THE GENETICS OF A VIVIPAROUS TOP-MINNOW PLATYPOECILUS; THE INHERITANCE OF TWO KINDS OF MELANOPHORES[1]*

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Received September 20, 1926

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INTRODUCTION

Although there have been only a few truly genetical studies of fishes, they have already contributed at least two new concepts to the science

[1] Paper No. 150, Department of Plant Breeding, CORNELL UNIVERSITY, Ithaca, New York. The work was conducted under the joint supervision of Professor A. C. FRASER and Professor G. C. EMBODY. To them I wish to express my thanks for laboratory equipment, information from frequent discussions and constant encouragement. Professor H. D. REED has led the research which has resulted in finding the morphological equivalents of the genetic factors. My thanks are also due to Dr. R. G. HARRIS, director of the Biological Laboratory, for placing at my disposal such equipment as was necessary for me to carry the experiments through the summer of 1925 at Cold Spring Harbor, Long Island.

* The GALTON AND MENDEL MEMORIAL FUND contributes the accompanying colored plate prepared from a painting by Miss LILA DVORAK.

of heredity. The first instance of crossing over in the X and Y chromosomes was demonstrated in fishes, almost simultaneously by SCHMIDT, 1920, by WINGE, 1922a, 22b, 23, and by AIDA, 1921. Secondly, the two types of sex-linkage have been reported as operating within the same sub-family; the XY type in Lebistes (SCHMIDT, 1920; WINGE, 1922a, 22b, 23) and the ZW in Platypoecilus (BELLAMY, 1922)—both genera within the sub-family Poeciliinae, the viviparous top-minnows. AIDA, 1921, has also reported the XY type of sex-linkage in Aplocheilus, a killifish belonging to the same order as the above genera. This paper deals with *Platypoecilus maculatus* and supports the hypothesis that the ZW type of sex-linkage operates in this species.

TAXONOMY OF PLATYPOECILUS

Under the genus Platypoecilus, proposed by GÜNTHER in 1866, there have been listed, at various times, the following species:

P. couchia	(Girard 1859)
P. maculatus	Günther 1866
P. mentalis	Gill 1876
P. quitzeoensis	Bean 1898
P. variatus	Meek 1904
P. nelsoni	Meek 1904
P. perugiae	Everman and Clark 1906
P. dominicensis	Everman and Clark 1906
P. tropicus	Meek 1907

The first to lose its position under the genus, Platypoecilus, was *P. quitzeoensis* when MEEK (1904) saw its identity with the genus Zoogoneticus and called it *Z. quitzeoensis*.

In 1907 REGAN further reduced the list of species by combining *P. variatus* with *P. maculatus*. In addition he showed that *P. mentalis* and *P. nelsoni* were synonyms of *Poecilia sphenops* Cuv. & Val.; later, 1913, he found it necessary to refer *Poecilia sphenops* to *Mollienisia*.

REGAN (1913) brought into general taxonomic employment what HUBBS (1924) termed "by far the most valuable of all the characters which have been used in the classification of these little fishes, namely, the structure of the gonopodium (this is the intromittent organ of the male, formed by the elongation and singular elaboration of rays 3, 4, and 5 of the anal fin)." On this basis REGAN was able to refer *P. perugiae* to *Limia vittata* (Gouchenot) and *P. dominicensis* to *Limia*. At the same time *P. tropicus* was found identical with *Mollienisia sphenops*.

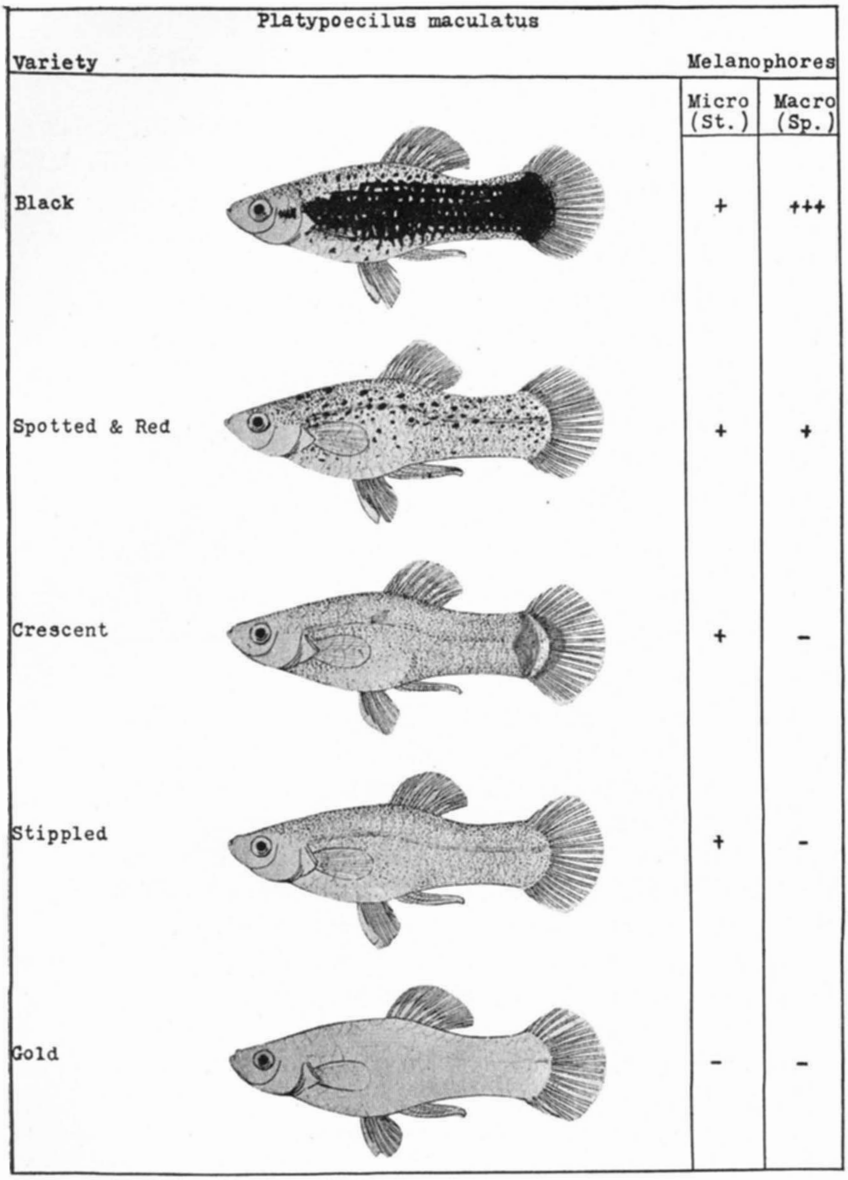


FIGURE 1.—A few varieties of *Platypoecilus maculatus*, (males) 1.4 natural size. The Red, Stippled and Gold were used in these studies. The pen and ink drawings were made by Miss MARY MEKEEL.

Two species now remain: *Platypoecilus couchia* and *P. maculatus.* The synonymy of the former is as follows:

Limia couchia Girard, Proc. Acad. Phila., 1859, p. 116
Poecilia couchii, Günther, Cat. vi, 1866, p. 347
Poecilia couchiana, Jordan and Gilbert, Synopsis, 1882, p. 348
Platypoecilus couchianus, Regan, Proc Zool. Soc. Lon. 1913, p. 977
Platypoecilus couchia, Gordon, Genetics **12**: p. 256

The synonomy of *Platypoecilus maculatus* is as follows:

Platypoecilus maculatus Günther, Cat. vi, 1866, p. 350
Poecilus maculatus, Regan, Bio. Cen. Amer. Pisces, 1907, p. 105
Platypoecilus maculatus, Regan, Proc. Zoo. Soc. Lon. 1913, p. 977

According to the latest revision (HUBBS 1924) the two species will be listed under the following groups:

Order: Cyprinodontes
 Family: Poeciliidae
  Sub-family: Poeciliinae
   Tribe: Xiphophorini
    Genus: Platypoecilus.

