THE CELLULAR EXPRESSION AND GENETICS OF TWO NEW GENES IN LEBISTES RETICULATUS

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THIS paper deals with the inheritance and phenotypic expression of two new pairs of alleles controlling color patterns in the cyprinodont fish, *Lebistes reticulatus*, commonly known as the guppy. Especial attention is given to the structures and arrangements of chromatophores as affected by the various gene combinations.

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The hereditary characters of Lebistes that have previously been analyzed are chiefly color characters, and most of these show X or Y chromosome inheritance or both—that is, crossing over between X and Y. For accounts of these see WINGE (1927), BLACHER (1927, 1928), GOODRICH (1929) (a summary) WINGE and DITLEVSEN (1938). Autosomal characters hitherto described include a color character Zebrinus (WINGE 1927), Abnomis (KIRPITCHNIKOFF 1935) (publication not accessible to us), Hunchback, which was studied by R. W. HARRISON and reported in abstract by GOODRICH *et al.* (1943). A character Fredlini, briefly described by HASKINS and DRUZBA (1938) has by personal conference been shown to be identical with our Golden and will be discussed below.

MATERIALS AND METHODS

These studies were begun in 1938. Our material has been obtained chiefly from M. MATSUNO; a fish fancier, who has been very helpful in supplying us with true breeding stock. Culture methods have been described by various writers (*cf.* WINGE 1927). Fertilization is internal, and sperm may remain in the female capable of fertilizing for eight months. A single mating will often serve for a number of litters that arrive at about 28-day intervals. It is therefore necessary to use virgin females. The sexes are separated for this purpose as soon as their characteristics can be distinguished, which is usually at about 48 days after birth (GOODRICH *et al.* 1934).

GENETIC ANALYSIS

The two mutant genes here reported are designated as Blond and Golden. After mutual comparison of stocks, DR. C. P. HASKINS has kindly adopted the above terminology by which Fredlini (HASKINS and DRUZBA 1938) becomes Golden. It was also shown that another strain present in his stocks is the same as our Blond. Our results show these to be independently assorting autosomal recessives, and this is confirmed by HASKINS's experiments (per-

sonal communication). Our genetic analysis will be first presented, followed by the detailed description of the phenotypes. Here it may be stated that Blond and Golden and the double recessive Cream are all lighter in color than the dark "Wild" type.

The evidence that Blond and Golden are each simple recessives is presented in tables 1 and 2, respectively. Counts were usually made within two days after

TABLE 1

Crosses of Wild×Blond

a. Segregation in F_2 from original cross Wild×Blond

(F_1 all, 77, Wild type)

	NO. OF MATINGS	TOTAL NO. OF LITTERS	WILD	BLOND	RATIOS
Observed	7	15	224	61	3.67:1
Calculated			214	71	3:1
b. Backcross (F_1 from Wild×Blond)×Blond					
Observed	9	14	182	155	1.17:1
Caclulated			168	168	1:1

TABLE 2

Crosses of Wild×Golden

c. Segregation in F_2 from original cross Wild×Golden

(F_1 all, 257, Wild type)

	NO. OF MATINGS	TOTAL NO. OF LITTERS	WILD	GOLDEN	RATIOS
Observed	6	15	230	74	3.1:1
Calculated			228	76	3:1
d. Backcross (F_1 from Wild×Golden)×Golden					
Observed	17	18	243	207	1.12:1
Calculated			225	225	1:1

birth, as is very desirable because of the high mortality of young fish. Sex cannot be distinguished at this time. Counts, however, of survivors showed an approximately equal distribution of sexes in each phenotype. For example, in the case of two litters raised to maturity from among those included in table 1b, from an original total of 53 born 41 survived, which were distributed as follows: 9 ♂, 12 ♀, Wild type; and 9 ♂, 11 ♀, Blond. In the case of the experiments on Blond progeny, tests by crossing with Blond stock were made to determine the existence of homozygous and heterozygous individuals in the wild type F_2. In five cases the fish was shown to be heterozygous, and in two homozygous. We have in no counts of survivors found an indication of sex linkage. Both the Blond and Golden stocks breed true. Our symbols for the

two pairs of alleles are B=dominant to Blond, b=Blond; G=dominant to Golden; g=Golden.

