females W4 and W5 with Palomino males P1 and P2 (Table 15), indicated that at the alevin stage the ratio of individuals with Wild and Palomino coloration was 1:1 (verified with the χ^2 test). Crossbreeding the Mottled female M2 and Wild females W2, W3, W5, and W6 with Wild males W1, W2, W3, and W4 (Table 15) produced alevins at a Wild to Palomino ratio of 3:1 in all the families (verified with the χ^2 test). This indicates that both the Mottled female M and her gynogenetic daughters (M1 and M2) have the heterozygotic genotype *Aa* locus *A* responsible for Xantoric coloration, which is inherited according to the mechanism described earlier.

Varied results were obtained from the analysis of the coloration of the alevins of meiotic gynogenetic progeny. In the first generation from the Mottled female M (Table 12), as in the second generation obtained from the W2, W4, and W5 females with Wild coloration (Table 15), the majority of the individuals in the families were of Wild coloration while the remainder were Xantoric. Even though the group of second generation meiotic gynogenetic progeny originated from the two gynogenetic M1 and M2 females with Mottled coloration, only a very few individuals were identified as Wild during the alevin period, while the remainder had Xantoric coloration (Table 15). The evaluation of the coloration of gynogenetic progeny was done based on skin pigmentation since attempts to segregate the embryos at the eyed-egg stage based on eye color were unsuccessful because of the wide range of eye color from black to red. Only in the case of meiotic gynogenetic progeny of Mottled female M was an attempt made to evaluate future coloration based on iris pigmentation; however, some of the progeny which were identified as having black eyes at the eyed-egg stage turned out to have Palomino coloration as alevins (Table 12). It is most probable that some Mottled individuals, which are predominately Xantoric in coloration, have black eyes (or eyes that are between red and black) in the eyed-egg stage and Palomino skin coloration in the alevin stage. In turn,

mottled individuals that are largely Wild in coloration are identified as Wild in both the eyed-egg and alevin stages. A similar situation was observed in the analysis of the number of fish of various coloration in the generation F_2 family from the crossbreeding of the gynogenetic female M1 with the male P1 (Table 16 and 17). In this group from the alevin stage until two years of age there was a distinct decline in the number of individuals that had been identified at the alevin stage as Wild and Palomino and a distinct increase in the number of individuals with Mottled coloration. Additionally, a portion of the progeny identified at the alevin stage as Wild with dark eyes became Mottled individuals. Most probably also among the fish identified as Palomino at the alevin stage, at the fingerling stage there appear fish of Mottled coloration from within the Wild-Xantoric group in which Xantoric coloration predominates, and fish of Mottled coloration from within the Palomino-Albino group. No Albino fish were noted among the adult individuals from generations F_1 and F_2 . However, among the progeny of generation F_2 , the influence of allele *b* responsible for Albino coloration was apparent since individuals with Mottled Palomino-Albino coloration were confirmed among the progeny (Table 16).

The Mottled trait does not begin to become apparent until the fingerling stage at a mean weight of about 20 g. First, individuals with Wild-Xantoric coloration begin to appear in nearly equal share of 50-50%. After achieving an individual weight of approximately 60 g, almost all of the fish that exhibit features of Mottled coloration do develop this trait. However, a few of the fish that are identified as Wild in the first year of rearing can turn out to be Mottled in the second year of life. This situation was observed when analyzing the quantity of Mottled and Wild fish in the progeny group of the Mottled female M from generation F_1 of meiotic gynogenesis. During the period between the first and second year of life the number of individuals with Wild coloration declined from 32 to 17, while fish with Mottled coloration increased from 19 to 20. At the same time, in the group of fish with Mottled coloration, the appearance of individuals with a distinct prevalence of Wild to Xantoric coloration (at a ratio of 90 to 10%) was observed, which may indicate that this coloration pattern does not appear until the second year of life (Tables 16 and 17).

The analysis of the number of Mottled fish observed in the different families at the current stage of the experiments do not permit determining the origin or the manner in which this trait is inherited. When Mottled individuals are crossbred with fish that

never had had any contact with this trait, it does not appear until the second generation. Its appearance is related to the co-occurrence of allele *a* responsible for Xantoric coloration. All of the Mottled individuals analyzed from the Wild-Xantoric groups have the heterozygotic genotype *Aa* in locus *A* responsible for Wild or Xantoric coloration.

