Melanomas in *Xiphophorus variatus* (Pisces, Poeciliidae) in the Absence of Hybridization

It is well known that several genes effecting the production of melanic patterns in fishes of the genus *Xiphophorus* cause the production of melanomas in certain hybrid crosses. ATZ[1] has reviewed the subject. No melanotic individuals have been collected from natural populations, however, and it is generally believed that hybridization is necessary for melanoma production. This paper reports two instances of melanomas occurring in non-hybrid *X. variatus* males.

Materials and methods. *X. variatus* individuals were collected from a number of localities in Tamaulipas, Mexico and were transported to New York where they were maintained in 20 by 25 by 40 cm aquaria and bred[2]. Broods were raised in 30 by 30 by 60 cm aquaria and the sexes were separated before the fish reached maturity.

P^1, P^2, and P^3 are three different pigmentation patterns consisting of variable numbers of black spots on the flanks of the fish. These patterns are controlled by codominant alleles at the same sex linked locus. The P^1 and P^2 alleles may be found on either the *X* or the *Y* chromosome, although in some natural populations it appears that the P^2 allele is found principally on the *X* chromosome and the P^1 on the *Y*[3]. The Gn pattern is a blackening of the gonopodium, the modified anal fin of the male, caused by a dominant allele at another sex linked locus. Although the allele is located on the *X* chromosome in this species, the pattern is under androgenic control and shows up only in males. P^1 and P^2 have been illustrated, and the genetics of all the patterns have been treated by various authors[3,4]. Fish were scored for pigmentation patterns at maturity unless otherwise stated.

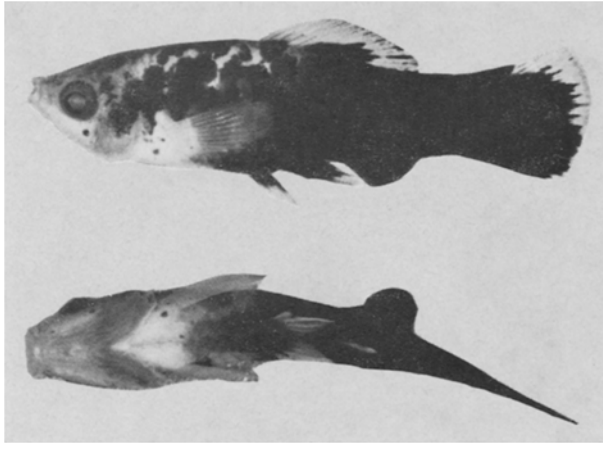

Fig. 1. Lateral and ventral views of N8-14. The melanoma is clearly visible on the caudal peduncle.

The P^2 allele is variable in its expression with the pattern consisting of anywhere from a few black spots to a black band along the flanks of the fish caused by the coalescence of numerous spots. Published photographs illustrate this variation[5]. I estimated the percentage of the body surface that was covered by the pattern in the following manner: using photographs of a fish, I counted the number of spots in various size classes on both of its sides. The product of the number of spots per class and the average area of a spot in that class was summed over all classes and the sum was divided by twice the area of the photograph of the fish.

Results and discussion. N8-14, one of the males I collected in 1970 that exhibited the P^2 pattern, developed atypically heavy pigmentation and clearly had a melanoma on its caudal peduncle (Figure 1) by June of 1972 when it was approximately 20 months old. This is the only known example of a *Xiphophorus* collected from a natural population exhibiting a melanoma. This fish could not have been a hybrid. The only other species of *Xiphophorus* that occurs in the Rio Sabinas system is *X. montezumae*[6] but this species does not occur in the Arroyo Sarco. I have collected intensively in that stream in 1966, 1967, 1970, and 1973 and the only *Xiphophorus* species present was *X. variatus*. DARNELL[6] collected in that area from 1950 to 1953 and reports the same thing.

As Table I shows, N8-14 was homozygous for the P^2 allele. Seven of its male offspring survive; 3 of these exhibit pattern enhancement while 4 exhibit normal pigmentation (Figure 2). Since half of these males should be homo-

[1] J. W. ATZ, Zoologica *47*, 153 (1962).

[2] Fish of pedigrees 2110 and 2112 were collected from the Arroyo Sarco, 2111 from the Arroyo La Flor and 2113 at Sta. Fe in July of 1967. N8-14 was collected as a juvenile from the Arroyo Sarco in December of 1970. DARNELL[6] and BOROWSKY[3] describe these localities. Pedigrees 2110–2113 and their offspring were maintained in the Genetics Laboratory of the New York Zoological Society through the courtesy of Dr. K. D. KALLMAN.

