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Genetic Linkage and Color Polymorphism in the Southern Platyfish (*Xiphophorus maculatus*): A Model System for Studies of Color Pattern Evolution

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ABSTRACT

Color pattern polymorphisms are widespread in animals, and are found within populations, among populations, and among species. The southern platyfish, *Xiphophorus maculatus*, represents one of the most extreme examples of color pattern polymorphism. Extensive research with this model system for melanoma formation has resulted in an understanding of the underlying genetic basis of over 40 sex-linked and autosomal color patterns, including alleles that code for melanin, pterin, and carotenoid coloration. Research has also found that genes that affect sex determination, timing of sexual maturation, and coloration are genetically linked on the sex chromosomes. In many animals, color patterns often show strong sexual dimorphism, with conspicuous coloration limited to males. Although some of the color pattern alleles are sex-limited in platyfish, many are expressed by both sexes. Despite the abundance of work on this model system, little is known about the evolutionary processes responsible for this diversity of color pattern alleles. This review discusses what is known about platyfish coloration, and the roles that genetic linkage and variation in environmental conditions within and among populations might play in the evolution and maintenance of the extreme color pattern polymorphism exhibited by this platyfish.

INTRODUCTION

EXTENSIVE COLOR PATTERN DIVERSITY exists in the animal world, with variation both within and among species. Such diversity likely has adaptive significance. Coloration can be important in reducing danger, with prey using coloration to either warn predators or remain cryptic to predators.[1] Cryptic coloration can also be important to predators, reducing the likelihood of their detection by potential prey. Coloration is also known to play a role in both mate choice and intrasexual competition.[2] For example, mating preferences in one sex for traits in the other sex appear to have favored the evolution of the colorful male sword in green swordtails, *Xiphophorus helleri*.[3,4] Intrasexual competition, primarily competition among males for access to females, appears to have favored the evolution of black vertical bars in males of the high-backed pygmy swordtail, *X. multilineatus*.[5]

Traits that increase an individual's attractiveness to the opposite sex may also increase its conspicuousness to predators.[6–8] Therefore, whereas sexual selection is predicted to favor the evolution of more conspicuous coloration, natural selection via predation should often favor the evolution of less conspicuous coloration. Conspicuous red and orange coloration has been found to be attractive to females in a number of animals, including a variety of fishes, for example guppies, sticklebacks, and swordtails.[9–11] In the guppy, *Poecilia reticulata*, females prefer males with larger orange spot coloration.[9] However, Endler[7] found that orange spots in guppies were smaller in populations with a high predation risk relative

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to populations with a low predation risk. Thus, natural and sexual selection have opposing effects on the evolution of coloration in guppies; female mate choice favors orange or red spots while predation disfavors these spots. In the same study, the size of black, blue, iridescent, and other colored spots also decreased when predation risk was increased. This suggests that not only is red coloration conspicuous to predators, but some black and iridescent color patterns are as well. In green swordtails, *X. helleri*, natural and sexual selection appear to act in opposition on the sword, a colorful male trait. While female green swordtails prefer long, multicolored swords,[3,12] males from populations with predatory fish have shorter swords than males from populations in which the predators are absent.[8]

The conspicuousness of a color pattern, whether to a conspecific or a predator, can be affected by environmental factors, such as ambient light conditions. For example, although female sticklebacks, *Gasterosteus aculeatus*, preferred red males when the light environment allowed the detection of red coloration, when a filter blocked the transmission of red coloration, females no longer exhibited the preference.[10] In the green swordtail, *X. helleri*, females prefer orange swords to green swords, but when the light environment is experimentally manipulated to reduce reflectance in the orange wavelengths, the preference is no longer detected in prep.[11] Likewise, in the guppy, when wavelengths that reflect orange are blocked, females no longer exhibit a preference for males with higher degrees of orange expression.[13] In an aquatic environment, variation in the spectral transmission of the water stemming from differences in color and opacity may also affect the perception of color patterns by individuals viewing from a distance, as some predators might do while deciding whether to attack. (Reflective color patterns like blue and iridescence are expected to be detectable at longer distances.[7]) Under the same water conditions, however, the visual perception of the same color patterns by receivers in close proximity, like females attending to courting males, could be affected to a lesser degree. Another environmental factor that can affect whether an organism is easily detected is the background; if there is a good match between an individual's color pattern and the background color pattern, there will be a reduced likelihood of detection,[7] especially at greater distances.

In addition to environmental effects on color pattern conspicuousness, animals may modulate the conspicuousness of color patterns using a variety of behavioral mechanisms, such as expressing coloration on body sections that can be facultatively hidden, using other individuals as a buffer between self and potential predators, and restricting activities to periods when potential predators are least active. For example, some reef fishes limit their activities during crepuscular hours (dawn and dusk) when predation risk is relatively higher,[14] and guppies from high predation risk populations tend to school more than guppies from low predation populations.[15]

Finally, the arrangement of color patterns on the body can affect the conspicuousness of color patterns; reds or yellows contrasted against black can be particularly conspicuous.[1] For any given population, then, the optimal color pattern phenotypes are a balance between different sources of selection, with the local biotic and abiotic environment playing an important role. The local environment, therefore, should be highly predictive of the color pattern variation in a population residing there. When environmental conditions are similar across a species' range, color patterns may also be similar. When environmental conditions are dissimilar across a species' range, color patterns may be very different across populations.

Of the diverse types of coloration expressed by animals, this review is primarily concerned with chromatophores found in the dermis, scales, integument, and eyes of fishes. Fox[16] makes a distinction between two types of chromatophores, those that contain biochromes and those in which colors result from purely physical properties, the schemochromes. Biochromes are true pigments, such as carotenoids (red, yellow, and orange), pterins (white, red, orange, and yellow), purines (white or silver), and melanins (reds, yellows, browns, and blacks). Chromatophores that contain the red and orange carotenoids are termed erythrophores, and those that contain the yellow

carotenoids are termed xanthophores. Most animals do not appear to synthesize carotenoids *de novo*,[17,18] but they can obtain carotenoid pigments in their diet, including plant material, algae, and other animals. The melanin pigments are contained in chromatophores termed melanophores. Melanin is a product of the oxidation of amino acids, such as tyrosine and tryptophan. A fourth type of biochrome, pterinophores, contain pterins that contribute to eye coloration as well as coloration of other fish tissues. Pterins can be synthesized in developing pterinophores by vertebrates and other animals.[19]

Chromatophores that do not contain true pigment, but instead produce structural colors, are called schemochromes; the color of schemochromes is the result of the interaction of light and cell structural configuration.[16] The detection of structural colors can be ephemeral. For example, detection of the chromatic effect of iridescent structural colors depends on the angle at which they are viewed; changes in angle can produce the "flashing" appearance seen in moving fishes.[16] Coloration of fish eyes, integument, and scales can involve such structural colors. The different types of biochromes and schemochromes can play off of a background of other types of chromatophores, the combined effect of which can result in complex color patterns, and a large degree of variation between individuals.

Several fish species have been developed as model systems for color pattern research, including the guppy, *P. reticulata*, and the southern platyfish, *X. maculatus*. While abiotic and biotic factors that affect color pattern variation has been the primary focus of guppy studies, the underlying genetic basis of color pattern variation has been a focus of study in southern platyfish. As a result, the southern platyfish is one of the few vertebrate systems in which the genetic basis of a large number of color patterns has been characterized. In this paper, I provide an overview of what is known about color pattern loci and alleles in the southern platyfish. I then highlight how this impressive body of knowledge can be used to investigate potential mechanisms responsible for producing the extreme degree of color pattern polymorphism found in platyfish.