NATIVE HABITAT AND VARIABILITY OF THE WILD FISHES

Platypoecilus is confined to Mexico, being most often collected in the southern, tropical fish-faunal zone. The most extensive field studies were made by MEEK (1904), who has made the following statement: "For example of extreme individual variation of color markings, I would refer to *Platypoecilus maculatus.*" This also has been the concerted opinion of all those who have collected it. Although guarding against unnecessary splitting of varieties into species, MEEK nevertheless described one new one; this latter species was placed into synonomy. It is of particular importance, in these studies in inheritance and variation, to know that this top-minnow has been singled out from the total number of 227 species described by MEEK from Mexico for its variability.

The first live specimens were imported into Germany in 1907 (GERLACH 1909[1]). The aquarists there, in describing the newly arrived fishes practically redescribed the types MEEK mentioned and illustrated. They have, however, added to the list of variable types; but it must be remembered that their descriptions were made from newly imported stocks. By 1912 practically all the patterns now known had been recorded.[2] The only

[1] Determination of species mentioned, confirmed by a specialist (LEONHARDT 1909).

[2] STANSCH (1911b) and SCHREITMULLER (1911) give good summaries of varieties newly imported up to that date. FINCK (1915) lists six imported varieties.

variety which owes its identity to the influence of domestication is the Gold, described in 1920, which has, in all probability, mutated from aquarium material.

The cause of variability of Platypoecilus cannot be traced to the effect of domestication but must be sought in its native habitat. Unfortunately up to this time no systematic field studies of this species have been made, but the general situation is presented by MEEK. Under the heading, "General Topography and Hydrography of Mexico" he says:

"The greater portion of Mexico is a plateau ranging in elevation from 3000 to 8000 feet above the sea.... The northern portion is the lower and the slope is to the northeast.... The eastern border of this plateau is formed by the eastern range of the Sierra Madre mountains leaving a plain to the east of varying width.... The western Sierra is exceedingly rough and its western slope very steep."

Under "Recent Geological Changes in Mexico and their General Effect on the Fish Fauna" he says:

"Geologically speaking, within more recent times the climatic and hydrographic conditions of Mexico have been less stable than in the Mississippi Valley. The central portion of Mexico has been subject to considerable volcanic disturbances which have continued to within recent times. The northern portion has, evidently at some former time, been much better watered than it is now." Many of "the rivers of this region never reach the sea and were, perhaps formerly, the tributaries of the Rio Grande, as is indicated by the character of the fish fauna and general topography." It is also held that "the western streams have cut their way back, captured the head-waters of the eastern stream, and with them their portion of the eastern fish fauna."

The rainfall is sufficiently variable to cause seasons of wet and dry weather. In the north the rains begin about the first of May and stop about October or November, while in southern Mexico, immediately to the north of the Isthmus of Tehuantepec, from which region Platypoecilus is most often reported, the rains continue into December and occasionally extend into February.

"In the height of the wet season many of the streams overflow, forming in depressions—shallow lakes, bayous and ponds. These later become dry, causing the destruction of many fishes."

MEEK further states that the viviparous killifishes give birth to young at the end of the dry season. Since they are well developed from the start, the young are able to withstand the keen competition in the thickly populated bodies of water; when the wet season sets in, they are in better condition to become widely distributed.

Commenting upon the ceaseless environmental changes in geologic and present periods, MEEK says: "Species of fishes in Mexico are undergoing changes, and the progress of making species of varieties is still actively in progress."

MCFARLANE (1923), after a study of the evolution and distribution of fishes believes that there is conclusive proof that Northern South America

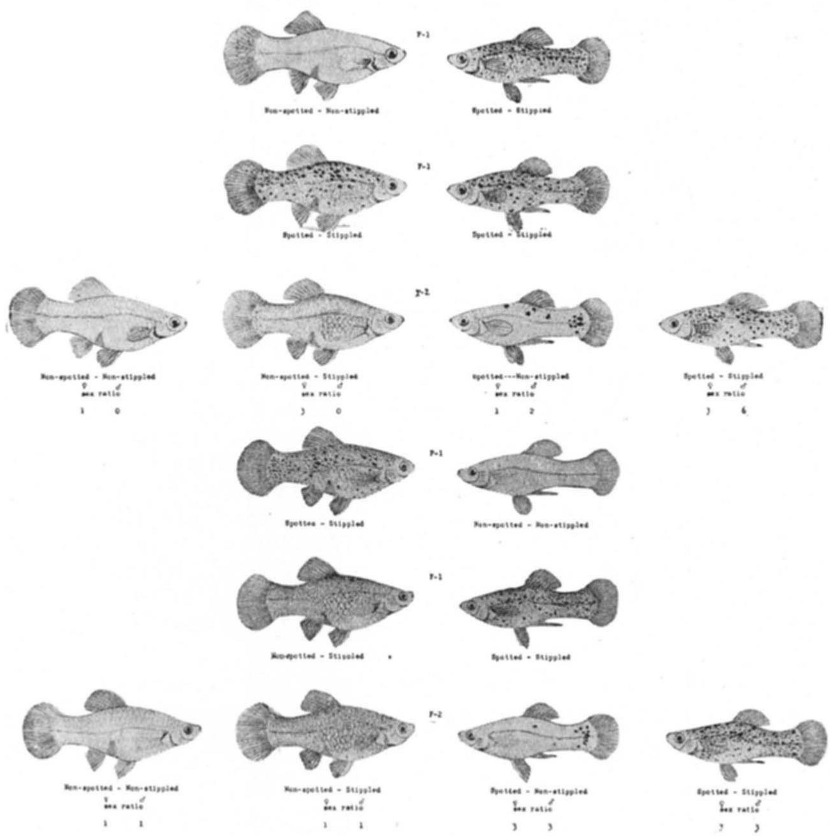


FIGURE 2.—The crosses diagrammed: above, Gold ♀ ×Red ♂; below, Red ♀ ×Gold ♂.

was the centre for the evolution of the Cyprinodontes, which at present are widely distributed.

Genetical studies of Platypoecilus should prove valuable in determining the nature of the variations. Individuals of a variable species may have become geographically isolated and thus may have developed several races. It will be interesting to study, thoroughly, the distribution of these fishes in their native habitat to determine the relation, if any, of a variety to its peculiar environment.

VARIETIES AND THEIR COLOR PATTERNS

In the aquarium, where the water is maintained at subtropical temperatures, *Platypoecilus maculatus* has adapted itself as well as if it had been carried to an isolated pool in its native habitat. This interesting little fish has taken the fancy of aquarists in Germany and in the United States who have fixed a wide variety of types. Domestication has not induced the production of any new forms, with the possible exception of the Gold. Through selection it has merely brought about a splitting up of a complexly marked animal into its various components. Fifteen types, most of which have already given indication of possessing individual genetic factors, have been identified. It is here proposed that these types be named. Since data is lacking which would supply the information necessary to establish these several varieties as sub-species, it would seem best to follow the accepted method in genetical nomenclature by using common names. Corresponding with the descriptions and their respective numbers contained in the list of GORDON (1926b) the following names are offered:

No.	*Name proposed*	*Synonomy*	*Pattern first described by*
1.	Shoulder blotch		Günther 1866
2.	Peduncular blotch		Günther 1866
3.	Ocellus		Meek 1904
4.	Crescent		Meek 1904
5.	Twin blotch		Meek 1904
6.	Dorsal black		Gunther 1866, Meek 1904
7.	Anal black		Gunther 1866, Meek 1904
8.	Ventrals black		Meek 1904
9.	Barred 2, 3, 4, 5.		Meek 1904
10.	Stippled platy	White[1]	Meek 1904
11.	Spotted platy	Pulchra[2]	Schreitmuller 1910 a, b, c.
12.	Blue platy		Schreitmuller 1910 a, b, c.
13.	Red platy	Rubra[3]	Finck 1911, Stansch 1911
14.	Black platy	Nigra[4]	Holmann 1912, Gerlach 1912
15.	Gold platy[5]	Immaculatus[6]	Struve 1920, Myers 1922

[1] Proposed by Bellamy 1922, 1924 (see page 276 for discussion).
[2] Proposed by Boulenger, adopted by Schreitmuller 1910c.
[3] Proposed by Stansch 1911a, b.
[4] Proposed by Gerlach 1912.
[5] Proposed by Struve 1920.
[6] Proposed by Myers 1922.

