THE GENETICS OF PLATYPOECILUS. II. THE LINKAGE OF TWO SEX-LINKED CHARACTERS[1]

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Of recent years investigators in the field of genetics have been giving increasing attention to fishes, especially those kinds which can be reared in aquaria. Among the latter the smaller killifishes of the order Cyprinodontes have proved well suited to inheritance studies. Certain species of this order present a variety of bright colors and a wide range of melanic patterns, as well as marked size differences, all of which characters attract the interest of the student of heredity. The small size of most of these fishes makes it possible to rear comparatively large numbers in a limited space. As an added advantage, they have a relatively short life cycle. To be sure there are difficulties attending their culture, such as exacting temperature requirements, problems in feeding and handling, as well as in

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disease control and in general aquarium management; but these can be overcome.

In an earlier paper, GORDON (1927) has discussed the taxonomy and life history of *Platypoecilus maculatus* and presented the results of a study of the inheritance of two types of melanophores: a micro-melanophore responsible for the character called *stipple* (S_t) and a macro-melanophore giving the character called *spot* (S_p). The Stippled race was shown to differ from the Non-stippled one by a single dominant autosomal gene, while the Spotted race differed from the Non-spotted by a dominant sex-linked gene. GORDON also confirmed the report of BELLAMY (1922) that in *Platypoecilus maculatus* sex determination is of the W Z type.

The present report deals with the factor for *red* body-color and its relation to the factor for *spots*. *Red* is shown to be sex-linked, thus confirming BELLAMY's findings. Data are presented which seem best explained on the basis of linkage between *spots* and *red*, with occasional crossing over between them in the W and Z chromosomes[4] of the female.

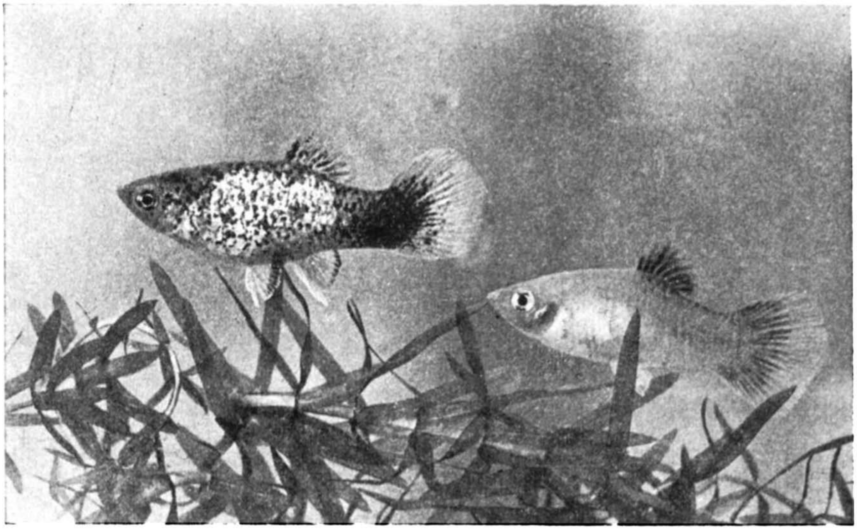

FIGURE 1.—*Platypoecilus maculatus*—about natural size. Left: Red, *spotted* female. Right: Gold male (*non-red, non-spotted*). The reflection of the light on the Red fish is due to an irridescence not genetically associated with the *red* character.

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[4] In general the writers favor the more recent practice of using XY to represent the heterogametic sex and XX the homogametic in both the Abraxas and Drosophila types of sex linkage. In this case, however, there seem to be certain advantages in the use of the earlier forms: WZ and ZZ.

MATERIALS

In these studies two strains of *Platypoecilus maculatus* were used: the Red or Rubra type and the Gold. Both of these have been described and pictured in color in an earlier paper (GORDON 1927).

The male of the Red variety is Brazil Red (RIDGWAY, plate 1) at maturity. In this sex the young fishes show no red color. The red pigmentation usually begins to appear at the stage when the anal fin has almost completed its transformation into the intromittent organ. Occasionally the assumption of red color may be delayed well beyond that time. In one or two cases males failed to take on color even though they were genetically Red. These fishes looked abnormal in other respects. An examination showed that their gonads were either abnormal or lacking. This seems to indicate a relation between normal male gonads and the capacity to develop red color. The delay in development of red by the male makes it necessary to keep the males for a comparatively long time in order to give all those which are genetically Red an opportunity to develop red color.

The female of the Red variety is a dull olive brown color, often becoming "Cinnamon Rufous" (RIDGWAY, plate 14). From BELLAMY'S description (1924) of his Red strain it would appear that the females were colored red like the males, but that the color developed much more strongly in the males. One of the writers has seen in the collections of aquarium fish fanciers, females of *Platypoecilus maculatus* which were distinctly reddish. It would seem possible that the same primary factor for red pigmentation is involved in both of these color phases, since both show sex linkage. Its expression in the mature fish may be influenced by environmental conditions, by sex hormones, or by a modifying gene or genes either in the autosomes or in the W or Z chromosomes.

From these descriptions it will appear that in the races used there is a sexual bicolorism, with *red* sex-limited in the males. The *red*-type females can usually be distinguished from those of the Gold race, with which they were crossed, by their brownish coloration. The designation *red-type female* is used here to refer to these brownish or olive brown females, not to fish which show distinct red color.