Background on the southern platyfish

The southern platyfish, *X. maculatus,* is a small, freshwater fish found in river drainages of northern Central America east of the continental divide, ranging from the Rio Jamapa, Veracruz, Mexico, to Belize and Guatemala (excluding the Yucatan Peninsula). Coloration in platyfish is highly variable, but the background is yellowish-beige to olive-grey in coloration. This background coloration consists of micromelanophores, xanthophores, and iridophores.[20] Platyfish are found in greatest numbers in ponds, ditches, small lakes, and streams, but can also be found in low numbers in larger rivers (personal observation). The sites at which platyfish occur vary in many ways, including the presence of piscivorous fish, the composition of sympatric species, water depth, substrate, type and amount of vegetation, elevation, temperature, current, amount of shade, and water color and opacity (personal observation).

Southern platyfish are poeciliids, a group of New World livebearing fishes with internal fertilization. During the day, male time is primarily spent foraging, attempting to attract female attention, courting females, chasing other males, and/or avoiding other males. Males are easily distinguished from females by the presence of the gonopodium, a specialized structure for transferring sperm to females. Sperm is transferred to females in the form of sperm packages containing numerous spermatozoa. Fertilization is internal, and females in my laboratory have produced offspring for up to 7 months after having been inseminated. Females produce numerous broods after reaching maturation, and each brood may contain offspring sired by more than one male. Sperm precedence has not been established for platyfish. In my laboratory, females have had as many as 13 broods in a 14–15 month period. Initially, broods tend to be smaller in the number of offspring, then tend to increase in number over time. However, female reproductive investment patterns appear to vary among families.[21] Young are self-sufficient at birth.

The southern platyfish has been studied for over 130 years. They were first named *Platypoecilus maculatus*;[22] this genus name refers to

both their flattened body and the numerous colors they exhibit. Platyfish were subsequently placed in the genus *Xiphophorus* with swordtails.[23] Several attributes of this system have made it ideal for both laboratory and field research, including a short generation time, high fecundity, ease of rearing and collecting, and the extensive background information that has steadily been generated on its natural history and a variety of measurable traits. In the past 60 years, platyfish have been used in genetic research focusing on sex determining mechanisms, age and size at sexual maturation,[20,24] melanin expression and melanoma formation,[25–32] and sex ratio evolution.[33] From this extensive body of work, the genetic basis of a surprising number of color patterns has been characterized.

THE GENETIC BASIS OF COLORATION IN *X. MACULATUS*

Like the guppy, another poeciliid, southern platyfish vary extensively in coloration. Female guppies do not express the conspicuous color patterns that are expressed in males, but in platyfish, both males and females express conspicuous color patterns. Studies of pigment cells in platyfish have established that a number of types of coloration are expressed, including black melanins, red, orange, and yellow carotenoids, and yellow, orange, red, and reddish brown pterins.[20,24,26–28,34] These studies have also characterized the genetic basis of at least 42 different color patterns (Table 1). (The adult expression of these patterns and the pertinent references are given in the Appendix.) Southern platyfish express additional color patterns, but the genetic basis of these has not been established (Table 2). (Note: Only naturally occurring coloration is discussed in this review.)

Of the numerous color patterns for which the genetic basis is known, some have been traced to autosomes while others have been determined to assort on the sex chromosomes. The sex determination locus and the pituitary locus (a locus with a major effect on the age and size at sexual maturation) are linked with many of these color patterns due to their location on the sex chromosomes;[24] one locus could thus affect the evolution of alleles at the linked loci. Because of genetic correlations, I will discuss the sex-linked color pattern loci in the context of the known variation at linked loci on the sex chromosomes.

Sex-linked color loci

A mechanism to maintain sexual dimorphism could be the location of the genes that either encode or regulate these traits on the sex chromosomes. In male heterogametic systems of sex determination (XX, XY), like those found in humans, guppies, and many mammals, the restriction of the Y chromosome to males can allow for the evolution of Y-linked traits expressed only in males. For example, in guppies, many conspicuous color patterns are Y-linked,[6] resulting in pronounced sexual dimorphism, with brightly colored males and dull females. In the southern platyfish, there is intraspecific variation in the mode of sex determination; some populations are male heterogametic (XX, XY), while others have a three factor system with three female genotypes (WX, WY, XX) and two male genotypes (XY, YY), similar to the multifactor sex determining system in several species of lemmings.[35] (For the purpose of this paper, the terms allele and factor are used interchangeably because selection is often expected to act similarly on them; factors are heritable units that act as alleles. However, a factor may actually represent a set of alleles that are in linkage disequilibrium. Several tightly linked loci are sometimes referred to as a supergene.)

The three sex chromosomes in platyfish are not cytologically heteromorphic, that is, they appear similar in size and shape, but by definition, the chromosomes that house the major sex determination loci are termed the sex chromosomes. The majority of populations of *X. maculatus* studied to date have three sex chromosomes producing five sexual genotypes, but two river drainages at the western end of the range lack the W chromosome and therefore exhibit only two sexual genotypes.[28,36–38] There is some evidence that the frequency of the X chromosome is low in two eastern river drainages, but this has been attributed to sampling error.[38]

TABLE 1. SOUTHERN PLATYFISH COLOR PATTERNS WITH ESTABLISHED GENETIC BASIS, ABBREVIATIONS, GENETIC LINKAGE, AND SEXUAL EFFECT (ADAPTED FROM BASOLO[65])

Color pattern	*Abbreviation*	*Linkage*	*Sex effect*
EYE COLOR			
Iris yellow	*ly*	Sex-linked	Sex-influenced
Iris red	*lr*	Sex-linked	Sex-influenced
BODY COLOR			
Red, Orange, and Yellow			
Mouth red	*Mr*	Sex-linked	Limited to males
Red anal fin spot	*ASr*	Sex-linked	Limited to males
Red flush	*Fr*	Sex-linked	Limited to males
Red nape	*Nr*	Sex-linked	Limited to males
Ruby throat	*Rt*	Sex-linked	Limited to males
Orange caudal peduncle-2	*CPo-2*	Sex-linked	Limited to males
Ventral orange	*Vo*	Sex-linked	Limited to males
Body red	*Br*	Sex-linked	Sex-influenced
Red background	*"R"*	Sex-linked	Sex-influenced
Orange caudal peduncle-1	*CPo-1*	Sex-linked	No difference
Yellow caudal peduncle	*CPy*	Sex-linked	No difference
Yellow anal fin spot	*Ay*	Sex-linked	No difference
Red vertical bars	*STr*	Sex-linked	No difference
Black			
Nigra	*N*	Sex-linked	No difference
Spot-sided	*Sp*	Sex-linked	No difference[a]
Stripe-sided	*Sr*	Sex-linked	No difference
FIN COLOR			
Red, Orange, and Yellow			
Red tail	*Tr*	Sex-linked	Sex-influenced
Dorsal red	*Dr*	Sex-linked	Sex-influenced
Anal red	*Ar*	Sex-linked	Sex-influenced
Yellow tail	*Ty*	Sex-linked	No difference
Black			
Spot dorsal	*Sd*	Sex-linked	No difference
Crescent	*C*	Autosomal	No difference
Complete crescent	*Cc*	Autosomal	No difference
Comet	*Co*	Autosomal	No difference
Comet	*Cò*	Autosomal	NA
Comet	*Có*	Autosomal	NA
Dot	*D*	Autosomal	No difference
Moon	*M*	Autosomal	No difference
Moon complete	*Mc*	Autosomal	No difference
One spot	*O*	Autosomal	No difference
Twin spot	*T*	Autosomal	No difference

No difference = Expressed to the same degree in males and females.
Sex-influenced = Expressed in both sexes, but to a higher degree in males than in females.
NA = Information not available.
[a]Appears to be sex-influenced in one population.
Note 1: The black sex-linked color pattern Sb is not presented per Kallman.[20]
Note 2: The designation as sex-influenced or sex limited is based on the collections to date.
Note 3: Refer to Appendix for references for individual color patterns.

