THE INHERITANCE OF THE CRESCENT AND TWIN SPOT MARKING IN XIPHOPHORUS HELLERI

ALICE MARRIN KERRIGAN

The Teachers College of the City of Boston, Boston, Massachusetts

Received January 12, 1934

INTRODUCTION

The viviparous teleost, *Xiphophorus helleri*, was first described and named by the naturalist, HECKEL, in 1848. The genus *Xiphophorus* has the lower rays of the caudal fin in the males prolonged into a long sword-shaped appendage which is sometimes as long as the rest of the fish. The anal fin of the male is modified into an intromittent organ, the gonopodium.

The classification of *Xiphophorus helleri*, according to HUBBS (1924) is as follows:

Order: Cyprinodontes

Family: Poeciliidae

Sub-family: Poeciliinae

Tribe: Xiphophorini

Genus: *Xiphophorus*

According to REGAN (1913), there are three species:

1. *X. montezumae*
2. *X. helleri*
3. *X. brevis*

This paper deals with *Xiphophorus helleri*, variety *rachovii*. This variety was thought by REGAN in 1911 to be a new species which he called *X. rachovii* in honor of ARTHUR RACHOW of Hamburg, Germany, who sent him the specimens. These fish had a pair of conspicuous blackish spots, one above and one below, at the base of the caudal fin. In his revision in 1913, however, REGAN considered them as a variety of *X. helleri*. I have found that some of these fish show two spots at the base of the caudal fin, while other animals have the spots continuous at the base of the caudal fin to give the appearance of a crescent. I have indicated the one with spots as twin spot, and the other as crescent.

BREEDING TECHNIQUE

In breeding *Xiphophorus helleri*, the fish were kept in small balanced aquaria. Unmated females were used for all crosses. This stock of immature virgin females was kept intact by removing the young fish which showed a thickening of the fore-margin of the anal fin. One or more females were placed with a single male. When the female showed signs of pregnancy,

she was removed to a tank well stocked with floating plants and fed with live food. After the birth of the fish, the female was removed and the young left in the aquarium.

Each young fish was placed in a 50 cc beaker and carefully examined with a hand lens. In transferring all fish, even the mature, I have followed the practice of keeping them in water. Either a specially constructed glass

FIGURE 1.—The mating of a heterozygous crescent male with a heterozygous crescent female.

cup-shaped container having a handle about twelve inches long or a glass beaker served as a transfer medium. After the examination of each fish, the young were returned to the container in which they were born. These young were allowed to remain in this container for at least two months before being removed to a larger one.

EXPERIMENTAL DATA

The stock used in the following experiments was obtained from F. W. SCHUMACHER, 753 Center Street, Jamaica Plain, Massachusetts, with the

exception of a crescent male (Series 13-1) which was purchased from WILLIAM TRICKER, INC., Saddle River, New Jersey. The work was begun in October, 1930.

A male crescent was mated with three females, two of which showed no crescent marking. The following experiments will prove the genotype of the male to be $Cc Pp$ and the female crescent $Cc PP$. The results of the mating of the crescent male with the crescent female is shown in the following table (see also figure 1).

TABLE 1

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT		NON-CRESCENT	
				MALES	FEMALES	MALES	FEMALES
11-1	F ₁	February 9, 1931	1	3	5	2	
11-1	F ₁	July 22, 1931	2	1	2		2
Total				11		4	
Expected on basis of $Cc \times Cc$ —3:1				11:25		3:75	

The factor for crescent marking is evidently dominant and the parents are heterozygous for this factor.

The genotypes of brood 1 of the F₁ as regards the crescent marking was next determined. A non-crescent male of F₁ mated with a crescent female of F₁ gave the results shown in table 2 (see also figure 2. In this drawing, the reciprocal cross is indicated).

TABLE 2

SERIES NUMBER	GENERATION	BROOD NUMBER	DATE	CRESCENT	NON-CRESCENT
21-1	F ₂	1	March 24, 1932	5	2
21-1	F ₂	2	April 24, 1932	7	6
21-1	F ₂	3	May 22, 1932	10	9
21-1	F ₂	4	July 9, 1932	16	24
21-1	F ₂	5	August 23, 1932	9	9
Total				47	50
Expected on basis of $Cc \times cc$ —1:1				48.5	48.5

This crescent female is, therefore, heterozygous for the crescent factor. The same male was mated with another crescent female giving the results in table 3.