The Gold variety of *Platypoecilus maculatus* was used as the other parent in these crosses, chiefly because the body exhibits no red color and no spots. GORDON (1927) gives the body color as deep Colonial Buff (RIDGWAY, plate 30), with the dorsal fin of the male Scarlet and that of the female LaFrance pink (RIDGWAY, plate 1). The males and females of the Gold race are quite distinct from those of the Red and no difficulty was experienced in classifying the progeny of the various crosses. Occasionally a few erythrophores may be seen in the Gold variety, forming a reddish saddle on the back, but these are confined to the region just below the dorsal fin and do not change the general yellowish coloration of the animal.

PARENT RACES TRUE-BREEDING

Preliminary studies of the Red variety and periodic examinations of the stock of it, have shown that is is a true-breeding race. Of more than 75 Red fish which have been reared, all the males have been red and the females have been the characteristic brown. These females in turn have given only red male and female offspring like themselves. In a similar test the Gold has proved to be a true-breeding race. Approximately 100 young of this variety have been raised.

CROSSES OF GOLD AND RED

In the following crosses between the Red and Gold races the numbers of fish reared to adult size are not always as large as might be desired. This was due chiefly to limitations imposed by the cultural requirements and to troubles with gill parasites. In order to rear these fishes successfully it was necessary to glass in a part of a large laboratory and to equip this smaller room with thermostatically controlled heating units and with artificial lighting. This limited the space available for aquaria. Then too, the delayed development of red color in the males made it necessary to keep them for a long time and to use for them aquaria which might have been occupied by other schools. In spite of these handicaps, however, the numbers are sufficiently large to be indicative of certain types of behavior.

TABLE 1

Cross of Gold ♀ (non-spotted, non-red) × Red ♂ (spotted, red).

$\mathbf{Z}\ r\ s_p\ \mathbf{W}\ r\ s_p \times \mathbf{Z}\ R\ S_p\ \mathbf{Z}\ R\ S_p$

GENERATION	PEDIGREES*	RED				NON-RED			
		Spotted		Non-spotted		Spotted		Non-spotted	
		♀	♂	♀	♂	♀	♂	♀	♂
F₁	14, 15	18 (20)†	22 (20)	0	0	0	0	1 (0)	0
F₂	28, 29, 30, 38	33 (28.5)	47 (57)	0	0	0	0	34 (28.5)	0

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* Separate records have been kept for each mating, but in most cases the data have been combined where they seemed clearly to show the same behavior.

† Calculated frequencies are shown in parentheses.

The cross Gold ♀ × Red ♂ gave an F_1 progeny of 40 red, spotted animals and 1 non-spotted, non-red female. In the first class there were about equal numbers of males and females. The single exceptional female died before it could be tested further. It is possible to account for this individual by assuming that an egg of the type $\mathbf{Z}\, r\, s_p$ was fertilized by a sperm which did not contain any Z chromosome. (W and Z in Roman bold type refer to chromosomes and are to be distinguished from the following factor symbols which are in italics). This second type of gamete could have been produced by non-disjunction in the male.

In the second generation, definite evidence of sex linkage is found. The data are in fairly good agreement with the calculated 1:2:1 ratio ($P = .1769$) and only females appear in the non-red, non-spotted class. These F_2 results suggest either that *red* and *spots* are caused by the same gene[5] or that two closely linked genes are involved and that there is little crossing-over between them.

TABLE 2

Cross of Red ♀ (red, spotted)×Gold ♂ (non-red, non-spotted).

$\mathbf{Z}\, R\, S_p\, \mathbf{W}\, r\, s_p \times \mathbf{Z}\, r\, s_p\, \mathbf{Z}\, r\, s_p$.

GENERATION	PEDIGREES	RED				NON-RED			
		Spotted		Non-spotted		Spotted		Non-spotted	
		♀	♂	♀	♂	♀	♂	♀	♂
F_1	16, 17, 36, 37	0	47 (49)	0	0	0	0	51 (49)	0
F_2	39, 40, 53, 54	14 (12)	13 (12)	0	0	0	0	13 (12)	9 (12)

The F_1 and F_2 results of the cross Red ♀ × Gold ♂ (table 2) bear out the assumption that sex-linked genes are operating. The F_1 shows criss-cross inheritance of the WZ type. While the F_2 frequencies are not large, they agree rather closely with the results calculated for 49 individuals. The 1:1 distribution within each sex is typical of the F_2 in a case of sex linkage where the F_1 shows criss-cross inheritance.

Two backcrosses of the F_1 females of table 1 (Gold ♀ × Red ♂) to pure recessive (Gold) males, yielded 85 young.[6] These were distributed in a manner similar to the F_1 shown in table 2. In both of these cases, the red,

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[5] It is not held, of course, that one gene is solely responsible for each of these characters, but rather that the *difference* between *red* and *non-red*, or *spotted* and *non-spotted*, is due largely to one gene. The terms "gene for *red*" and "gene for *spots*" are used for convenience.

[6] The complete data may be found in table 3 on the page following.

spotted females (P_1 and F_1, respectively) seem to be heterozygous for the dominant sex-linked genes for *red* and *spots*. Farther on, genetical evidence will be presented which indicates that the W chromosome is present in the female of this species, but that it usually carries only recessive genes. None of the above results admit of an explanation on the basis of autosomal genes which are in the heterozygous condition in the P_1 female.

TABLE 3

Backcross of the red, spotted F_1 ♀ of the cross Gold ♀ × Red ♂ (table 1) to Gold ♂ (non-red, non-spotted).