The difference in sex determination across populations could result in differences in color pattern expression, particularly in females. Because sex limitation of conspicuous coloration could be achieved by the location of the color pattern loci on the Y chromosome in XX:XY populations, costly conspicuous traits could be reduced in females. However, with a W, X, Y system, the chromosomes for both male genotypes are shared with females; restriction of loci for conspicuous traits to a single chromosome is thus not sufficient to prevent female expression. Rather, alternate mechanisms must evolve in populations with three sex chromosomes in order to restrict conspicuous traits that benefit males but are

Table 2. Common Color Patterns in Adult Southern Platyfish for Which the Genetic Basis Has Not Been Well Studied

Color pattern	*Abbreviation*	*Sex effect*
BODY COLOR		
Melanin		
Black shoulder spot	*Bss*	Expressed in both sexes
Vertical stripes	*Vs*	Limited to males
Iridescence		
Blue iridescence	*Bi*	Expressed in both sexes
Green iridescence	*Gi*	Expressed in both sexes
Yellow iridescence	*Yi*	Expressed in both sexes
Violet iridescence	*Vi*	Expressed in both sexes
FIN COLOR		
Melanin		
Anal fin fringe	*Aff*	Limited to females
Dorsal fin fringe	*Dff*	Limited to females
Pelvic fin fringe	*Pff*	Limited to females
Black gonopodium	*Bg*	Limited to males
Reflective white		
Anal fin stripe	*Afs*	Limited to females
Caudal fin stripe	*Cfs*	Expressed in both sexes
Pelvic fin stripe	*Pfs*	Expressed in both sexes

Note: Other than the shoulder spot and black gonopodium coloration, I have named the color patterns (field and laboratory data).

costly to females from being expressed in females.

In southern platyfish, a suite of color pattern alleles (Table 1) assort at loci in close association with the sex-determining locus on the sex chromosomes, and thus are sex-linked. Variation at sex-linked coloration loci can be sex-limited, sex-influenced, or expressed to the same degree in both sexes. Loci that occur on sex chromosomes are termed sex-linked. Sex-linked traits that are expressed in only one sex are termed either male sex-limited or female sex-limited. Sex-influenced traits are expressed in both males and females, but to a lesser degree in one sex. In southern platyfish, there appear to be at least five sex-linked color pattern loci that control: (1) red and yellow iris coloration involving pterin pigments; (2) red and yellow body and fin coloration involving carotenoid pigments or carotenoid plus pterin pigments; and (3) black body and fin coloration involving melanin.[28,37] The color pattern alleles or factors that assort at these loci are dominant, and the wild-type alleles, which are the most common alleles at both the sex-linked and autosomal color pattern loci at all locations, are recessive. If a different dominant allele is at the homologous locus on each sex chromosome, both are expressed, unless the expression of an allele is sex-limited.

Sex-linked eye coloration

Red and yellow eye coloration in platyfish is not sex-limited, but is more strongly expressed in males. The wild-type phenotype for eye coloration is a glistening whitish silver color with a hint of yellow. To my knowledge, the types of chromatophores in platyfish eye coloration have not been established, however, for the whitish silver iris, likely candidates are purine biochromes, and perhaps pterin biochromes. Both are known to contribute to eye coloration in fishes,[39] and fish are known to synthesize both types of pigments.[40] Two codominant alleles have been described for eye coloration, one of which produces a dense yellow iris (*Iy*) and the other a red iris (*Ir*). Likely pigment cell candidates for these color patterns are biochromes containing red or yellow carotenoids, biochromes containing red or yellow purines, or biochromes containing both types of pigments, all of which are found to affect eye coloration in fishes.[39] Because the angle of light

striking the platyfish iris can alter its appearance (personal observation), iridescent schemochromes may also be involved.

Red, orange, and yellow body and dorsal fin coloration

Seven red and orange sex-linked color pattern alleles/factors assort at one to two loci on the sex chromosome, are expressed only in males, and code for body coloration. In addition, five sex-linked alleles/factors result in sex-influenced red color patterns (two body and three fin color patterns), all of which are expressed to a greater degree in males than females. An additional five red, yellow, and orange sex-linked alleles are expressed to the same degree in males and females.

Based on the pigment cells that comprise them, the sex-linked body and dorsal fin color patterns can be grouped into the red, orange, and yellow patterns, and the black melanin patterns. Goodrich et al.[41] identified several types of chromatophores in southern platyfish. They found xanthophores with two different carotenoids, lutein and zeaxanthin, both of which are xanthophylls producing yellow coloration. They also found a red pigment that was not a carotenoid that their analyses indicated was erythropterin, a pterin pigment. Some chromatophores, which they designated "xantho-erthrophores" appeared red in coloration. These chromatophores were characterized by a concentration of yellow pigment at the center of the cell surrounded by the red pigment. They concluded that their "xantho-erythrophore" was a xanthophore in which erythropterin co-occurred with lutein and zeaxanthin, and thus that red or orange coloration was due to erythropterin in platyfish. From a hybridization study, they concluded that the genetic factors for the color patterns were genetically dominant.

Subsequent researchers have examined the red and yellow color patterns of platyfish further.[34,42,43] The distribution of the pigments in pterinophores, as Valenti and Kallman[34] termed them, was lutein at the center and pterinosomes containing drosopterin at the periphery. They concluded that the red coloration stems from the pterin, drosopterin. The discrepancy between the different studies may be accounted for in several ways. First, the source population for the platyfish studied by the two groups may have been different. Second, hybrids between southern platyfish and other *Xiphophorus* spp. can be artificially produced and are common in the aquarium trade. While the origin of the fish studied by Kallman and Valenti[34] was known, the origin of the fish studied by the Goodrich group is not clear. If they were hybrids, an allele or alleles coding for erythropterin could have been introduced by hybridization into the platyfish that they studied. Matsumoto[42] examined the ultrastructure of integumentary pterinosomes in platyfish. When carotenoids and pterins co-occurred in the xanthophores, he found that the pteridines were located in cytoplasmic organelles termed pterinosomes, and that carotenoids were located in the endoplasmic reticulum or in cytoplasmic vesicles. The internal structure of the pterinosomes containing drosopterin was composed of concentric lamellae.