TABLE 3

SERIES NUMBER	GENERATION	BROOD NUMBER	DATE	CRESCENT	NON-CRESCENT
21-2	F ₂	1	May 22, 1932	19	0
21-2	F ₂	2	June 24, 1932	13	0
21-2	F ₂	3	August 7, 1932	12	0
Total				44	0
Expected on basis of $CC \times cc$				44	0

The female is, therefore, homozygous for the crescent factor, while the former female is heterozygous.

The other non-crescent male of brood 1 of F₁ was mated with another crescent female, giving the results shown in table 4.

FIGURE 2.—The mating of a heterozygous crescent male with non-crescent females.

TABLE 4

SERIES NUMBER	GENERATION	BROOD NUMBER	DATE	CRESCENT	NON-CRESCENT
22-1	F ₂	1	April 24, 1932	3	4
22-1	F ₂	2	May 19, 1932	3	5
22-1	F ₂	3	June 28, 1932	16	15
22-1	F ₂	4	August 15, 1932	10	7
Total				32	31
Expected on basis of CcXcc—1:1				31.5	31.5

This female is heterozygous for the crescent factor. The genotypes of the three females may be represented by Cc , Cc , and CC . C = dominant crescent gene and c = the recessive non-crescent gene.

The two female non-crescents of the brood died when half grown, so that the male crescents were mated with non-crescent females obtained in the breeding of *Xiphophorus helleri*, which showed no crescent markings. The males were removed from the brood at the appearance of the thickening of the anal fin. The males were kept in a twenty gallon tank and the females in a twenty-five gallon tank.

The data on the breeding of the two non-crescent animals is as follows:

TABLE 5

SERIES NUMBER	GENERATION	FEMALE NUMBER	DATE	BROOD NUMBER	NUMBER OF NON- CRESCENT YOUNG
12	F ₁	1	April 30, 1931	1	10
12	F ₁	1	July 22, 1931	2	5
12	F ₁	2	April 14, 1931	1	23
12	F ₁	2	July 13, 1931	2	10
12	F ₁	3	August 17, 1931	1	15

Total					63
-------	--	--	--	--	----

Of the 63 non-crescent offspring, only 51 lived to reach maturity, even though they were kept in fairly large containers. Of the 51, 20 were females and 31 were males.

The male crescents of the F₁ of series 11-1 were mated with females of series 12. There were only two crescent males since one died. One crescent male mated with a non-crescent female gave the results in table 6.

TABLE 6

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	NON-CRESCENT
23-1	F ₂	March 24, 1932	1	22	0
23-1	F ₂	April 30, 1932	2	26	0
23-1	F ₂	June 20, 1932	3	18	0

Total				66	0
Expected on basis of $CC \times cc$				66	0

This male is evidently homozygous for the crescent factor.

The other male crescent mated with a female of series 12 gave the results in table 7.

TABLE 7

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON- CRESCENT
24-1	F ₂	August 17, 1932	1	5	1	8
24-1	F ₂	October 6, 1932	2	17	6	24
24-1	F ₂	December 6, 1932	3	8	3	16

Total				30	10	48
Expected on basis of 3:1:4				33	11	44

This same male crescent mated with another female of series 12 gave the results in table 8 (see also figure 2).

TABLE 8

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON- CRESCENT
24-2	F ₂	June 20, 1932	1	1	2	1
24-2	F ₂	August 5, 1932	2	2	1	1
24-2	F ₂	September 7, 1932	3	2	2	6
24-2	F ₂	November 15, 1932	4	0	0	2
Total				5	5	10
Expected on basis of 1:1:2				5	5	10

This same male crescent mated with a third female of series 12 gave the results in table 9 (see also figure 2).

TABLE 9

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON- CRESCENT
24-3	F ₂	August 23, 1932	1	1	0	1
24-3	F ₂	October 6, 1932	2	1	1	4
Total				2	1	5
Expected on basis of 1:1:2				2	2	4

This male is heterozygous for the crescent factor. Of the five crescent fish tested in the F₁ of series 11-1, three are heterozygous and two are homozygous for crescent marking. The genotype of the females may be represented by *Cc*, *Cc*, and *CC*,—the males by *CC* and *Cc*.

There is, however, another factor that is introduced—that of twin spots. In this case, the crescent at the base of the tail fin is incomplete. The black pigment appears only in the dorsal region, and the ventral region, and the area between the two is without pigment, thus giving the effect of two spots. Twin spots appeared in the last case (see tables 7, 8, and 9) when the crescent male was bred with females from series 12.

Another crescent male (purchased from TRICKER's) when mated with a female from series 12, gave the results in table 10, in which no twin spot fish appeared (see also figure 2).