TABLE 3

Crosses of Wild×Golden

Segregation in F_2 from original cross Blond×Golden (F_1 all wild)

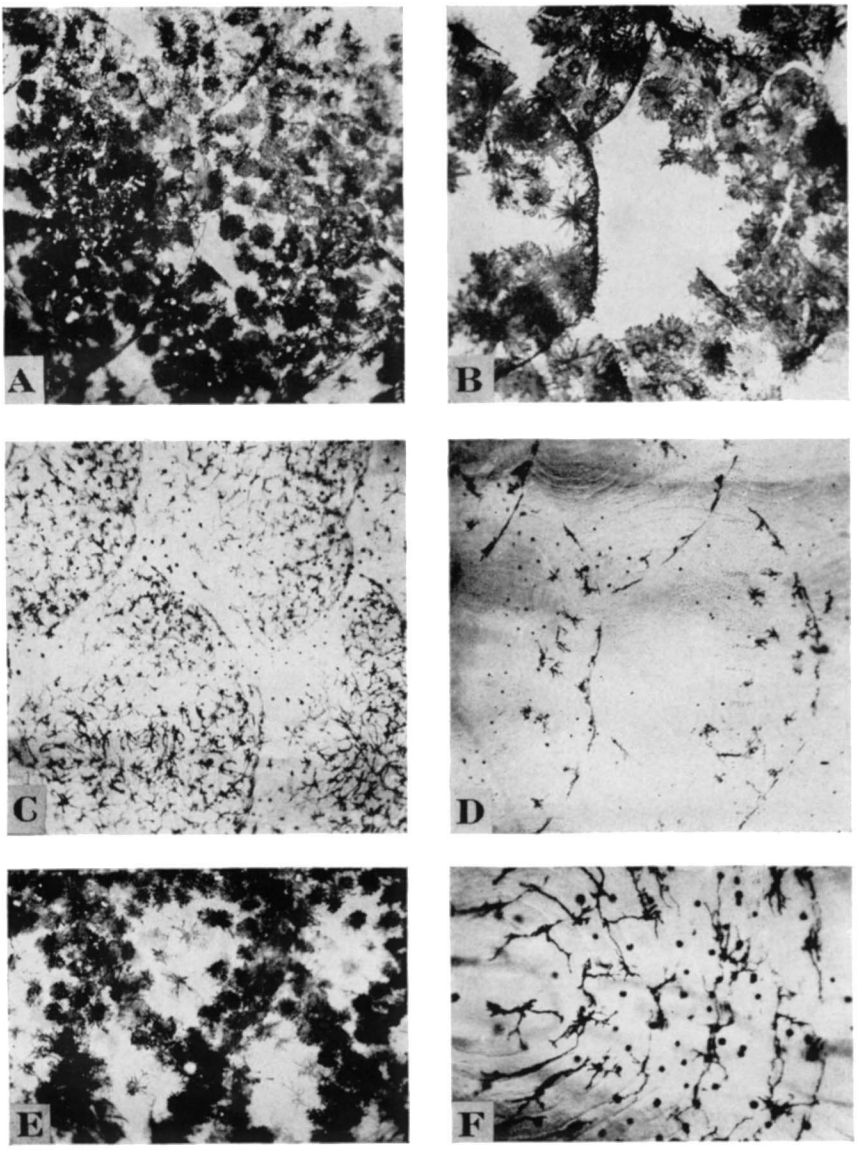
MATING NO.	WILD	BLOND	GOLDEN	CREAM	NO. OF LITTERS
122	27	9	11	1	3
123	30	10	9	3	6
124	4	3	2	1	1
126	43	19	14	3	6
133	48	13	13	4	5
135	43	16	9	7	8
136	16	6	5	1	3
137	16	4	8	1	3
138	65	18	19	4	7
139	19	10	6	2	5
141	23	13	16	4	3
142	14	8	8	4	4
143	16	3	4	1	5
144	14	5	6	2	3
Observed totals	378	137	130	38	62
Calculated distribution	384.3	128.1	128.1	42.7	