Valenti and Kallman[34] also investigated why the expression of red coloration is limited to males in most cases, and found that males in which the gonads were removed had a weaker expression of the red color pattern dorsal red (*Dr*) and a decrease in the overall amount of drosopterin compared to males that had intact gonads (controlling for surgery effects with a sham treatment). They concluded that males express this red pattern to a stronger degree than females due to androgenic hormones. For females, they found no difference in the amount of drosopterin in homozygotes (*DrDr*) versus heterozygotes (*Dr+*); all females, however, had much less drosopterin than males. They also found that the expression of red coloration varied with the amount of red pigment per cell, not the number or position of pigment cells. Since this study, advances have been made in methodologies used to detect hormonal control of trait expression. A follow-up hormone replacement study in which the gonads of all test subjects were removed, and then administering supplemental androgenic hormones to one subset and mock supplements to the other subset would be useful to confirm these findings, and perhaps extend our under-

standing of the regulation of color pattern expression.

Kallman[20] suggests that the chromatophores responsible for the "pure" yellow color patterns like anal yellow (*Ay*), tail yellow (*Ty*), and caudal peduncle orange (*CPo*) are pterinophores that contain carotenoids and pterins, but that the pterins do not produce a color. He has found that the expression of these yellow patterns does not differ for the sexes. Kallman also suggests that some red color patterns, like dorsal red (*Dr*) and anal red (*Ar*), can be found across the range of *X. maculatus*, while others like red flush (*Fr*) are only found in a single river system. However, although certain color patterns may be widespread, Kallman[37] suggests that some of these do not share the same genetic basis. The dorsal red (*Dr*) pattern is an example. This color pattern is found at the eastern and the western edges of the platyfish range. Although the dorsal red phenotypes from each area are virtually identical, hybridization studies indicate that the *Dr* in the Rio Jamapa drainage has a different genetic basis than the *Dr* in the Belize River drainage.

Some of the pigment cells that are affected by the sex-linked color pattern alleles contain both carotenoids and colorful pterins, while others appear to contain either carotenoids, or, carotenoids and noncolorful pterins. In a sexual selection context, carotenoid pigments have been heralded as indicators of superior male fitness;[44–46] the degree to which a male is able to express carotenoids has been suggested to be positively correlated with a male's overall quality since higher levels of expression require males to either obtain more or higher quality food, or more efficiently process acquired food. By mating with males with a high degree of carotenoid expression, a female and/or her young might then gain either direct or indirect benefits. Although current studies of indicator traits in animals have focused on carotenoid-based color patterns, females might also be able to assess male quality based on red, orange and yellow coloration produced by pterins, as pterins have been shown to have positive fitness effects. For example, Norris and Simmons[47] treated nutritionally anemic juvenile salmon, *Oncorhynchus tschawytscha*, with xanthopterin, and found that this pterin could reverse the anemia. Likewise, xanthopterin has been found to hinder tumor formation in mice.[48] In addition to these studies, pterins are known to be important in a number of biochemical pathways.[19] Thus, both carotenoid- and pterin-based color patterns could be expected to affect male attractiveness to females. Lastly, as many animals can synthesize yellow, orange and red pterins, but often cannot synthesize carotenoids, pterins may be favored by mechanisms of mate choice not involving traits that indicate foraging ability, such as preexisting receiver biases.[4,49]

Black macromelanophore body and dorsal fin coloration

Four sex-linked black color patterns have been described, all of which are expressed to the same degree in males and females.[20,28,34,37] (However, one of the 12 spot-sided alleles, *Sp8*, is expressed to a greater degree in males.[20]) These color patterns appear to be controlled by several tightly linked loci, each having several alleles. Crossover evidence suggests that at least two of the alleles, one for a spotted dorsal fin (*Sd*) and one for a zigzag pattern along the flank called stripe-sided (*Sr*), assort at different loci on the sex chromosomes. Whether spot-sided (*Sp*: small spots along the flank) and nigra (*N*: large, irregular blotches along the flank) assort at one of these two loci, or a third linked locus has yet to be established.[20] All of the black sex-linked color patterns are produced by macromelanophores, which are large, have a high density of melanin, and produce a dark black color pattern. A second set of black patterns is formed by micromelanophores, which are much smaller and have a more diffuse appearance. The loci controlling the expression of these pigment cells are on autosomes and are discussed in the next section.

Whereas some melanin patterns in other *Xiphophorus* spp. are facultatively expressed,[5] the patterns coded for by the macromelanophore alleles are not. The overall degree of expression, however, may increase with age or environmental conditions. For example, platyfish in my laboratory that are maintained in tanks with natural sunlight express the macromelanophore patterns stripe-sided and

spot-sided to a stronger degree than those raised under broad spectrum artificial lighting (personal observation). The degree of expression can also vary depending on whether a male is homozygous or heterozygous. For example, males from the Rio Jamapa that are homozygous for stripe-sided (*SrSr*) more strongly express the color pattern than do males that are heterozygous.[20]

Kallman[20] suggests that macromelanophore patterns from different river drainages that appear to be identical actually have different genetic bases. In fact, for every river system that he sampled, there were two spot-sided (*Sp*) phenotypes (the Rio de la Pasion had three). As of 1975, 12 different alleles for the spot-sided pattern had been identified. In addition, work he conducted on the spot-dorsal pattern (*Sd*) indicated that not only is the *Sd* pattern different between river systems, but so are its modifiers. In addition, all mutations in the *Sd* allele that have been identified have resulted in an increased expression of this pattern. It thus appears that selection has independently favored the evolution of dorsal spots and spots along the flank in multiple platyfish populations. This could be the result of similar mating preferences in females, similar responses to coloration in a male competition context, similar levels of predation risk, similar water conditions, and/or similarities in other environmental factors across populations.

Out of 235 adult platyfish from a Belize River drainage population with predatory cichlids that I sampled (unpublished field data), two macromelanophore patterns occurred at similar frequencies in males and females (nigra occurred at a frequency of 0.26 in males and 0.20 in females; stripe-sided occurred at a frequency of 0.15 in males and 0.11 in females). If a specific color pattern is equally beneficial to the sexes, we would expect to see the frequencies to be similar for both sexes, as with nigra and stripe-sided. Conversely, if a pattern is costly to one sex, but benefits the other sex, we expect to see different frequencies of the pattern in females and males. This could explain the difference in expression of spot-dorsal (0.15 in males versus 0.02 in females) in this population. Three possible explanations for the low frequency of spot-dorsal in females are genetic drift, differential predation on individuals with these patterns, and a female but not a male mate choice preference for these patterns.

Sex-linkage relationships

The precise order of the loci as well as the actual number of loci on the sex chromosomes that affect coloration has not been fully determined, however, available information provides some insight into the position of the color pattern loci on the sex chromosomes. The sex-linked pigment alleles have been used in heritability studies to demonstrate that there are indeed three distinct sex chromosomes (W, X, Y) in southern platyfish.[28,36] Morphological differences for the three sex chromosomes are not cytologically apparent,[38,50] but the sex chromosomes appear to be acrocentric,[51] and the sex-determining locus/loci appear(s) to be located in close proximity to the centromere.[20] The color pattern loci and the pituitary locus (another sex-linked locus) would then be located between the sex-determining locus and the telomeric region. The loci for sex-determination, red and yellow coloration, age at sexual maturation (pituitary locus), and macromelanophore coloration are closely linked, and, breeding experiments and crossing over occurrences provide some evidence about the relative sequence of these loci. The melanophore pattern spot-dorsal (*Sd*) and the dorsal red (*Dr*) color pattern have been separated,[34] therefore they appear to assort at different loci. Crossover occurrences indicate that the locus for anal fin yellow (*Ay*), is between the macromelanophore locus and the red locus at which the mouth red allele (*Mr*) assorts.[20] Valenti and Kallman[34] reported that the locus for mouth red (*Mr*) is located between the locus for a red dorsal fin (*Dr*) and the macromelanophore locus. Gordon[52] reported a 1% recombination rate for the macromelanophore locus and the sex-determining locus. Recombination rates of less than 0.002 are reported for the pituitary alleles and red color pattern alleles.[36] Recombination rates of less than 0.01 are suggested for the pituitary alleles and melanophore alleles (Kallman: personal communication), suggesting that the pituitary locus lies closer to the red pigment pattern loci than the macrome-

lanophore or the yellow loci. Tight linkage between the sex determining locus, the macromelanophore locus and a locus for red and yellow coloration is supported by molecular studies.[53,54]