TABLE 10

SERIES NUMBER	GENERATION	DATE	NUMBER	CRESCENT	NON-CRESCENT
13-1	F ₁	May 19, 1932	1	8	7
13-1	F ₁	August 13, 1932	2	9	11
Total				17	18
Expected on basis of <i>Cc</i> × <i>cc</i> —1:1				17.5	17.5

The results in table 6 in which a crescent male again was mated with a female from series 12 showed no twin spots in the offspring. It would, therefore, appear that there were two kinds of non-crescent animals as regards this factor.

This twin spot factor was earlier observed when the original male crescent (*P*) of parent series 11-1 was mated with non-crescent females. The following results were obtained:

TABLE 11

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	NON-CRESCENT
11-2	F ₁	April 10, 1931	1	0	3
11-2	F ₁	May 10, 1931	2	5	2
11-2	F ₁	June 22, 1931	3	6	6
11-2	F ₁	July 31, 1931	4	0	5
Total				11	16
Expected on basis of $Cc \times cc$ —1:1				13.5	13.5

The twin spots were not distinguished when the young were born. Evidently the figures listed for non-crescent include some animals having twin spots, because on December 14, 1931, there were only 6 survivors of these 27 animals. Observation showed that one had a complete crescent marking, two had the crescent marking interrupted to give the twin spot effect, and three had no crescent or spotted marking on the tail fin. The two twin spot animals and the one non-crescent died before the sex was determined. The fish remaining were:

Crescent

1 ♀

Non-crescent

1 ♀, 1 ♂

When this same male (*P*) was mated with another non-crescent female, the following results were obtained.

TABLE 12

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	NON-CRESCENT
11-3	F ₁	May 8, 1931	1	5	11
11-3	F ₁	July 4, 1931	2	3	2
Total				8	13
Expected on basis of $Cc \times cc$ —1:1				10.5	10.5

December 14, 1931, there were 12 survivors, as follows:

Brood Number	Crescent	Twin Spot	Non-Crescent
1	2 ♀	3 ♀	3 ♀, 1 ♂
2	1 ♀	2 ♀	0
Total	3 ♀	5 ♀	3 ♀, 1 ♂

A twin spot male mated with a non-spot female gave the following results:

TABLE 13

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON- CRESCENT
15-1	F ₁	July 2, 1931	1	1 ♀	1 ♀	8 ♀, 1 ♂
15-1	F ₁	August 10, 1931	2	1 ♀	0	1 ♂
15-1	F ₁	May 22, 1932	3	9	7	20
15-1	F ₁	July 1, 1932	4	10	8	20
15-1	F ₁	August 15, 1932	5	9	7	15
15-1	F ₁	October 10, 1932	6	4	5	9
Total				34	28	74
Expected on ratio 1:1:2				34	34	68

FIGURE 3.—The mating of a heterozygous twin-spot male with a heterozygous twin-spot female.

The genotypes of brood 1 were next demonstrated. The twin spot female mated with a twin spot male gave the results in table 14 (see also figure 3).

This same twin spot male mated with a non-crescent female of brood 1 gave the results in table 15 (see also figure 4).

FIGURE 4.—The mating of a heterozygous twin spot male with non-spot females.

TABLE 14

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	TWIN SPOT	NON-CRESCENT
25-1	F ₂	July 6, 1932	1	4	3
25-1	F ₂	August 15, 1932	2	10	3
25-1	F ₂	September 26, 1932	3	18	7
25-1	F ₂	November 14, 1932	4	8	3
Total				40	16
Expected on basis of 3:1				42	14

TABLE 15

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON- CRESCENT
25-2	F ₂	August 5, 1932	1	4	3	9
25-2	F ₂	September 18, 1932	2	8	7	20
25-2	F ₂	November 13, 1932	3	5	6	9
Total				17	16	38
Expected in ratio 1:1:2				17.5	17.5	35

This same twin spot male mated with another non-crescent female of brood 1 gave the results in table 16 (see also figure 4).

TABLE 16

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	TWIN SPOT	NON- CRESCENT
25-3	F ₂	August 18, 1932	1	2	3
25-3	F ₂	September 27, 1932	2	8	10
25-3	F ₂	November 23, 1932	3	12	14
Total				22	27
Expected on basis of 1:1				24.5	24.5

The crescent female of brood 1, series 15-1, mated with a male non-crescent of the same brood gave the following results:

TABLE 17

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON- CRESCENT
26-1	F ₂	June 14, 1932	1	2	3	5
26-1	F ₂	August 3, 1932	2	4	3	7
26-1	F ₂	September 15, 1932	3	7	6	20
Total				13	12	32
Expected on ratio 1:1:2				14.5	14.5	29

When born, the ration of brood 1 was 8 non-crescent and 2 crescent. The twin spot characteristic was clearly visible a month later. Likewise brood 2, when born, consisted of 4 crescent, 10 non-crescent, and brood 3 consisted of 7 crescent, 26 non-crescent.

