COLOUR INHERITANCE AND SEX DETERMINATION IN *LEBISTES*

By Ø. WINGE and E. DITLEVSEN
Carlsberg Laboratory, Copenhagen, Valby

Received 16.xi.46

GENERAL INTRODUCTION

ABOUT 25 years have passed since the small, viviparous tropical teleostean fish, *Lebistes reticulatus*, was first employed for genetic studies.

Lebistes reticulatus (Peters) Regan, the " millions fish " or " guppy " is well known to all aquarium fanciers. It is characterised by a very

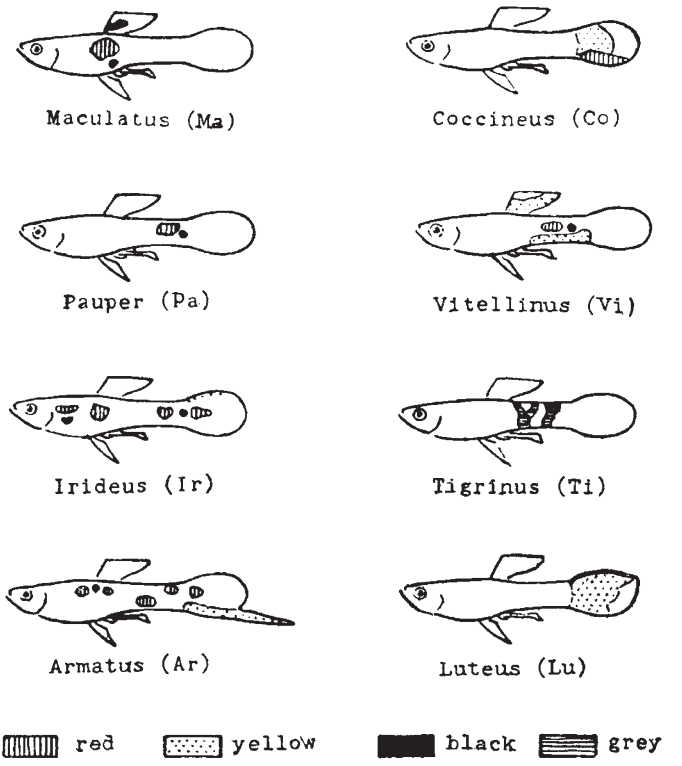


FIG. 1.—The effects of some colour genes in males of *Lebistes reticulatus*; at the left are seen some of the " absolutely Y-linked " genes; at the right some genes that may occur in X as well as in Y.

conspicuous sex difference. The female is normally about 5 cm. long, the male only about 3 cm., and the difference is rather pronounced in other respects also. Thus the male is more slender, and its anal fin is transformed into a copulating organ. Further the colour of the female is generally a rather inconspicuous greyish-brown, whereas

the male is provided with beautiful red, yellow and black spots and configurations on the body and often on the dorsal and caudal fins too ; the caudal fin may also be elongated.

The studies carried out on *Lebistes* concern the inheritance of the colour pattern and sex determination. *Lebistes reticulatus* has the male with XY and the female with XX. It was the first organism in which a Y-linked inheritance was demonstrated (Schmidt, 1920 ; Winge, 1921) ; two rather different colour patterns, *Ir* and *Ma*, were found on crossing two strains continually to be transmitted from father to son, grandson and so on, and never to be inherited through the mother. Subsequently by thorough genetic analysis more than 20 colour genes have been demonstrated (Winge, 1922, 1927 ; Blacher, 1927, 1928 ; Kirpichnikow, 1935). Most of them are X-linked or Y-linked (see fig. 1). The genes are dominant in the male. The female normally presents no colour pattern, even though it possesses genes for colour homozygotically.

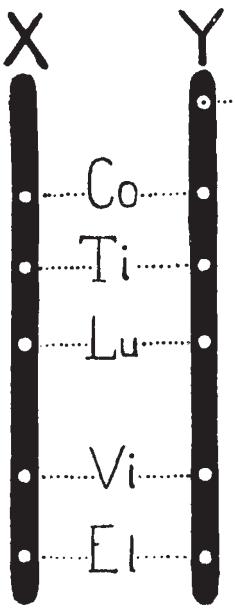


FIG. 2.—Map showing the position of some genes in X and Y.

Crossing-over between X and Y has been demonstrated in several cases (Winge, 1923, 1927, 1934). It was found that some genes are always Y-linked, while others may occur in X as well as Y. The Y chromosome of the male *Lebistes* contains always one of the absolute Y-linked genes that are to be looked upon as allelomorphs, *e.g. Ma*, *Ir*, *Pa* or *Ar*. Other genes—as for instance *Ti*, *Lu*, *Co*, *Vi*, *El*—may occur in X as well as in Y. It has been possible to map the sex chromosomes (see fig. 2). The maximum cross-over percentage was found to be only 10. It is reasonable to assume that X and Y are mostly homologous, as exchange of genes may take place ; and the difference between them may hardly amount to much more than the one gene, *Ma*, or its allelomorphs. Possibly the colour gene itself is identical with a superior male-determining gene ; possibly the genes are merely strongly linked. So far it has not been possible to demonstrate any morphological difference between the X and Y chromosomes. All the chromosome pairs—a total of 23—are very nearly of the same size.

Genes located in the autosomes are *Zebrinus*, concerning a special colour pattern of the male (Winge, 1927), *Gold* and *Blond*, concerning the ground colour of the entire body in both sexes (Haskins and Druzba, 1938 ; Goodrich, Josephson, Trinkaus and Slate, 1944), and some genes that have nothing to do with the colour pattern.

X-linked and Y-linked inheritance of colour genes and crossing-over between X and Y have been found also in some kindred fishes :

Aplocheilus (Aida, 1921) and *Platypoecilus maculatus* (Bellamy, 1928; Fraser and Gordon, 1928-29; and Gordon, 1927). The latter has female heterogamy in domesticated stocks whereas male heterogamy has recently been observed in wild populations (Gordon, 1947).