Finally, no field fish have been collected that express any of the co-dominant, sex-linked color pattern alleles on the W chromosome; rather all field females with a W allele have been found to express the recessive, wild-type color pattern on the W chromosome.[20] However, viable crossovers have occurred in the laboratory resulting in a color-marked W chromosome, which females express. The evidence that such females are absent in the field suggests that selection may favor females that lack most of the sex-linked color patterns.

Autosomal micromelanophore tail-spot patterns

The genetic basis of eight micromelanophore tail-spot patterns has been identified (Table 1). These color patterns occur either on the caudal peduncle or on the caudal fin. (The genetic basis of an additional two patterns, *Có* and *Cò*, has been suggested, but not verified by breeding experiments.) Like the sex-linked color patterns, the autosomal tail fin patterns are determined by codominant alleles, with a recessive wild type. The occurrence of three tail-spot patterns in individual fish indicates that the micromelanophores patterns involve at least two closely linked loci.[20]

Expression of the micromelanophore tail-spot patterns is not facultative, but can intensify over time. In addition, during the ontogeny of some micromelanophore patterns, they resemble an alternate micromelanophore pattern, but over time, the true phenotype becomes apparent. Such transitions from one state to another may indicate the influence of a modifier gene. For example, a tail-spot modifying factor, *Cg*, extends the twin-spot pattern (*T*) to produce what is called the Guatemala crescent pattern (*TCg*).

Field data suggest that at least some micromelanophore tail-spot patterns can be common in platyfish populations. In a sample of 235 adults from a Belize River drainage population with predatory cichlids (the same population discussed above in the macromelanophore pattern section), 73% of the fish expressed at least one micromelanophore pattern (unpublished field data). The frequencies of the patterns did not differ significantly between the sexes (males: 63/92 = 68%; females: 109/143 = 76%). One tail-spot pattern, one-spot (*O*: a spot in the center of the caudal peduncle near the caudal vein) was fairly common (males: = 46%; females: = 52%) while others were rare (crescent (*C*): males: = 1%; females: = 1%). Interestingly, complete crescent (*Cc*) was at a frequency of 0.18 in males and 0.21 in females. (Gordon and Gordon[27] did not find *C* in the Belize River, but they did find *Cc*.) One explanation for the difference in frequencies of the similar patterns, *C* and *Cc*, is that both patterns equally improve the likelihood of survival (avoid detection by predators), but random genetic drift could have driven one pattern to lower frequencies while alternate alleles at the same locus were driven to higher frequencies. Alternatively, selection may act differently on the patterns. No individuals in the population that I sampled expressed more than two tail-spot patterns. Some allelic combinations, however, were more common than others: *O-M* occurred at a frequency of 0.12; *Cc-O* occurred at a frequency of 0.09; and *Cc-D* occurred at a frequency of 0.05. Other combinations occurred at lower frequencies.

Additional color patterns

A number of color patterns for which the genetic basis has not been well studied also occur in southern platyfish (Table 2). (Because the genetic basis of these color patterns has not been well studied, it is not clear which, if any involve genetic polymorphisms.) These include melanin patterns, iridescent patterns, and patterns likely involving structural colors other than iridescence (unpublished field data). Three melanin patterns involve coloration at the distal tips of the two unpaired fins, dorsal fin fringe (*Dff*) and the anal fin fringe (*Aff*), and the paired pelvic fins, pelvic fin fringe (*Pff*). The two unpaired fin patterns appear in juveniles, 1–20 days after birth (personal observation). All adult females retain these patterns, but adult males do not. The melanin pattern vertical stripes (*Vs*) appears within 20–30 days of birth,

but then disappears in all but the smallest adult platyfish. (This pattern may be similar to or the same as the pattern "par marks".[55]) Another melanin pattern, possibly the same as black shoulder spot (*Bss*),[55] appears as platyfish mature, and is more common in adult females then males in the populations that I have sampled (unpublished field data). Adult male platyfish can have black gonopodium (*Bg*) coloration that varies in expression: anywhere from the distal one-third of the gonopodium to the entire gonopodium may be black.

A reflective pearly white stripe can be present on the anal fin (*Afs*), the caudal fin (*Cfs*) or the paired pelvic fins (*Pfs*). The caudal fin stripe and the pelvic fin stripe can be expressed in both males and females, while the anal fin stripe is only expressed in mature females. Such coloration could result from pigment cells containing pterins, purines, schemochromes, or a combination of these.

Four different types of iridescent colors occur in platyfish populations: blue, green, yellow, and violet. These schemochrome colors could be the result of guanine packed guanophores. The schemochromes can be quite prominently expressed on the scales (and perhaps the underlying dermis) along the flank in both males and females. Although these iridescent color patterns are not well understood, anecdotal information suggests that at least one may be heritable; a female I collected from a Belize River drainage population and returned to the laboratory produced 93 male offspring in a single brood, all of which expressed a similar patch of iridescent scales in the same position along the flank, but the mother did not express this coloration. Alternatively, the shared expression could have resulted from environmental conditions experienced by the female during gestation of these offspring.

Color pattern summary

The palette of color patterns in platyfish is set against a background of yellowish-beige (the wild-type coloration). Some of the alleles may produce patterns that allow an individual expressing them to blend into the background, while others likely make individuals quite conspicuous. In addition, whereas a particular black pattern against the yellowish-beige background alone may serve a function in crypticity, the same black pattern against a red, yellow or orange pattern could increase conspicuousness,[1] and thus the detection of the individual by others. The evidence that similar macromelanophore patterns producing similar phenotypes have arisen in different populations indicates that there has been convergence on certain phenotypes, thus there are likely similar environmental conditions favoring similar phenotypes across populations. In high predation risk populations, one would expect a decrease in the frequencies of color pattern alleles or combinations of alleles that produce highly conspicuous coloration, and an increase in those alleles that decrease detection by platyfish predators. If sexual selection favors conspicuous coloration in platyfish, the frequency of conspicuous alleles should be higher in populations with few predators relative to populations with many predators, all else being equal, as has been demonstrated for the guppy.

FUTURE DIRECTIONS

The extensive use of *X. maculatus* as an experimental system has led to an understanding of the genetic basis of over 40 color patterns as well as the establishment of genetic lines with known variation at the sex-linked and the autosomal color pattern loci. In addition, field research has yielded information about the distribution of and geographic variation in the different color pattern alleles. Information on the genetics of coloration in platyfish has proven instrumental in the study of variation at the sex determination locus and at the pituitary locus, and, knowledge about the melanin locus has facilitated the study of factors that can cause melanoma formation. Although almost no work has been done on the evolutionary processes affecting color pattern evolution in platyfish, it would be an outstanding system for such studies. First, because of the work on color pattern genetics, it is possible to examine not only temporal and spatial changes in phenotypic expression of color patterns, but also temporal and spatial changes in allele frequencies. Second, because of the high heritability of

the color patterns, selection on color patterns should result in rapid evolutionary responses, thereby minimizing one of the common problems of using a vertebrate system to study evolutionary processes.

