THE INTERACTION OF GENES IN *LEBISTES RETICULATUS*

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THIS paper continues studies begun by GOODRICH *et al.* (1944) on the cellular analysis of certain genetic characters in *Lebistes reticulatus* (Peters). In the first paper the chromatophore patterns of two new autosomal recessive characters, blond and golden, and the double recessive, cream, were compared with the pattern of the wild type. Here a study is made of the effect of combining the maculatus gene with these autosomal genes. Maculatus was first noted by SCHMIDT (1920) and named with further description by WINGE (1922). This gene is carried by the Y chromosome and its most distinctive phenotypic effect is a conspicuous black spot on the dorsal fin of the male.

MATERIALS AND METHODS

Our stocks were supplied by kindness of C. P. HASKINS who had obtained them from WINGE. The stock we have used is also recorded as carrying the genes coccineus and vitellinus which are sex-linked ($CoVi_xMa_y$). These have been maintained in mass culture which has been necessary in order to provide sufficient numbers for experimental work. We also obtained a stock carrying maculatus only (O_xMa_y). But this has not supplied sufficient material for our use. SCHMIDT (1920) described the maculatus character as a "large black spot set in yellowish white." WINGE (1922) further noted the presence of a large red side spot below and in front of the dorsal fin and a black anal dot. Our analysis has been largely confined to a study of the cellular composition of the spot on the dorsal fin.

Our method has been to introduce the maculatus gene by appropriate crosses into the wild, blond, golden and cream stocks. The phenotypes were then studied and the presence of the maculatus gene was later confirmed by crosses to the wild stock. In all such cases the spot on the dorsal fin reappeared showing all typical characteristics. The details of these crosses are summarized in the accompanying table. As maculatus is a Y chromosome character only males are listed among the offspring.

DESCRIPTION OF PHENOTYPES

The characteristic wild (BG or $++$), blond (bG or $b+$), golden (Bg or $+g$) and cream (bg) are controlled by autosomal independently segregating genes (GOODRICH *et al.* 1944). It is important to note that the melanophores in these three types vary in size, form and number. The purpose of our experiments was to discover how the maculatus gene utilizes or reacts with the cell types controlled by the autosomal genes. A description of each phenotypic combination follows.

TABLE I

RECORD NUMBER	CROSS	TOTAL YOUNG	MALES MATURING
517	wild maculatus ♂ × normal wild ♀ +/+ +/+ O_x/ma_y × +/+ +/+ O_x/O_x	26 wild	8 wild maculatus (1) +/+ +/+ o_x/ma_y
542	(1) × normal wild ♀ +/+ +/+ O_x/ma_y × +/+ +/+ O_x/O_x	21 wild	10 wild maculatus +/+ +/+ O_x/ma_y
519	wild maculatus ♂ × blond ♀ +/+ +/+ O_x/ma_y × b/b +/+ O_x/O_x	30 wild	15 wild maculatus (2) +/b +/+ O_x/ma_y
522	(2) × blond ♀	82 wild	31 wild maculatus
523	+ /b +/+ O_x/ma_y × b/b +/+ O_x/O_x	80 blond	+ /b +/+ O_x/ma_y 44 blond (maculatus) (3) b/b +/+ O_x/ma_y
	(3) × normal wild ♀	65 wild	
529			27 wild maculatus
533			
541	b/b +/+ O_x/ma_y × +/+ +/+ O_x/O_x		+ /b +/+ O_x/ma_y
518	wild maculatus ♂ × golden ♀ +/+ +/+ O_x/ma_y × +/+ g/g O_x/O_x	35 wild	15 wild maculatus (4) +/+ +/g O_x/ma_y
533, 534	(4) × golden ♀	21 wild	11 wild maculatus
537, 538		37 golden	+ /+ +/g O_x/ma_y 16 golden (maculatus) +/+ g/g O_x/ma_y (5)
543	+ /+ +/g O_x/ma_y × +/+ g/g O_x/O_x		
539	(5) × normal wild ♀ +/+ g/g O_x/ma_y × +/+ +/+ O_x/O_x	76 wild	13 wild maculatus +/+ +/g O_x/ma_y
520	wild maculatus ♂ × cream ♀ +/+ +/+ O_x/ma_y × b/b g/g O_x/O_x	15 wild	6 wild maculatus (6) +/b +/g O_x/ma_y
525, 526	(6) × cream ♀	38 dark	6 wild maculatus
527		59 light	+ /b +/g O_x/ma_y 6 blond b/b +/g O_x/ma_y 2 golden +/b g/g O_x/ma_y 1 cream b/b g/g O_x/ma_y (7)
556	(7) × wild ♀ b/b g/g O_x/ma_y × +/+ +/+ O_x/O_x	80 wild	10 wild maculatus +/b +/g O_x/ma_y