$Z\ R\ S_p\ W\ r\ s_p \times Z\ r\ s_p\ Z\ r\ s_p$

GENERATION	PEDIGREES	RED				NON-RED			
		Spotted		Non-spotted		Spotted		Non-spotted	
		♀	♂	♀	♂	♀	♂	♀	♂
F_2	33	0	12	0	0	0	0	10	0
	52	1*	29	0	0	0	0	33	0
Total		1	41	0	0	0	0	43	0

* This exceptional individual (52–20) has been tested further. See tables 6 and 8.

These backcrosses constitute good proof of the sex linkage of both *red* and *spots*. The F_1 females, although they do not show red color, presumably have received genes for *red* and *spots* from their male parents, and they are considered to be of the genotype $Z\ R\ S_p W\ r\ s_p$. When these females are crossed to Gold (non-red, non-spotted) males, the appearance of red, spotted male offspring and non-red, non-spotted females shows that the F_1 females were carrying the genes for *red* and *non-red*, *spotted* and *non-spotted* in the chromosomes which differentiate between maleness and femaleness.

One exceptional female (pedigree 52-20) appeared among the progeny: a red, spotted animal. She was mated to two different males and the results of those matings are given in tables 6 and 8. This red, spotted female seems best explained on the basis of crossing over between the W and Z chromosomes of the F_1 female (table 3). Evidence to support this belief will be presented farther on.

The backcross of the F_1 non-spotted, non-red females resulting from the cross Red ♀ × Gold ♂ (table 2) to Gold males, gave only non-spotted, non-red fishes in F_2 with males and females in approximately equal numbers.[7] Such results are to be expected if the P_1 red, spotted female (table 2) fails to transmit either *red* or *spots* to her female offspring.

[7] See table 4 on page following.

TABLE 4

Backcross of the non-red, non-spotted F_1 ♀ of the cross Red ♀ × Gold ♂ (table 2) to Gold ♂ (non-red, non-spotted).

$Z\ r\ s_p\ W\ r\ s_p \times Z\ r\ s_p\ Z\ r\ s_p.$

GENERATION	PEDIGREES	RED				NON-RED			
		Spotted		Non-spotted		Spotted		Non-spotted	
		♀	♂	♀	♂	♀	♂	♀	♂
F_2	49, 50	0	0	0	0	0	0	10	15
	71, 72	0	0	0	0	0	0	35	47
	99, 100	0	0	0	0	0	0	25	22
Total		0	0	0	0	0	0	70	84

A cross of the red, spotted F_1 male of table 1, to a red, spotted, stock female, gave red, spotted fish and non-red, non-spotted ones. If the genes for *red* and *spots* are sex-linked, all of the F_2 males should have been spotted and red, and the females should have been of two kinds: spotted and red, and non-spotted and non-red. For some unknown reason, most of these fish died before becoming differentiated as to sex. Two red, spotted animals turned out to be males and a single *non-spotted fish also developed into a male.* Later on this non-spotted animal took on full *red color.*[8] Both the development of red color and the assumption of maleness by this non-spotted fish were contrary to theoretical expectations. This exceptional type will be referred to later on under the number 32–11.

TABLE 5

Cross of the red, spotted F_1 ♂ of the cross Gold ♀ × Red ♂ (table 1) to a red, spotted stock female.

$Z\ R\ S_p\ W\ r\ s_p \times Z\ R\ S_p\ Z\ r\ s_p.$

PEDIGREE	RED		NON-RED	
	Spotted	Non-spotted	Spotted	Non-spotted
32	14	1	0	2

THE EXCEPTIONAL FEMALE (NO. 52–20) OF TABLE 3

In the backcross of an F_1 red, spotted female (Cross = Gold ♀ × Red ♂) with a Gold male, a single female appeared which was red and spotted. Attention has been called to this above. Several possible explanations of this exception suggest themselves: (1) that it was due to non-disjunction, (2) that gene mutation caused it, (3) that it resulted from a case of sex

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[8] See table 5.

reversal, and (4) that it was due to crossing over between W and Z in the F_1 female.

The parents of the cross reported in table 3 may be represented as in figure 2.

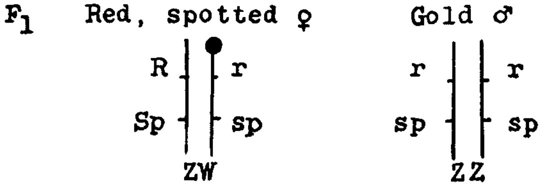

FIG. 2.

1. *Non-disjunction*

Case (a)

If the $Z\,R\,S_p$ gamete of the female shown above had been fertilized by a *zero* type of sperm, (the result of non-disjunction in the male), it would have given a fish of the type $Z\ R\ S_p\,O$. This might very well have been female in sex, judging from the case in *Drosophila melanogaster*, where the XO type is male and where the mode of sex determination is the reverse of that in Platypoecilus. The fact that an XO male in Drosophila is sterile does not necessarily mean that a ZO female in Platypoecilus would also be sterile.

Case (b)

If non-disjunction had occurred in the W and Z chromosomes of the F_1 female and these had gone together into the egg, a subsequent fertilization with the $Z\,r\,s_p$ sperm of the Gold male would have given a fish of the constitution $Z\ R\ S_p\,W\,r\,s_p\,Z\,r\,s_p$. The corresponding type in Drosophila, namely XXY, is female in sex. This ZZW fish might be expected to be a male on the same basis, because of the presence of two Z chromosomes. This type of reasoning does not prove, however, that non-disjunction in the F_1 female would produce F_2 exceptional males instead of females. A fish of the constitution ZZW, should exhibit secondary non-disjunction and subsequent tests should throw light upon this possibility.