With this wealth of genetic information, what types of questions can be addressed with platyfish? An obvious direction of inquiry would be to investigate factors that have been found to be important in geographic variation and color pattern evolution in other animals. For example, studies examining how sources of selection, such as predation, sexual selection, environmental factors that interfere with the transmission and reception of signals, amount of cover, and aquatic vegetation, etc., affect color pattern variation in guppy populations[56,57] could be conducted with platyfish. However, the platyfish system adds a novel component to such studies: the quantification of differences in allele frequencies among field populations rather than simply measuring differences in spot number, size and intensity. In addition, laboratory studies could track change in allele and genotype frequencies in experimental populations under different types and levels of selection, as has been done using variation at the sex determination locus to study the dynamics of sex ratio evolution[33] and sex ratio stability in platyfish.[58] For example, populations of platyfish varying in the presence or absence of predators could be established with known frequencies of known color pattern alleles to investigate the effect of predation on the evolution of color pattern alleles.

Beyond the advantage of tracking evolutionary changes in allele frequencies, the southern platyfish offers the potential for insight into mechanisms of evolution that few, if any, other systems offer. Of the many different ways the platyfish system is uniquely suitable to address fundamental but unexplored evolutionary questions, I will highlight three: (1) the evolutionary consequences of sex-limited color pattern expression; (2) the evolutionary consequences of male versus female heterogamety; and (3) the evolutionary consequences of size-linked color pattern expression.

Directly testing the evolutionary consequences of sex limitation can be difficult because few animals show variation in the extent of sex limitation for a given trait (e.g., populations in which the trait is sex-limited and populations in which the trait is not sex-limited). Females can be made to express conspicuous traits in some animals through hormonal manipulations[59,60] or phenotypic manipulations.[61] However, while such manipulations may allow assessment of the costs and benefits to females of expressing conspicuous traits, they do not allow the direct evaluation of how sex limitation affects the evolutionary dynamics of trait evolution, since the female traits produced by such manipulations are not heritable. Such experiments can be conducted relatively easily with platyfish by constructing XX:XY populations in which the sexes differ in the presence of a conspicuous color pattern. For example, a set of populations could be established in which half the Y-chromosomes have a hypothetical color pattern allele (C), half the Y-chromosomes lack the allele (N), and all X-chromosomes lack the allele (N). These populations would thus consist of two male genotypes (X_NY_C and X_NY_N) and one female genotype (X_NX_N). A second set of populations could be established in which both males and females show variation in the expression of a color pattern. For example, populations could be established in which all the Y-chromosomes lack a color pattern allele (N,) half the X-chromosomes have the allele (C), and half the X-chromosomes lack the allele (N). These populations would thus consist of two male genotypes (X_CY_N and X_NY_N) and three female genotypes (X_CX_C, X_CX_N and X_NX_N). It would then be possible to test how sex limitation affects color pattern evolution under different sources and levels of selection. A second approach to investigate sex-limitation with the platyfish system would be to determine how variation at the sex determination locus affects the degree of color pattern expression by males and females in field populations. All else being equal, if conspicuous coloration is favored in males due to mate choice, females should be more colorful in three-factor populations than two-factor populations because in three-factor populations one female genotype (WY) shares the Y chromosome with males and expresses color pattern alleles carried by the Y chromosome.

The role that sex chromosomes can play in protecting the loss of sexually selected male

traits could also be investigated in platyfish. Based on a literature search and the outcome of a mathematical model, Reeve and Pfennig[62] recently proposed that female heterogamety (WY:YY) is more likely to result in the preservation of male traits important to sexual selection than male heterogamety (XX:XY). Platyfish present a suitable system with which to experimentally test a prediction from this model, as well as test potential extensions of the model because it would be relatively easy to establish populations that are either male or female heterogametic. This is only possible for systems such as the platyfish that have multiple sex determination factors.

The fitness consequences of expressing a color pattern may depend upon an individual's size. For example, larger individuals may have a lower risk of predation than smaller individuals because they are too large for a predator to capture or handle. As a result, larger individuals may benefit from expressing a conspicuous color pattern that would be costly to express for a small individual. The sex-linked color pattern loci in platyfish are near the pituitary locus/loci, a locus that affects age and size at sexual maturation. This locus plays a regulatory role in gene activation. Variation at the pituitary locus (initially termed P-locus, subsequently changed to PIT locus) appears to control the timing of the differentiation of the gonadotropic zone of the pituitary gland, resulting in physiological activity that triggers gonad maturation.[24,63,64] This in turn affects the age and size at which an individual matures, and thus able to first reproduce. Nine PIT alleles in platyfish have been characterized thus far, with PIT genotypes varying in the age at sexual maturation from 10 weeks to as much as 2 years.[24] However, the PIT locus does not appear to affect the rate of growth; individuals with different PIT genotypes have similar growth rates to maturation. As a side effect of the consistency in growth rate, the PIT locus also affects the size at which an individual matures; early maturing genotypes are smaller and late maturing genotypes are larger. The ultimate outcome of the PIT alleles differs for the sexes. Males grow little after reaching maturation, thus males with PIT genotypes for early maturation are small for life. In females, growth is indeterminate and size appears to have a greater effect on sexual maturation than age. However, female southern platyfish express variation at the PIT locus, with some female genotypes maturing in 8–10 weeks and others taking well over a year to mature. The possibility that variation at the PIT locus for large size allows conspicuous color pattern expression could be examined in the laboratory or field by looking at the correlation between size and overall conspicuousness. In addition, linkage disequilibrium between PIT alleles and color pattern alleles could be assessed.

SUMMARY

The southern platyfish, *X. maculatus,* has proven to be an excellent model system in the study of melanoma formation, sex ratio evolution, intraspecific variation in the sex-determining mechanism, and sexual maturation. This model system is now poised to become a major model system for the causes and consequences of genetic polymorphism for coloration and for genetic polymorphism in general. Studies directed at investigating why such extreme polymorphism in coloration is present in platyfish populations promise to yield not only information about color-pattern evolution in platyfish, but also provide information applicable to other species concerning differences in patterns of evolution when sources of selection are directed at one sex as opposed to both sexes.