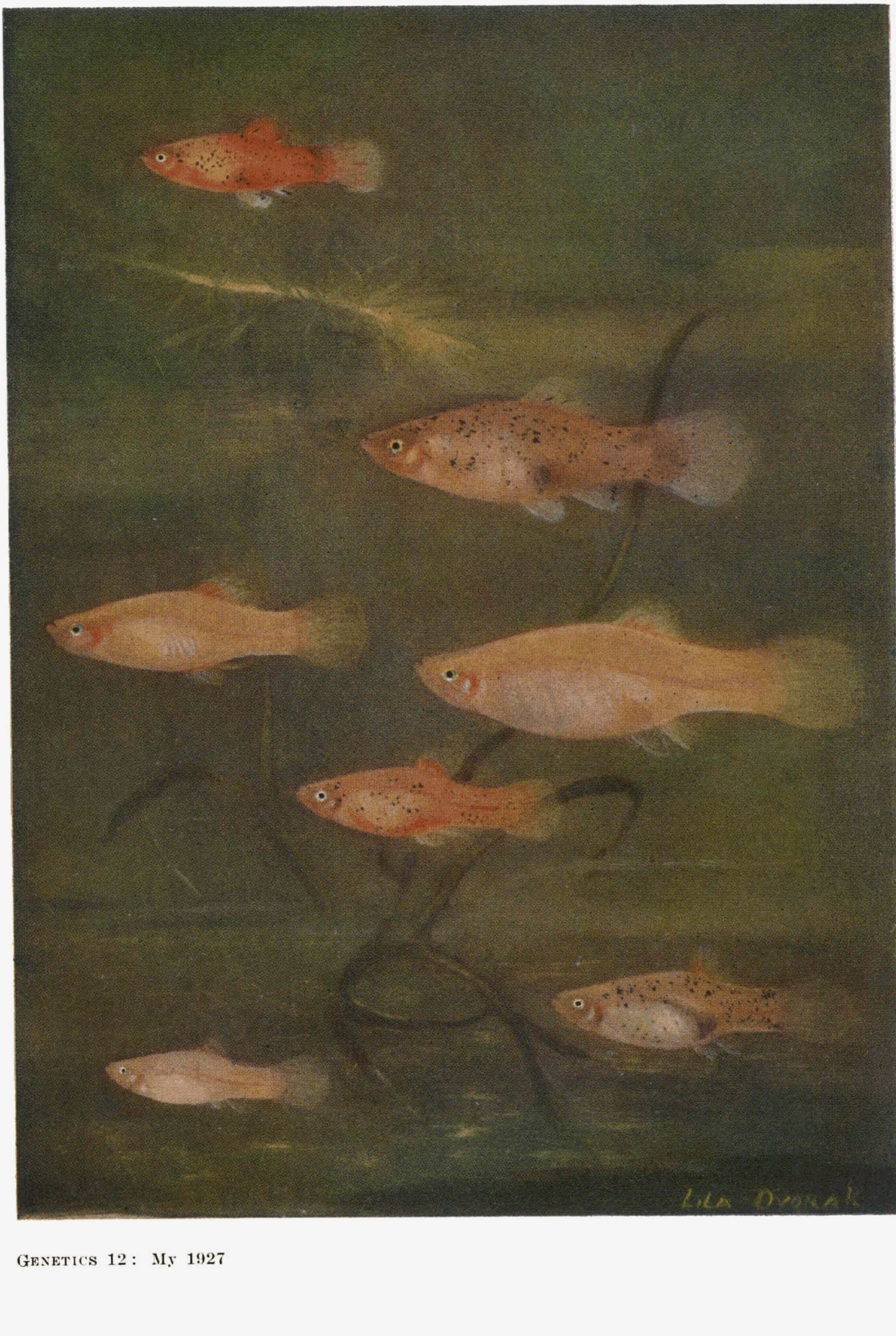
In general the varieties owe their individuality to the presence or absence of the various color pigment cells: the black or melanophore; the yellow, or xanthophores; and the red, or erythrophores. Of melano-

Legend for Plate 1

PLATYPOECILUS MACULATUS

1. Red ♂
2. Red ♀
3. Gold ♂
4. Gold ♀
5. F_1 hybrid ♂: P_1=Red ♂, (1) Gold ♀, (4)
   and
   F_1 hybrid ♂: P_1=Gold ♂, (3) Red ♀ (2)
6. F_1 hybrid ♀: P_1=Red ♂, (1) Gold ♀, (4)
7. F_1 hybrid ♀: P_1=Gold ♂, (3) Red ♀, (2)

Figures numbered from top to bottom. Nos. 1, 5 and 6 natural size; nos. 2, 3 and 4 slightly more, No. 7 less, than natural size. No. 5 should have more red since the animal drawn was a young male.


Lila Dvorak

phores there are several types,[1] a genetical study of two of which is to be presented in this report. The first and most obvious type is a large intensely black kind which occurs irregularly over the body of the animal. These cells are found in the Spotted and also in practically all of the Red varieties. When they occur in great numbers even to the point of blanketing the sides of the animal they form the Black variety. Since this type of cell is the largest, and may be seen with the unaided eye, the term macro-melanophore may be applied to it.

The next type is one which will be referred to as micro-melanophore, or commonly to be spoken of as "stipple"—stipple because of the effect of many small melanophores operating together to give a general grayish-olivacious coloration. The individuality of the pigment cells, lost to the unaided eye, may readily be observed with the aid of low magnification.

When compared under high magnification, micro- and macro-melanophores appear to differ not only in size but also in their morphology (see figures 3, 4, 5). And this is significant because long before morphological differences were discovered, genetical studies made it evident that two types of melanophores were operating: the stipple type acting as an autosomal character and spot showing sex linkage.

Recently in a preliminary study of the morphology of the melanophores a third type has been observed, a companion type to stipple. They are typically like *Ameba radiosa* when expanded (see plate 2), and appear to be due to a factor operating in a manner similar to stipple. So far every Stippled animal studied showed the third type as well as stipples.

The following varieties used in genetical work may be characterized by the following table:

	Red	*Spotted*	*Black*	*Stipple*	*Gold*
Melanophores					
a. Stipples	+	+	+	+	–
b. Spots	+	+	+++	–	–
Erythrophores	+	–	–	–	–
Xanthophores	+	+	+	+	+

The Stippled variety

The general ground color of both sexes of this type is the Dark Olive Buff of Ridgway's color chart, 1912, plate 40. This color is in the main the one most commonly seen both in this group and in other fishes. It is a highly protective type of coloration. GÜNTHER (1866), for its original description, called it brownish olive; to MEEK (1904), it was olivacious; to BRIND (1919), cold straw; to BADE (1923), bluish gray to olive. This general olivaceous ground color is undoubtedly due to the presence

[1] See abstract on melanophores of Platypoecilus, GORDON (1926a).

of the dot-like micro-melanophores which occur in great numbers all over the body overlying the xanthophores. Only when the Stippled variety is observed under the low power of a binocular do the individual melanophores become clearly evident. On the mid-region immediately above the belly, these small pigment spots arrange themselves in a net-like formation, seen best in the adult females (see figure 3). Along the back, the snout, and the greater part of the tail region, the micro-melanophores are generally distributed. They are found, also, at the border of the anal fin and along all the fin rays.

In general the male is slightly brighter than the female. His dorsal fin at its origin is the Flaming Scarlet of Ridgway, plate 1. The female is generally duller and her dorsal fin is the La France Pink of Ridgway, plate 1. On the latter in the lower mid-region of the body between the anal and pectoral fins there is a brownish-black stain-like spot known to the fish fanciers as the sign of pregnancy. It is due to the black pigmented peritoneum appearing through the thin body wall at that particular place. It should rather be known as the sign of maturation, since it not only may be seen in virgin females, but in some well formed adult males as well. Other than this mark the Stippled variety when pure lacks all the patterns mentioned above. However, practically any or all patterns may be found in combination with it. As such, it becomes known under the various names of the markings.