The sex determination and the localisation of the sex genes in *Lebistes* have been elucidated especially by studies on XX males and XY females which have now and then appeared in the material (Winge, 1932, 1934). Sex-determining genes of differing potency, some pulling in a female direction, others in a male, are found distributed over a majority of the autosomes. The Y chromosome contains a strong male-determining gene closely linked to, or perhaps identical with, the absolutely Y-linked colour gene. Probably the X chromosome has a corresponding female-determining gene.

Here it will be appropriate briefly to mention the appearance of XX males. As stated above, the female *Lebistes* is colourless even

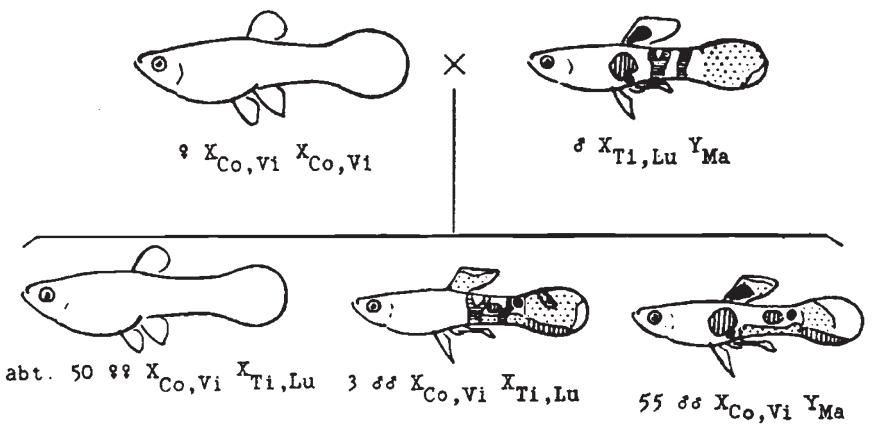


FIG. 3.—A cross between an $X_{Co,Vi}$ $X_{Co,Vi}$ female and an $X_{Ti,Lu}$ Y_{Ma} male, giving in addition to ordinary XY males three $X_{Co,Vi}$ $X_{Ti,Lu}$ males

though she possesses genes for colour pattern. Within certain races, however, the female may sometimes show a trace of colour pattern, which might possibly be interpreted as evidence of a certain degree of masculinity. It must be pointed out, however, that as a rule the sexual differentiation in males and females is very clear-cut in *Lebistes*. Only exceptionally are hermaphroditic or intersexual individuals observed. In this respect there is a pronounced difference between *Lebistes* and, for instance, *Lymantria*. Crossing of two races, in which some of the females had a tendency to show colouring, gave—besides about 50 females and 55 XY males—3 deviating males, who quite unquestionably had the formula XX (see fig. 3). Through inbreeding for several generations (back-crossing of the daughters to XX males) we finally obtained a race with about 50 per cent. XX males and 50 per cent. XX females.

The origin of the XX males has to be explained as due to the accumulation of so many male-determining genes in the autosomes

that the development could proceed in a male direction even in the absence of Y. As the outcome is equal numbers of coloured XX males and uncoloured XX females, and as the inheritance of the X-linked genes is now no longer sex-linked but merely Mendelian, we may reasonably assume that now a pair of autosomes has become decisive in sex determination. The new sex balance was less firmly established than the normal. It was rather susceptible to external factors, so that in a cold and dark season there were rather considerable deviations from 50 per cent. of either sex.

In another *Lebistes* family on one occasion XY females appeared. Crossing of a female of the formula $X_O\ X_O$ (*i.e.* with no colour genes in the X) with a male of the formula $X_{Li}\ Y_{Ma}$ gave—besides several ordinary $X_{Li}\ X_O$ females and $X_O\ Y_{Ma}$ males—a few females with a

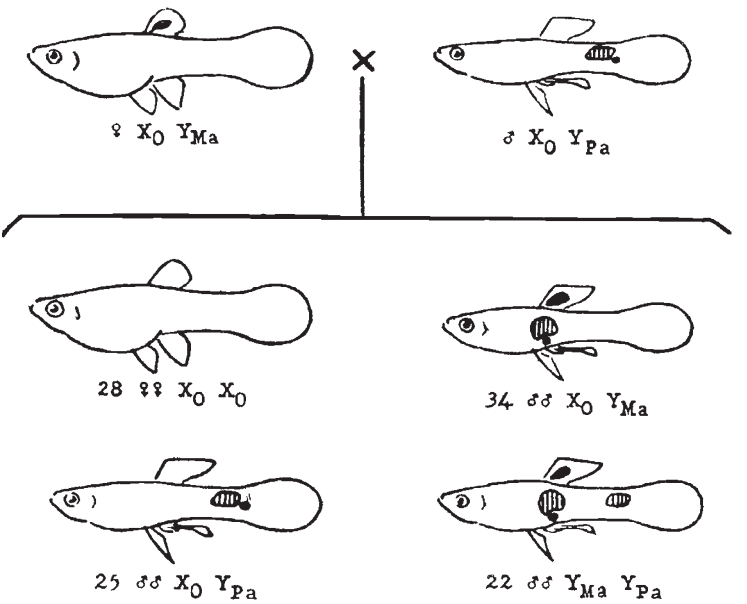


FIG. 4.—A cross between an XY female and an XY male.

spot on the dorsal fin which presumably was due to the *Ma* gene. By crossing such females with males containing another gene in the Y chromosome it could be shown that the females actually had the formula $X_O\ Y_{Ma}$ (see fig. 4). This, as will be noticed, resulted among others in viable $Y_{Ma}\ Y_{Pa}$ individuals, which, on crossing with ordinary females, gave entirely male progeny.

The origin of XY females is explainable like that of XX males ; but here it is XY individuals which have accumulated so many female genes in the autosomes that, in spite of the presence of the strong sex-determining gene in Y, the development has changed into a female direction. That YY individuals may be viable and fertile agrees very well indeed with the fact that the crossing-over experiments have shown the difference between X and Y to be slight.