2. *Mutation*

The exceptional red, spotted female of table 3 could have been produced by mutation, but from what is known of the frequency of gene mutations this possibility seems remote. It would necessitate a coincidental mutation in two genes, r and s_p, either in the W chromosome of the F_1 female or in the Z chromosome of the Gold male, unless R–S_p and r–s_p are each one gene. The writers believe that R and S_p are better accounted for by assuming two distinct genes.

3. *Sex reversal*

There is also a possibility that this exceptional female was genetically a male, but that it later became transformed into a female by a process of sex reversal. A number of cases of this kind have been reported. Essenberg (1923 and 1926) described cases of sex reversal in a closely related fish *Xiphophorus helleri*. Crew (1927) refers to a number of reported cases of hermaphroditism in fishes, which he is inclined to believe may represent transition stages in the process of sex reversal. Enough work has been done, at least, to show that the phenomenon of sex reversal is probably not an uncommon one among fishes, and particularly among those forms related to the genus Platypoecilus.

Crew (1927) says regarding individuals which have been transformed as to sex.

> "If in a group there is a sex-chromosome, sex-determining mechanism (for example, XY:XX) and if this mechanism can be over-ridden, then in that group it can be expected that there will be individuals genotypically of one sex, phenotypically of the other, and that these when mated to individuals in which sexual genotype and phenotype are in agreement will produce offspring among which there will be a preponderance of the sex to which the transformed parents genotypically belonged (that is, a transformed female functioning as a male will yield a preponderance of females; a transformed male functioning as a female will yield a preponderance of males)." (p. 438).

If the exceptional female of table 3 was genotypically male, she must have been of the constitution $\mathbf{Z}\ R\ S_p\ \mathbf{Z}\ r\ s_p$. Such a female when crossed with a normal (ZZ) would give only male offspring, barring other cases of sex reversal. In as much as this female gave 8 females among her offspring in one cross (table 6) and 15 females in the progeny of another cross (table 8), it seems very unlikely that she could have been an F_1 male in which sex reversal had taken place.

4. *Crossing over*

Crossing over between the W and Z chromosomes in the F_1 female of table 3, would have given a red, spotted female among the progeny of the backcross. The F_1 female was assumed to be of the constitution $\mathbf{Z}\ R\ S_p$ $\mathbf{W}\ r\ s_p$. If the genes for *red* and *spots* were transferred to the W chromosome by crossing over in this F_1 female, and the egg which received the W chromosome was fertilized by a sperm bearing $\mathbf{Z}\ r\ s_p$, the resulting fish would be a female of the constitution $\mathbf{Z}\ r\ s_p\ \mathbf{W}\ R\ S_p$. Subsequent tests of this exceptional female (tables 6 and 8) lend strong support to the belief that she was actually of this constitution.

By the time this female no. 52 – 20 was recognized as an exceptional type, she had already mated with a red, spotted male of the same F_1 family. The results of that mating are given in table 6.

TABLE 6

Cross of the exceptional red, spotted female of table 3, to a male of the same family (No. 52).

PEDIGREES	RED				NON-RED			
	Spotted		Non-spotted		Spotted		Non-spotted	
	♀	♂	♀	♂	♀	♂	♀	♂
52-20×52-11	8	8	0	0	0	0	0	6

The males of pedigree 52 were red and spotted (see table 3) and presumably of the genotype $Z\ R\ S_p\ Z\ r\ s_p$. If the exceptional female (52-20) had been like the ordinary red, spotted females and of the constitution $Z\ R\ S_p\ W\ r\ s_p$, a cross with one of the F_1 males should have given red, spotted males and females, and non-red, non-spotted females only (no crossovers considered here). On the other hand if female 52-20 had been produced by crossing over between chromosomes W and Z in such a way as to place genes R and S_p on the W chromosome, a cross with a male of family 52 would have given results as follows:-

$Z\ r\ s_p\ W\ R\ S_p \times Z\ R\ S_p\ Z\ r\ s_p$.

F_2

$Z\ r\ s_p\ Z\ R\ S_p$—red, spotted ♂.
$Z\ r\ s_p\ Z\ r\ s_p$—non-red, non-spotted ♂.
$Z\ R\ S_p\ W\ R\ S_p$—red, spotted ♀.
$Z\ r\ s_p\ W\ R\ S_p$—red, spotted ♀.

The results shown in table 6 check with the assumption that female no. 52-20 was of the genotype $Z\ r\ s_p\ W\ R\ S_p$. They also rule out the possibility that such a female was produced by non-disjunction of type 1a (above), that is, that her genotypic constitution was $Z\ R\ S_p\ 0$. Non-disjunction of type 1b would give a fish of the genotype $Z\ R\ S_p\ W\ r\ s_p\ Z\ r\ s_p$, probably a male. If it were possible for such a fish to be a female a cross with a male of family 52 would give a number of non-spotted, non-red females in the next generation.

It would seem, therefore, that the results in table 6, effectively eliminate suggestions 1a, 1b, and 3, regarding the genotype of the exceptional female, 52-20. This table does, however, clearly support assumption 4, namely that the exceptional female resulted from a crossing over between the W and Z chromosomes in her maternal parent. Two other tests furnish additional evidence of a similar nature.

The red, spotted females (table 6) arising from the cross ♀ 52-20× ♂ 52–11, were mated to Gold (double recessive) males. Table 7 gives the results of these matings. The offspring are classified only for *spots*, since none of them are as yet old enough to develop red color. Judging from the high degree of association between *red* and *spots* in the preceding crosses, most if not all of the spotted young will probably be red, and the non-spotted will be non-red.