The Spotted variety

This variety was first described by SCHREITMULLER (1910b, 1910c) from newly imported aquarium specimens. Since he worked with live material his description is more complete than others previously presented, as far as coloration is concerned. Clearly being different from the typical *Platypoecilus maculatus* SCHREITMULLER (1910b) called this variety *Platypoecilus sp.*?; later BOULENGER of the BRITISH MUSEUM proposed the varietal name of *P. maculatus* var. *pulchra* and this was accepted in his 1910c paper.

The ground color of this variety is generally, as in the former, the Dark Olive Buff of Ridgway. It has a strong tendency to take on a brownish cast and often intergrades with Red in color. On the other hand it may lose some of its olivaceousness and become paler and sometimes yellowish. But the character of most significance in this variety is the presence of large black pigment spots distributed, with varying intensification, irregularly over the body. They are usually in greatest preponderance on the upper half of the body and the whole tail region, but rarely extend to the tip of the snout, the operculum, the belly or fins. These macro-melanophores are different from the micro-melanophores, found in Stippled, although the latter pigment cells are also contained in the present variety and may be seen in parts of the body not covered by the former. The Spotted variety as such is now rarely seen in aquarium specimens, being undesirable from the breeder's point of view. All of its characteristics are preserved, however, in the Red variety which is merely the Spotted variety plus erythrophores. A fuller description of Red is given below.

The Red variety[1]

In this variety sexual dimorphism is well exhibited in the adult animals, but when immature, both sexes are almost indistinguishable. The adult females are generally dull, much like Spotted but often become Cinnamon Rufous of Ridgway plate 14. The males of the best strains when fully mature are the brilliant Brazil Red of Ridgway plate 1. In most cases the red begins to appear when the transformation of the anal fin into intromittent organ is almost completed. In some fishes, however, the full red color is delayed in its appearance until several months later, and many become deeply red with age. The red pigment is usually associated with a spotted type of animal, that is, most Reds in addition to possessing erythrophores, also have micro- and macro-melanophores. There is a tendency for a reduction of the melanophore number but individual variations are great and often increased numbers above the average may be found. It seems to be a general rule, however, that when macro-melanophores and erythrophores occur together, and the micro-melanophores are wanting, that there are decidedly fewer macro-melanophores present than when both kinds of melanophores are present together with erythrophores.

The Black variety

This variety may be considered as an extreme phase of the black spotted form. So closely set are the macro-melanophores on the sides of this variety that the spotted effect is entirely lost. A black blanket-like band of melanophores covers the animal from immediately behind the eye to the base of the caudal fin. The back, tail, belly and head regions are mostly free of macro-melanophores, but here also, as in the varieties already discussed, micro-melanophores are found. At the extremities of the black band, detached macro-melanophores are visible, showing their relation to the spotted form. The ground color of the pure Black is similar

[1] OSSWALD (1913) says that red specimens were obtained directly from Mexico.

LEGEND FOR PLATE 2

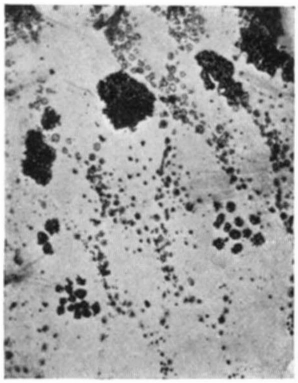
FIGURE 3.—A portion of the skin of *Platypoecilus maculatus*, under low magnification, showing spots, irregularly distributed, and stipples arranged to form a net work.

FIGURE 4.—Stipples (micro-melanophores) under high magnification: a. expanded (0.8 mm); b. partially contracted, (when fully contracted 0.3 mm.).

FIGURE 5.—Spots (macro-melanophores) under high magnification; a. expanded (2.4 mm.); b. contracted (1.2 mm.).

FIGURE 6.—The third type of melanophores adhering to the scale covering: a. expanded (0.8 mm.); b. contracted (0.2 mm.); c. entire scale under low magnification showing third type. (Note lines of growth in scales in a, b, c.).

Photo-micrographs made with assistance of Doctor D. J. LEFFINGWELL.



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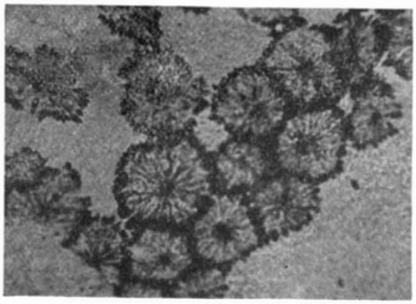

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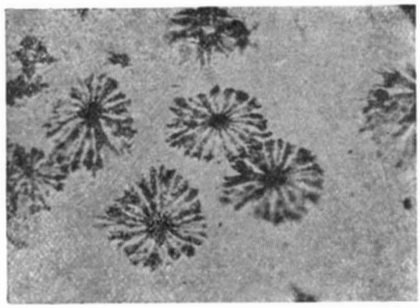

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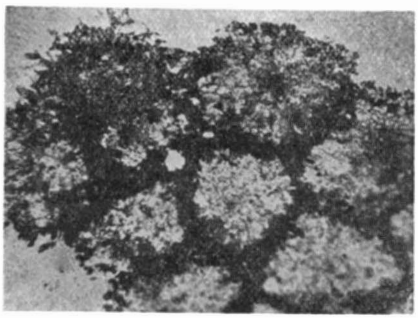

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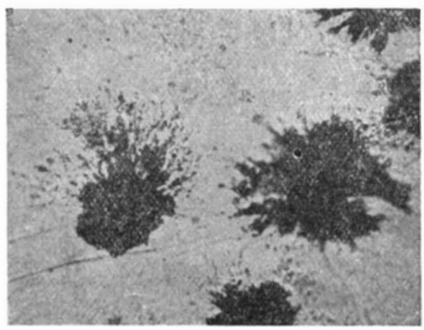
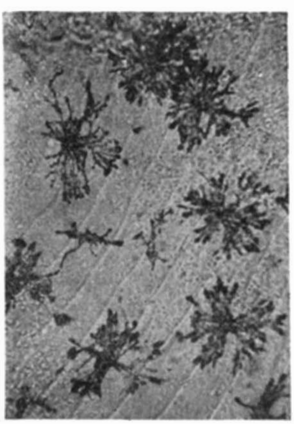


a b



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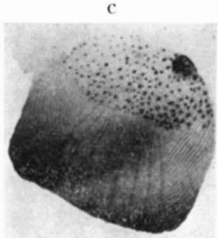

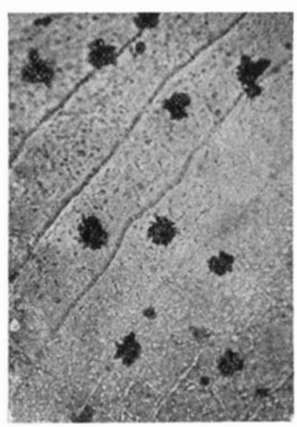

b a

to that in Stippled and Spotted, generally olivaceous. When crossed with certain varieties it may become red pigmented and the scales may become iridescent as in the Blue variety (MYERS 1922).

The Gold variety

This variety was first described and brought to the attention of Berlin aquarists by STRUVE (1920). Sachs pointed out that the variety lacked melanophores but retained its xanthophores (1922). The latter reviewed Professor TORNIER'S experiments on Axolotl eggs to show how pigmentless forms may arise, but apparently did not believe that the same factors were operating here. MYERS (1922) also noticed the lack of melanophores in the Gold variety and remarks, "No Goldplaty the author has examined has been spotted." (Platy is often used in common terminology for Platypoecilus.) Not only is the Gold variety non-spotted but it is also non-stippled. MYERS' color description is as follows: clear transparent yellow, slightly tinged with pale orange; eyes greenish blue, dorsal fin flushed red." For standard comparison the body color may be referred to as Deep Colonial Buff Ridgway plate 30; the dorsal fin of the male, Scarlet; and of the female La France Pink, both in Ridgway plate 1; eyes, Turquoise Green, plate 7.