Blond and Golden

These two types were crossed, and the F_1 were all wild. The F_2 in each of 14 matings (62 litters) (table 3) produced Wild, Blond, Golden, and a new type, Cream, which gave a total of 378 Wild, 137 Blond, 130 Golden, and 38 Cream, or approximately the 9:3:3:1 ratio. One mating not included in the above total (our no. 125) gave all Wild (total of 9). While it is possible that this was due either to chance variation of the mendelian ratio or to an exceptional case requiring explanation, it seems more probable to us that it is an error in our mating records. F_1 wild type from the original cross have also recently been mated with Cream (the double recessive), and although numbers are as yet small, they confirm our interpretation. Two matings (three litters) have given total of 8 Wild, 11 Blond, 12 Golden, 8 Cream, which appears to be a first approximation of the expected 1:1:1:1 ratio. From these combined results we conclude that the two characters Blond and Golden are controlled by two independently assorting autosomal genes. In work as yet unpublished except in abstract (GOODRICH *et al.* 1943) R. W. HARRISON has shown that another gene, Hunchback, is autosomal and is also not linked to either Blond or Golden.

Cellular Analysis of Phenotypes

Our chief interest in this study lies in the comparison of the effect produced by the various gene combinations on cells and cell arrangements. The cells



EXPLANATION OF PLATE I

A–D Photomicrographs of cell pattern of the four phenotypes taken on dorsal midline slightly anterior to dorsal fin. ×43.

A, Wild; B, Golden; C, Blond; D Cream.

(C and D show clearly both dendritic and punctate melanophores).

E From side of body of Wild to show diamond pattern. ×39.

F From dorsal aspect of punctate type to show punctate and dendritic melanophores. ×80.

studied have been chromatophores—chiefly melanophores. Of these we recognize the following types or categories: (1) The dendritic melanophores (fig. 1 A–D). These are exceedingly irregular in form, and much variation is found. Most frequently there are two or three irregular processes proceeding from a relatively small cell body. They are practically always located in the dermis superficial to the scale and frequently may be lined up at the edge of a scale. (2) The corolla melanophores (fig. 1, E–F). These are so named because, in their configuration, they often resemble a composite flower with petal-like processes proceeding from a disc-like center (SUMNER and WELLS 1933). These are located in the dermis, beneath the scales. (3) The punctate melanophores (fig. 1, G–H). We believe these to be homologous with the corolla type for reasons mentioned later. They are small rounded cells, occasionally extending short processes. (4) The xanthophores. These are exceedingly delicate in outline and difficult to study.

The following are descriptions of the various phenotypes based on the appearance of the females. The female is considerably larger than the male and is uniformly colored, in contrast to the varied color patterns of the male. The distinctive color patterns of the male are due to sex-linked or Y chromosome genes (see WINGE 1927) and do not appear in the female except in cases of sex reversion, but the ground pattern of the male is essentially similar to that of the female. Our description is of the female pattern, or ground pattern, and is based chiefly on the dorsal aspect shortly anterior and posterior to the dorsal fin. There seems to be complete dominance. Variation in the heterozygote has not been observed. The Wild type (*B G*) female macroscopically is of a gray-brown color and is characterized by a diamond-shaped pattern (Plate I, E) within the meshes of which may be seen other somewhat more sparsely and irregularly distributed melanophores. On the dorsal aspect the coverage by melanophores is more uniform and the diamond pattern only dimly visible (Plate I, A). The diamond pattern is formed of the corolla melanophores (fig. 1, E), varying from 42μ to 119μ in diameter when expanded, and are located at the edges of dermal pockets in which the scales are inserted. From this it follows that they are normally observed on the body through the next anterior overlying scale. The irregularly distributed melanophores are of two types—the corolla type, deeply located beneath the scales, and the dendritic form (fig. 1, A) in the superficial dermis. Both types of cells are present at birth. Counts were made of numbers of melanophores visible in a measured area (0.9 sq mm) on various regions of the body (table 4), which gives an average of 120.1 per 0.9 sq mm.