As emphasised in 1934 it should be possible by suitable inbreeding to obtain a change in the mechanism of sex determination which would result in a strain with XY females and YY males, and thus we should obtain a change from male heterogamy to female heterogamy. For this purpose the inbreeding was continued, and thus we made an interesting discovery. Even though the Y_{Ma} Y_{Pa} males were fully viable and fertile the Y_{Ma} Y_{Ma} type was lethal, which must be due to a recessive lethal gene in the Y chromosome, close to the *Ma* gene. Naturally this lethal gene contributes to maintain the normal sex determination within the Y_{Ma} race (Winge and Ditlevsen, 1938).

The main points as to the distribution of the genes on X, Y and autosomes in *Lebistes* and as to the manifestation of the genes as hitherto observed may be summarised as follows :—

Genes	Inheritance	Gene manifestation observed in	
		Males	Females
Maculatus	Y	XY, YY	XY
Pauper	Y	XY, YY	
Armatus, Oculatus, Iridescens, Aureus, Variabilis, Ferrugineus, Sanguineus, Gladigerens, Bimaculatus	Y	XY	
Coccineus, Vitellinus, Tigrinus, Luteus .	X and Y	XY, XX	
Cinnamomeus, Minutus, Elongatus, Solaris, Lutescens, Purpureus	X and Y	XY	
Lineatus	X (and Y ?)	XY, XX	
Flavus	X (and Y ?)	XY, XX	XX
Gold, Blond, Coecus, Abnormis, Curvatus .	Autosomes	XY	XX
Zebrinus	Autosomes	XY, XX	

1. Y is never empty for *colour genes*. At least one colour gene is present ; while X and autosomes *may* be empty.
2. All genes are *dominant* in males, except the two autosomal recessives—*Gold*, Blond, and probably also the autosomal Coecus and Abnormis.
3. X-linked colour genes except *Flavus* never manifest themselves in females, not even when homozygotically present.
4. Old females may sometimes faintly show the colour genes which normally appear only in males.
5. YY males with two identical Y-chromosomes are probably always *lethal*. This, however, has so far only been demonstrated in Y_{Ma} Y_{Ma}.

In *Aplocheilus latipes* Aida has reported XY females as well as XX and YY males, but his explanation of the sex determination deviates somewhat from the one given here (Aida, 1936).

Goldschmidt (1937) has criticised our conception of the localisation of the sex genes, which he tries to bring into closer harmony with the prevailing view. Later on, in the section on sex determination, we shall deal with this criticism.

In 1944, for various reasons, it was decided to stop the *Lebistes* experiments in the Carlsberg Laboratory and dispose of the fishes. Lately we had been occupied, among other things, with a couple of genes which, in contrast to most of those hitherto studied,

manifested themselves both in females and in males—*Flavus* and *Gold*. Now, after the war, we have received a paper by Goodrich, Josephson, Trinkaus and Slate (1944) that brings together many of the same facts we had found concerning the *Gold Lebistes*. Even though this section of our communication, therefore, on several points may appear as a repetition of the findings reported by Goodrich *et al.*, we still thought that we ought to present it.

I. THE "FLAVUS" GENE

Some years ago Dr Anton Bruun of Copenhagen called our attention to a *Lebistes* strain, the female of which was characterised

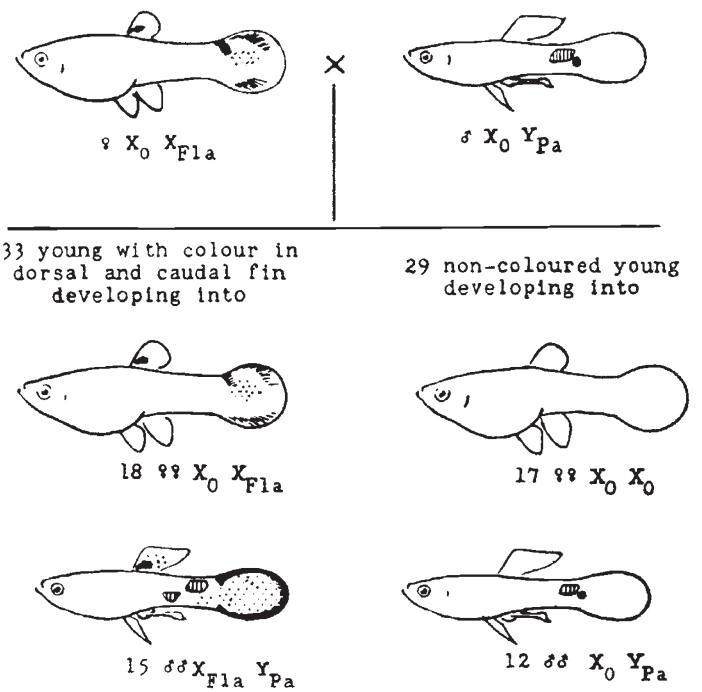


FIG. 5.—Cross between "coloured" female $X_O X_{Fla}$ and male of the Pauper race, $X_O Y_{Pa}$.

by the possession of colour in the dorsal fin and tail-fin. Mr Harry Andersen, printer, of Elsinore, was kind enough to give us some specimens of this strain.

In these "coloured" females the caudal fin, especially its middle part, is rather distinctly yellow with a blackish margin, while the dorsal fin presents a somewhat effaced black spot, often together with a little yellow (fig. 5). In the males of this strain (*cf.* fig. 6) the caudal fin is very intensely yellow, bordered with black, and the dorsal fin is yellow with a black spot; a few red spots are seen on the sides of the body. This colour pattern, with the strong contrast between yellow and black, is one of the most decorative encountered in *Lebistes*.

