THE HEREDITARY BASIS FOR MELANOSIS IN HYBRIDS OF MEXICAN KILLIFISHES

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In the *American Journal of Cancer* for April, 1931, there has just appeared a paper by the writer on the "Morphology of the heritable color patterns in the Mexican killifish, Platypoecilus." It is the first of a series of three papers, two of which are to appear in the July number of that journal. The present communication summarizes the chief features of the two unpublished papers: one is concerned with the hereditary basis for the occurrence of melanosis in hybrids between certain genetic varieties of Platypoecilus and Xiphophorus, and the other discusses the morphology of the melanotic hybrids with respect to the melanotic state in general and melanotic tumors in particular.

The Melanotic State.—Melanosis in hybrid fishes (*Platypoecilus maculatus* by *Xiphophorus hellerii*) is a condition characterized by an excessive growth of large black pigment cells. Normal tissues of the dermis are invaded and replaced by black chromatophores. The degree of melanosis shown by the individual hybrid fish varies. For purposes of description the advance of melanosis will be mentioned as occurring in a system of three states of intensity. As intermediate stages between these three states of melanosis have been observed within fishes of a given brood, it is believed that all of the melanotic hybrids express different states of the same pigmentary disturbance.

The initial state of melanosis is characterized by the replacement of the normal tissues in the dermis by macromelanophores. Large areas are invaded but the growth process of melanophores does not involve the loss of body parts. The initial stage may be recognized on the day of birth of the hybrids and there is every reason to believe it may be detected even during the period of larval development within the oviduct of the gravid female.

In the second progressive state of melanosis there is a further invasion of the dermal regions, accompanied by a deterioration of the structural elements themselves. The scales and fin rays are divested of their soft parts and there is a noticeable loss of rays especially in the dorsal and caudal fins. Correlated with the loss of dermal areas is the invasion of subcutaneous tissues by macromelanophores and the replacement of connective tissues associated with muscle and bone.

The third state of melanosis may be characterized by the development of large melanotic overgrowths in various parts of the body. In some cases the tumor is the size of the head while in others the entire posterior part of the body is greatly enlarged. Histological preparations of these melanotic overgrowths reveal neoplastic features. (On this point our diagnosis is confirmed by the following well-known workers in cancer, who have examined the material: Professor James Ewing, Dr. Francis Carter Wood and Dr. James B. Murphy.)

Relation of Color Pattern of Parent to Type of Hybrid Offspring.—An analysis of the results of fish fanciers and geneticists reveals the fact that whenever the pulchra, rubra and under certain conditions nigra (black) varieties of *Platypoecilus* are used in generic crosses with the swordtail, *Xiphophorus*, melanosis develops in one half or all of the hybrid fishes.

On the other hand when varieties of the platyfish, other than the pulchra, rubra and nigra are used, no comparable melanotic conditions appear among the generic hybrids. The following varieties have been tried and have given negative results with respect to melanosis: gold, stippled, one-spot, twin-spot and crescent platyfish.

An explanation of the above results may be gained by a study of the morphology and genetics of the pigmentary equipment of the various color patterns in Platypoecilus. In an extended study of the morphology of the heritable color patterns in Platypoecilus and Xiphophorus, the writer (1929, 1931) analyzed all the parent types which have been used in these intergeneric crosses. The following table summarizes the pigmentary elements involved and the varieties in which they are found:

TABLE 1

ANALYSIS OF THE HERITABLE COLOR PATTERN ELEMENTS IN THE VARIETIES OF PLATYPOECILUS

	GENETIC RACE OF PLATYPOECILUS	MELANOPHORES		ERYTHROPHORES	
		MACRO SPOTS[2]	MICRO STIPPLES[3]	*Rf*[4] RED FINS	*R* RED BODY
1.	Gold platy*	..	..	*Rf*	..
2.	Red platy*	..	..	*Rf*	*R*
3.	Stipple platy*	..	*St*	*Rf*	..
4.	Stipple, crescent	..	*St*	*Rf*	..
5.	Stipple, one-spot*	..	*St*	*Rf*	..
6.	Stipple, twin-spot*	..	*St*	*Rf*	..
7.	Red, spotted*	*Sp*[1]	..	*Rf*	*R*
8.	Nigra	*Sp*	*St*	*Rf* or *rf*	..
9.	Pulchra	*Sp*	*St*	*Rf* or *rf*	..
10.	Rubra*	*Sp*	*St*	*Rf*	*R*

[1] The presence of the symbol *Sp* indicates that spots (macromelanophores) appear in the variety.

[2] Spots are made up of a group of macromelanophores.

[3] Stipples refer to micromelanophores.

[4] *Rf* is the genetic symbol for red fins. (Its inheritance is poorly known.)

* Indicates that these varieties have been used in the experiments at Cornell University laboratory.

The Hereditary Basis for Color Patterns.—In 1927 the writer showed genetically that there were two kinds of melanophores in the rubra platy. The pigmentary elements which are responsible for large black spots are macro-melanophores and their hereditary behavior is conditioned by a sex-linked dominant factor (*Sp*). The small black pigment cells responsible for the underlying stippling and olivaceous color, the micromelanophores, are conditioned by an autosomal dominant factor (*St*).

The nigra pattern formed by macromelanophores, arranged in a dense row along the sides, is conditioned by a dominant sex-linked gene (*N*) according to Bellamy (1928) and this is confirmed by unpublished data obtained in this laboratory.

The red body color due to erythrophores is conditioned by the sex-linked dominant factor *R* (Fraser and Gordon, 1929), while the red fin complex seems to be due to an autosomal dominant factor *Rf*, although Kosswig thinks it is sex-linked.