The Golden (*B g*) female exhibits a more clear-cut but also more variable pattern than any of the other types. The difference is due to the combination of two factors: first, the larger size of both dendritic and corolla type melanophores (fig. 1, B, F), the latter varying from 70μ to 210μ in diameter; secondly, the melanophores are more definitely concentrated in the underlying diamond pattern and also above this at the edges of the scales, producing a superimimposed scalloped design which tends to accentuate the underlying diamond network. The interstices of the mesh are often clear of melanophores (Plate I,

B). The pattern may vary considerably in different parts of the body and in different individuals. This is especially noticeable in half-grown fish and gives a coarsely mottled appearance. In this respect the Golden type resembles certain conditions in the goldfish (GOODRICH and HANSEN 1931; GOODRICH and ANDERSON 1939). Neither type of melanophore is present at birth but usually appears during the first week thereafter. This is subject to considerable variation. Counts show only about 50 percent as many melanophores per unit area as in the Wild and Blond (table 4).

TABLE 4

Counts of melanophores per measured area (0.9 sq mm) in each of three regions of body in the four phenotypes.

LENGTH IN MM OF FISH TO CAUDAL PEDUNCLE																AVER.
		WILD *B G*														
		28	26	23	22	29	26	26	22	23	26	25	24	22	22	
Region*	A	142	121	157	137	77	97	116	101	76	130	79	131	112	144	115.7
	B	163	147	175	155	102	100	111	128	91	131	97	128	112	126	126.1
	C	181	151	147	139	80	91	118	103	86	109	94	122	113	124	118.4
																120.1
		BLOND *b G*														
Length		27	28	27	27	32	29	26	21	31	28	26	24	25	21	
Region*	A	116	140	111	152	89	65	125	140	149	86	155	112	106	109	118.2
	B	120	123	107	135	122	122	70	117	117	101	161	115	121	124	118.2
	C	143	158	115	166	111	97	86	118	150	120	142	139	106	139	127.8
																121.4
		GOLDEN *B g*														
Length		22	29	25	23	24	22	22	25	28	27	26	29	27	27	
Region*	A	70	64	75	69	32	49	58	57	32	61	38	75	60	79	57.7
	B	86	75	81	72	65	45	51	69	58	61	52	63	61	51	62.1
	C	84	91	65	64	42	40	51	46	52	50	48	64	65	61	58.7
																59.5
		†CREAM *b g*														
Length		25	30	23	20	29	32	27	26	24	30	29	31	22	23	
Region*	A	7	33	20	15	1	1	2	1	0	0	0	0	4	0	6.0
	B	14	27	14	8	0	1	2	1	0	0	0	0	0	0	4.8
	C	0	9	13	1	0	0	1	0	0	0	0	0	0	0	1.7
																4.2

* The regions in which measurements were made were all dorsal to the lateral line and as follows: A, posterior to operculum; B, dorsal to anus; C, 3 mm anterior to caudal peduncle.

† Counts on Cream were inadvertently made using a different magnification from other counts and the error not discovered until after death of fish used. As no other fullgrown fish were available, corrected counts based on comparative areas and corrected to the nearest whole number appear in table, except that the last two counts were made on two fish which have since then matured. For comment on variability in Cream see text.