In crossing experiments this pattern was found to involve a single

X-linked gene. This gene is dominant in both sexes, although it manifests itself less distinctly in the female. Further, it manifests itself earlier than the X-linked and Y-linked colour genes hitherto known. Even when the young are only about one month old they can be separated into " coloured " (with a dark spot in the dorsal fin, and somewhat black and yellow at the upper and lower margins of the caudal fin) and " uncoloured " (with colourless fins like normal *Lebistes* offspring). We designate the gene here concerned as *Flavus* (*Fla*).

The culture from which our fish originated was not " pure," therefore our first females were heterozygous, X_O X_{Fla}. Later on

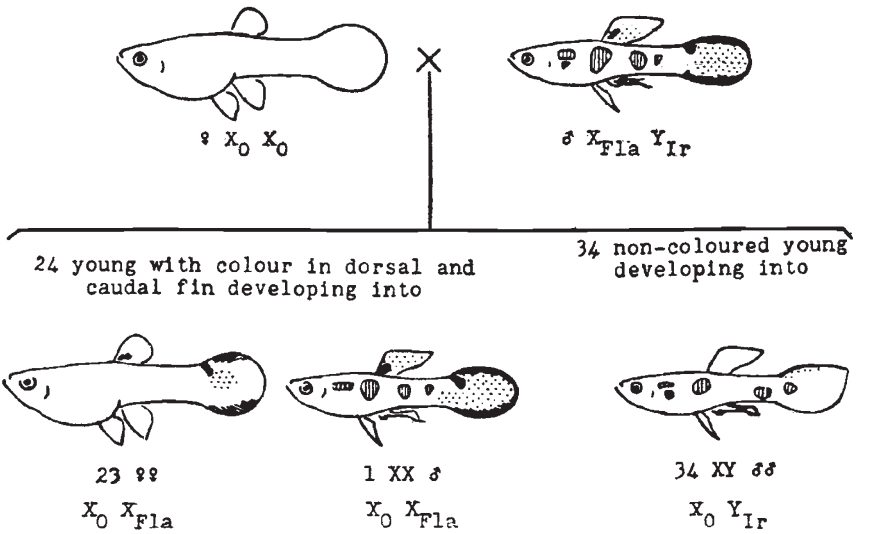


FIG. 6.—Cross of X_O X_O female to X_{Fla} T_{Ir} male showing X-linked inheritance and segregation of an X_O X_{Fla} male.

we succeeded in producing homozygous females, X_{Fla} X_{Fla}, with an external resemblance to the heterozygotes.

Crossing of *Fla* ♀ with X_OY_{Ma} ♂ ($X_OX_{Fla} \times X_OY_{Ma}$)
(♀ 2345 × ♂ 2346)

gave :

Fla	Non-*Fla*
12 ♀♀ 11 ♂♂	10 ♀♀ 12 ♂♂

Crossing of *Fla* ♀ with X_OY_{Pa} ♂ ($X_OX_{Fla} \times X_OY_{Pa}$) see fig. 5, gave :

	Fla		Non-*Fla*	
	♀♀	♂♂	♀♀	♂♂
♀ 2309 × ♂ 2347	2	1	1	1
♀ 2384 × ♂ 2385	3	3	3	2
♀ 2403 × ♂ 2404	0	2	0	0
♀ 2420 × ♂ 2421	13	9	13	9
	18	15	17	12

♂ of the *Fla* strain crossed with X_OX_O ♀ ($X_OX_O \times X_{Fla}Y$) (♀ 2310 × ♂ 2285), *cf.* fig. 6, gave :

Fla	*Non-Fla*
23 ♀♀ 1 XX ♂	0 ♀♀ 34 ♂♂

As is evident from the figure, the single *Fla* male did not differ from its father in appearance. It had not only the same yellow and black pattern in the caudal fin and dorsal fin, but also exactly the same red spots on the body. Subsequent experiments proved this son (♂ 2367) to be an XX male. The non-*Fla* sons show that, in addition to the X-linked *Flavus* gene, ♂ 2285 possessed a Y-linked gene that seems identical with the gene *Ir* (Winge, 1922, 1927). Other males, by the way, showed a Y-linked colour pattern somewhat different from this.

Crossing of non-*Fla* ♀, segregated from the *Fla* strain, with *Fla* ♂ ($X_OX_O \times X_{Fla}Y$) gave :

	Fla		Non-*Fla*	
	♀♀	♂♂	♀♀	♂♂
♀ 2322 × ♂ 2323	41	0	0	49
♀ 2335 × ♂ 2336	7	0	0	4
♀ 2364 × ♂ 2365	7	0	0	4
	55	0	0	57

Crossing of *Flavus* mutually gave :

(*a*) Three matings in which the female evidently was heterozygous ($X_OX_{Fla} \times X_{Fla}Y$) :

	Fla		Non-*Fla*	
	♀♀	♂♂	♀♀	♂♂
♀ 2333 × ♂ 2334	5	11	0	8
♀ 2374 × ♂ 2375	7	5	0	9
♀ 2412 × ♂ 2413	19	8	0	9
	31	24	0	26

(*b*) Four matings in which the female was homozygous ($X_{Fla}X_{Fla} \times X_{Fla}Y$) :

	Fla		Non-*Fla*	
	♀♀	♂♂	♀♀	♂♂
♀ 2355 × ♂ 2375	15	13	0	0
♀ 2355 × ♂ 2399	1	0	0	0
♀ 2457 × ♂ 2458	16	24	0	0
♀ 2510 × ♂ 2511	3	4	0	0
	35	41	0	0

With the *Flavus* type we have an X chromosome characterised by a gene that is easy to recognise in the female as well as in the male. It seemed natural, therefore, to try to introduce this gene in a strain without a Y chromosome, where both females and males have two X chromosomes, in order to study its sexual effect.