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SEX-LINKED COLORATION

Eye Coloration

Iris yellow (*Iy*): Yellow coloration of the iris. Sex-influenced: expressed in males and females, but expression is stronger in males. Occurs in the Rio Jamapa, Rio Usumacinta, Belize River, and Sibun River drainages.[20,28]

Iris red (Ir): Red coloration of the iris (masks *Iy*). Sex-influenced: expressed in males and females, but expression is stronger in males. Occurs in the Rio Papaloapan, Rio Jamapa, Belize River, and Sibun River drainages.[20,28]

Red and Orange Body and Fin Coloration

Mouth red (*Mr*): Lower jaw and throat region colored red. Sex-limited: expressed in males. Occurs in the Lake Peten Basin and the Rio Tonala, Belize River, and August River drainages.[20,28, 37]

Red anal spot (*ASr*): Red spot above the gonopodium sometimes extending dorsally for two or three scale rows. Sex-limited: expressed in males. Replaces *Ay* geographically. Occurs in the Rio Jamapa drainage to the Rio Tonala drainage.[20]

Red background ("*R*"): Small irregular red spots at the base of the caudal fin, the ventral part of the caudal peduncle, the gonopodium, and the anterior fin rays of pelvic fins. May resemble red stripe of *X. helleri* (K. Kallman, personal communication). Expressed in males and females, but may be expressed in males to a greater degree. Occurs in the Belize River drainage only.[20]

Red flush (*Fr*): A reddish flush along the anterior, ventral part of the flank from under the eyes to the insertion of the gonopodium. Sex-limited: expressed in males. Occurs in the Rio Papaloapan drainage only.[20]

Red nape (*Nr*): Dull, red nape (saddle-shaped) anterior to dorsal fin. Most strongly developed in mid-dorsal line. Sex-limited: expressed in males. Occurs in the Belize River drainage.

Ruby throat (*Rt*): Red throat area similar to *Mr*. Sex-limited: expressed in males. Occurs in the Rio Papaloapan drainage.[20]

Orange caudal peduncle-2 (*CPo-2*): Strong red pigmentation of the caudal peduncle. Sex-limited: expressed in males. Occurs in the Belize River drainage.[20,28]

Ventral orange (*Vo*): Orange coloration along ventral most scale rows extending from area of the heart to the insertion of anal fin. Often appears with *Sp-8* (K. Kallman, personal communication). Sex-limited: expressed in males. Occurs in the Belize River drainage.[20,28]

Anal fin red (*Ar*): Anal fin or gonopodium colored red or orange. May also "bleed" onto pelvic fin above anal fin and along mid-ventral line as far back as caudal fin base (may be visible only microscopically). Sex-influenced: coloration may be more intense in males than in females. Occurs in the Rio Papaloapan, Rio Jamapa, Belize River, and Sibun River drainages. Genetic basis appears to be different for the Rio Jamapa and Belize River drainages.[20,28,37]

Body red (*Br*): Strongly developed coloration along the flank behind the operculum. Some populations exhibit only a faint orange wash behind the operculum. Sex-influenced: expressed in males and females, but expression may be stronger in males. Occurs in the Rio Usumacinta and Belize River drainages.[20,28]

Dorsal fin red (*Dr*): Dorsal fin is colored orange-red, most intense along the base. Also, faint orange spots may occur below dorsal fin, dorsal part of caudal peduncle, dorsal part of caudal fin, and proximal part of anal and pelvic fins (may be visible only microscopically). Sex-influenced: coloration may be more intense in males than in females. Occurs in the Rio Papaloapan, Rio Jamapa, Belize River, and Sibun River drainages. Genetic basis appears to be different for the Rio Jamapa and Belize River drainages.[20,28,37]

Red tail (*Tr*): Caudal fin is red. Sex-influenced: expressed in males and females, but can be more intense in males. Occurs in the Belize River drainage.[68]

Orange caudal peduncle-1 (*CPo-1*): May give rise to bright yellow or red pigmentation of the caudal peduncle. Expressed in both sexes. Occurs in the Lake Peten Basin and the Belize River and Sibun River drainages.[20,28,68]

Red vertical bars (*STr*): Red vertical stripes on the flank anteriorly and above the mid-lateral line. Expression of this trait with regard to sex is under question; expressed in both males and females, but female may show reduced expression. Occurs in the Rio Papaloapan drainage.[20]

Yellow Body and Fin Coloration

Yellow anal spot (*Ay*): Area above anal fin is yellow, but may also be expressed elsewhere. Expressed in both sexes. Replaces *ASr* geographically. Occurs in the Rio Usumacinta to the August River drainages, excluding the New River drainage.[20,28]

Yellow caudal peduncle (*CPy*): Golden-yellow coloration of caudal peduncle. Expressed in both sexes. Occurs in the Rio Jamapa, Rio Grijalva, Rio Usumacinta, Belize River, and Sibun River drainages.[20,28,68]

Yellow tail (*Ty*): Caudal fin colored golden-yellow. Expressed in both sexes. Occurs in the Rio Jamapa, Rio Coatzacoalcos, Rio Usumacinta, and Belize River drainages.[20,28,68]

Black Macromelanophore Body and Dorsal Fin Coloration

Nigra (*N*): Irregular black blotches extending from behind the operculum along the flank to the base of the caudal fin (solid band expression occurs in Rio Grijalva and Rio Coatzacoalcos, but is rare). Expressed in both sexes. Occurs across platyfish range, excluding the Rio Jamapa drainage.[20,27,28,36]

Spotted dorsal fin (*Sd*): Irregular black spots in the dorsal fin, which may extend into the body, forming a dorsal saddle-like appearance (similar to that seen in Rio Papaloapan drainage in *X. helleri)*. May also "spillover" onto flank anterior to the dorsal fin (visible macroscopically or microscopically-only). Older fish may develop spots near dorsal origin of the caudal fin. Expressed in both sexes. Occurs in the Rio Jamapa drainage to the Belize River drainage, but it appears that there are different modifiers for the Rio Jamapa and Rio Coatzacoalcos drainages. Genetic basis appears to be different for the Rio Jamapa and Belize River drainages.[20,27,28,37]

Spot sided (*Sp*): Small irregular spots along the flank; spots may be faint to intensely black. Expressed in both sexes. There are two different spot pattern types. Type A is restricted to the flank below the dorsal fin and the caudal peduncle and is composed of from one to over 100 spots. Type B: Speckled "salt and pepper"-like appearance with hundreds of small spots covering the fish from head to tail.[20,27,28,36]

The following alleles have been described:

Sp-1– Type A spot pattern described for the Rio Jamapa drainage.

Sp-2– Type A spot pattern described for the Rio Coatzacoalcos drainage.

Sp-3– Type A spot pattern described for the Rio Grijalva drainage.

Sp-4– Type B spot pattern described for the Rio de la Pasion, Rio Usumacinta drainage.

Sp-5– Type A spot pattern described for the Rio de la Pasion, Rio Usumacinta drainage.

Sp-6– Spot pattern is restricted to the flank area above the mid-lateral line and anterior to the dorsal fin, described for the Rio de la Pasion, Rio Usumacinta drainage.

Sp-7– Type A spot pattern described for the Belize River drainage consisting of small spots ventrally, heaviest in the caudal peduncle, and do not occur anterior of the dorsal fin.

Sp-8– Type B spot pattern expression appears to differ for the sexes, described for the Belize River drainage and possibly the Rio Coatzacoalcos drainage.

Sp-9– Type B spot pattern described for the Rio Coatzacoalcos drainage.

Sp-10– Type A spot pattern in which the spots are larger and fewer than *Sp-7*, and most spots appear on the body with a concentration above the midlateral line and a few on the dorsal fin, described for the Rio de la Pasion, Rio Usumacinta drainage.

Sp-11– Type B spot pattern described for the Rio Jamapa drainage.

Sp-12– Type B spot pattern described for the Rio Tonala drainage. Absent from the Lake Peten Basin and the Rio Hondo drainage.