The Blond (*b G*) female is of a uniform yellow shade. The melanophores are not visible macroscopically. The corolla type cells of the Wild are replaced by much smaller punctate cells (fig. 1, G; Plate I, C, F). These are found both in the diamond pattern beneath the scales and deeply located in the interstices of the mesh, a distribution identical with the corolla type melanophores in the Wild and Golden types. These cells as usually observed are concentrated, and only rarely and then usually only by special treatment (see below) have any of them been seen in the expanded or dispersed condition. Then they form one or two blunt short processes (fig. 1, G). The punctate cells vary from 7μ to 14μ concentrated and about twice this when dispersed. In addition there is the usual complement of superficial dendritic cells (fig. 1, C) but these average

TABLE 5

	WILD	GOLDEN	BLOND	CREAM
Genotype	*B G*	*B g*	*b G*	*b g*
Melanophores per unit area 0.9 sq mm	120.1	59.5	121.4	4.2
Diameter of corolla and punctate cells expanded	Corolla 42μ–119μ	Corolla 70μ–210μ	Punctate 7μ–14μ	Punctate 7μ–14μ

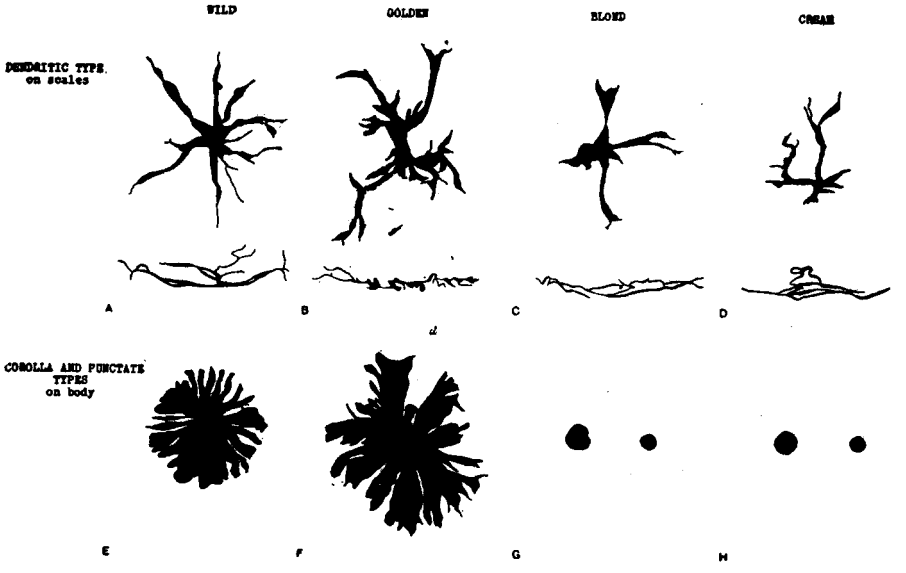


FIG. 1.—Forms of melanophores. Magnification same in all types, ×450. A–D Dendritic type: upper row, as they appear on central part of scale; lower row, at edge of scale. E–F Corolla type. G–H Punctate type. A E from Wild, B F from Golden, C G from Blond, D H from Cream.

smaller than those in the Wild or Golden types. The cell counts show a number comparable with that of the Wild form, the average per unit area being 118.1 (table 4). Both types of cells are usually present at birth. The xanthophores are fainter and probably smaller than those in the Wild.

The Cream female (*b g*) is a uniform warm yellow color and no chromatophores are macroscopically visible. The melanophores are most frequently few in number (Plate I, D) and sometimes are almost completely absent in a given area. They consist of small punctate cells (fig. 1, H) tending to follow the diamond pattern beneath the scales, while a few dendritic superficial cells (fig. 1, D) are located near the edges of the scales. Both types of cells may be present at birth. Recently we have found in our cultures some individuals having a larger number of cells, which, nevertheless, do not resemble Blond, since the cells are restricted to the edges of scales on the dorsal aspect of the body. Counts are given in table 4, and here the second and third listed cases are this latter type. There is also much variability in the different areas of a single fish. The Cream gives a positive reaction to the Dopa test, discussed below, indicating the presence of "colorless" melanophores and that therefore the fish may be light colored because of some disarrangement of normal metabolic processes. The Cream is not a hardy stock and is difficult to maintain in homozygous condition.