[3] R. L. BOROWSKY, PH. D. thesis, Yale University (1969).

[4] K. D. KALLMAN and J. W. ATZ, Zoologica. *51*, 107 (1966). – K. D. KALLMAN and R. BOROWSKY, Heredity *28*, 297 (1972).

[5] J. W. ATZ, Op. Cit., the female in figure 7 illustrates mild expression of the P^2 gene. K. D. KALLMAN and J. W. ATZ, Op. Cit., figure 1 illustrates strong expression of the P^2 gene.

[6] R. M. DARNELL, Publs Inst. mar. Sci. Univ. Tex. *8*, 299 (1962).

Table I. Summary of crosses establishing the genotypes of the fisch referred to in the text

Parents and presumed genotypes		F_1	F_1 patterns					
			Females			Males		
Females	Males	Pedigree	+	P^2	P^3	+	P^2	P^3
2111-1 $X_{P^2}X_{P^3}$	2112-11 X_+ Y_+	2122		3	3		14	9
2111-2 $X_{P^2}X_+$	2113-11 $X_{Gn}Y_+$	2123	6	3		2	7	
2110-1 $X_{P^2}X_+$	2110-12 X_+ Y_{P^2}	2185	12	16			23	
N9-1 X_+ X_+	N9-11 X_{P^2} Y_+	N18		8		8		
N18-2 $X_{P^2}X_+$	N8-14 X_{P^2} Y_{P^2}	N32		21			12	

The F_1 fish exhibited either P^2, P^3 or no pattern (+). The numbers in the columns at the right give the number of offspring resulting from each cross that exhibited a particular pattern.

Table II. Summary of crosses establishing the genotypes of males 2185-11 through 2185-17

Predicted P_1	Parents and presumed genotypes		F_1	F_1 patterns					
				Females			Males		
Male genotype	Females	Males	Pedigree	+	P^1	P^2	+	P^2	P^2Gn
$X_{P^2}Y_{P^2}$	2151-1 X_+ X_+	2185-11 $X_{P^2}Y_{P^2}$	2339			48		55	
X_+ Y_{P^2}	2137-1 X_+ X_+	2185-12 X_+ Y_{P^2}	2403	7		1[a]		5	
X_+ X_{P^2}	2123-1 X_+ X_{Gn}	2185-13 X_+ Y_{P^2}	2343	11				20[b]	
X_+ Y_{P^2}	2132-1 X_+ X_{P^1}	2185-14 X_+ Y_{P^2}	2502	1	1			5[c]	
$X_{P^2}Y_{P^2}$	2126-7 $X_{Gn}X_{Gn}$	2185-15 X_+ Y_{P^2}	2342	16					12
$X_{P^2}Y_{P^2}$	2185-1 X_+ X_+	2185-16 $X_{P^2}Y_{P^2}$	2378			9		15	
???[d]	2151-3 X_+ X_+	2185-17 $X_{P^2}Y_{P^2}$	2351			23		25	

I predicted the genotypes of these fish on the basis of their phenotypes and these predictions are given in column 1. Column 2 gives the genotypes of the females to which they were mated. Column 3 gives the genotypes of the males as determined from analyses of the phenotype ratios of the offspring which are given at the right. [a] presumably this fish is the result of a crossover during spermatogenesis. [b] At the time of scoring, 15 of these fish were adult and 11 of these exhibited the Gn pattern. [c] The P^2 pattern masks the P^1 pattern. [d] This fish had exhibited intermediate expression so no firm prediction had been made.

