

Genetics of Melanomas in Fishes

V. The Reappearance of Ancestral Micromelanophores in Offspring of Parents Lacking These Cells*

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In previous papers in this series (5, 8, 13) it has been shown that spontaneous malignant melanomas are produced in the hybrid offspring of the black-spotted platyfish, *Platypoecilus maculatus*, and the swordtail, *Xiphophorus hellerii*. The melanomas are evoked genetically in hybrids by the interaction of the sex-linked, heritable factor for macromelanophores, *Sp*, of the platyfish with a series of *Sp* modifiers, *A* and *B*, of the swordtail. These melanomas may be said to be produced experimentally because the platyfish and the swordtail probably do not crossbreed under natural conditions in Mexico. The author (10) has collected nearly 10,000 specimens of these two species in their native habitat without finding a single natural hybrid, despite the fact that both species were often found living side by side.

TWO KINDS OF MELANOPHORES

In the fishes studied here, an important distinction has been drawn between a relatively small melanophore, 0.3 mm. in size, and a considerably larger one which may attain a size of 1.2 mm. at its widest dimension. The smaller, micromelanophores, are found normally in the dermal areas of the integument and in the tissues that surround the large blood vessels and the spinal cord; they are found in the meninges of the brain, in the lining of peritoneum, and in the retina. The larger, macromelanophores, in normal fish, are restricted in their distribution, being found only in scattered areas of the corium.

* Through the courtesy of Dr. Robert Chambers of New York University an opportunity was presented for the study of fish melanomas by the tissue culture technic. Cultures were made of the melanomas that were obtained from the hybrids of a black-spotted platyfish and an albino swordtail. These are described in the paper that follows (14).

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The micro- and macromelanophores differ not only in their distribution and in morphology but also in the method of inheritance, the micromelanophore factor, *St*, being autosomal while the macromelanophore factor, *Sp*, is sex-linked.

The most striking distinction between these two melanophores is made evident in the development of melanomas in the hybrids of the black-spotted *Platypoecilus maculatus* with other species. Melanomas have appeared repeatedly in a Mendelian proportion of hybrid broods, but they appear only in those fish which are genetically dominant for macromelanophores, *Sp*. Micromelanophores, *St*, in the absence of macromelanophores, are incapable of evoking the melanomas. However, the presence of micromelanophores as well as of the increased numbers of macromelanophores, *Sp St*, intensify the melanotic tumor in the hybrid fish. Further details of the part played by each type of melanophore are given by the author (5, 8).

MELANOMAS WITH KNOWN TYPES OF MELANOPHORES

For the purpose of the tissue culture study (14), it had been planned to use melanomas from fish hybrids that had only one type of corial melanophore, the macromelanophores. The author (8) pointed out that melanomatous fish of this kind could be developed by mating a spotted platyfish (a *Platypoecilus maculatus* variety, possessing macromelanophores, *Sp*, but no micromelanophores, *st*) with a golden swordtail (a *Xiphophorus hellerii* variety lacking both types of melanophore, *sp st*). It was believed that the interpretation of the cell types in tissue cultures could be clarified by the use of material having only one kind of melanophore.

At the time when plans were completed for the culture of fish tissue, melanotic hybrids of a spotted platyfish with the golden swordtail were not available.

Instead, we had highly melanotic hybrids between the spotted platyfish and the albino swordtail¹ (Fig. 1).

At first it had seemed to us that the substitution of an albino for the golden swordtail would not alter the melanophore complex of the melanotic hybrid since neither the albino nor the golden swordtail possesses micro- or macromelanophores;—at least, they do not show them phenotypically. We now have evidence, however, that the albino swordtail is genetically dominant for micromelanophores, *StSt*, but it does not show

This conclusion has been reached independently by Kosswig (18) who employs the term *f* for the golden and *F* for the wild type. Simple Mendelian inheritance was also found by Kosswig (17) when he crossed the albino swordtail (*ii*) with the dominant wild type (II). Our unpublished data confirm Kosswig's results on this point. Kosswig, in 1935, employed the term *pp* for the albino and *PP* for the wild type, but since, in 1931, Gordon and Fraser used the symbols *P* and *p* in their earlier study of a dominant series of alleles in *Platypoecilus* for entirely different characters, *I* and *i* have been substituted in this paper for *P* and *p*.