Maculatus combined with wild

The dorsal fin of the wild type shows characteristic rosette type melanophores averaging from 70 to 120 micra in diameter. These are distributed so that although cell processes may be in contact the individual cells are clearly distinguishable (fig. 1).

When maculatus is introduced a dense accumulation of melanophores forms a spot covering from one half to three quarters of the area of the dorsal fin (fig. 2). Histological sections show this to be several cell layers in thickness in contrast to the single layer of melanophores of the wild type. The cells are so crowded that cell boundaries are merged. Even when the pigment is concentrated by treatment with adrenalin only a few peripheral cells become individually distinguishable. The melanophores appear to be both larger and more numerous than in the wild non-maculatus type. The black spot is surrounded by an area crowded with xanthophores and iridocytes. The presence of large numbers of xanthophores may be due to the coccineus vitellinus complex present in these crosses as noted above. By transmitted light this zone appears deep yellow but by reflected light it is bright bluish green.

Maculatus combined with blond

The dorsal fin of the blond type carries small rounded or "punctate" melanophores from nine to 15 micra in diameter (fig. 3). These are relatively evenly distributed and never form cell processes of any sort. It will be noticed in the table that when the male No. 2, heterozygous for wild and blond and carrying maculatus in the Y chromosome, is crossed with a blond female two male phenotypes are found as expected—the wild showing the maculatus spot and the blond without it. In this blond form there is no aggregation of melanophores of any type (fig. 4). Frequently, however, there is a massing of xanthophores and iridocytes in from one to three areas and melanophores are largely absent in these spots. There is such an area toward the middle of the right side of the fin in figure 4. A red spot on the side of the body is similar to that associated with maculatus but the anal dot is absent. WINGE (1922) noted that in the wild maculatus this dot was sometimes "invisible."

Maculatus combined with golden

In the golden the coralla type melanophores of the body region are larger and less numerous than in the wild form. On the dorsal fin, however, they seem to vary from 70 to 130 micra in diameter indicating that they are about the same size as in wild. The distribution on the body is most irregular, producing a variegated pattern. This condition also extends to the dorsal fin where the melanophores tend to accumulate in clusters, which sometimes (fig. 5) resemble the maculatus spot. When however the maculatus gene is introduced no change is produced. There is merely the same variability as in the golden type. Figure 6 which shows the fin from a fish carrying the maculatus gene is well within the range of variations of the golden without maculatus. Some with maculatus had no spots. The suppression of the maculatus spot was noted by HASKINS and DRUZBA (1938) who stated that the factor Fredlini (later identified as our golden) inhibited the expression of the maculatus gene. Some counts have indicated that there may be a smaller number of melanophores than in the ordinary golden type. Xanthophores and iridocytes are localized in the lower anterior quarter of the fin. Both the red spot and anal dot are pres-

ent on the side of the body. As in the case of blond the table shows two classes of males, wild maculatus and golden, appearing in the backcross with golden.