TABLE 7

Test of the red, spotted females of table 6 by crossing to a (r s_p) Gold male.

PEDIGREE OF ♀ TESTED*	SPOTTED YOUNG	NON-SPOTTED YOUNG	PROBABLE GENOPTYE OF PARENT
121–1	13	0	W Z S_p S_p
121–2	3	4	S_p s_p
121–3	3	4	S_p s_p
121–5	41	0	S_p S_p
121–6	85	0	S_p S_p
121–7	69	0	S_p S_p

*Females 121–4 and 121–8 died before producing any young fishes.

If the exceptional female 52–20 was of the constitution $Z\ r\ s_p\ W\ R\ S_p$, her red, spotted female offspring shown in table 6 should have been of two kinds: $Z\ R\ S_p\ W\ R\ S_p$ and $Z\ r\ s_p\ W\ R\ S_p$. The first of these when mated to a Gold male should give only red, spotted young, males and females. The second type should give non-red, non-spotted male offspring and red, spotted female offspring. Females no. 121–1, 121–5, 121–6 and 121–7 are undoubtedly of the first type. They represent an unusual genetic condition: homozygosity of sex-linked genes in the heterogametic sex. Females 121–2 and 121–3 are clearly of the second class mentioned above. Both kinds of females are of further interest because they seem to carry dominant, sex-linked genes in the W chromosome and they should (barring subsequent crossing over) transmit these genes to their daughters and thence to their granddaughters and so on, in a manner similar to the one-sided inheritance of *maculatus* in *Lebistes reticulatus* (SCHMIDT 1920 and WINGE

1922) and *bobbed* in *Drosophila melanogaster* (STERN 1926). The present case, however, would be one of one-sided inheritance in the female line.

After the exceptional red, spotted female (52–20) of table 3 had given birth to her young by the mating with the F_1 red, spotted male (see table 6), she was crossed with a Gold male. The results of that cross are given in table 8.

TABLE 8

Cross of the exceptional red, spotted female (table 3) to a Gold (r, s_p) male.

PEDIGREES	RED SPOTTED		NON-RED NON-SPOTTED	
	20		28	
	♀	♂	♀	♂
52–20×99–11	15	1	0	17

If female 52–20 was genetically $\mathbf{Z}\ r\ s_p\ \mathbf{W}\ R\ S_p$, a cross with a Gold male should have given red, spotted daughters (like the mother) and non-red, non-spotted sons (like the father). Fifteen spotted females and seventeen non-spotted male offspring support this conclusion regarding the genotype of female 52–20. The single, red, spotted male may be the result of crossing over between the W and Z chromosomes of this exceptional female to place the genes for *spots* and *red* back on the Z chromosome. At least it is possible to account for it on this basis.

These tests of female 52–20 seem clearly to support the original assumption that she was of the constitution $\mathbf{Z}\ r\ s_p\ \mathbf{W}\ R\ S_p$. Such a fish could only have been produced by crossing over between the W and Z chromosomes of the F_1 female.

EXCEPTION NUMBER 2.

In the discussion of the results given in table 5, reference is made to a non-spotted, *red* male which appeared in the offspring of an F_1 male heterozygous for *red* and *spots*, crossed to a stock red, spotted female. The parents in this cross may be represented as follows: $\mathbf{Z}\ R\ S_p\ \mathbf{W}\ r\ s_p \times \mathbf{Z}\ R\ S_p\ \mathbf{Z}\ r\ s_p$. The exceptional red, non-spotted male (32–11) is thought to have been produced by the union of a sperm of the constitution $\mathbf{Z}\ r\ s_p$ with an egg carrying $\mathbf{Z}\ R\ s_p$. The odds seem strongly against its having been produced in any other way. The female gamete in this case could have been produced in either one of two ways: by crossing over between the W and Z chromosomes of the female parent, with the break coming between the genes R and S_p; or by mutation of S_p to s_p on the Z chromosome of the female. Either process would give the same end result, and it is hard to see how