A non-crescent female of brood 1, series 15-1, mated with the original male (*P*) of series 11-1, proven heterozygous for crescent marking, gave the results in table 18.

TABLE 18

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON- CRESCENT
11-4	F ₂	June 13, 1932	1	1	1	3
Expected on ratio 1:1:2				1.25	1.25	2.50

A female twin spot mated with a male non-spot gave the results in table 19 (see also figure 4. In this drawing, the reciprocal cross is indicated).

TABLE 19

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON- CRESCENT
16-1	F ₁	March 21, 1932	1	28	0	26
16-1	F ₁	May 4, 1932	2	11	0	15
16-1	F ₁	June 13, 1932	3	11	0	13
Total				50		54
Expected on ratio 1:1				52		52

These fishes had a decided crescent marking, and there were no twin spot animals.

DISCUSSION

Crescent and twin spot marking

An examination of the preceding results shows clearly that the factor for crescent marking is dominant over that for non-crescent or lack of marking. There must be, however, two different types of non-crescent animals, since a heterozygous crescent mated with a non-crescent will give crescent and non-crescent offspring in a ratio of 1:1, as results in tables 2 and 4 indicate. On the other hand, a heterozygous crescent mated with a non-crescent gives crescent, twin spot, and non-crescent animals (see tables 7, 8, and 9). A factor is introduced which evidently interacts with one present to give a different phenotype. If the factors for crescent, twin spot, and non-crescent were triple alleles, we might expect the crescent factor to be dominant over twin spot and non-crescent, and, in turn, twin spot dominant over non-crescent, as is the case in the fresh water fish, *Aplocheilichthys latipes* (AIDA 1921), in which the three colors, brown, variegated red, and red form a triple allelic series. Any two, when crossed, show in the F₂ a 3 to 1 segregation. Brown is dominant to variegated red as well as to red, while variegated red is dominant to red. A similar mode of inheritance was described by IBSEN (1919) in respect to black, tortoise, and red color in guinea pigs which are produced respectively by complete extension, partial extension, and non-extension of black pigment.

The three factors, crescent, twin spot, and non-crescent do not act in this way in *Xiphophorus* and, consequently, are not allelic. It is clearly a case of interaction of factors in which the factors belong to different allelic pairs. The crescent characteristic is due to two factors, one for color *C* and pattern *P*. The twin spot characteristic is due to the same pigment *C* but the pattern is reduced (*p*). Therefore, the genotype of the homozygous crescent could be represented by *CCPP*, the genotype of the homozygous twin spot could be represented by *CCpp*, and consequently, the homozygous non-crescent or non-spot animal could be *ccPP* or *ccpp*.

An examination of the results given in the tables of the various crosses will determine whether or not this is the correct explanation.

In table 1, the parents were proven to be heterozygous for the crescent factor. Twin spots did not appear in the F_1 . However, in F_2 , twin spots do appear (see tables 7, 8, and 9) so that one of the parents must be heterozygous for P as well as for C . The parent heterozygous for P is the male, since tables 11 and 12 give the results of the mating of this male with non-crescent females in which twin spot offspring appear. This male then would have to be heterozygous for factor P . The genotype of the male parent in table 1 is, therefore, $CcPp$, and the genotype of the female parent is $CcPP$.

Diagram to illustrate series 11-1, table 1.

P		$CcPP$ ♀		×	$CcPp$ ♂	
				↓		
Gametes		♂	Cp		Cp	
	♀				cP	
	CP		$CCPP$ Crescent		$CCPp$ Crescent	
					$CcPP$ Crescent	
					$CcPp$ Crescent	
F_1	cP		$CcPP$ Crescent		$CcPp$ Crescent	
					$ccPP$ Non-Crescent	
					$ccPp$ Non-Crescent	
Phenotypic Ratio in F_1			Genotypic Ratio in F_1			
3 $C-P-$			crescent			
			1 $CCPP$ crescent			
			1 $CCPp$ crescent			
			2 $CcPP$ crescent			
			2 $CcPp$ crescent			
1 $ccP-$			non-crescent			
			1 $ccPP$ non-crescent			
			1 $ccPp$ non-crescent			

The phenotypic ratio is in agreement with results in table 1.