Maculatus combined with cream

Cream results from the presence of both golden and blond. The melanophores are few in number and chiefly of the punctate type. This is a very non-viable type and it has been difficult to carry through crosses to demonstrate the effect of the maculatus gene. In one case, however, it has been carried to completion and the progeny tested (see table 1). In this case the backcross of the heterozygous wild maculatus (6) with a cream female is expected to give four classes which were all realized but not in equal numbers probably due in part to differential viability. No effect of the maculatus gene while present in cream has been noted on the dorsal fin. The red spot, however, appears on the side of the body.

HORMONE EXPERIMENTS ON MACULATUS

A limited number of tests were made similar to those of previous investigators (see review by REGNIER 1938, and subsequent papers by BERKOWITZ 1937, 1938, 1941) to determine the relation of the maculatus spot to sex hormones. Female hormone (estradiol) was fed to the young of a litter from wild maculatus stock. All eight that survived to maturity showed female characteristics and no maculatus markings. The feeding of the hormone was then discontinued and two assumed male characteristics and the maculatus spot appeared. Thus it was indicated that in this litter there were six genetic females and two genetic males. The upper half of the dorsal fin of an adult male which was being fed with the female hormone was removed by a cut through the maculatus spot. The spot did not re-form when the fin regenerated above the cut and the part remaining below disintegrated.

The male hormone (methyl-testosterone) was similarly fed to a litter of young and at maturity all surviving were male. Of these some showed the spot which was absent in others. This result would of course be expected as the female genotypes would not carry the maculatus gene. Male hormone was fed to adult females of the maculatus stock. There was some disintegration of melanophores in the dorsal fin and brighter colors appeared but no formation of a spot. When male hormone was fed to adult males not carrying maculatus there was a deepening of colors but no formation of a spot. These tests seem to confirm the hypothesis that the female hormone suppresses the development of the maculatus spot and that possibly the male hormone is necessary for its formation.

DISCUSSION

The blond maculatus phenotype appears intermediate so far as the spot is concerned between the combinations with wild and with golden. The melanophores are not massed but the locus of the spot appears marked by an assemblage of xanthophores and iridocytes. This suggests that some morphogenetic factor may be in operation but that it does not utilize the type of melanophores

DESCRIPTION OF PLATE

Photomicrographs of dorsal fins magnified about $\times 14$ are here reproduced. Unfortunately it has not been possible to obtain all fins of the same degree of development and all similarly expanded. Also figure 5 shows an unusual form, probably due to a genetic modification, but the markings are typical.

FIGURE 1.—Wild.

FIGURE 2.—Wild maculatus.

FIGURE 3.—Blond.

FIGURE 4.—Blond maculatus. Lighter region right of center is area occupied by iridocytes that are only visible by reflected light.

FIGURE 5.—Golden—one variation.

FIGURE 6.—Golden maculatus—one variation.

that are available. It is hoped that studies in progress on the development and regeneration of the maculatus spot may give some information about factors that are involved. It appears, however, that the maculatus gene and those dominant to blond and to golden and the proper balance of hormones to control the secondary male sex characters must all be present to produce the maculatus spot.

We have noted that our maculatus stock carried the coccineus vitellinus genes (WINGE 1922). The principal effect on the dorsal fin is to produce more xanthophores. While this should be borne in mind we do not feel that it vitiates our essential results.

SUMMARY

1. Maculatus, a Y chromosome gene of *Lebistes reticulatus* which in the wild type produces a black spot on the dorsal fin, has been crossed into mutant stocks having different body color patterns of chromatophores controlled by autosomal genes.

2. Maculatus combined with blond produces no spot on dorsal fin but there is an aggregation of iridocytes and xanthophores.

3. Maculatus combined with golden produces no effect on the dorsal fin.

4. Hormone tests have been made which indicate that the female sex hormone can suppress the maculatus spot and that the male hormone may be necessary for its development.

5. The maculatus gene, the genes dominant to blond and golden, and the male sex determiners must be present to permit development of the maculatus spot.

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