The evaluation of Mottled fish growth in generation F_2 indicated that the growth rate of these fish is distinctly lower in comparison to fish with Wild coloration. In five groups of gynogenetic progeny in the second year of their life, fish with Wild coloration had a higher mean weight than did Mottled fish of Wild-Xantoric coloration. This difference was highly significant in three groups (Table 17).

In two generation F_2 families created by crossbreeding the Mottled M1 and the Wild W5 females with the Palomino P1 male, among the individuals with Wild, Palomino, and Mottled Wild-Xantoric (W-K) and Mottled Palomino-Albino (P-A) coloration patterns, the highest mean weight was attained by individuals with Wild coloration. In the $M1 \times P1$ family, the Mottled fish of both patterns had the lowest mean weights. The lowest mean weight of Mottled W-K fish in this family was highly significantly lower than the mean weight of fish with Wild coloration and significantly lower than the mean weight of fish with Palomino coloration, while the mean weight of Mottled fish from the P-A group was significantly lower only than the mean weight of fish with Wild coloration. In the $W5 \times P1$ family, the lowest mean weight was attained by Mottled fish from the P-A group, and Mottled fish from the W-K groups attained a slightly higher mean weight than the fish from the Palomino group. No significant differences were detected in the mean weight of fish from the various coloration groups in this family (Table 17). In the case of Mottled fish, no definitively higher growth rate was confirmed at this stage of the experiment in Wild-Xantoric individuals in relation to those of Palomino-Albino coloration, which could suggest the results of experiment obtained earlier in which the growth of Wild, Palomino and Albino fish were compared (Tables 9 and 10).

Analyzing the survival of fish with Mottled coloration is difficult since this trait does not appear until four to 16 months following hatching, and the first four months of rearing are usually when the highest mortality occurs.

Comparative analysis of the mottled pattern of the M2 Mottled female of the first gynogenetic generation (F_1) with that of ten of her progeny from the second gynogenetic generation (F_2) indicated that there was great similarity in the share of particular

patches and colors on the bodies of the fish examined. The coloration on the body surfaces of all these fish was predominately Xantoric as opposed to Wild coloration. The estimated correlation coefficient for body coloration distribution on the Mottled fish examined indicated that the coloration of five individuals from among the progeny is very similar to that of the mother. Four individuals from among the progeny were singled out as having mutually similar coloration and a significantly larger number of patches of Wild coloration in comparison to that of their mother (Table 18).

The analysis of the mottled coloration of ten individuals of meiotic gynogenetic second generation progeny indicated that the pattern of coloration is similar in 50% of the individuals. Based on a genetic study of individuals obtained through meiotic gynogenesis, Allendorf et al. (1986) reported a degree of homozygosity of about 44%.

Based on these results it is possible to put forward the hypothesis that Mottled coloration noted at DSR Rutki is of genetic origin and that the pattern of Mottled coloration in fish may be determined by many genes within a cumulative system (quantitative heredity).

6. SUGESTIONS FOR FURTHER RESEARCH

The experiments conducted at Rutki on the Xantoric coloration of rainbow trout have provided information regarding the types of such coloration and their inheritance. Simultaneously, this research has also raised new question that require further investigation. There is no confirmation of the hypothesis that the co-action of allele *B* responsible for Palomino coloration and allele *b* responsible for Albino coloration is additive (co-dominating). The first observation of individuals with known genotype locus *B* as the homozygotes *BB* (Fig. 6) and heterozygotes *Bb* (Fig. 8) suggest that this is additive gene action. Crossbreeding androgenetic Palomino males with a known homozygotic genotype of *BB* at locus *B* with Palomino females of a known heterozygotic genotype of *Bb* and Albino females of a *bb* genotype and observations of the coloration of their progeny should provide answers for this question.

The initial results regarding Mottled coloration presented in this work suggest that it was confirmed for the first time at Rutki that the mutation responsible for the occurrence of this coloration, which is passed from one generation to the next, is hereditary. Using Mottled fish of both sexes (this is the first opportunity to crossbreed individuals

with Mottled coloration) obtained from generation F_2 as well as their sibs and half-sibs of different coloration further crossbreeding will be conducted. The appropriately planned experimental crossbreeding of fish of a known origin and the analysis of the coloration of groups of progeny should help to determine the inheritance mechanism and the value of the biological traits of fish with mottled coloration. The Mottled trait will also be used to as a marker to create and observe clonal lines of rainbow trout by using meiotic gynogenesis and androgenesis. The clonal lines of Mottled fish obtained should also aid in verifying the hypothesis that the pattern of Mottled coloration (share of particular spots and color saturation on the fish bodies) is a quantitative trait.