Results in table 2 indicate that the genotype of the two fish must be either

$$\begin{array}{l}
 CcPP \text{ crescent} \times ccPP \text{ non-crescent} \\
 \text{or} \\
 CcPp \text{ crescent} \times ccPP \text{ non-crescent} \\
 \text{or} \\
 CcPP \text{ crescent} \times ccPp \text{ non-crescent}
 \end{array}$$

to give a 1:1 ratio of crescent and non-crescent offspring in F_2 . The crescent fish cannot have the genotype $CCPP$ or $CCPp$, since the F_2 would be all crescent. Neither could the mating be $CcPp$ crescent $\times$ $ccPp$ non-crescent—since twin spot fish would be expected in the F_2 in the ratio of 3 crescent, 1 twin spot and 4 non-crescent. Of the 97 fish, 8 should be twin spot and no twin spot fish were obtained.

Table 3 indicates that the genotypes of the two parents must be either

$$\begin{array}{l} CCPP \text{ crescent} \times ccPP \text{ non-crescent} \\ \text{or} \\ CCPP \text{ crescent} \times ccPp \text{ non-crescent} \\ \text{or} \\ CCPp \text{ crescent} \times ccPP \text{ non-crescent} \end{array}$$

to give all crescent offspring. The genotypes of the two parents could not be $CCPp \times ccPp$ since the offspring would be 3 ($C-P-$) crescent to 1 ($C-pp$) twin spot. The crescent in table 2 is heterozygous for factor C , while that in table 3 is homozygous for this factor.

Results in table 4 show that this mating is similar to that in table 2, and there are the same genotypic possibilities. Results in table 6 are similar to those in table 3, and the genotypic possibilities are the same.

Results in tables 7, 8, and 9 indicate that the crescent parent must have the genotypic formula $CcPp$ in order to have twin spot offspring. The non-crescent females have either the genotypes $ccPp$ or $ccpp$. They could not have the genotype $ccPP$, since in this case no twin spot offspring could appear. If the genotype of the non-crescent female is $ccPp$, the expected ratio would be 3 crescent, 1 twin spot, and 4 non-crescent, while if $ccpp$, the ratio would be 1 crescent, 1 twin spot, and 2 non-crescent. The figures in table 7 seem to indicate that this female is $ccPp$, since the results approximate a 3:1:4 ratio. The female in table 8 must have the genotype $ccpp$ since 5 twin spot offspring were obtained out of a total of 20. The females in tables 7 and 8 are, therefore, genotypically different as regards the P factor as the ratio of the results shows. The female in table 9 also has the genotype $ccpp$, since the results, although small, indicate a 1:1:2 ratio.

In tables 11 and 12, the male parent (P) series 11-1 is proven to have the genotype $CcPp$. The non-crescent females have either $ccPp$ or $ccpp$ genotypes. Figures would tend to indicate, particularly in table 12, that the genotype of the non-crescent female is $ccpp$, since there are 5 twin spot fish to 3 crescent. If the parent had the $ccPp$ genotype, the ratio should be 3 crescent to 1 twin spot. The 5 twin spot to 3 crescent ratio more closely approximates the 1:1 ratio of twin spot and crescent expected if the genotype of the female is $ccpp$.

It is possible to account for the fact that, from the mating of a non-crescent individual with a twin spot, crescent offspring are obtained. In table 13, the twin spot male parent must be $Ccpp$, since non-crescent offspring are obtained. The only genotype for the non-crescent female parent to have is $ccPp$ to give crescent and twin spot offspring, as the diagram below indicates.

<i>P</i>	<i>Cc</i> <i>pp</i>	×				
	♂		<i>ccPP</i>	<i>ccPp</i>	<i>ccpp</i>	
	Twin spot		♀	♀	♀	
			Non	Non	Non	
			↓	↓	↓	
	gametes	♀	<i>cP</i>	<i>cP</i>	<i>cp</i>	<i>cp</i>
	♂		<i>CcPp</i>	<i>CcPp</i>	<i>Cc</i> <i>pp</i>	<i>Cc</i> <i>pp</i>
	<i>Cp</i>		Crescent	Crescent	Twin spot	Twin spot
<i>F</i> ₁						
	<i>cp</i>		<i>ccPp</i>	<i>ccPp</i>	<i>ccpp</i>	<i>ccpp</i>
			Non-crescent	Non-crescent	Non-crescent	Non-crescent

Since the twin spot female of the *F*₁ is *Cc**pp*, the genotype of the male twin spot must be *Cc**pp* to give the results in table 14 of twin spot to non-crescent offspring in the ratio of 3:1. The non-crescent female in table 15 must have the genotype *ccPp*, since the ratio obtained is 1 crescent, 1 twin spot to 2 non-crescent when mated with this one male those genotype is *Cc**pp*. Likewise, the genotype of the female in table 16 must be *ccpp* to give a ratio of 1 twin spot to 1 non-crescent offspring when mated with this same male.