As demonstrated previously (Winge, 1934), we have to assume that the X chromosome contains a sex-determining gene, pulling in a female direction. It seemed conceivable indeed that the new X chromosome would be of another potency than the X chromosomes already present in the XX race. Therefore numerous crossing experiments were made, partly of the aforementioned XX ♂ 2367 and some other $X_{Fla}X$ males, obtained later, with females of the XX strain, partly of *Fla* females with XX males of our XX strain. In spite of extensive inbreeding it was not possible to attain 50 per cent. males, but several matings yielded a few XX males.

In the following counts the total formula of the XX females or males has not been given, as the exact formula for other colour genes was not known in all cases.

Crossing of Non-*Fla* XX females with X_OX_{Fla} males gave :

	Fla		Non-*Fla*	
	♀♀	♂♂	♀♀	♂♂
♀ 2366 × ♂ 2367	36	0	35	0
♀ 2383 × ♂ 2367	14	0	15	0
♀ 2430 × ♂ 2367	28	0	23	0
♀ 2441 × ♂ 2367	7	0	6	0
♀ 2450 × ♂ 2367	14	0	14	1
♀ 2463 × ♂ 2367	6	1	9	0
♀ 2469 × ♂ 2367	23	0	26	0
♀ 2483 × ♂ 2367	8	0	6	0
♀ 2410 × ♂ 2411	19	0	16	0
♀ 2429 × ♂ 2411	28	2	23	1
♀ 2436 × ♂ 2411	5	0	4	0
♀ 2443 × ♂ 2411	8	0	9	0
♀ 2459 × ♂ 2411	25	1	25	1
♀ 2464 × ♂ 2411	22	3	22	0
♀ 2487 × ♂ 2411	14	0	12	0
♀ 2496 × ♂ 2477	9	0	14	0
♀ 2503 × ♂ 2504	1	0	1	0
♀ 2507 × ♂ 2508	1	1	3	2
♀ 2506 × ♂ 2367	5	2	2	0
Total	273	10	265	5

Fla females ($X_{Fla}X_O$) with Non-*Fla* XX ♂♂ gave :

	Fla		Non-*Fla*	
	♀♀	♂♂	♀♀	♂♂
♀ 2343 × ♂ 2344	23	0	19	0
♀ 2431 × ♂ 2432	9	0	12	0
♀ 2433 × ♂ 2452	32	0	33	0
♀ 2435 × ♂ 2351	11	0	8	2
♀ 2442 × ♂ 2398	3	0	3	0
♀ 2444 × ♂ 2398	8	0	10	0
♀ 2447 × ♂ 2438	4	0	4	0
♀ 2453 × ♂ 2454	13	2	14	3
♀ 2455 × ♂ 2422	10	0	6	0
♀ 2474 × ♂ 2475	15	0	11	1
♀ 2484 × ♂ 2485	13	0	12	0
♀ 2488 × ♂ 2489	11	0	13	0
♀ 2497 × ♂ 2498	0	0	0	1
♀ 2499 × ♂ 2500	3	1	4	1
♀ 2509 × ♂ 2486	0	0	1	0
♀ 2517 × ♂ 2498	5	0	3	0
Total .	160	3	153	8

Thus among 877 individuals in all 26 XX males were segregated out, namely 13 with *Fla* and 13 without.

Unfortunately the numbers for XX males are only small. But as *Flavus* and non-*Flavus* males appear in the same number the new X chromosome cannot in the potency of its sex-determining gene be so different from the genes already present as to influence sex determination.

2. THE "GOLD" GENE

The yellow *Lebistes* has been described by Haskins and Druzba (1938), and more thoroughly by Goodrich, Josephson, Trinkaus and Slate (1944). These authors found the yellow to be due to a recessive autosomal gene. In males as well as females of this race the whole body has a translucent yellow tone. This yellow *Lebistes* is designated as "fredlini" among fanciers. Goodrich *et al.* prefer to designate it as *Gold*, and they employ the symbols *GG* = Wild Type, *gg* = *Gold*. They have given a description of the effect of the gene on the chromatophores, chiefly the melanophores. In Wild Type the numerous melanophores form a "diamond-shaped pattern within the meshes of which are seen other somewhat more sparsely and irregularly

distributed melanophores." The pattern results from the fact that the chromatophores follow the scales. In the *Gold Lebistes* the melanophores are far fewer in number, larger in size and limited more exclusively to the net pattern; the newborn *Gold* has no visible melanophores. Counting of the melanophores gave about half as many in *Gold* as in Wild Type. Goodrich *et al.* have also described two other recessive types with defective melanophores, which they designate as "Blond" (*bbGG*) and the double-recessive "Cream" (*bbgg*).

In general our studies, which were carried out independently of these investigations, gave the same result. Our laboratory obtained its material, designated as "fredlini," from the Malmö Aquarian Society in Sweden through the courtesy of Mr Edvin Brorsson, teacher, Chairman of the Society.

The difference between the *Gold* strain and the normal-coloured is already conspicuous at birth, the normal being born with numerous melanophores on the body, while the *Gold* is entirely lacking in visible melanophores in the skin (see fig. 7). Gradually, as the *Gold* fish grow up, they get a certain number of melanophores on the upper part of the body. When by means of a knife a scale is removed cautiously from the back of an adult fish, a little of the epidermis will adhere to the posterior free part of the scale, and under the microscope this gives a fairly distinct picture of the chromatophores—though not of those that are situated most deeply, as they are not removed by the detaching of the scale. Figs. 8 and 9 show scales of two females who measured 36 and 40 mm. in length—a normal type and a *Gold*. The difference in the number of melanophores is very striking. The xanthophores are somewhat more numerous in the *Gold* fish than in the normal. Counting of melanophores and xanthophores on the scales was commenced but given up again, as it was found to be very

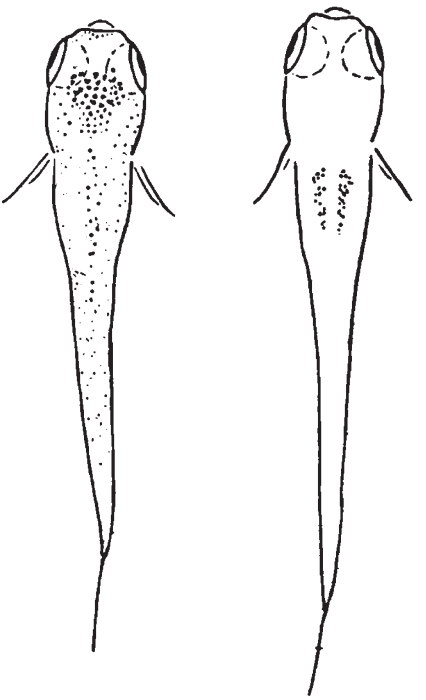


FIG. 7.—To the left, newborn normal (grey) young; to the right newborn *Gold* young—for illustration of the melanophores. The *Gold* young possesses no visible melanophores in the skin, but a few melanophores on the surface of the air bladder. (Enlarged.)

