GENETICS OF THE SIAMESE FIGHTING FISH, BETTA SPLENDENS[1]

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BETTA SPLENDENS more commonly known as the Siamese fighting fish has been popular in aquariums of western Europe and America for over 35 years. Its domestication and consequent inbreeding antedates the introduction into the West by 60 or 70 years. Selection for pugnacity, long fins (see Figure 1), and bright colors over this long period has produced a number of phenotypes, none of which is very similar to the short-finned wild form from the sluggish rivers and flooded rice paddies of Thialand (SMITH 1945).

The aquarium Betta is noted for its brilliant and varied colors. These are produced by three pigments, lutein (yellow), erythropterin (red), and melanin (black) (GOODRICH, HILL and ARRICK 1941) and by scattering of light through small hexagonal crystals (GOODRICH and MERCER 1934) giving steel blue, blue, or green. Each kind of pigment is contained in a distinct cell type, xanthophores, containing yellow, erythrophores red, and melanophores black. There are no chromatophores containing two pigments such as the xanthoerythrophores of *Xiphophorus helleri*. The reflecting cells responsible for iridescent blues and greens are known as iridocytes or guanophores and they are more superficial than the other chromatophores.

Since the pigment granules may be greatly dispersed in the many branched pseudopods or clumped into a small knot in the center of the chromatophores, the color of any single fish may vary over a wide range of shades, and may do so in a matter of seconds. Frightened or sick fish are quite pale with no dispersion of pigment whereas fighting and courting fish are at the other extreme.

In Bettas there seems to be an almost complete continuum in shades from light brown to almost pure black. The same condition exists with respect to red and also to the amount of iridocyte produced color. This almost infinite variety of phenotypes makes the separation of spawns into classes difficult, but examination of many large spawns has shown that with the "correct" criteria and method of examination the apparent continuum falls into a few large blocks within which, however, there still seem to be continua. The differences between the large groups are traceable to the segregation of a few genes which we may call major ones.

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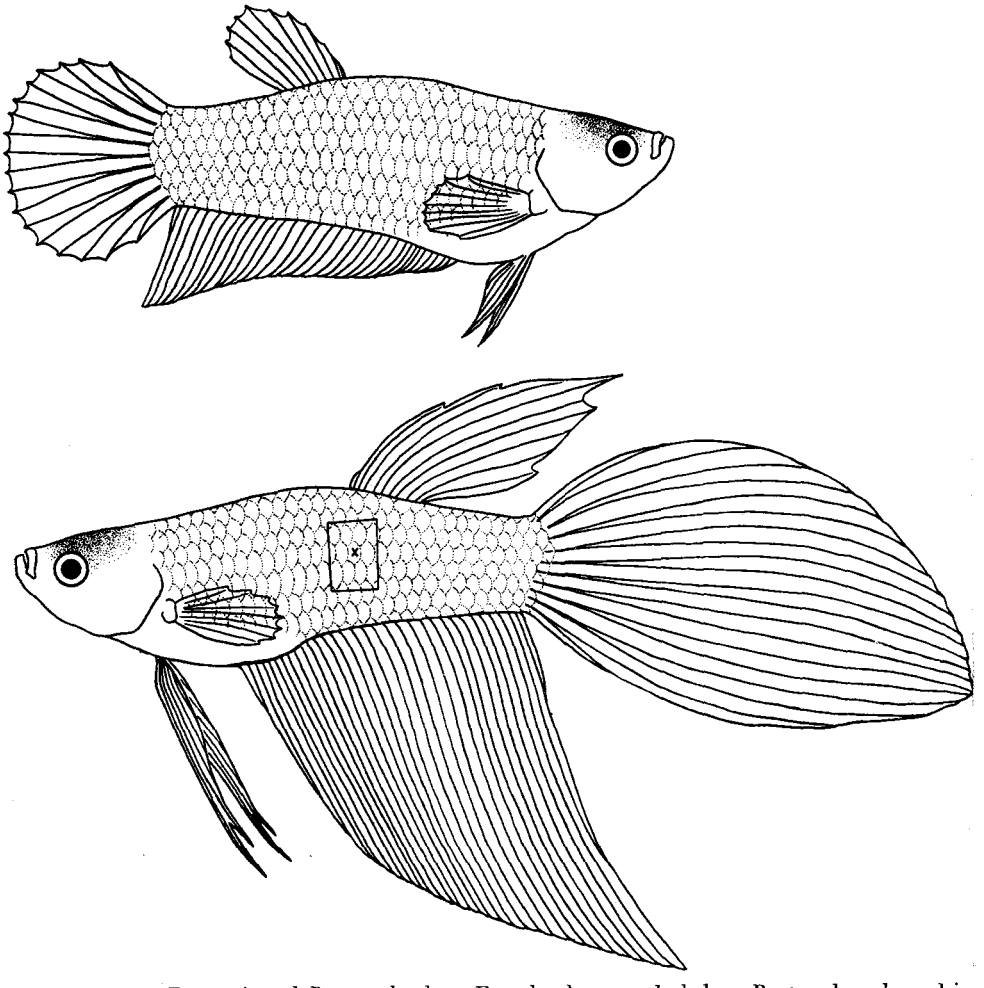