The crescent female of the *F*₁ of series 15-1 has the genotype *CcPp*, and mated with a non-crescent of the *F*₁ gives offspring which approximate a 1:1:2 ratio rather than a 3:1:4 ratio of crescent, twin spot, and non-crescent fish, as is indicated in table 17. The non-crescent genotype is, therefore, *ccpp* rather than *ccPp*. The non-crescent female of *F*₁ which was mated with the male (*P*) of series 11-1 whose genotype was proven to be *CcPp* probably has also the genotype *ccpp*. Although too small for certainty, the figures in table 18 seem to be more in accord with the 1:1:2 ratio, which would indicate the non-crescent genotype to be *ccpp*.

The results in table 19 of the cross of a twin spot and non-crescent to give only crescent and non-crescent offspring may be explained as follows. The twin spot must have the genotype *Cc**pp*. The only other possible genotype is *CC**pp*, and in this case there could be no non-crescent offspring. The only genotype the non-crescent parent can possibly have is *ccPP* to give the results in table 19 of crescent and non-crescent offspring.

It has been found possible to explain the results of all crosses on the basis of interaction of factors. The crescent marking is due to two factors, *C* which evidently indicates color pigment, and *P* the pattern. The crescent phenotype is then *C-P-*, in which *P* represents a supplementary gene which acts only in conjunction with *C* to produce this crescent marking. When *P* is found in a homozygous recessive condition *pp* in the presence of *C*, the twin spot phenotype appears *C-pp*, as if the patterns were not as extended or complete as in the previous condition *C-P-*. In the non-

crescent animals, color never appears and the factor for pattern is concealed. The genotypes of these fish are *ccpp*, *ccPP*, and *ccPp*.

The hair pattern determiner demonstrated by CASTLE in guinea pigs is similar to this in which a supplementary gene acts only in conjunction with some other gene to bring about a modification. The wild agouti color of the hair of certain guinea pigs is due to the fact that pigment is distributed along the length of each hair in a definite pattern. The tip of a single hair is black followed by a band of yellow, while most of the proximal part is a leaden color. The distribution of the pigment is such as to give the characteristic gray or "agouti" color to the coat when taken as a whole. CASTLE showed that upon crossing a solid black guinea pig, possessing pigment but no pattern (*CCaa*) with a white albino pig having no pigment (*ccAA*), the offspring reverted to the ancestral agouti or pattern type (*CcAa*) thus proving that the pattern must be carried in this case by the white or albino guinea pig as a factor independent of the color necessary for its expression. The same is true of *Xiphophorus*, the non-crescent animal carrying the factor which is necessary for interaction with the color factor to produce either crescent or twin spot marking.

The spotting of piebald mice is another illustration of this same type of interaction of factors. CUÉNOT discovered that such spotting is due to the absence of a uniformity gene which, if present, causes color to be uniformly distributed over the entire coat. Both of these independent allelic genes are transmitted through albino animals, although in the albino both are hidden due to the absence of pigment upon the presence of which their visibility depends, just as in *Xiphophorus* the non-crescent may carry *pp* or *P* — which produces different phototypes in the presence of *C*.

There are numerous other illustrations of the interaction of factors to produce different phenotypes. There are in both rabbits and mice factors which interact to produce various coat colors, spotting, clumping of hairs, and amount of pigment of the iris. It is possible to have the factors segregated, and they may occur in any combination.

Sex linkage

Because of the lack of space and equipment, it was not possible to segregate the offspring of all the fish until they had reached maturity in order to determine the sex. However, the evidence from table 1 would indicate that the factors for crescent and twin spot markings are carried by autosomal genes.