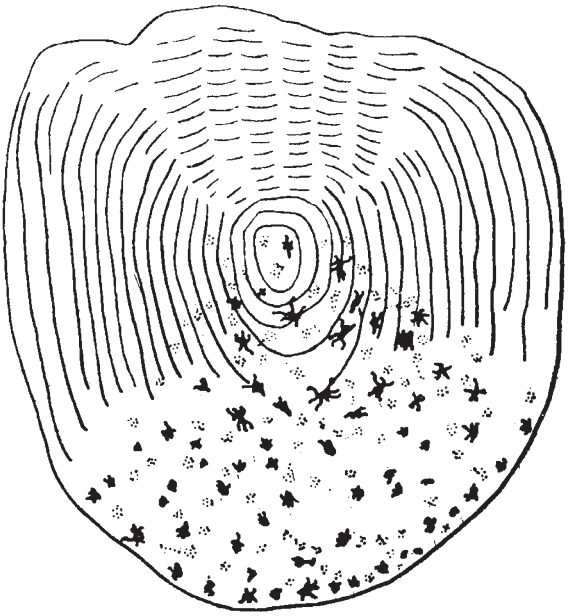


FIG. 8.—Scale of normal (grey) female (Wild Type) with the free posterior margin pointing downwards. Melanophores black, xanthophores dotted. (Greatly enlarged.)

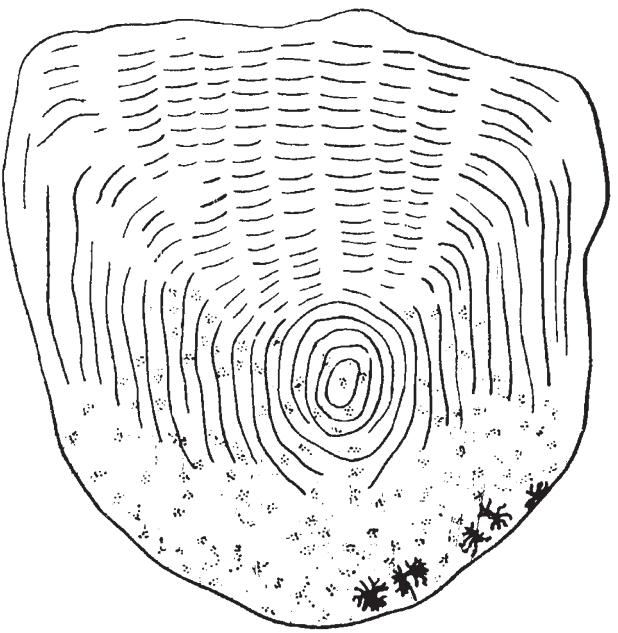


FIG. 9.—Scale of *Gold* female (*gg*) with the posterior margin pointing downwards. Melanophores black, xanthophores dotted. (Greatly enlarged.)

difficult to count the xanthophores quite accurately; a few exact counts are given in table 1.

TABLE 1

Melanophore and xanthophore counts in Gold *and normal females*

		M	X
Gold ♀ No. 1.	Scale No. 1	16	110
	,, ,, 2	2	101
	,, ,, 3	1	70
	,, ,, 4	0	62
Gold ♀ No. 2.	,, ,, 1	20	117
	,, ,, 2	19	160
	,, ,, 3	26	90
	,, ,, 4	2	77
	,, ,, 5	27	156
Gold ♀ No. 3.	,, ,, 1	6	129
		119	1072
Gold average		*11·9*	*107·2*
Normal ♀ No. 4.	Scale No. 1	48	109
	,, ,, 2	53	79
	,, ,, 3	67	81
	,, ,, 4	32	60
Normal ♀ No. 5.	,, ,, 1	70	99
	,, ,, 2	59	101
	,, ,, 3	36	41
Normal ♀ No. 6.	,, ,, 1	64	79
		429	649
Normal average		*53·6*	*81·1*

The outcome of our genetic experiments is quite in keeping with the findings reported by the authors cited above—that *Gold* is due to a recessive autosomal gene. Crossing of *Gold* with normal (= Wild Type) gives exclusively normal in F_1.

Crossing of F_1 individuals mutually gives 3 normal : 1 *Gold*.

(*a*) F_1 of normal ♀ × *Gold* ♂ crossed together (♀ 2098 × ♂ 2099) gave

normal		*Gold*	
24 ♀♀	27 ♂♂	13 ♀♀	7 ♂♂

(*b*) F_1 of *Gold* ♀ × normal ♂ crossed together (♀ 2303 × ♂ 2304) gave

normal		*Gold*	
9 ♀♀	20 ♂♂	6 ♀♀	10 ♂♂

When to this we add some young ones that were not countered for sex, besides the outcome of a cross in which the male was XX, we have altogether :

(Total, 217)	164 normal	53 *Gold*
(Theoretically :	163 ,,	54 ,,).