The DSR also has adult specimens with coloration that differs from Wild and Xantoric (Goryczko and Dobosz 2004), namely:

- metallic blue, which appears in fingerlings, – adult fish attain sexual maturity;
- blue, which appears in fry – adult fish do not attain sexual maturity, they are sterile;
- white – adult fish attain sexual maturity.

All of these fish of various coloration have dark eyes. Experiments will be undertaken with the aim of determining the cause, the origin, and the breeding value of these forms of coloration.

7. SUMMARY AND CONCLUSIONS

1. The appearance of Xantoric coloration in rainbow trout is caused by a mutation that leads to the occurrence at locus A of allele a , which is recessive to the dominant allele A responsible for Wild coloration. The homozygotic aa combination at this locus causes the disappearance in the fish skin of melanophores (pigmentation cells), which create melanin (black pigment).
2. Within Xantoric coloration, the hereditary fish colorations of Palomino and Albino also occur. At Locus B allele B is responsible for Palomino coloration, while allele b , which in the homozygotic bb is responsible for Albino coloration in fish. Allele B which occurs in the homozygotic combination BB in androgenetic fish influences distinctly darker Palomino coloration in comparison with fish that are known to have the heterozygotic Bb .
3. Locus A is epistatically dominating in relation to locus B .

4. During the eyed-egg stage and the early hatch stage, Palomino and Albino fish have red irises. During post-larval (alevin) development, the iris in Palomino fish becomes dark, while it remains red in Albino fish throughout life.
5. The alleles responsible for coloration in Palomino and Albino fish have a negative pleiotropic impact on the growth, survival, and nonspecific immunity indices. The worst indices for these traits are noted in Albino fish, while those of Palomino fish are between the values for Wild and Albino fish.
6. Most of the individuals with Mottled (Wild-Xantoric) coloration observed at Rutki did not pass this trait to their progeny. In the literature, these fish are described as mosaics.
7. By applying the gynogenesis method, Mottled females that pass this trait to their progeny were identified; this is the first known case of the inheritance of the Mottled trait.
8. After crossbreeding the progeny (first generation) of the Mottled female, Mottled individuals were confirmed in two of six families.
9. Mottled coloration appears between four and 16 months following hatching in both individuals which were identified in the alevin stage as Wild and Palomino.
10. The type of coloring and the patterns of patches in Mottled fish is highly variable and range from Wild coloration with only a few patches of Xantoric coloring to Xantoric coloration with very few patches of Wild coloring and even Palomino coloration with patches of Albino coloring.
11. The results obtained allow putting forward the hypothesis that the mutation identified as responsible for the appearance of Mottled traits in the rainbow trout at Rutki is inheritable and connected to the occurrence of allele *a* responsible for Xantoric coloration.
12. In all groups of progeny in which the occurrence of Mottled and Wild individuals was confirmed, the Wild fish exhibited faster growth rates than did the Mottled fish.
13. In the second generation of Mottled fish that were reproduced by meiotic gynogenesis, significant similarity was noted in both the Mottled pattern and shades of color among the progeny as well as among them and the Mottled coloration of the mothers.

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9. SUMMARY

During selection work on rainbow trout at the Department of Salmonid Fisheries at Rutki, it was noted that in some of the cultivated strains fish occurred whose coloration differed from the wild coloration and ranged from yellow to dark brown. Additionally, a strain was obtained that was fully yellow. All of these coloration varieties were described as Xantoric. By appropriately crossbreeding within and between these strains, it was proved that the recessive allele *a* at locus *A* was responsible for Xantoric coloration in contrast to the dominant allele *A* that is responsible for Wild coloration. Further studies revealed that within the Xantoric coloration, hereditary Palomino and Albino coloration also occurs. Allele *B* at locus *B* is responsible for Palomino coloration in contrast to allele *b* that, in the homozygote *bb*, is responsible for Albinism. Allele *B*, which is responsible for Palomino coloration in the homozygote *BB*, influences the distinctly darker color that occurs in comparison with heterozygotic *Bb* fish. It was also confirmed that locus *A* is epistatically dominant in relation to locus *B*.

It was observed that during the eyed-egg stage and the early hatch development that the irises of Palomino and Albino fish are red, but that in later developmental alevin stage the irises of Palomino fish darken while those of the Albino remain red. The alleles responsible for the Palomino and Albino colorations have a negative pleiotropic impact on growth, survival, and nonspecific immunity. Albino fish exhibited the worst values of the indices for these traits, while those for Palomino fish were intermediary between the Albino and Wild fish.