Platypoecilus and Lebistes, both belonging to the same sub-family, Poeciliinae, show different types of sex linkage. WINGE proved that Lebistes shows the XY type, while BELLAMY showed that Platypoecilus has the WZ type; that is, the female is heterogametic for sex, while in the XY

type the male is heterogametic for sex. AIDA, in his studies of inheritance in *Aplocheilichthys latipes*, another small fish of the order Cyprinodontes, showed that the XY type of inheritance held here, and further that a gene for red body color (*R*) was carried both by the X and Y chromosomes. He found, also, that crossing over of the *R* gene occurred between the X and Y chromosomes in the offspring of the backcrosses between heterozygous brown or red males and white females, and also matings of heterozygous red males with heterozygous red females. WINGE found in *Lebistes reticulatus* that the Y chromosome carried recessive genes. FRASER and GORDON found in *Platypharodon maculatus* the WZ type of inheritance, and showed that the W chromosome usually bears only recessive genes.

There are two possibilities to consider if the WZ type of inheritance functions in *Xiphophorus*, providing that the factors *C* and *P* are sex-linked.

Case 1—If the W chromosome carries the recessive genes or none.

Case 2—If the W chromosome carries the dominant genes.

In table 1, the parents have the genotypes *CcPp* and *CcPP* for male and female respectively.

DIAGRAM OF CASE 1

Let the W chromosome be represented by | and carrier of either recessive genes or none as regards *C*.

There are no non-crescent males, yet two were obtained in table 1. These could not be obtained if this type of inheritance held unless crossing over took place. There were two males obtained out of a total of 15 animals, and this possibility of crossing over, therefore, seems slight.

CASE 2

The W chromosome carrier of the dominant genes:

There would be no non-crescent females, yet two were obtained in table 1. The only explanation would be that crossing over took place, which does not seem probable since two non-crescent females were obtained out of a total of 15 animals. Further, if dominant *C* is carried on the *W* chromosome, all the female offspring should carry this gene, no matter what genes are introduced in the male inheritance, and all the females would have the crescent marking,—the exception being if crossing over between the *W* and *Z* chromosomes should take place.

From the results in table 1, it would appear that the *WZ* type of sex inheritance does not operate here.

If the *XY* type of inheritance is common to *Xiphophorus*, there are again two possibilities.

Case 1. If the *Y* chromosome carries recessive genes or none.

Case 2. If the *Y* chromosome carries the dominant genes.

There were, however, two non-crescent females obtained in this mating, and these again could be accounted for only if crossing over took place, which is not very probable, since two females were obtained out of 15 animals. Also, table 6 indicates that this type of inheritance, as regards these factors, does not hold here. A crescent male mated with a non-crescent female gave all crescent offspring. If there were a sex-linked character, and the *Y* chromosome a carrier of recessive genes or none, half of the offspring should be non-crescent as the diagram below indicates.

To obtain all crescent offspring, the *C* factor would have to be carried in the Y chromosome.

CASE 2

If the Y chromosome is a carrier of the dominant genes.

In this case, there would be no non-crescent males, two of which were obtained out of 15 animals. Further, all the males would transmit this gene for crescent, and all males would be crescent, despite the gene introduced by the females which is not true.

None of the possible cases of sex inheritance agrees with the results obtained, and it seems evident that the factors for crescent and twin spot marking are carried by autosomal genes.

SUMMARY

The results of the matings of the *Xiphophorus helleri*, variety *rachovii*, in respect to crescent and twin spot markings indicate that:

1. The factor for crescent marking is not carried by non-crescent parents as is proven by series 12.
2. The crescent marking is inherited as a dominant factor.
3. The factor for crescent marking is carried by an autosome. It is, therefore, not sex-linked.
4. Crescent marking is produced by the interaction of two factors *C* and *P*.
5. Twin spot marking is produced by the interaction of two factors, *C* and *p*.
6. Non-crescent or non-spot fish differ as regards the *P* factor. As regards the genotypes of these fish, these are *ccpp*, *ccPP*, and *ccPp*.

LITERATURE CITED

- AIDA, TATUO, 1921 On the inheritance of color in a fresh water fish; *Apllocheilus latipes*, Temmick and Schlegel, with special references to sex-linked inheritance. *Genetics* 6: 554-573.
- BADE, E., 1923 and 1930 *Das Süßqasser Aquarium*, 1023 pp. Berlin: Fritz Pfenningstorff.
- BELLAMY, A. W., 1922-1923 Sex-linked inheritance in the teleost, *Platyepocilus maculatus* Günth. *Anat. Rec.* 24: 419-420.
- 1924 Bionomic studies on certain teleosts (Poeciliinae) *Genetics* 9: 513-529.
- CASTLE, W. E., 1924 *Genetics and eugenics*, 3rd ed. Cambridge.
- DEAN, B., 1923 A bibliography of fishes. The American Museum of Natural History, N.Y.