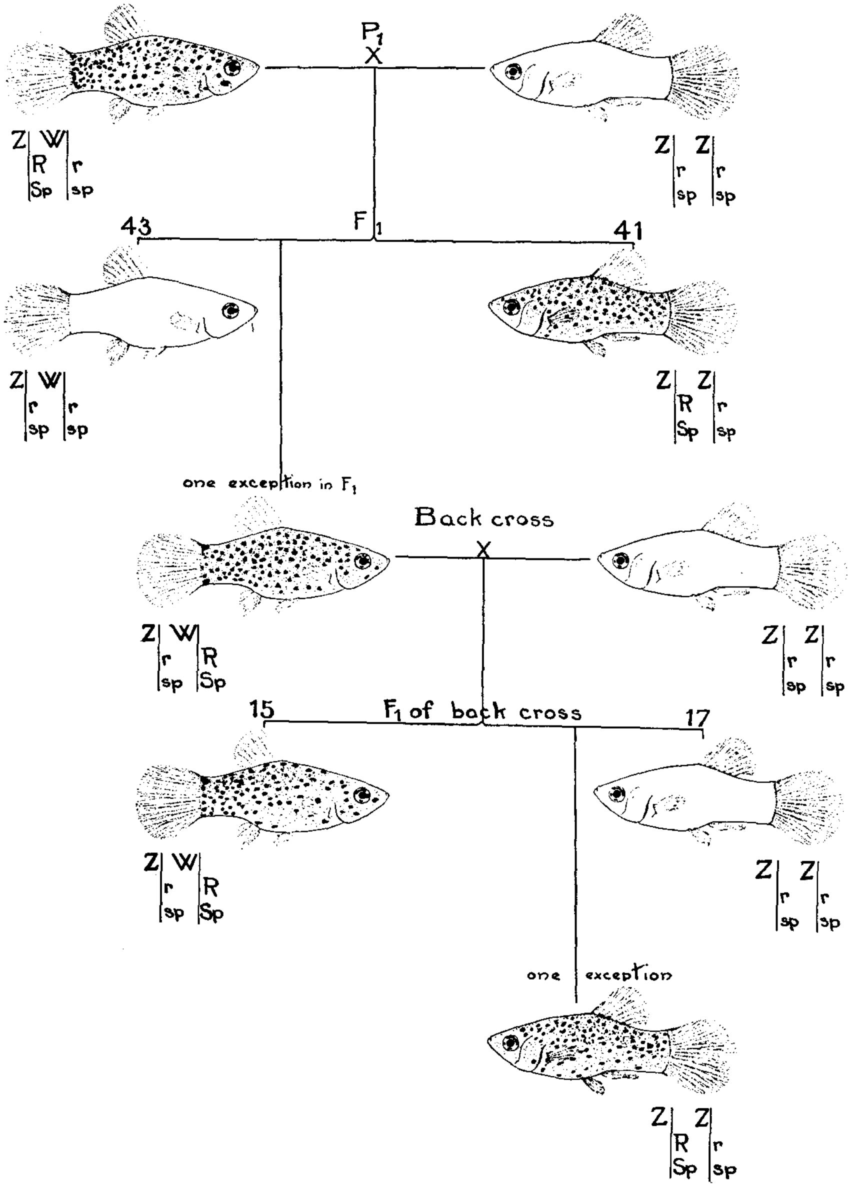

FIGURE 3.—This illustrates the breeding behavior of a normal, red, spotted female (pedigree number 15–2) when crossed with a double recessive, gold, male (pedigree number 20–11). It shows definite criss-cross inheritance of two sex-linked genes R and S_p in F_1. A single exception occurred: a red, spotted female (pedigree number 52–20). This individual's appearance is accounted for by crossing over in the sex chromosomes of the P_1 female, resulting in a transfer of the genes R and S_p from the Z to the W.

The exceptional red, spotted F_1 female is backcrossed to a double recessive male (pedigree number 99–11). The results show a different sort of inheritance: the dominant genes R and S_p are transmitted from mother to daughter and their recessive allelomorphs r and s_p from father to son. Again an exception appeared: a red, spotted male. It is accounted for by crossing over in the sex chromosomes of the F_1 exceptional female, resulting in the transfer of the dominant genes R and S_p from the W back to the Z.

Note: Females are to the left of the cross (X), males are to the right.

one could tell with any degree of certainty which one did occur. However, in view of the fact that crossing over seems to have occurred between the W and Z chromosomes of a female of Platypoecilus in the case of female 52–20, the writers are disposed to consider this male (32–11) as very probably the result of a similar process.

A cross of this exceptional male (32–11) to a Gold female gave results which showed that the male was heterozygous for *red* and was genetically *non-spotted*. The results of this cross are shown in table 9.

TABLE 9

Mating of Gold ♀ (stock) × the non-spotted, red ♂ of table 5.

PEDIGREES	RED NON-SPOTTED		NON-RED NON-SPOTTED	
	♀	♂	♀	♂
42–2×32–11 ♀ ♂	5*	13	20	12

* In this cross the females were hard to classify for red and non-red.

DISCUSSION

The data presented above show that the characters *red* and *spots* are sex-linked. From a theoretical standpoint these characters might be considered as due to either one of two conditions: first, a single gene influencing both *red* and *spots*, with the various combinations of these characters comprising a multiple allelomorphic series; secondly, a distinct gene for each character with a rather close linkage of the two. The first case is shown not to apply here by the appearance of new combinations of the characters *red* and *spots*. A linkage of the genes for *red* and *spots* is best shown by a close association of these characters in heredity, with occasional breaks between them, and the data given above answer this requirement.

AIDA (1921) seems to have been the first to report crossing over between unlike sex chromosomes (X and Y). In his studies of inheritance in *Aplocheilus latipes*, another small fish of the order Cyprinodontes, he obtained evidence that a gene for red body-color (*R*) was carried both by the X and the Y chromosomes. This gene was found to crossover from X to Y, or Y to X in heterozygous animals. Several types exceptional with reference to color and sex seem best explained on that basis.

WINGE (1923), working with *Lebistes reticulatus*, another Cyprinodont fish, reported crossing over of a factor for elongated caudal fin (elongatus: *e*) from the X to the Y chromosome, and by further crossing over, a return of *e* to the X chromosome. More recently (WINGE 1927) has described five genes (luteus, elongatus, minutus, vitellinus, and cinnamomeus) which have crossed over in different ways from X to Y, Y to X, or X to X, with crossing over occurring in both sexes.