Gold ♀ crossed with normal F_1 ♂ (from *Gold* ♀ × normal ♂) gave :

normal		*Gold*		
27 ♀♀	19 ♂♂	24 ♀♀	1 XX ♂	25 ♂♂

As the laboratory had a strain with the autosomal gene *Zebrinus* (*Ze*), which is dominant in the male while it does not manifest itself in the female (*cf.* Winge, 1927), it seemed natural to let this gene get together with *Gold* in order to see whether the two genes were located in the same pair of chromosomes. For this purpose, normal F_1 males were produced heterozygous for *Gold* as well as *Zebrinus*. *Gold* ♀ × normal F_1 ♂ with *Ze* (*ggzeze* × *GgZeze*) gave, considering only the males

	normal		*Gold*	
	with *Ze*	without *Ze*	with *Ze*	without *Ze*
(Total, 50)	9	18	12	11

In cases of free combination we would expect 12·5 : 12·5 : 12·5 : 12·5. If, on the other hand, the two genes belonged to the same pair of chromosomes, and if no crossing-over took place, we would expect to get 25 normal with *Ze* and 25 *Gold* without *Ze*.

Normal F_1 ♀ without *Ze* × normal F_1 ♂ with *Ze* (that is *Ggzeze* × *GgZeZe*) gave :

	normal		*Gold*	
	with *Ze*	without *Ze*	with *Ze*	without *Ze*
(Total, 30)	9 (11·25)	11 (11·25)	6 (3·75)	4 (3·75)

Here, too, we meet with a free combination, and thus we may take it that *Gold* is not located in the same chromosome pair as *Zebrinus*. As the chromosome which contains *Zebrinus* already previously has been designated (Winge, 1927) as No. 1, it might be appropriate to designate the chromosome pair that contains the *Gold* gene as No. 2. Whether the gene *Blond*, described by Goodrich *et al.*, is located in chromosome 1 or in another pair of chromosomes cannot be settled of course before *Blond* also has been crossed with *Zebrinus*.

In the *Gold* Y_{Ma} males as a rule the *Maculatus* pattern is not typical as the black spot in the dorsal fin most often is absent—a fact that has been pointed out also by Haskins and Druzba.

Of 70 yellow Y_{Ma} males in our experiments only 7 had a really black spot on the dorsal fin, while 2 showed a trace of a spot, and in all the rest the dorsal fin was practically colourless. Naturally, it is not very surprising that a gene with such a strong effect on the melanophores has influence also upon the *Maculatus* pattern. So far no rule has been ascertained for the occasional occurrence of the spot on the dorsal fin.

Nor is the *Zebrinus* pattern quite typical in the *Gold* males even though it can be made out here.

3. THE "MACULATUS" GENE

In all the numerous crossing experiments carried out by us with the *Maculatus* gene, it has always appeared as a unit. In 1927 some selection experiments were reported with this gene, in which males with a particularly insignificant black spot on the dorsal fin were selected through several generations, and in this way some X_OY_{Ma} males were obtained in which the black spot was altogether absent. Still, one and the same male was able with one female to give spotless sons, with another female, spotted sons. So this phenomenon was undoubtedly ascribable to the interaction of minor factors affecting the spots, not to any change in the *Maculatus* gene itself. In *Gold* males with *Ma* a distinct spot on the dorsal fin is most often absent, but this indeed is due to the defective development of melanophores in general, and the spot reappears on crossing with normal. In 1933, however, an instance of a truly hereditary alteration of the *Ma* gene was recorded.

♀ 1662 × ♂ 1663
($X_OX_{Co,\,Vi}$) | (X_OY_{Ma})

16 ♀♀

22 ♂♂
- 1 $X_OX_{Co,\,Vi}$
- 11 $X_{Co,\,Vi}Y_{Ma}$
- 9 X_OY_{Ma}
- 1 (♂ 1768) with only a part of *Ma*

♂ 1768 (see fig. 10) had a red spot on the side, but no trace whatever of any spot on the dorsal fin. On crossing with X_OX_O

FIG. 10.—♂ 1768, X_O $Y_{Part\ of\ Ma}$ and brother with normal *Ma* pattern.

females he gave 8 daughters and 11 sons, all of which quite resembled the father. Of the 11 sons 2 were mated with X_OX_O females and gave respectively 20 and 16 sons, all without any trace of a spot on the dorsal fin, just like ♂ 1768. A daughter of ♂ 1768 was crossed with a normal X_OY_{Ma} ♂ and gave 17 sons, which all showed a normal *Maculatus* pattern with a well-defined black spot on the dorsal fin.

In order to preserve the altered *Maculatus* conveniently, some daughters and sons of ♂ 1768 were placed together in a tank, so

that they would be able to maintain themselves by inbreeding. This strain kept constant from 1935 to August 1941, when 6 males were drawn ; they all presented the same appearance as ♂ 1768. There can be no doubt that here we are dealing with a true hereditary alteration of the *Maculatus* gene. Unfortunately, indeed, it cannot be decided whether this represents a mutation or a cross-over.

Blacher (1928) regards *Ma* as composed of at least two genes, one producing the black spot in the dorsal fin and the other producing the red side-spot on the body. The correctness of this interpretation has not, however, been demonstrated by him through cross-over or other experiments but only through the observation of few individuals with deviating colour pattern. Such variants, of course, do not allow us to draw any conclusions as to the existence of a gene or a gene complex.

4. SEX DETERMINATION

A thorough account of the sex determination has been given in a previous paper (Winge, 1934). As pointed out at that time, we have to assume that sex-determining genes—some pulling in a female direction, others in male—are distributed over a great many of the autosomes, with superior sex genes in the X and Y chromosomes. Accordingly the XY females would be individuals that contain a lot of autosomal sex genes pulling so strongly in the female direction that, in spite of the presence of the Y chromosome, the development has yet been female. Conversely, the XX males would have a lot of autosomal sex genes pulling sufficiently in the male direction.