When the albino swordtail (*ii*) is mated to a golden swordtail

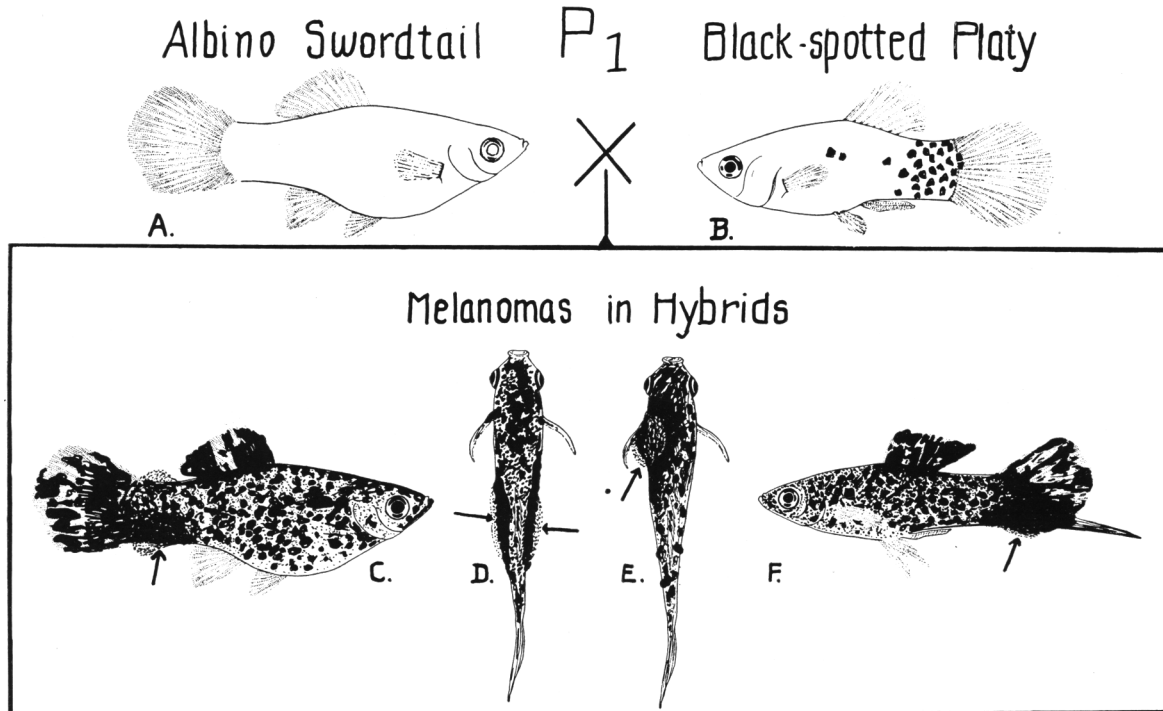


FIG. 1.—Origin of melanomas in hybrid fishes. When an albino swordtail, without black pigment (upper left), is mated with a macromelanophore-carrying platyfish (upper right) their hybrid offspring (lower line) give rise to melanomas. Neither the albino swordtail nor the spotted platyfish parents show any micromelanophores; nevertheless their melanomatous offspring (F_1) have these small pigment cells in addition to the macromelanophores. This is demonstrated by the genetical analysis in the text.

these pigment cells in the presence of a homozygous recessive inhibitor of melanin pigment, *ii*.

Since it is difficult to distinguish between micro-melanophores and macromelanophores under conditions of extreme melanosis, the evidence for the presence of micromelanophores in the spotted platyfish \times albino swordtail hybrid has been determined by a genetic method of analysis.

The author (6, 7) has shown that when the wild type, olive-green swordtail (*StSt*) is mated with the aquarium-developed, black-eyed, golden mutant (*stst*) the wild type is dominant.

¹These were kindly provided by Mr. Fred Flathman, an aquarist of Woodhaven, Long Island, New York; duplicate hybrids were reared at the New York Aquarium.

(*stst*), the author (9) has found that the original color of the wild swordtail, with its full complement of micromelanophores, is restored as follows:

Albino swordtail \times Golden swordtail = Wild swordtail
 No micromel. \times No micromel. = Micromelanophores.
 $ii\ StSt \times II\ stst = Ii\ Stst.$

When *Ii Stst* swordtails are mated *inter se*, these ratios appear, approximately, in F_2 : 9 wild type, 3 golden and 4 albinos.²

The genetic constitution of the fish bearing melanomas used in the present tissue culture studies may therefore be expressed in the following manner:

Albino swordtail \times Black-spotted platyfish = Melanotic hybrid.
 $ii\ StSt\ spsp\ AA\ BB \times II\ stst\ SpSp\ aa\ bb = Ii\ Stst\ Spsp\ Aa\ Bb.$

²Unpublished data.

I represents normal coloring, the wild gray; *i* represents a recessive inhibitor of melanin pigment development; in a homozygous state *ii* denotes albinism.

St represents the dominant factor for micromelanophores, the wild gray color; *stst*, when in a homozygous recessive state, represents an animal that is golden, black-eyed. However, *StSt ii* denotes albinism.