PHYSIOLOGICAL STUDIES

Dopa Reaction

Previous experiments (GOODRICH 1933) showed that *Oryzias latipes* responds positively to the 'Dopa' test, and since then it has been the practice in this laboratory to try the test on all light-colored mutants studied here, using Oryzias as a control. These have included varieties of the goldfish, *Carassius auratus*, *Betta splendens*, *Macropodus opercularis*. No other fish, however, had given a positive reaction until the test was applied to Cream. Tests on Blond and Golden were negative. In Cream, however, a few brown cells became visible having a configuration similar to other chromatophores. Dopa is 3:4 dihydroxyphenylalanine, which is considered to be a transformation product in the change from tyrosine to melanin (RAPER 1928). If cells show a darkening when tested, it has been interpreted as indicating that the necessary oxydase for the next step in melanin production is present but that the chromogen now supplied by Dopa had been absent. In our tests Oryzias was used as a control and gave the usual clear-cut reaction. The results in Cream were not so pronounced but nevertheless were sufficiently definite to reveal a few previously undetected chromatophore-like cells. The results have to be interpreted with caution, since work by FIGGE (1940) has indicated that under certain conditions of the reduction oxidation reaction many connective tissue cells may respond by melanogenesis. Here, however, the form of the cells seems to indicate that they were true melanoblasts. This then may indicate that the Cream fish is lighter colored either because the necessary chromogen is not present (GOOD-

RICH 1933) or because there does not exist the right oxidation-reduction potential for the enzyme to act (FIGGE 1940).

Chromatophore Reactions

GOODRICH and TRINKAUS (1940) reported that the melanophores in Blond apparently failed to show the usual responses to various stimuli and therefore might be considered as physiologically inert cells. These cells seemed to be unresponsive to light and dark backgrounds, denervation, intermedin, ergotamine, KCl, NaCl, to all of which the wild type melanophores readily respond by dispersion or concentration of pigment. Further tests by these and other agents have been made. It has been concluded that the dendritic superficial cells do clearly respond and that the punctate cells also show occasional reactions. These latter, however, are so small and the response so slight that they make very unfavorable material for nice observations. Many further experiments were tried, including electrical stimulation, injection of acetylcholine, etc. Since the results do not certainly show that the Blond cells are physiologically exceptional, the details of these extensive experiments are not reported here. However, it seems highly probable that the punctate cell is relatively sluggish in its reaction.

DISCUSSION

The charts (table 5, fig. 1) outline the most striking characteristics of the four phenotypes. It will be noticed that the Wild is in some respects intermediate between the Golden and Blond. The Golden has the larger melanophores, next is the Wild, and those of the Blond are the smallest. On the other hand, the melanophores are least numerous in the Golden type, there being about twice as many in the Blond and Wild. The Blond cells, especially the punctate type, are relatively sluggish in physiological responses compared with the Wild and Golden. The normal Wild type color pattern may be considered as a resultant of the interaction of the opposed tendencies of the two dominant genes. The double recessive Cream is relatively negative in its characteristics, both in regard to number and development of cells. The positive result of the dopa reactions suggests defective metabolism as a partial cause of the condition in the Cream type.

SUMMARY

The genetics of two new autosomal characters, Blond and Golden, in the guppy, *Lebistes reticulatus*, has been described.

The four phenotypes, Wild, Golden, Blond, and Cream, have been analyzed to show the differential effects of the various gene combinations on type, size, number, arrangement, and physiological reactions of the melanophores concerned.

The size, number, and arrangement of chromatophores in the Wild type appear to be in part the resultant of opposed tendencies of the two dominant genes.

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