It seems advisable to make a reservation, although it is but a slight one, to the statement that this variety is without traces of external melanophores. A few scattered melanophores of the stipple type may be made out, with the aid of a binocular, upon the back and sides. These cells are all that are left from the normally pigmented form like Stipple, from which it has probably originated. It will be shown (p. 275) that Gold differs from Stipple apparently in only one specific heritable factor, that for 'stipples'. The Gold variety has probably arisen as a recessive mutation. It is hardy and prolific in protected containers, but it is hardly possible that it can survive in the wild habitat, for it is decidedly conspicuous.

This variety has tentatively been named *Platypoecilus maculatus immaculatus* by MYERS (1922b), but the author is doubtful of its subspecific rank.

CULTURAL METHODS[1]

In Mexico, Platypoecilus is found in the tropical fish faunal zone, the fourth and southernmost, the region which lies immediately north of the Isthmus of Tehuantepec. It can not survive our winters without artificial heat. A constant temperature of 24° C has been found suitable

[1] Further details may be found in GORDON (1926d).

for breeding these fishes.[2] This has also been the experience of most of the commercial tropical fish fanciers and of BELLAMY (1924). At this temperature, maturation is hastened and seasonal reproductive habits destroyed, the young being produced every month in the year. It has previously been pointed out that in the wild habitat the fish spawn at the close of the dry season, which in the region of the tropical fish faunal zone extends from April to May.

Viviparity in Platypoecilus presents some difficulties which must be thoroughly appreciated and overcome before successful and accurate work can be carried on. In rearing a school of young fishes for future mating, constant observation is made to detect and isolate those fish which show beginnings of change in the anal fin. Slight thickening of the fore-margin of the anal fin is indicative of rapid modification of that fin into the male intromittent organ. It is by means of this fin that internal fertilization is effected. (The mode of mating in viviparous species of top-minnows has been described by WINGE, 1922 and BELLAMY, 1924). Obviously, differentiating males are not allowed to remain with females because of the likelihood of promiscuous mating. Furthermore, as has been pointed out by GERSCHLER (1914), females once mated, hold sufficient spermatozoa in the folds of their oviducts to serve for the fertilization of many schools of fishes. He records as many as seven schools having been so produced, each at approximately one month's interval. WINGE (1922), reporting on *Lebistes reticulatus*, a viviparous fish of the sub-family Poeciliinae (as is Platypoecilus) says:

"Some spermatozoa remain in the oviduct where they are able to keep alive for several months, and when the female, a month after fertilization, has cast her young, these spermatozoa can fertilize the eggs which have matured in the meantime."

It has been found advisable, therefore, to use unmated females for all crosses.

One to several females are crossed with a single male. These are placed in a well planted battery jar, eight inches high and six in diameter,which serves for mating purposes. Signs of pregnancy are evidenced by a swelling and distension of the belly in the females. At this time each female is given a separate jar thickly planted with Vallisneria, Elodea and, most important, Riccia. This last plant grows just beneath the surface and

[2] One evening the temperature in the incubator accidently rose from 24°C at 11 P.M. and reached 41°C at 8 A.M. the next morning, thereby killing an important group of fishes. Other battery jars further away from the source of heat had a temperature of 35°C and while the animals were distressed no further losses were experienced. DAVENPORT (1908) does not list any fishes surviving above 40°C.

its dense mesh-like branches offer the newly born fishes an excellent hiding place during their first few hours of utter helplessness. To further prevent cannibalism, which must be carefully guarded against, the parent is given an abundance of food. It is at this particular time that live food, especially Daphnia and Enchytraeus, becomes almost indispensable since quantities may be fed without danger of polluting the water of the aquarium. It further aids in attracting the attention of the parent away from the young fishes. When the young are expelled from their mother's body they straighten out from their previously coiled position and attempt to swim upwards, seeking shelter in the thick vegetation. Within an hour or two their slightly extended yolk sac is entirely absorbed. Most of the members of the newly born school will emerge during the early morning hours, but the entire complement will not usually be expelled until evening. The female is allowed to remain with her young until that time and then removed leaving the progeny in the aquarium. Here they remain for a month or two and then if larger containers are available they are given more room.

In most cases, a count of the offspring is taken about six to eight weeks after birth. At that time they are quite strong and are able to survive a good deal of rough treatment. The period of maturation varies greatly but averages eight months, at which time sex data may be taken.

While it was undesirable to do so, it became necessary occasionally to rear the young to adults in the same small battery jars. As many as ten have been raised in such containers but complete and normal development was slow and difficult to obtain. Because of lack of space only the best specimens were selected after counts were made, and reared to maturity. Only where sex linkage was evident was a special effort made to raise the entire school to maturity. In any given school it is soon evident that individual variation in general vigor and ability to grow is great. Under the best possible conditions a number of young soon drop out and many others never mature. It may be that the present cultures have many inherent lethal factors which inbreeding is bringing out. On the whole, however, barring accidents, when given plenty of room, proper temperature of 24° C and good food, the fishes respond very well.

EXPERIMENTAL DATA[1]

In the present series of experiments the following strains have been used: Gold, Stippled and Red. They may conveniently be distinguished by these characters:

[1] Summary of data may be found in GORDON (1926c).

	Gold	Stippled	Red
Melanophores			
a. Stipples	−	+	+
b. Spots	−	−	+
Erythrophores	−	−	+

It is evident from the above that there are three characters which may be studied experimentally, that is, micro-, macro-melanophores and erythrophores. The latter while possible of factorial analysis, have not been extensively studied in these experiments. One or two items of interest that have been worked out with respect to them will be discussed below. The most significant results relate to the melanophores, and the presentation and discussion of data concerning their mode of inheritance follows.

The stocks used

The varieties used in the present experiments proved upon testing to be homozygous for the characters studied. This testing, by matings within the strain, did not precede the varietal crosses, but was run in parallel with them. The pedigree numbers of the original mated pairs and their homozygous progeny are given in table 1. In no case was there a deviation from the parental type in F_1, F_2 or in F_3. In the progeny of Red, there was a slight variation in the number of macro-melanophores exhibited, occasionally more, sometimes less than in the P_1, but it is significant that they all possessed spots.

The cross of Gold by Red

Direct: Gold ♀ by Red ♂

The first inter-varietal crosses were made on May 14, 1924. Two homozygous Gold females were mated to homozygous Red males—each cross was run independently and the results recorded separately. These crosses involve factors for *spots*, *stipples*, and *red* (erythrophores)—the Red variety bringing in all the three factors and the Gold variety bringing in none.

The F_1 results are summarized from table 2:

P_1 GOLD ♀ (Pedigree 10 of school born 1-21-24) NO SPOTS NO STIPPLES
RED ♂ (" 11 " " " 1-28-24) SPOTTED STIPPLED

F_1	Spotted Stippled	Non-spotted Stippled
Observed	118	1
Expected	*119*	*0*

It is quite apparent from the above that the factors for *spotting* and *stippling* are dominant over the absence of such pigmentation. From this one series of experiments alone, one could not differentiate between the two types of melanophores. It might appear that stipples are merely smaller spots or that spots are merely aggregates of stipples, the factors for the melanophores being one and the same. The process of differentation will become evident in the reciprocal cross, described below.

Limitation of space prevented the rearing of the entire F_1 population to maturity. The progeny in the third school of cross, 10–1×11–11 and 10–2×11–12 were all discarded as soon as the count was taken on them (see table 2). Sex ratios were found not to be significant in this series.

Up to the present time the sex ratio for the spotted-stippled class in F_1 is as follows: 18 females and 22 males. The latter upon full maturity are red in color but not quite as brilliant as their father, the color being Mars Orange of Ridgway plate 2. The F_1 females are similar in color to a homozygous Red female; that is, olivaceous. Since the assumption of red pigment is a secondary sex character of the male it is not surprising that color differences are evidenced. The factor for *red* color, then, is dominant over *non-red*.

The aberrant F_1 non-spotted stippled fish appeared singly in a total of 119 individuals. It was reared with great care and, when apparently a mature female, was mated to an F_1 male (pedigree 15–11) of known fertility but no progeny were ever produced. It died (unexpectedly) in its eleventh month—unfortunately the internal organs spoiled too rapidly for purposes of further study. A plausible explanation is offered below for its occurrence.