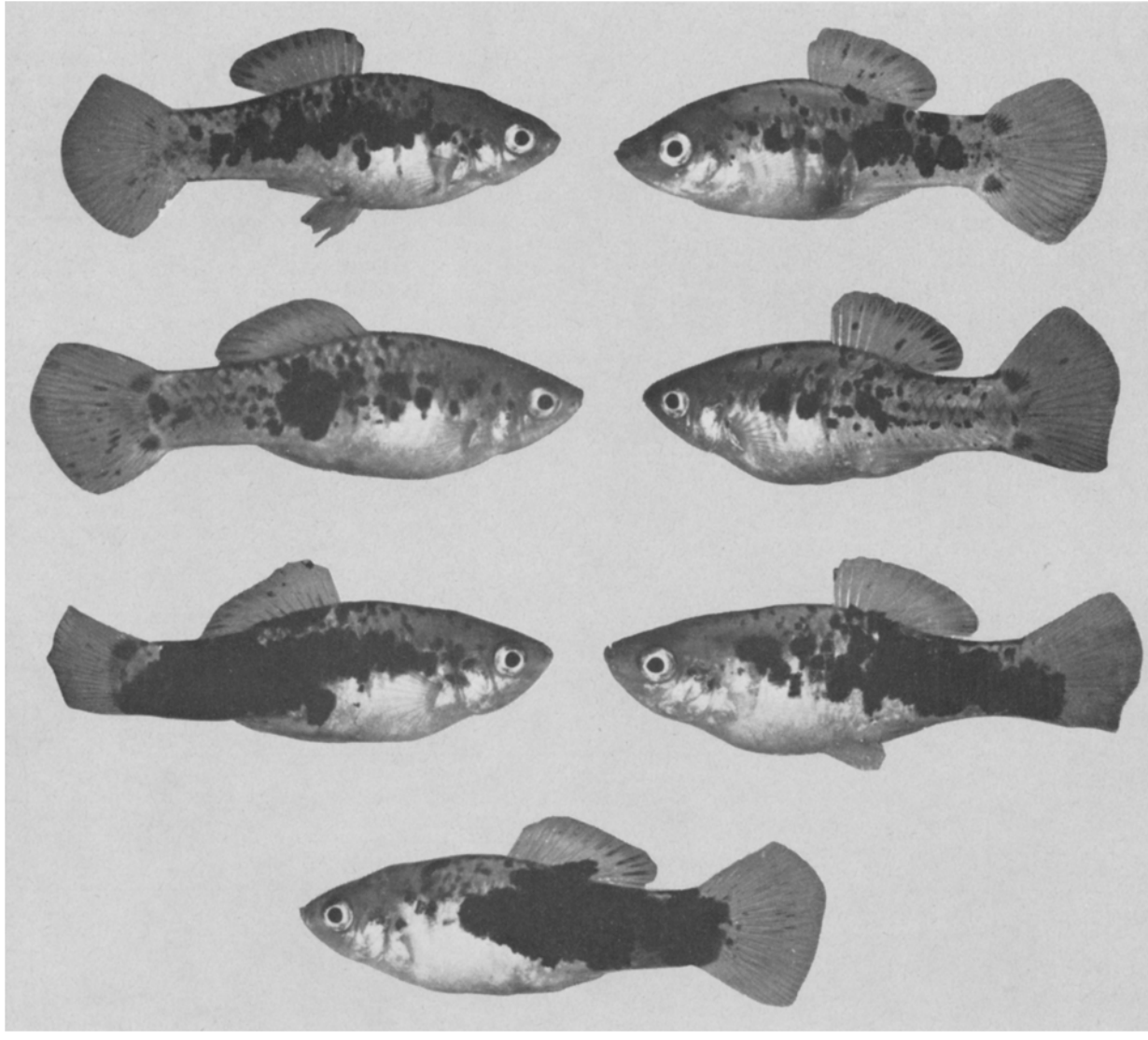

Fig. 2. The 7 surviving male offspring of N8-14. The upper 4 fish exhibit typical P^2 patterns while the lower 3 fish exhibit enhanced patterns similar to that of N8-14.

zygous and the other half heterozygous, I am attempting to breed these males in order to determine whether there is a correlation between genotype and phenotype. The female siblings exhibit a considerable range of variation in the expression of the P^2 allele but the variation is continuous. These fish are being bred as well.

There is reason to suspect that such a correlation may exist in males. The 2185 pedigree contained both heterozygous and homozygous males and although none of these males exhibited atypical pigmentation, as a group they showed a bimodal distribution of degree of pattern expression. I classified the males of 1 brood according to the degree of pattern expression and predicted that those individuals with poor expression would prove to be heterozygous and those with good expression, homozygous. Seven of these fish were successfully bred (Table II) and 5 of the predictions proved true, 1 proved false and in the 7th case the fish was considered to have been intermediate so no firm prediction had been made[7].

Population hybrids in this species may also exhibit enhancement of the P^2 pattern. The 2122 pedigree was derived from a female collected in Arroyo La Flor and a male collected in Arroyo Sarco. These two localities within the Rio Sabinas system are 8 km apart as the fish swims. Two 2122 males developed melanomas and 1 exhibited atypical pattern enhancement by the age of 20 months. Eleven other P^2 males in this pedigree did not show atypical pigmentation but not all of these survived long enough for this to have developed. Figure 3 illustrates one of the melanotic individuals.

