THE DEVELOPMENT OF MENDELIAN CHARACTERS IN APLOCHEILUS LATIPES*

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The Japanese freshwater fish, *Aplocheilus latipes*, offers certain special advantages for the study of the development of Mendelian characters. A genetic analysis made by Aida[1] has shown a clearly defined Mendelian inheritance of color characteristics. Aplocheilus is oviparous while other fish of which such an analysis has been made are viviparous. Hence this is the only fish of which the genetics is in part known and which readily permits of a study of the development of Mendelian characters from fertilization to the adult condition. Also the color effects are produced by pigment cells which may be identified early in development.

The genetic constitution of the color types here considered may be indicated by the following formulae based on the analysis made by Aida:

Wild (homozygous)	BBYY
Yellow (homozygous)	bbYY
White (homozygous)	bbyy
Variegated (homozygous)	B'B'YY

Microscopic studies of the scales of these fish have shown that the colors are produced by three types of pigment cells. These are the melanophores, the brown chromatophores and the yellow chromatophores or xanthophores. The cells may also be differentiated by their characteristic form and by the relative solubility of the contained pigment. In ninety-five per cent alcohol the melanin is practically insoluble while the pigment of the brown chromatophores will dissolve in about eight minutes and that of the yellow chromatophores in three hours.

The Mendelian effects are due to the varied development of the melanophores and the xanthophores while the brown chromatophores remain nearly constant in all types. A study of the pigment cells of the wild type (BBYY) shows that the color, a greenish brown, is produced by the presence of both melanophores and xanthophores. Superficial inspection of the yellow type (bbYY) seems to indicate that only xanthophores are present. It is, however, possible by special treatment to show that melanophores with slight development of pigment are abundantly present. The method used was to remove scales from fish anaesthetized by chloretone and to treat these while fresh with a strong solution of adrenalin. This causes a concentration of pigment in cells rendering faintly colored cells more visible. These scales were then fixed in 95% alcohol which dissolved pigment in

cells other than the melanophores thus rendering confusion impossible. The scales were mounted in balsam and counts of the melanophores within a uniform measured area from each scale were made. The results showed that the melanophores were present in the scales of the yellow fish in numbers comparable with those of the wild type. Similar studies of the white fish (bbyy) demonstrated the presence of melanophores in abundance. These observations are summarized in the chart, figure 1, in which are plotted the results of counts of chromatophores from 100 scales of each type of fish obtained from the region of the pectoral fin. Here it will be noted that the modal class of chromatophore counts is the same for all three types but that more scales with larger counts are found among the yellow and white types than in the wild type. Apparently the large size of the in-

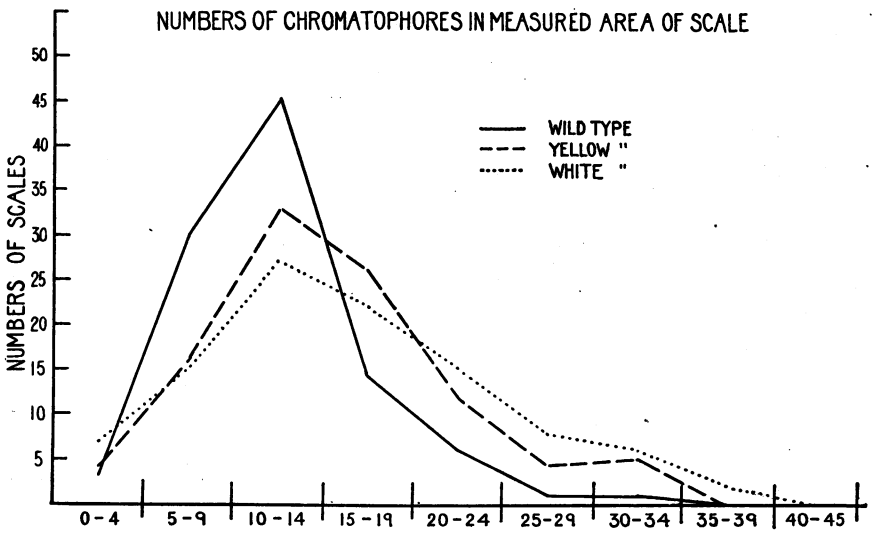

FIGURE 1

dividual cells in the wild form limits the number which occupy a given area. Also an examination under oil-immersion lens of fresh scales from white fish revealed the presence of a network of cells similar in position, form and size of granules, to the xanthophores of the yellow and wild types but usually without any yellow pigment. It is thus indicated that the Mendelian gene produces its effect, not by controlling the number of pigment cells produced but by regulating the amount of pigment in cells always present.

Studies on earlier stages in the life history have been made. Young fish were examined shortly after hatching and counts of melanophores visible on the dorsal surface were made. The earlier counts seemed to indicate that the color effects were correlated with different numbers of

chromatophores rather than with the development of pigment in cells. The wild type showed the greatest number of melanophores, the variegated less and the yellow the least. Here again, however, treatment with adrenalin revealed that there were approximately equal numbers of melanophores in all three types. The contraction of pigment in cells otherwise invisible showed their presence in areas which previously seemed unoccupied.

Observations on embryonic stages have shown that in the case of crosses between wild types heterozygous for the black factor (BbYY) and yellows (bbYY) that the Mendelian ratios could be distinguished at two days after fertilization or at the stage of about eleven somites. Examples of this are the counts of eggs obtained from an aquarium containing one wild (heterozygous) male and two white females. Of 206 eggs 104 showed pigmentation and 102 were unpigmented. Table 1 shows counts of melanophores in eggs of a single spawning from a similar mating.

TABLE 1

COUNT OF CHROMATOPHORES FROM SINGLE BATCH OF EGGS THREE DAYS AFTER FERTILIZATION FROM CROSS OF YELLOW FEMALE BY WILD MALE (HETEROZYGOUS)

Body region	22	20	16	20	25	24	12	19	20	0	0	0	0	0	0	0
Tail region	25	22	14	27	19	22	24	20	38	0	0	0	0	0	0	0
Yolk (dorsal)	34	20	33	40	27	37	30	32	35	0	0	0	0	0	0	0
Yolk (ventral)	34	24	36	27	28	60	32	32	20	1	0	0	1	0	2	0
Total	115	86	99	114	99	143	98	103	113	1	0	0	1	0	2	0

As far as observations have been pushed back in embryonic history the three types of pigment cells remain distinct. There has been no indication that one type may arise from another. This indicates that the differentiation must take place at a stage in cell lineage proceeding the formation of pigment.

Studies of scales of variegated fish show results in harmony with those of the preceding types discussed. Faintly pigmented melanophores are present in the light areas in numbers equal to those of the more deeply pigmented types in dark areas. This condition seems to indicate a physiological differentiation between the darker and the lighter melanophores, that is, that some types possess a greater melanin-producing power than others. This lends weight to the suggestion that a similar distinction exists between the melanophores of the wild and the yellow-white types. A hormone activity might account for the latter case but could hardly be construed to be a complete explanation of the development of a variegated pattern as hormones would be carried to all parts alike.

All of the observations seem in harmony with the hypothesis that the Mendelian gene produces its effect not by controlling "division energy," regulating numbers of cells but by controlling the amount of melanin

to be elaborated in cells always present and further, that the definitive action is at some stage in cell lineage preceding the earliest appearance of pigment.

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[1] Aida, T., *Genetics*, Baltimore, 6, 554–73 (1921).