Reciprocal: Red ♀ by Gold ♂

The reciprocal crosses were also set up on May 14, 1924. Two homozygous Red females were mated to one Gold male, the progeny of each female being recorded separately. (Only one Gold male was available at the time). The results from these two crosses and two others made later (for details see table 2) are as follows:

P_1-1 RED ♀ (Pedigree 11 of school born 1-28-24) SPOTTED STIPPLED
GOLD ♂ (" 10 " " " 1-21-24) NO SPOTS NO STIPPLES

F_1	Spotted Stippled	Non-spotted Stippled
Observed	70	86
Expected	*78*	*78*

Here the F_1 results point to a clear cut distinction between micro- and macro-melanophores—the latter occurring in a ratio of 1:1 while the former are completely dominant. Morphological studies have confirmed the assumption, first drawn from genetical evidence that stipples and spots are actually as well as apparently different. (See photomicrographs).

The purity of the Red might be brought to question—although the stock proved, by test, to be pure. Ratios of 1:1 would be expected if the females were heterozygous for the *spot* factor. If the ratios of the sexes, male to female, become 1:1 in each class, both in the Spotted Stippled and Non-spotted Stippled, it could be assumed that the females (there were four in all, since two more were added later) were heterozyous. But such is not the case and the evidence from sex ratios is conclusive.

Realizing the importance of the ratio exhibited in the young fishes, special effort was made to rear all the progeny of these crosses. The young of pedigree 16 and 17 were therefore sorted out phenotypically, counted, and given more room, about ten being placed in a jar. The school born 12–1–24 being small was kept separate. In this last school there were originally five Spotted Stippled fishes and six Non-spotted Stippled. Of these eight were reared to maturity and the sex ratio has been found as follows:

PEDIGREE 16 SCHOOL 12-1-24	SPOTTED STIPPLED		NON-SPOTTED STIPPLED	
	♀	♂	♀	♂
Observed	0	2	6	0
Expected[1]	*0*	*2*	*6*	*0*

Due to an unfortunate accident one night all the rest (38) were killed when they were only four months old so that sex ratios were not obtained. (See footnote 2, page 266). As soon as was possible, this reciprocal cross, Red female by Gold male, was repeated. Six months later the first schools appeared under pedigree 36 and 37. Data on sex are as follows:

PEDIGREE	SPOTTED STIPPLED		NON-SPOTTED STIPPLED	
	♀	♂	♀	♂
36	0	7	12	0
37	0	38	33	0
Observed total	0	45	45	0
Expected[1]	*0*	*45*	*45*	*0*

Here, then, is a clear case for sex-linkage, as against that for autosomal heterozygosity in the female. As yet no exceptions have occurred. Mature

[1] Assuming sex linkage.

Spotted Stippled fishes have always turned out to be males and Non-spotted Stippled fishes have remained female-like, many of the latter producing schools of young. The situation with respect to sex-linkage is similar to that found in Abraxas and in poultry, namely, the ZW type.

Males of pedigree No. 16, 36, and 37 tended to become *reddish* just as did the males of the direct cross. This is significant since the female parents of 16, 36, and 37 were Dark Olive Buff and the male parents were Deep Colonial Buff, neither showing red. But it will be recalled that *red* is a male secondary sex character and as such is carried by the olive colored female of the Red strain.

An explanation may now be offered for the appearance of the aberrant sterile, Non-spotted Stippled female in the F_1 of the direct cross. Non-disjunction in the P_1 male of the Z chromosomes carrying the sex-linked factor for *spots* to give a zygote with no Z chromosome would account for the non-spotted condition in this F_1 daughter. The factor for *stipples* being carried by a different set of chromosomes was not affected, so that while the female was *non-spotted*, it was nevertheless *stippled*. The chances of obtaining a ZZW or ZZZ individual are very small since comparatively few individuals have been reared to maturity.

The F_2 (Direct cross)

As a result of four matings a total F_2 population of 320 individuals was obtained. Summarized from table 3, the results are as follows:

P_1 Gold ♀×Red ♂ (F_1 spotted stippled ♀×spotted stippled ♂).

F_2	SPOTTED STIPPLED	NON-SPOTTED STIPPLED	SPOTTED NON-STIPPLED	NON-SPOTTED NON-STIPPLED
Observed	175	73	52	20
Expected (9:3:3:1)	*180*	*60*	*60*	*20*

Observed	all spotted	227	all non-spotted	93
Expected (3:1)		*240*		*80*
Observed	all stippled	248	all non-stippled	72
Expected (3:1)		*240*		*80*

Further proof of the individuality of the micro-and macro-melanophores is obtained from the F_2 data of the direct cross (above), as well as from the F_1 data of the reciprocal cross. In the F_2, four phenotypes appear, presenting two new types: the Non-spotted Stippled and the Spotted Non-stippled. The four phenotypes in the F_2 indicate that two pairs of factors are operating. The 9:3:3:1 ratio observed (it is not a perfect agreement, to be sure, but significant) points to dihybridism in the F_1 parents.

Due to various causes, this group did not produce as many mature specimens as one might have desired. Particularly striking is the low number of adults in the two Non-stippled classes. Whenever stipples are wanting, the animals are gold colored at birth, and are easily recognized by their flashing brightness. In protected containers, of course, they are not subjected to attacks by predators which naturally would seek out the forms most easily seen. Other factors[1] have been responsible for the low vitality of this class, shown by the F_2 adult populations reported below. The stippled fishes, whether spotted or not, are olivaceous at birth.

In the F_2 population the Non-spotted Stippled type is obtained which is similar to the F_1 females of the reciprocal cross. Theoretically not only should these fishes be similarly colored but similarly sexed. As far as data are available, none of the non-spotted animals have transformed into males, all that have grown to maturity are typically female. Sex data, such as are available, are in agreement with the hypothesis that the *stipple* factor is of autosomal origin and the factor for *spot* shows sex-linkage of the ZW type.

The present results of the examination of the sexes[2] for the F_2 are:

	SPOTTED STIPPLED		NON-SPOTTED STIPPLED		SPOTTED NON-STIPPLED		NON-SPOTTED NON-STIPPLED	
	♀	♂	♀	♂	♀	♂	♀	♂
Observed	28	41	32	0	5	6	2	0
Expected numbers	*21*	*42*	*21*	*0*	*7*	*14*	*7*	*0*
Expected ratio	*3*	*6*	*3*	*0*	*1*	*2*	*1*	*0*

The F_2 (Reciprocal cross)

F_2 (Reciprocal cross). P_1 Red ♀×Gold ♂; F_1 non-spotted stippled ♀×spotted stippled ♂.

F_2	SPOTTED STIPPLED	NON-SPOTTED STIPPLED	SPOTTED NON-STIPPLED	NON-SPOTTED NON-STIPPLED
Observed	76	87	16	29
Expected (3:3:1:1)	*78*	*78*	*26*	*26*
Observed	all spotted	92	all non-spotted	116
Expected (1:1)		*104*		*104*
Observed	all stippled	163	all non-stippled	45
Expected (3:1)		*156*		*52*

[1] Ancyrocephalus, a parasitic flat-worm, has been observed in the gills of many sick fishes.

[2] Further data on the sexes now available would permit the increasing of the values as presented in this table. Similarly, the values given in sex ratio tables, which follow, may now, also, be extended.

The summarized results from table 3 given above, show the method of dihybrid segregation of the F_2 progeny in new proportions. The F_1 females, being recessive for the *spot* factor, when crossed with F_1 males heterozygous for the same factor, should yield a 1:1 ratio. The observed results 92:116 approach this. Since both females and males ued in these crosses were heterozygous for *stippling*, a 3:1 ratio would be expected in the F_2 for this factor. The observed results 163:45 tend to support the assumed conditions.