I estimated the percentage of surface area covered by the P^2 pattern for adult fish of the 2122 and 2123 pedigrees and found that the pattern was significantly better expressed in males[8]. Although the 3 fish destined to develop atypical pigmentation were included in this analysis, this may indicate that males are more prone to develop melanomas than are females. That is, it may be significant that all 4 examples of atypical pattern enhancement reported here involved males. This is particularly interesting because SICILIANO, PERLMUTTER and CLARK[9] report that in hybrids of *X. maculatus* and *X. helleri* which have inherited the Sd gene of the former species and therefore develop melanomas, males develop the melanomas earlier than do females. KALLMAN[10] points out that the Sp^8 gene in *X. maculatus* produces a prominent black spot in males that does not show up in females. The P^1 pattern of *X. variatus* also has better expression in males than in females[3].

There is only one other reported case of melanomas in non-hybrid *Xiphophorus*; the Sc gene in an inbred strain of *X. montezumae cortezi* causes their production in some of the carriers[11]. The present report indicates that melanomas may be normal, although very rare events in platyfish populations.

It is interesting to speculate on the consequences of melanomas in natural populations of these fish. The P^2 gene appears to be found only rarely on the *Y* chromosome in the Rio Sabinas populations but is found on both the *X* and the *Y* chromosome in the population inhabiting its tributary, the Arroyo Sarco. Since crossing over between the gonosomes occurs in *Xiphophorus*[12], the bias in the main river populations indicates that *Y* chromosomes bearing the P^2 allele are selectively eliminated. The lack of bias in the Arroyo population indicates that the selective agent at work in the river populations is not at work in the Arroyo populations. From the observations I have reported it appears that homozygous males have the highest risk of developing melanomas and such a factor would tend to limit the P^2 allele to the *X* chromosome. The melanomas reported in this paper developed only in older fish and this could account for the difference between the 2 populations. Annual flooding appears to wash adults from the steeply banked Arroyo Sarco[3] so most males would not live long enough for melanomas to develop. In the main river with its broad flood plains, the impact of flooding is decreased and enough males would survive to an age at which the melanoma factor could become important[13].

Zusammenfassung. Nachweis von genetisch bedingten Melanomen bei Wildfängen der reinen Art des Zahnkarpfen *Xiphophorus variatus*, wobei es sich eindeutig nicht um einen Artbastard handelt.

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26 April 1973.

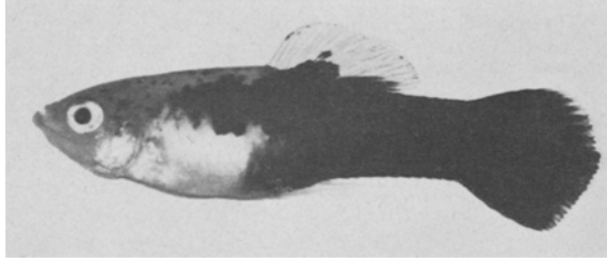

Fig. 3. One of the 2122 males with a melanoma. The nodule was on the side of the fish and is not visible in this photograph but the enhancement of the P^2 patern is clear.

[7] $P = 0.11$.

[8] For the 2122 pedigree: 3 females, range of 4.3–7.3%, median = 7.2%, 10 males, range of 4.2–36.5%, median = 8.8%, $p = 0.143$ (WILCOXON's *t*-test). For the 2123 pedigree: 3 females, range of 1.7–2.4%, median = 1.9%, 4 males, range of 2.5–21.3%, median = 7.25%, $p = 0.029$. The significance of the combined results evaluated by means of the KOLMOGOROV-SMIRNOV statistic: $p = 0.05$.

[9] M. J. SICILIANO, A. PERLMUTTER and E. CLARK, Cancer Res. *31*, 725 (1971).

[10] K. D. KALLMAN, Zoologica *55*, 1 (1970).

[11] K. D. KALLMAN, Zoologica *56*, 77 (1971).

[12] P. A. MACINTYRE, Am. Nat. *95*, 323 (1961).

[13] This work was supported in part by grant No. CA 06665 of the USPHS and in part by a grant from the Arts and Sciences Research Fund of New York University. I thank Dr. K. D. KALLMAN for the provision of facilities and his advice.

Action of Antituberculosis Drugs on Human Leukocyte Chromosomes in vitro

Due to the high incidence and prevalence in the developing countries, tuberculosis invariably represents the most frequently found infectious disease in the world. Therefore millions of patients must continuously undergo long-term treatment with antituberculosis drugs in triple or double combinations. Moreover, many healthy individuals with a high risk of catching tuberculosis are treated with isoniazid (INH) as primary or secondary chemo-