The other patterns involved in intergeneric crosses: stippled, one-spot, twin-spot and crescent are formed by micromelanophores. Specific differences in melanophore distribution determine the pattern characteristics in each variety. Each pattern is conditioned by a distinct dominant autosomal gene (Gordon 1927, 1931).

Relation of Color Pattern of Parents to Type of Hybrid Offspring.—Each genetic race of Platypoecilus has been crossed with Xiphophorus in order to determine which color varieties are associated with the occurrence of melanosis in the hybrid progeny. The varieties tested are listed in table 1, to which have been added several strains employed by other workers. In the first series of crosses made at this laboratory the following results were obtained:

TABLE 2

XIPHOPHORUS FEMALE	×	PLATYPOECILUS MALE	=	TYPES OF HYBRIDS MELANOTIC* 1	2	3	NON-MELANOTIC	TOTAL
Stipple (*St'St'*)		Gold (*RfRf*)		0	0	0	8 red-finned, stipple	8
Stipple (*St'St'*)		Red (*RR*)		0	0	0	13 red, stippled	13
Stipple (*St'St'*)		Rubra (*RrSpspStst*)		5	0	0	6 stippled	11
Male		Female						
Stipple (*St'St'*)		Rubra (*RRSpSpStSt*)		30	12	3	0	45
								77

* The melanotic fishes are separated into three classes according to the state of melanosis.

As table 2 indicates, when the gold or red platyfish are used as parents only non-melanotic hybrids result; but when the rubra platy is used melanosis appears in all of those hybrids which have inherited the factor for macromelanophores (*Sp*). Three states of melanosis are indicated. Haussler (1928) and Kosswig (1929) have provided further data on some of these crosses which are in agreement with the facts presented here.

Since the parent platyfish which have given melanotic hybrids (pulchra, rubra, nigra) have all possessed both types of melanophores (table 1) it seemed desirable to test each melanophore factor (*Sp* and *St*) to determine which one is necessary for melanosis in the hybrids. A lead in this direction has been obtained from the work of Gerschler (1914) who crosses the stipple platy (*St*) with Xiphophorus and reported no melanotic hybrids. It would seem, then, that the macromelanophores (*Sp*) were alone responsible for melanosis in hybrids. The latter conclusion has been put to an experimental test by using the following special strain of Platypoecilus, recessive for the stipple factor but heterozygous for spots:

XIPHOPHORUS MALE	×	PLATYPOECILUS FEMALE	=	TYPES OF HYBRIDS
Stipple (*St'St'*)		Red, spotted (*RrSpsp*)		6 melanotic (2d state), 6 stippled

From the above cross it may be seen that it is the factor for macromelanophores which is necessary for the production of melanotic hybrids. It is brought about by the interaction of the *Sp* factor for macromelanophores of Platypoecilus with the modifying factors contributed to the hybrids by Xiphophorus. Further evidence for this has been obtained from the following series of crosses:

In table 2 it has been pointed out that a rubra platy female when crossed with a stippled (normal type) Xiphophorus male produced forty-five melanotic hybrids. The same rubra platy female parent was then crossed to a stippled platy male and as a result of this second mating produced two other broods containing thirty-two spotted fishes. But in none of these latter fishes were there any indications of melanosis. These data show that the genus Xiphophorus contributes certain factors which interact with the macromelanophore factor of Platypoecilus with the result that pigmentary abnormalities develop in the hybrids between them.

The modifying factor of Xiphophorus are represented in non-melanotic hybrids as well as in the melanotic ones. This has been determined by crossing a non-melanotic hybrid back to a rubra platy with the result that melanotic fishes were obtained. Previous workers (Preuss, Haussler, Kosswig) have shown that melanotic hybrids, when crossed back to either parent species, produce a number of melanotic offspring. An attempt has been made to determine the genetic nature of the Xiphophorus modifying factors but no positive results have been obtained as yet.

Summary.—1. The spotted varieties of Platypoecilus (pulchra, rubra, nigra) carry the hereditary factors for *macromelanophores* and when mated to Xiphophorus give rise to melanotic hybrids.

2. Varieties of the platyfish characterized by the possession of *micro-*

melanophores (stipple, one-spot, twin-spot, crescent) or erythrophores (red, gold) when mated to Xiphophorus give rise to normal hybrids.

3. The spotted varieties of Platypoecilus provide the pigment cells (macromelanophores) while Xiphophorus contributes the modifying factors which alter the normal development of the macromelanophores in generic hybrids.

4. Three states of melanosis are recognized in the melanotic hybrids. The third state is characterized by distinct melanotic overgrowths which reveal neoplastic features histologically.

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THE RESPONSES OF THE DEEP-SEATED MELANOPHORES IN THE FROG TO ADRENALIN AND PITUITRIN

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The color changes of cold-blooded vertebrates are caused chiefly by the activity of the melanophores in the skin. Consequently, the responses of these cells have aroused much interest. Frogs placed on a white background or in complete darkness become light-colored due to a contraction of their melanophores. The same result is achieved when adrenalin is injected. If the animals are kept on a black background or are injected with pituitrin, they turn dark through an expansion of the pigment cells of the skin. So far as I know, such observations have not been extended to the melanophores in the deeper parts of the animal. In fact, these cells have received very little attention. Such deep-seated pigment cells are found in the connective tissue of the following regions: the membranous covering of the leg muscles, of the muscles of the back, of the inside of the body wall, the mesenteries, the pericardium, the pleura, the lining of the sub-dermal lymph sacs especially next the skin. The