FIGURE 1.—Domesticated *Betta splendens*. Female above, male below. Rectangle enlarged in Figure 2. x = scale over standard area for counting melanophores.

The apparent continua within the large groups are very likely due to a number of minor factors but so far this has not been adequately tested.

MATERIAL AND METHODS

If light is reflected from the iridocytes at an obtuse angle rather than approximately 90°, green may look blue and blues look purplish. Hence the "correct" method of examination is to light the aquarium from the front. The "correct" criteria include classifying any specimen that shows green at any angle, green even though it may seem blue with other lighting since blue fish never appear green. Steel or dull blue is not easily confused with green or (bright) blue. In fish with few or no guanophores on the body and fins, a fleck of iridocyte produced color can nearly always be detected at the base of the eye.

In order to obviate the difficulties of color classification due to movement of pigment granules it seemed advisable to make counts of pigment cells. If a scale is removed from the body (Figure 2), the covering of epidermis and dermis on

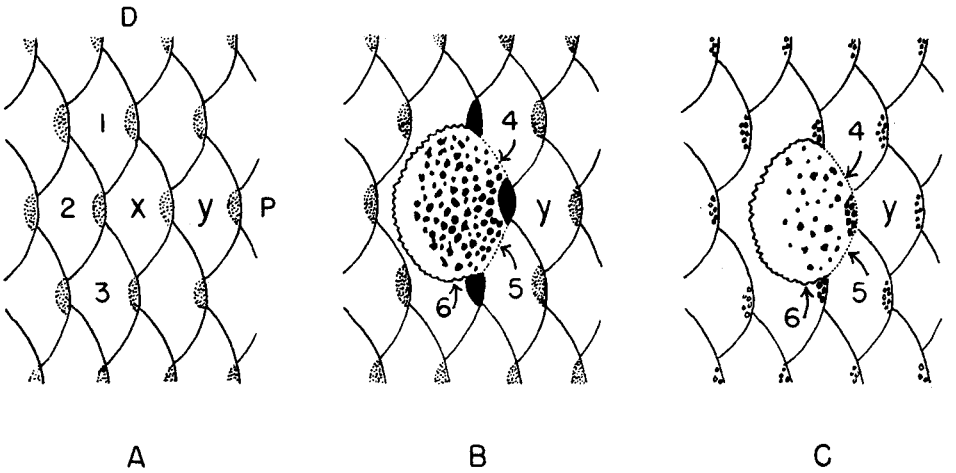

FIGURE 2.—Enlarged view of scales within rectangle in Figure 1. A. Fish of genotype *C–B–* with all scales intact. Chromatophores other than those of the intermediate zone have been omitted. B. Same fish as in A but with scales 1, 2, 3, and x removed. C. Fish of genotype *C–bb* with scales 1, 2, 3, and x removed. In B and C melanophores of the deep zone under scale x have been added. D = dorsal, P = posterior. x = scale over the standard area for counting, see Figure 1. The postero—dorsal and postero—ventral limits of the standard area are designated by arrows in scales 4 and 5. These limits are determined by the line of emergence of scales 4 and 5. Arrow 6 designates edge of dermis which has been torn away to reveal anterior limit of melanophores in standard area.

the free (posterior) portion remains intact. These layers over the anterior part remain on the body below the next more anterior scale. The line at which the separation of the dermis takes place is quite precise and corresponds to the line at which the scale emerges from the stratum of fat cells. GOODRICH and SMITH (1937) in making counts of chromatophores in the paradise fish designated the dermis with its chromatophores which remain on the scale upon removal from the body, the superficial zone. The dermis which remains on the body is divisible into intermediate and deep zones. In the Betta the intermediate zone is so heavily pigmented with corolla or large punctate type melanophores (see GOODRICH *et al.* 1944) that in most phenotypes individual cells cannot be made out and the presence of xanthophores and erythrophores cannot be detected.