- ESSENGERG, J. M., 1923 Sex differentiation in the viviparous teleost, *Xiphophorus helleri* Heckel. Biol. Bull. **51**: 46-96.
- 1926 Complete sex-reversal in the viviparous teleost *Xiphophorus helleri*. Biol. Bull. **51**: 98-111.
- FRASER, A. C. and GORDON M., 1928 Crossing over between the W and Z chromosomes of the killifish *Platyopocilus*. Science N.S. **67**: 470.
- 1929 I. The genetics of *Platyopocilus*. II. The linkage of two sex-linked characters. Genetics **14**: 160-179.
- GOODRICH, H. B., 1929 Mendelian inheritance in fish. Quart. Rev. Biol. **4**: 83-99.
- GORDON, M., 1926a Melanophores of *Platyopocilus*, the top-minnow of geneticists. Abstract No. 71, Anat. Rec. **34**: 138.
- 1926b Variation in the tropical viviparous killifish, *Platyopocilus*. Abstract No. 133, Anat. Rec. **34**: 166.
- 1926c Inheritance in fishes. Abstract No. 150, Anat. Rec. **34**: 172.
- 1926d Tropical aquarium fish-culture in industry and scientific research. Trans. Amer. Fish Soc. **56**.
- 1927 The genetics of a viviparous top-minnow *Platyopocilus*; The inheritance of two kinds of melanophores. Genetics **12**: 253-283.
- 1928 Pigment inheritance in the Mexican Killifish, J. Hered. **19**: 551-556.
- 1931a Morphology of heritable color patterns in Mexican Killifish, *Platyopocilus*. Amer. J. Cancer **15**: 732-787.
- 1931b Hereditary basis of melanosis in hybrid fishes. Amer. J. Cancer **15**: 1495-1523.
- HODGE, A. E., 1927 Tropical aquarium fishes. How to breed and rear them. New York: Frederick A. Stokes Company.
- HUBBS, C. L., 1924 I. Studies of the fishes of the order Cyprinodontes. II. An analysis of the genera of the Poeciliidae. Mus. Zool. Misc. Pub. Univ. Michigan, Ann Arbor, Mich. **13**: 5-11.
- IBSEN, H. L., 1919 Tricolor inheritance. The triple allelomorphic series in guinea pigs. Genetics **4**: 597-606.
- INNES, W. T., 1929 Goldfish varieties and tropical aquarium fishes. Philadelphia: Innes and Son, 223 pp.
- JORDAN, D. S. 1925 Fishes. New York: D. Appleton & Co.
- JORDAN, D. S. and EVERMANN, 1896 The fishes of North and Middle America. U.S. Natl. Mus. Bull. **47**.
- JORDAN, D. S. and GILBERT, C. H., 1882 Synopsis of fishes of North America. U.S. Natl. Mus. Bull. **16**: 384.
- McFARLANE, J. M., 1923 The evolution and distribution of fishes. New York: Macmillan and Co.
- MEEK, S. E., 1901 A contribution to the ichthyology of Mexico. Field Col. Mus. Zool. Series P. **63**: Vol. III. No. 6.
- 1904 The fresh water fishes of Mexico north of the Isthmus of Tehuantepec. Field Col. Mus., Zool. Series **5**: XXII-252 pp.
- 1907 Notes on fresh water fishes from Mexico and Central America. Field Col. Mus., Zool. Series **7**: 133-157.
- MORGAN, T. H., 1926 Recent results relating to chromosomes and genetics. Quart. Rev. Biol. **1**: 166-211.
- REGAN, C. T. 1907 (1906-1908) Pisces Biologia Centrali Americana XXXII-203 pp. 26 pls. 2 maps. London: Dulau.
- 1911 On some fishes of the family Poeciliidae. Ann. Mag. Nat. Hist. (8) VIII. p. 373.
- 1913 A revision of the cyprinodont fishes of the sub-family Poeciliinae. Proc. Zool. Soc. London **2**: 977-1018, figs. 168-173.
- SINNOTT, E. W. and DUNN, L. C., 1925 Principles of genetics. New York: McGraw-Hill Book Co. Taschenkalender für Aquarien und Terrarienfreunde. 1932.
- WINGE, O., 1922a A peculiar mode of inheritance and its cytological explanation. J. Genet. **12**: 12: 137-144.
- 1922b One-sided masculine and sex-linked inheritance in *Lebistes reticulatus*. J. Genet. **12**: 145-162.
- 1923 Crossing over between and X and Y chromosomes in *Lebistes*. J. Genet. **13**: 201-217.