Goldschmidt (1937) has criticised our theory and tried to make the experimental data comply with the older conception of sex determination, employing the old familiar formulas, MMFF in the female and MMF in the male, where F is stronger than M but weaker than MM. The appearance of the XX male would then be due to the circumstance that (either by presence in the stock, or by mutation, or by appropriate crosses as in *Lymantria*) there was introduced either a weak F or a strong M. In his detailed account, Goldschmidt employs the formula MMF_wF_w for the XX male, in which, however, M is assumed to be constant and hence is left out. He then gets :

1. Accidental female with XX male :

$$FF \times F_wF_w = \text{all the young } FF_w = ♀♀.$$

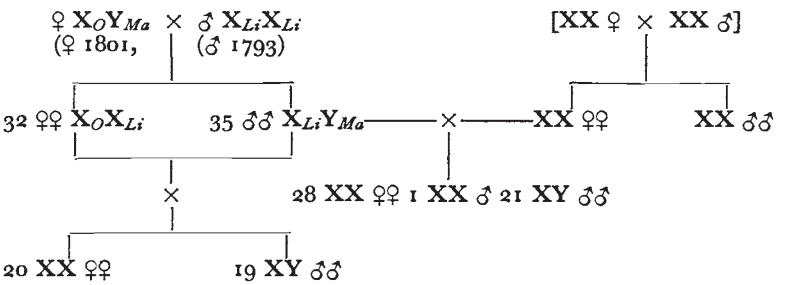
2. Back-crossing :

$FF_w \times F_wF_w = FF_w$ and $F_wF_w = 50$ per cent. ♀♀ and 50 per cent. XX ♂♂.

Here, however, two facts must be remembered. Firstly, it was not at all the first back-cross that yielded 50 per cent. XX males ; after the second back-cross only 1 XX male turned up among 31 individuals and only the third back-cross resulted in 50 per cent. of either sex. Secondly, we found no sex heterozygosity with regard

to the X chromosome. It had just been demonstrated by experiment that in the XX race the inheritance of the colour genes associated with the X chromosome is not sex-linked. In the XX race the X's are to be regarded as autosomes (*cf.* Winge, 1932). Goldschmidt tries to explain the XY females correspondingly, wishing to parallel both cases to *Lymantria*. It was therefore of interest to see what might be the result of crossing an XY female with an XX male.

A female of the formula $X_O Y_{Ma}$ (♀ 1801) was crossed with a male of the formula $X_{Li}X_{Li}$ (♂ 1793), and two of their sons were crossed with females of the XX strain. In addition a son and a daughter of ♀ 1801 × ♂ 1793 were crossed together. Slightly schematised this experiment looks as follows :—



When in this pedigree the detailed formulas have not been given it is because several genes (*Co*, *Vi*, *Ti*, *Lu*) were introduced from the XX race, which gave a rather complicated segregation including crossing-over. The main point is that the Y chromosome was marked with *Ma*, so that XX and XY individuals could easily be distinguished.

The present results agree fully with the assumption of multiple sex genes in the autosomes and particularly strong sex-determining genes in X and Y. On crossing an XY female, possessing a particularly strong female autosomal set of genes, with an XX male which has a particularly strong male autosomal set of genes we get an F_1 with marked heterozygosity. The strongly female autosomes from the mother meet with a set of strongly male autosomes from the father, and these two sets balance each other so that the sex determination now depending on the X and Y chromosomes becomes normal both in the sons and in the daughters.

It was to be expected that the sex determination would be normal also in F_2, at any rate as long as the number of individuals was not very great ; it is not surprising that a single XX male may segregate out when an F_1 male is mated to a female with strongly male autosomes.

On the other hand, if we were to explain the abnormal sex types by assuming variation in the strength of a single gene we would undoubtedly have to expect a segregation of XY females and XX

males in F_2. If, as suggested by Goldschmidt in a private communication to the senior author in 1935 concerning the XX males, as in the case of *Lymantria* we were to introduce a plasmatic factor **M** and assume this to vary from one race to another and that a particularly weak **M** was present in the XY female, we would have to expect that in the inbred F_2 one-half of the XY individuals would be females ; and on crossing an F_1 male with a female of the strongly male race we would have to expect one-half of the XX individuals to be males. But it does not turn out this way.

So we will have to maintain that the view of the sex determination advanced by us (Winge, 1934) is correct. The Y chromosome contains a strong male-determining gene, while the X chromosomes contain a female-determining gene, and sex-determining genes of varying potency are furthermore distributed over the autosomes in such a way that the sex determination normally depends on X and Y. XX males appear when, through recombination including crossing-over, particularly strong male-determining genes have accumulated in the autosomes ; and XY females appear when sufficiently strong female-determining genes have accumulated in an XY individual.

It will be appropriate here to recall that a quite similar distribution of the sex-determining elements has been found in *Melandrium* (Winge, 1931). In this plant, furthermore, the study of polyploid forms has shown that the Y chromosomes contain strong male-determining genes (Warmke and Blakeslee, 1939 ; Westergaard, 1940). According to Westergaard, in *Melandrium* the sex is determined by " a very strong male-determining element in Y and a female-determining element distributed in the X chromosome and in the autosomes. Moreover, at any rate, some of the autosomes must contain male-determining genes." As will be noticed, this corresponds rather closely to the distribution of the sex genes in *Lebistes*.

SUMMARY

1. Two genes in *Lebistes* are shown to manifest themselves in both the male and the female :—

(i) *Flavus*, appearing as a black and yellow colour pattern in the dorsal fin and caudal fin, is localised in the X chromosome. It is dominant in both sexes.

(ii) *Gold*, already described by Haskins and Druzba and by Goodrich *et al.*, characterised by a much smaller number of melanophores than in the normal Wild Type, is due to a recessive gene in an autosome pair other than the one that has formerly been shown to contain *Zebrinus*.

2. The *Maculatus* gene is shown to have undergone a change whereby the black spot on the dorsal fin completely disappears.

3. The outcome of a cross between the two abnormal sex types XY ♀ and XX ♂ agrees very well with the view previously advanced by the senior writer concerning the localisation of the sex genes.

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