Sp indicates the presence of macromelanophores; *sp* in the homozygous state represents lack of macromelanophores.

A and *B* represent multiple factors of the swordtail which act in conjunction with *Sp* for macromelanophores of the platyfish to change the growth habits of these melanophores from the normal to the pathological. Further details concerning the multiple effects of *A* and *B* may be found in Gordon (8).

The first use of the albino factor in the production of melanomas in fish hybrids was made by Breider (1, 2). His results were later discussed by Kosswig (18). Breider (1) reported the mating of a black-banded platyfish to a swordtail. The black female hybrid was backcrossed to an albino swordtail and then a black offspring was backcrossed again to an albino. Later, Breider (2) mated a black-spotted red hybrid to an albino swordtail. All these melanotic hybrids were derived from platyfish parents that had both micro- and macromelanophores. Thus the genetical analysis, outlined above, could not be made from their experiments alone.

The great influence of *i* in the homozygous state upon the phenotypic expression of black spots, *Sp*, and of black band, *N*, both of which contain macromelanophores, and of the wild pattern, *St* (micromelanophores) is explained by Breider and by Kosswig. They also discuss the role of *i* in the formation of colorless tumors.

SIMILARITY OF MELANOMAS

The melanotic tumors in the spotted platyfish \times albino swordtail hybrids of the F_2 (Fig. 2) are histologically similar to other spotted platyfish-swordtail hybrids described by Reed and Gordon (19), Haussler (15), Smith, Coates, and Strong (20), and Gordon and Smith (12). They are essentially similar, also, to melanomas found in hybrids between the spotted platyfish, *P. maculatus*, and the other species of *Platy-poecilus*: *couchianus*, *xiphidium*, and *variatus* described by Gordon and Smith (13). In every case on record involving a platyfish hybrid combination, melanomas have always been associated with the presence of macromelanophores derived from *P. maculatus*.

Other types of pigment-bearing cells may occasionally be seen in platyfish \times swordtail hybrid melanomas such as xanthophores and xantho-erythrophores. They have their origin in either the platyfish or swordtail parent or both according to Goodrich, Arrick, and Hill (3). Even the albino swordtail has these two types of chromatophores in a much reduced number. They seem to take no essential part in the formation of the neoplasm. In a rare case Kosswig (16) found

an erythrophoroma, but the genetic basis of this tumor was not established.

SUMMARY

Melanomas in hybrids of the platyfish and the swordtail are said to be experimentally produced because these two species have never been found to cross-breed in their native habitat in Mexico.

The fish melanoma used in tissue culture studies, described by Grand, Gordon, and Cameron (14) were obtained by mating a spotted platyfish with an albino swordtail.

In terms of melanophore content, the platyfish has macromelanophores only. These cells are inherited and are referred to a sex-linked, dominant factor, *Sp*.

The albino swordtail is without a trace of melanophores, small or large; that is, they do not show the melanophores phenotypically. It has been found, how-

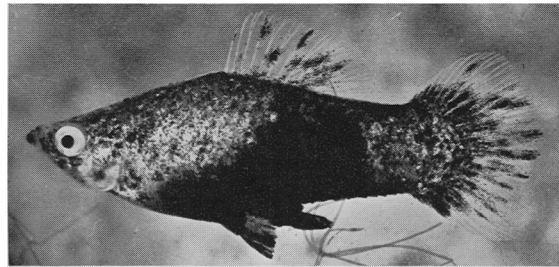


FIG. 2.—Melanoma in hybrid fish. The lower half of the mid region of the body bears a melanotic tumor, although the entire integument is involved. A dorsal view of this specimen may be seen in Fig. 1 D. This individual and the others in Fig. 1 were the source material for tissue culture studies of fish melanomas (14).

ever, that the albino swordtail, genotypically, like the wild type, is dominant for micromelanophore. These cells are inherited through an autosomal factor, *St*. The albino swordtail does not show its micromelanophores because of the presence of a homozygous recessive factor, *ii*, an inhibitor of melanin. The albinos used in these experiments have the genetic constitution *StSt ii*.

The hybrid offspring of a spotted platyfish (macromelanophores only) and an albino swordtail (no visible melanophores) have two kinds of melanophores: a) macromelanophores and b) micromelanophores. This combination is brought about by the interaction of the factors *Sp*, *St*, and *I* all of which are represented in the melanomatous hybrids of the first generation.

The melanomas are produced by the interaction, specifically, of the factor for macromelanophores, *Sp*, of *Platy-poecilus maculatus* with the *Sp* modifying factors *A* and *B* of *Xiphophorus hellerii*.

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