Among the fish cultivated at DSR Rutki, individuals with distinct patches on their bodies were noted and described as Mottled. Generally, this color type was not inherited by the progeny. Nevertheless, in one instance, the gynogenetic progeny of a Mottled female inherited this trait. Initial studies of using genome engineering (meiotic and mitotic gynogenesis, androgenesis), the masculinization of females, and tagging fish with PITs permit considering that the mutation responsible for the occurrence of Mottled traits that were confirmed at DSR Rutki is hereditary and connected to the occurrence of Xantoric coloration. In the second generation of meiotic gynogenetic Mottled fish, similarities were confirmed concerning Mottled coloration within the progeny as well as among the progeny and the mother.

The studies conducted indicated that although the color forms of rainbow trout at DSR Rutki have similarities with descriptions in the literature, they exhibited a different inheritance mechanism. Additionally, for the first time hereditary traits of Mottled coloration in rainbow trout were identified.

10. STRESZCZENIE

W Zakładzie Hodowli Ryb Łososiowatych Rutki w trakcie prac selekcyjnych prowadzonych na pstrągu tęczowym, zaobserwowano w niektórych z hodowanych szczepów występowanie ryb o ubarwieniu różnym od Dzikiego, o odcieniach od żółtego po ciemny brąz. Oprócz tego pozyskano szczep charakteryzujący się w całości ubarwieniem żółtym. Wszystkie te formy ubarwienia określono jako Ksantoryczne. Dzięki przeprowadzeniu odpowiednich krzyżowań tak w obrębie, jak i pomiędzy szczepami ustalono, iż za ubarwienie Ksantoryczne odpowiada recesywny allel a w lokus A w stosunku do dominującego allelu A odpowiedzialnego za ubarwienie Dzikie. Dalsze badania ujawniły, iż w obrębie ubarwienia Ksantorycznego występuje dziedziczne ubarwienie Palomino i Albino. Za ubarwienie Palomino odpowiada allel B w lokus B przeciwstawnie do allelu b , który w homozygotcie bb odpowiada za ubarwienie Albino. Allel B odpowiedzialny za ubarwienie Palomino w homozygotcie BB wpływa na wyraźnie ciemniejsze ubarwienie w porównaniu do ryb o heterozygotycznym układzie Bb . Stwierdzono także, że lokus A jest epistatycznie dominujący w stosunku do lokus B .

Zaobserwowano, iż w okresie zaoczkowania i wczesnych stadiów rozwoju wylęgu ryby Palomino i Albino charakteryzują się czerwonym ubarwieniem tęczówki oka, z tym, że w późniejszych stadiach rozwojowych u ryb Palomino następuje jej ciemnienie, zaś u Albino pozostaje czerwona. Allele odpowiedzialne za ubarwienie Palomino i Albino mają negatywny plejotropowy wpływ na wzrost, przeżywalność i odporność nieswoistą, przy czym najgorsze wskaźniki tych cech wykazały ryby Albino, zaś Palomino pośrednie pomiędzy Albino a rybami ubarwionymi Dzikie.

Wśród ryb hodowanych w Rutkach zaobserwowano także osobniki z wyraźnymi plamami na ciele określane jako Łaciate (Mottled). Najczęściej ten typ ubarwienia nie był przekazywany potomstwu. Jednakże w jednym przypadku gynogenetyczne potomstwo samicy Łaciatej zachowało tę cechę. Wstępne badania z zastosowaniem metod inżynierii genomowej (gynogenezy mejotycznej i mitotycznej, androgenezy), maskulinizacji samic oraz znakowanie znaczkami mikroprocesowymi PIT pozwalają przypuszczać, iż stwierdzona w Rutkach mutacja odpowiedzialna za występowanie cechy Łaciatości jest dziedziczna i powiązana z występowaniem ubarwienia Ksantorycznego. W drugim pokoleniu gynogenotów mejotycznych ryb Łaciatych stwierdzono podobieństwo ubarwienia Łaciatego w obrębie potomstwa jak i pomiędzy nim a matką.

Przeprowadzone badania wykazały, iż występujące w ZHRŁ Rutki formy barwne pstrągów tęczowych pomimo podobieństwa z opisywanymi w literaturze, wykazały odrębny sposób dziedziczenia. Także po raz pierwszy zidentyfikowano dziedziczną cechę Łaciatości u pstrąga tęczowego.