Crossing over between the like chromosomes of the homogametic sex has long been known to occur in Drosophila and in fowls. The work with fowls has shown that crossing over can take place between the Z chromosomes of the male. GOODALE (1917) reported crossing over between the gene for barred pattern and that for silvering of the hackle, when F_1 males were backcrossed to recessive females. HALDANE (1921) confirmed this and gave a crossover percentage for *B* and *S* of 34.6 ± 3.6. SEREBROVSKY (1922) added a third sex-linked gene, "suke", for retarded development of feathering in chicks, and showed that this gene is linked with the factors for bar and silvering (which he called "trage" and "tuge"). Crossing over between these occurred in the male, but not in the female. This is to be expected, of course, if in the female there is no mate for the Z chromosome. (HANCE (1926) reports an unpaired sex chromosome in the female of fowls). AGAR (1924) and HALDANE and CREW (1925) have confirmed the findings of SEREBROVSKY, the latter workers reporting an increase in percentage of crossing over as the males got older.

The present work with Platypoecilus seems to include the first case of crossing over between the sex-chromosomes of an heterogametic female (that is, between W and Z). Crossing over between the X and Y (or W and Z) chromosomes in the heterogametic sex is of considerable theoretical interest; first, from the standpoint of its effect upon the sex-determining mechanism, and secondly, as it affects the study of sex-linkage.

The more recent theory of BRIDGES (1922 and 1925) regarding the genetical nature of sex appears to have been well received by geneticists. (This theory has proved helpful to an understanding of the possible genetic nature of all characters). Evidence that the autosomes play a part in the determination of sex is convincing. The consequent assumption seems warranted that there are many genes for maleness and femaleness scattered through the whole chromosome complex, and that the sex of the individual is determined by the algebraic sum, so to speak, of all these genes. According to this theory the sex chromosomes still control the situation. When two of them are present, the overbalance of femaleness in the two X's is sufficiently greater than the overbalance of maleness in the autosomes to make the individual a normal female. When only one X is present, the balance is decidedly in favor of maleness. As the writers understand this theory, the members of any pair of autosomes are genetical equivalents with respect to these genes for sex, and crossing over between them should cause no change in the sex of the individuals receiving the chromosomes which have interchanged parts. In the same way the two X's are genetical equivalents with respect to the sex genes and crossing-over between them should produce no new types of chromosomes as far as the genes for sex are concerned. The X and Y chromosomes, however, are genetically different with respect to the sex genes. Crossing over between these chromosomes should result in a wholly new type of internal balance and consequently an appreciable number of intersexes.

Neither AIDA nor WINGE mentions the occurrence of intersexes in the course of his experiments and none were detected in the present work with Platypoecilus. This raises a question as to the nature of the sex-determining mechanism in this group of fishes.

AIDA speaks of the crossing over of the *R* gene from the Y to the X chromosome, but does not subsequently explain what would be the role of such a chromosome, part X and part Y. WINGE simplifies matters by assuming that the X chromosome in Lebistes carries a gene for femaleness and the Y chromosome, a gene for maleness. These are allelomorphic and hence cannot both lie in the same chromosome. In the various crossings over, that chromosome which receives the gene for maleness becomes the Y chromosome. This implies a belief that the genetic difference between maleness and femaleness in Lebistes lies chiefly in one pair of genes. (Later, in reply to a criticism, WINGE (1927) modifies his earlier conception as follows: "In Lebistes, also, the autosomes contain dispositions towards both male and female sex characters; the presence or absence of the *Y* gene is only that which *normally* regulates the sex determination." The statement that the autosomes influence sex is unsupported by published evidence.)

In Platypoecilus the evidence from sex linkage shows that there is one pair of chromosomes which differentiates between maleness and femaleness. There is crossing over between the W and Z chromosomes of the female and no intersexes result. Hence the conclusion seems warranted that the regions in which crossing over occurs are not regions of *difference* between W and Z, at least with respect to any very potent sex factors. (MORGAN (1926) says: "This means no more than that one part only of the sex-chromosomes has the function of an X– or Y–chromosome. . . . .") If there are sex genes in these regions, they must be the same in both the W and Z chromosomes. The portions of W and Z which are similar (for sex genes) are thus essentially autosomal in nature, as MORGAN (1926) has pointed out. The writers are assuming, tentatively, that the difference between W and Z lies in a very few genes which tend to remain associated in heredity, either because of close proximity or some influence which prevents the occurrence of breaks between them. As in the case of WINGE's assumption, that chromosome which gets one set of genes is genetically the W chromosome and its mate is the Z.

While there is no evidence that the autosomes of Platypoecilus play a part in sex determination, the above assumptions can be brought into harmony with such a belief if it is so desired. The autosomes can be assumed to carry an overbalance of genes for femaleness, but not in great amount. It is necessary to assume that the genes in the area which distinguishes Z from W are very potent and that the balance in Z is decidedly toward maleness. Values will have to be adjusted to allow for the possible appearance of intersexes between the true conditions of maleness and femaleness.