Stripe-sided (*Sr*): Horizontal black rows along the flank above the midlateral line and in front of the dorsal fin that follow the scale reticulation forming a zig-zag appearance (also may include the posterior, ventral part of the caudal peduncle). Expressed in both sexes. Occurs in the Rio Jamapa, Rio Papaloapan, Rio Coatzacoalcos, Rio Grijalva, Rio Usumacinta, and Belize River drainages.[20,27,28]

COLORATION CONTROLLED BY AUTOSOMAL LOCI

Black Micromelanophore Caudal Peduncle and Fin Coloration

Crescent (*C*): A crescent-shaped black blotch just posterior to the caudal nerve. Expressed in both sexes. Occurs in the Rio Papaloapan drainage and possibly the Belize River drainage.[20,66]

Crescent complete (*Cc*): A black crescent-shaped blotch just posterior to the caudal nerve plus a blotch just anterior to the caudal nerve. Expressed in both sexes. Occurs in the Rio Jamapa drainage to Belize River drainage.[20,66]

Comet (*Co*): One black stripe along the dorsal margin of caudal fin and one black stripe along the ventral margin of the caudal fin; both stripes taper posteriorly and rarely extend to the distal tip of the caudal rays. Expressed in both sexes. Occurs in all drainages, excluding those from the Rio Tonala eastward.[20,66]

Còmet lower stripe (*Cò*): A rare pattern consisting of one black stripe along the ventral margin of the caudal fin which tapers posteriorly and rarely extends to the distal tip of the caudal rays. Gordon[20] suggested that this pattern is coded for by an allele belonging to the autosomal tail-spot allelic set, but this has not been verified through genetic crosses. Specific locales unknown at this time.[20]

Cómet upper stripe (*Có*): A rare pattern consisting of one black stripe along the dorsal margin of the caudal fin which tapers posteriorly and rarely extends to the distal tip of the caudal rays. Gordon[20] suggested that this pattern is coded for by an allele belonging to the autosomal tail-spot allelic set, but this has not been verified through genetic crosses. Specific locales unknown at this time.[20]

Dot (*D*): A small black dot at the center of caudal peduncle adjacent to the caudal nerve (often confused with *O*, but *D* is much smaller). Expressed in both sexes. Occurs throughout range.[20,66]

Moon (*M*): A large black blotch covering the portion of caudal peduncle just anterior to the caudal nerve. Expressed in both sexes. Occurs throughout range, excluding the Rio Jamapa drainage.[20,66]

Moon complete (*Mc*): A large black blotch covering the portion of caudal peduncle just anterior to the caudal nerve plus a spot on the dorsal and ventral margin of the caudal fin adjacent to the caudal nerve. Occurs throughout range, excluding the Rio Jamapa drainage.[20,66]

One spot (*O*): One black spot at the middle of the caudal peduncle adjacent to the caudal nerve. Expressed in both sexes. Occurs throughout range.[20,66]

Twin spot (*T*): Black spots on the dorsal and ventral caudal margin that overlap the caudal nerve. Expressed in both sexes. Occurs throughout range.[20,66]

Autosomal Modifier of Micromelanophore Expression

Guatemala crescent (*TCg*): A modifier gene that extends the expression of *T* into a black blotch that runs along the caudal nerve on the caudal peduncle. Expressed in both sexes. Only known from the Lake Peten Basin.[66,67]

COLOR PATTERNS—GENETIC BASIS UNKNOWN

Body Coloration

Black shoulder spot (*Bss*): Fuzzy black window-shaped patch on the flank starting anterior to the insertion of the first dorsal ray, always flanked by two iridescent spots (usually *Yi* or *Bi*). Not sex-limited. Present in the Belize River and the Rio Papaloapan drainages.* This pattern is also present in genetic lines Jp 163 A and B from the Jamapa River drainage. (Reference 34 and Basolo field data)

Vertical stripes (*Vs*): One to several narrow black vertical stripe(s) with tapered ends. Expressed by small males. Present in the Rio Papaloapan drainage.* (Basolo field data)

Blue iridescence (*Bi*): Speckled blue structural scale coloration appearing anywhere along the flank from just above and posterior to the pectoral fins to below the dorsal fin. Coloration occurs in one to five rows, each row consisting of one to many iridescent specks. Expressed in both sexes. Present in the Belize River and the Rio Papaloapan drainages.* (Basolo field data)

Green iridescence (*Gi*): Speckled green structural scale coloration appearing anywhere along the flank from just above and posterior to the pectoral fins to below the dorsal fin. Coloration occurs in one to five rows, each row consisting of one to many iridescent specks. Expressed in both sexes. Present in the Belize River and the Rio Papaloapan drainages.* (Basolo field data)

Yellow iridescence (*Yi*): Speckled yellow structural scale coloration appearing anywhere along the flank from just above and posterior to the pectoral fins to below the dorsal fin. Coloration occurs in one to five rows, each row consisting of one to many iridescent specks. Expressed in both sexes. Present in the Belize River and the Rio Papaloapan drainages.* (Basolo field data)

Violet iridescence (*Vi*): Speckled violet structural scale coloration appearing anywhere along the flank from just above and posterior to the pectoral fins to below the dorsal fin. Coloration occurs in one to five rows, each row consisting of one to many iridescent specks. Expressed in both sexes. Present in the Belize River and the Rio Papaloapan drainages.* (Basolo field data)

Fin Coloration

Anal fin fringe (*Aff*): Black coloration along the distal margin of the anal fin, often expressed to a greater degree closer to the ventrum, with decreasing expression for those rays further removed from the ventrum. Expression limited to females. Expressed by all females in the Belize River and the Rio Papaloapan drainages.* (Basolo field data)

Dorsal fin fringe (*Dff*): Black coloration along the distal margin of the dorsal fin, often expressed to a greater degree closer to the dorsum, with decreasing expression for those rays further removed from the dorsum. Expression limited to females. Expressed by all females in the Belize River and the Rio Papaloapan drainages.* (Basolo field data)

Pelvic fin fringe (*Pff*): Black coloration along the distal margin of the pelvic fin. To date, only seen in females. Occurs in the Belize River and the Rio Papaloapan drainages.* (Basolo field data)

Black gonopodium (*Bg*): Black coloration of the male gonopodium that varies in expression from less than one-third to the entire gonopodium. Occurs in the Belize River drainage.* (Basolo field data)

Anal fin stripe (*Afs*): Reflective white coloration occurring along the distal margin of the anal fin. Limited to females. Occurs in the Belize River and the Rio Papaloapan drainages.* (Basolo field data)

Caudal fin stripe (*Cfs*): Reflective white coloration occurring along the ventral margin of the caudal fin. Expressed in males and females. Occurs in the Belize River and the Rio Papaloapan drainages.* (Basolo field data)

Pelvic fin stripe (*Pfs*): Reflective white coloration occurring along the distal margin of the pectoral fins. Expressed in males and females. Occurs in the Belize River and the Rio Papaloapan drainages.* (Basolo field data)

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*Pattern occurs in this drainage, but may be found to occur in other drainages as well.

Note: Reference 67 provides photographs and reference 27 provides line drawings illustrating many of the black color patterns.

This article has been cited by:

1. Alexandra L. Basolo , William E. Wagner Jr. . 2006. Genetic Variation in Maternal Investment Patterns in Platyfish Xiphophorus maculatus . *Zebrafish* **3**:3, 339-345. [Abstract] [PDF] [PDF Plus]
2. 2006. Recent Papers on Zebrafish and Other Aquarium Fish Models. *Zebrafish* **3**:2, 253-261. [Citation] [PDF] [PDF Plus]