The sexes of the F_2 of the reciprocal cross: The animals making up the F_2 total, given above, came from two groups of F_1 crosses. Those of pedigree 39 and 40 were obtained quite early from some of the few surviving F_1 fishes, 16–1, 16–2 and 16–11 of the school born 12–1–24. About two-thirds of these have matured and the table given below shows the result of the sex count. The remaining F_2 progeny derived from pedigree 36 and 37 were obtained much later (see table 3) and are too young for one to determine sex.

Data on sexes of the F_2 of the reciprocal cross of the pedigree 39 and 40 (the total F_2 population of this group being 70) follows:

	SPOTTED STIPPLED		NON-SPOTTED STIPPLED		SPOTTED NON-STIPPLED		NON-SPOTTED NON-STIPPLED	
	♀	♂	♀	♂	♀	♂	♀	♂
Observed	10	9	9	5	4	4	4	4
Expected numbers	*9*	*9*	*9*	*9*	*3*	*3*	*3*	*3*
Expected ratio	*3*	*3*	*3*	*3*	*1*	*1*	*1*	*1*

It will be seen that the F_2 ratios obtained above are quite different from those obtained in the direct cross (see page 271). Here every class has equal numbers of the two sexes whereas in the former case there were no males obtained in the two Non-spotted groups.

The backcrosses

With respect to the direct cross, Gold female by Red male, the F_1 progeny were subjected to the four possible backcrosses. While the numbers obtained are small, and possibly not admitting of final analysis of the situation, they tend to corroborate the general hypothesis already formulated: the autosomal nature of the *stipple* factor and the sex linked nature of the factor for *spots*.

In backcross A (table 4) an F_1 hybrid female heterozygous for *spots* and *stipples* was crossed with a double recessive male, Gold. The summarized results are as follows:

	SPOTTED STIPPLED		NON-SPOTTED STIPPLED		SPOTTED NON-STIPPLED		NON-SPOTTED NON-STIPPLED	
Observed	8		17		13		7	
Expected (1:1:1:1)	*11*		*11*		*11*		*11*	
Observed	all spotted		21		all non-spotted		24	
Expected (1:1)			*22*				*22*	
Observed	all stippled		25		all non-stippled		20	
Expected (1:1)			*22*				*22*	
	Sex data							
	♀	♂	♀	♂	♀	♂	♀	♂
Observed	0	7	9	0	0	5	1	0
Expected	*0*	*5*	*5*	*0*	*0*	*5*	*5*	*0*

The low number of mature animals reared was directly accounted for by the presence of the parasitic gill flatworm. It is seen, however, that all the *Spotted* fish are *males* and the *Non-spotted, females.*

Backcross B is the reciprocal of the above (A). A double recessive female, Gold was crossed with an F_1 hybrid male, heterozygous for *spots* and *stipples*. The results are:

	SPOTTED STIPPLED		NON-SPOTTED STIPPLED		SPOTTED NON-STIPPLED		NON-SPOTTED NON-STIPPLED	
Observed	20		42		24		28	
Expected (1:1:1:1)	*28*		*28*		*28*		*28*	
Observed	all spotted		44		all non-spotted		70	
Expected (1:1)			*57*				*57*	
Observed	all stippled		62		all non-stippled		52	
Expected (1:1)			*57*				*57*	
	Sex data[1]							
	♀	♂	♀	♂	♀	♂	♀	♂
Observed	5	4	7	5	4	4	6	5
Expected	*5*	*5*	*5*	*5*	*5*	*5*	*5*	*5*

[1] Sex data taken June 11, 1926, many yet too young. (See footnote to page 272).

The ratios in the above cross are similar to the former for the various class groups; but here the sexes occur in equal proportion in each class.

Backcrosses C and D are interesting when compared. They show the influence of the sex linked factor which appears in a new proportion. In C when an F_1 hybrid female is crossed with a homozygous Spotted and Stippled (Red) male all the progeny are, as expected, Spotted and Stippled, (see table 4 C). If, however, the reciprocal of C is made (as in D) a different result is obtained:

	SPOTTED STIPPLED	NON-SPOTTED STIPPLED
Observed	14	3
Expected (3:1)	*12*	*4*

Since the female is considered normally heterozygous for Spots (due to sex linkage) and the male is known to be heterozygous for the same factor, the ratio of 3:1 is expected.

Unfortunately many of this school died before sexes became differentiated.

The cross of Gold by Stipple

The individuality of *stipples* is conclusively shown in the following series of experiments and the mode of their inheritance is brought out clearly, uncomplicated by the factor for *spots*.

Two homozygous Gold females were mated to two homozygous Stipple males. These crosses were made in duplicate just as in the former series. The F_1 results as given in table 5, show all Stippled offspring (64).

In a similar manner, the reciprocal crosses were set up in duplicate, and the totals in the F_1 showed similar results: all Stippled fishes (83).

Carried through the next generation the F_1 hybrids of both the direct and reciprocal crosses produced a total of 105 F_2 fishes, up to the present time. These form a ratio approaching 3:1 (for details see table 5.)

A case of simple Mendelian inheritance is illustrated above, one factor pair being contrasted; *stipples* are completely dominant over the *lack of stipples*. As the frequencies will indicate, the sexes of the F_1 occurred approximately in the proportion of 1:1, the totals being 28 females to 32 males.

GENERAL DISCUSSION

The experiments reported here, were not undertaken with the purpose of checking up on work already done. The fact that Platypoecilus was being studied, elsewhere, was realized only after the present experiments were well under way. The brief abstract of the work of Bellamy (1922) first came to notice only after his later publication in Genetics, November, 1924, referred to it. In the abstract it was stated that the female Platypoecilus was heterogametic for sex.

His data (1922), he claimed, "disagree in several important respects with the findings of Aida (1921) and Winge (1922)." The most important respect obviously is the type of sex linkage found in the different species —females of Platypoecilus being heterogametic for sex would naturally fall under the ZW type, while Aida and Winge hold to the XY type for Aplocheilus and Lebistes respectively. Notwithstanding the fact that Lebistes is contained in the same sub-family as is Platypoecilus, and all three fishes belong to the order, Cyprinodontes, it must be remembered that these are different species. It cannot be assumed that what applies to Lebistes or to Aplocheilus must necessarily be true of every Cyprinodont.[1] While, heretofore, sex linkage types have been confined to no lower than ordinal groups—XY for Diptera and ZW for Lepidoptera in insects—as more species of animals are studied it is conceivable that a group lower in scale than a sub-family, might be found to contain species exhibiting different types of sex linkage.

The work reported in this paper has been largely derived from the use of the Gold Platypoecilus—a variety not figured nor described previously in genetical work. Its recessiveness has proved to be a most useful tool in testing dominant factors: *stipples*, *spots* and *red*. It is now being used to test *black*, *crescent*, *blue* and others.

That the female is the heterogametic sex in Platypoecilus is Bellamy's hypothesis (1922, 1924) and it is supported by the present investigations. But exception must be made to the following statement: that the factors *W*, *N*, *P*, *R*, (of Bellamy) constitute a multiple allelomorphic series. Before presenting the evidence, the synonomy of the varietal terms must be set down.

White = Stippled, considered by Bellamy as a triple recessive.
Pulchra = Spotted, " " " " dominant for spots only.
Rubra = Red, " " " " " " red pigment.
Nigra = Black, " " " " " " intense black.

The factor for *stipples* was not considered by Bellamy. It is present in all four of his types and could only have been tested with Gold which lacks this character. The variety White merely represents a form recessive to *P*, *R*, and *N* (*spots*, *red* and *black*). The name White is not a fortunate one since the animal is not really white but possesses both micro-

[1] Compare with Morgan (1926b) p. 205.

melanophores and xanthophores. It is best to reserve the term White for one without color pigment. (Pure white forms have been described in Aplocheilus by TOYAMA 1916, ISHIWARA 1917, and AIDA 1921).

BELLAMY says (p. 523) that "males may be heterozygous for any two of the four characters, *W*, *P*, *R*, or *N;* and the female may carry the determiners for one only." This cannot account for the existence of the *spotted-red* (pulchra-rubra) female. These fishes transmit both *spots* (pulchra) and *red* (rubra) to their sons when mated to a Gold male, a double recessive, (table 2 and page 269).