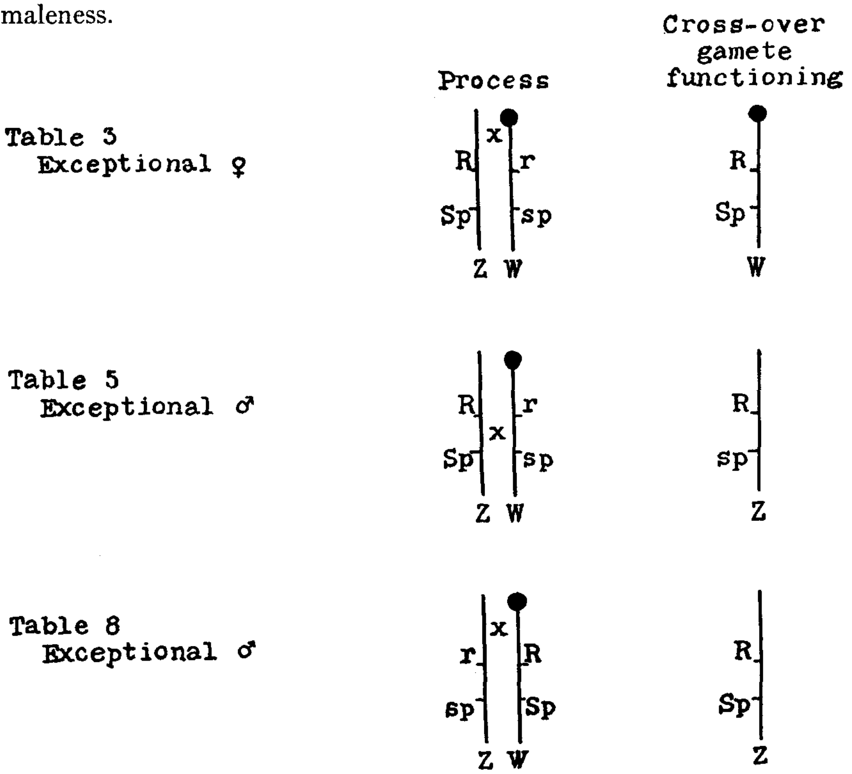

FIG. 4.

The diagrams in figure 4 show where crossing over is believed to have taken place in the course of this work with Platypoecilus. The darker area at the end of one chromosome distinguishes the W chromosome from the Z. It can be thought of as that portion in which Z and W are unlike with respect to important sex genes, although there is no evidence that such a region is located at the end of the chromosome.

Crossing over between the W and Z chromosomes of the female in Platypoecilus may give peculiar types of offspring in which the sex linkage relations are confusing. A normal red, spotted female of *Platypoecilus maculatus* is presumably of the constitution $Z\ R\ S_p\ W\ r\ s_p$. Such a female when crossed with a non-red, non-spotted male gives red, spotted sons and non-red, non-spotted daughters in F_1 and both types equally represented by males and females in F_2. If however, the genes R and S_p cross over together from the Z to the W chromosome, it is possible to get by subsequent crosses two types of females: $Z\ R\ S_p\ W\ R\ S_p$, that is a female homozygous for dominant sex-linked genes, and $Z\ r\ s_p\ W R\ S_p$, which is just the reverse of a normal female of the red variety. The first type of female when crossed with a Gold male ($r\ s_p$) would give all red, spotted offspring in F_1, and in F_2 (not considering crossovers), 3 red, spotted fish to 1 non-red, non-spotted one, the latter occurring only in the males. Such behavior is characteristic of the XY type of sex linkage. These results are similar to those obtained in the F_2 of the cross red-eyed female Drosophila X white-eyed male, where the F_2 recessives (whites) are all males. Here then is the unusual situation of an organism which normally exhibits the WZ type of sex determination, but which in some individuals can show the XY type of sex linkage. Fortunately the reciprocal of the cross listed above gives the WZ type of sex linkage.

AIDA obtained results similar to these in his studies of the sex-linked gene for red body color in Aplocheilus. His cross of a white female ($X r\ X r$) with a red male ($X R\ Y R$) gave in F_2 (Experiment 7) 3 reds to 1 white, with only females in the white class. This behavior, however, is characteristic of the WZ type of sex determination. AIDA speaks of these as "peculiar results" but does not point out their importance from the standpoint of studies on sex linkage.

From these facts it would seem to be permissible to make the following generalizations. (1) Where, of a pair of sex-determining chromosomes (XY or WZ), the heterochromosome (Y or W) does not bear any genes which are dominant over recessives on the X (or Z) chromosomes, the genetical evidence from sex linkage furnishes a reliable index of the mode of sex determinaton. (2) Where both sex chromosomes in the heterogametic sex may carry dominant, sex-linked genes, an individual of the constitution XY or WZ can be homozygous dominant and tests for sex linkage are likely to indicate a sex-determining mechanism just the reverse of that which actually operates.

CHROMOSOMES

DOCTOR BARBARA MCCLINTOCK of the Department of Botany, CORNELL UNIVERSITY, has kindly made some preliminary studies of the chromosomes of the male of *Platypoecilus maculatus*, using both sections and Belling smears. The chromosomes in these preparations are numerous and small in size, so that it is hard to make an accurate count of their number without a much more intensive study of this material.

SUMMARY[6]

1. Data are presented on the results of crosses between the Red, *spotted*, and the Gold races of *Platypoecilus maculatus*.
2. The characters *red* and *spots* in the Red race are apparently due to distinct dominant genes both of which are carried on the sex chromosome, the W chromosome usually bearing only recessive genes.
3. Occasionally crossing over occurs between the W and Z chromosomes of the female to interchange these dominant genes with their recessive allelomorphs. It has not yet been determined whether crossing over takes place between the two Z chromosomes of the male.
4. The transference of dominant genes from the Z to the W chromosome gives rise to females which exhibit one-sided feminine inheritance.
5. In some of these experiments females were obtained which, although they were of the heterogametic sex, nevertheless carried dominant sex-linked genes in the homozygous condition.
6. The nature of the sex-determining mechanism is considered.

POSTSCRIPT

Soon after the above manuscript was sent to the editor of Genetics, A. W. BELLAMY's second paper on Platypoecilus arrived at this laboratory (Genetics 13: 226–232, 1928). The problems and interpretations contained in this paper are essentially the same as those which have previously been communicated by him. They do not, therefore, require any additional comment at this time.

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