Very little need be said here of the XY type of sex determination in Lebistes and Aplocheilus. They have been reviewed by CASTLE (1921) and, recently, in greater detail, by CREW (1925) and MORGAN (1926). AIDA's work (1921) on Aplocheilus confirms the results of an earlier experiment by TOYAMA (1916) with respect to sex linkage. AIDA, however, has accumulated a much stronger body of evidence and his conclusions are significant—some two thousand fishes were reared to maturity, a wonderful cultural project. ISHIWARA (1917) had also worked on Aplocheilus (*Oryzias latepes*) but obtained no indication of sex linkage. This may probably be explained by the fact that his varieties were Yellow-black, (*YB*); Yellow, (*Yb*); Blue, (*yB*); and White, (*yb*); whereas AIDA worked with the factor *R* for red pigment in addition to those above —and *R* the factor for red pigment is the only one that shows sex linkage.[1]

It appears, then, that within the same order, Cyprinodontes, and within the same sub-family, Poeciliinae, different species exhibit the two types of sex linkage. The evidence is primarily genetical. For a more complete solution of the problem, it will be interesting and important to have cytology contribute its share of information.

SUMMARY

1. *Platypoecilus maculatus* is an extremely variable species in its native habitat. It was first introduced to the aquarists in 1907. By selective methods true breeding types have been isolated.

2. The varieties, Gold, Stippled and Red have been used in the present experiments.

3. The above varieties owe their individuality to the presence or absence of the various color pigments:

A. Erythrophores, red pigment cells, are controlled by the dominant factor *R*, found in the variety, Red.

[1] ISHIMARA cites ISHIKAWA (1913) as having crossed black×yellow to get black F_1 and three blacks to one yellow in F_2. No sex linkage was evidenced.

B. Melanophores, of which there are two types, differing not only genetically but morphologically: (*a*). macro-melanophores, or spots, controlled by the dominant factor S_p found in the variety, Red. (*b*). micro-melanophores, or stipples controlled by the dominant factor S_t found in the varieties, Red and Stipple.

The variety Gold is recessive for R, S_p, and S_t.

4. S_p is sex linked; S_t is borne by an autosome.

5. The female is heterozygous and the male is homozygous for sex.

6. Platypoecilus has the ZW type of sex inheritance, Lebistes has the XY type—both belong to the sub-family, Poeciliinae, of the order Cyprinodontes.

7. The native habitat and the taxonomy of Platypoecilus are discussed; the cultural methods which were employed in this study are given elsewhere.

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TABLE 1

Pedigrees of homozygous stocks.

P_1	F_1			F_2			F_3		
	Pedigree Number	Total	How crossed	Pedigree Number	Total	How crossed	Pedigree Number	Total	Grand Totals
Gold	10	30	10–3×10–12	18	40	18–2×18–12	35	21	91
Red	11	33	11–3×11–13	19	17	19–1×19–11	26	22	72
Stipple	12	51	12–3×12–13	24	19				70

Gold and Stipple stocks obtained from Mr. WAGNER, New York City; Red from Mr. RABENAU Brooklyn, New York.

TABLE 2a

The direct cross (Gold ♀, non-spotted, non-stippled × Red ♂, spotted, stippled).

P_1			F_1					
GOLD ♀ PEDIGREE	×	RED ♂ PEDIGREE	PEDIGREE	SCHOOLS			SPOTTED STIPPLED	NON-SPOTTED STIPPLED
10–1		11–11	14	8	16	24	18	0
				10	28	24	30	0
				12	1	24	20	0
10–2		11–12	15	8	20	24	17	0
				10	28	24	13	1
				12	1	24	20	0
							118	1

TABLE 2b

The reciprocal cross (Red ♀, spotted, stippled, × Gold ♂, non-spotted, non-stippled).

P1 RED ♀ PEDIGREE	×	P1 GOLD ♂ PEDIGREE	F1 PEDIGREE	F1 SCHOOLS			F1 SPOTTED STIPPLED	F1 NON-SPOTTED STIPPLED
11–1		10–11	16	10	29	24	11	16
				12	1	24	5	6
11–2		10–11	17	10	7	24	6	12
19–3		18–12	36	4	19	25	7	14
19–4		18–13	37	4	19	25	15	13
				5	28	25	17	17
				7	3	25	9	8
							70	86

TABLE 3a

The F_2 of the direct cross (P_1, Gold ♀ × Red ♂, table 2a).

F1 SPOTTED STIPPLED PEDIGREE ♀	×	F1 SPOTTED STIPPLED PEDIGREE ♂	F2 PEDIGREE	F2 SCHOOLS			F2 SPOTTED STIPPLED	F2 NON-SPOTTED STIPPLED	F2 SPOTTED NON-STIPPLED	F2 NON-SPOTTED NON-STIPPLED
14–1		15–11	28	2	25	25	16	4	11	1
				4	26	25	15	7	3	1
14–2		15–12	29	3	15	25	15	7	8	4
				5	1	25	13	4	1	0
14–3		15–13	30	3	20	25	20	3	7	2
				5	15	25	14	12	2	1
15–1		16–11	38	7	2	25	18	4	2	0
				11	9	25	24	8	5	2
				12	19	25	40	24	13	9
							175	73	52	20

TABLE 3b

The F_2 of the reciprocal cross, (P_1, Red ♀ ×Gold ♂, table 2b).

F-1		F2						
NON-SPOTTED STIPPLED PEDIGREE ♀	SPOTTED STIPPLED PEDIGREE ♂	PEDIGREE	SCHOOLS	SPOTTED STIPPLED	NON-SPOTTED STIPPLED	SPOTTED NON-STIPPLED	NON-SPOTTED NON-STIPPLED
16–1	16–12	39	8 18 25	6	4	5	0
16–2	16–12	40	9 20 25	2	8	2	2
			1 10 26	16	10	4	11
37–1	36–11	53	6 29 26	14	21	1	7
			7 27 26	18	17	0	3
37–2	36–11	54	7 14 26	6	13	0	3
			8 11 26	14	14	4	3
				76	87	16	29

TABLE 4

The backcrosses.

PEDIGREE ♀ PEDIGREE ♂	NEW PEDIGREE	NUMBER OF SCHOOLS	SPOTTED STIPPLED	NON-SPOTTED STIPPLED	SPOTTED NON-STIPPLED	NON-SPOTTED NON-STIPPLED
Cross A F_1 ×Gold 14–5 18–11	33	2	8	17	13	7
Cross B Gold×F_1 18–1 14–12 35–1 16–11	34 44	1 4	2 18	3 39	3 21	3 25
		Total	20	42	24	28
Cross C F_1 ×Red 14–4 19–11	31	2	50	0	0	0
Cross D Red ×F_1 19–2 14–11	32	1	14	3	0	0

TABLE 5a

The cross of Gold by Stipple (Gold ♀, non-stippled × Stipple ♂, stippled).

P₁		F₁			
GOLD PEDIGREE ♀ ×	STIPPLE PEDIGREE ♂	PEDIGREE	STIPPLED	STIPPLED	NON-STIPPLED
10–3	12–11	20	3 1 25	25	0
10–4	12–12	21	3 1 25	21	0
			4 15 25	18	0
				64	0

TABLE 5b

The reciprocal cross (Stipple ♀, stippled × Gold ♂, non-stippled).

P₁		F₁			
STIPPLE PEDIGREE ♀ ×	GOLD PEDIGREE ♂	PEDIGREE	SCHOOLS	STIPPLED	NON-STIPPLED
12–1	10–12	22	1 1 25	18	0
			1 17 25	12	0
			3 1 25	25	0
12–2	10–13	23	1 8 25	28	0
				83	0

TABLE 5c

F_1 inbred to give F_2.

F₁		F₂			
PEDIGREE ♀	PEDIGREE ♂	PEDIGREE	SCHOOLS	STIPPLED	NON-STIPPLED
20–1	20–11	45	12 27 25	12	2
			1 23 26	24	4
20–2	20–11	46	12 13 25	15	5
22–1	20–11	47	1 16 26	8	2
			12 12 25	11	3
22–2	20–11	48	1 23 26	15	4
				85	20