The deep zone is well delimited, posteriorly by the heavily pigmented intermediate zone, dorsally and ventrally by folds of skin from which scales emerge and anteriorly by the lack of chromatophores deep below the next more anterior intermediate zone. Thus, anterior to each scale there is a deep zone of chromatophores which can be used as a unit area.

Since all counts were made on adult fish, measured areas were not used and chromatophores of the whole deep zone were counted in order to eliminate differences traceable to inhomogeneities within this area.

To eliminate the differences in the number of chromatophores per unit area which are dependent upon position on the fish, the fifth row of scales counting ventrally from the anterior edge of the dorsal fin was adopted as the standard region for counting (scale labelled x in Figure 1).

RESULTS AND DISCUSSION

A light bodied phenotype or group of phenotypes known as Cambodia has been traced to a single recessive gene by GOODRICH and MERCER (1934) and independently by UMRATH (1939). Fortunately, in both papers the recessive was designated *c* and the normal allele *C*. Work of EBERHARDT (1941) and WALLBRUNN (1948) corroborate the earlier findings. It is important, however, to clarify the nature of the action of this gene since the earlier papers state that it produces an "unvollstaendige Albino" or a "light pink bodied fish."

Cambodia (*cc*) males are occasionally as red as any *C* fish with the only difference being a suppression of black on both the body and fins. Cambodias are not albinos since the eye is fully pigmented with melanin. True albinos are known in the Betta but they are either blind or nearly so, which makes both survival and spawning very difficult; hence no albino strain has been established and the relationship between albinism and the *C* locus has not been tested.

In some strains of Cambodia a considerable amount of black forms on the bodies of adults as a very regular series of dots. Careful examination reveals these to be due to melanophores in the intermediate zone but never in the deep zone and only rarely in the superficial. This black spotting on Cambodia is apparently inherited but has not been investigated because of the irregular time of appearance, sometimes developing only after the rather advanced age of 18 months.

Red develops considerably later in ontogeny on Cambodias than on dark fish and female Cambodias never develop red on the body although their fins are as red as those of males.

In 1948, a brilliant red male Betta was purchased by the author. Phenotypically this fish seemed to be intermediate between *cc* and *C*–. In a test for allelism with *c*, the male was mated to a Cambodia female completely devoid of black on the body. Their offspring (spawn #100) were quite uniform in body color. Seventy-three fish grew to maturity and they all were deep red, much darker (contained more black) than their father (Table 1).

The backcross (#105) of a dark female from #100 to her bright red father gave the two shades of red in the ratio of 1:1 (22 dark: 13 bright); while the F_2 (#107) gave 16 dark red:8 bright red:11 Cambodia. If the three spawns (#107, 109, and 152) in which the three colors, dark red, bright red, and Cambodia, appear from matings of dark × dark, are taken together, the totals are 106:33:49 which is unusually close to 9:3:4 (105.8:35.2:47.0). This would also fit the 2:1:1 ratio but

TABLE 1

Inheritance of bright red (bb)

Spawn No.	Phenotype		Parental Genotype (deduced)		Phenotypes of offspring		
	Male	Female	Male	Female	Dark	Bright	Cambodia
100	Bright	Cambodia	*CCbbVVriri*	*ccBBVvriri*	73	0	0
101	Bright	Dark	*CCbbVVriri*	*CcBBVVRiRi*	13	0	0
103	Dark	Dark	*CCBBVVriri*	*CcBbVvriri*	69	0	0
105	Bright	Dark	*CCbbVVriri*	*CcBbVvriri*	22	13	0
106	Bright	Bright	*C–bbVvriri*	*C–bbVVriri*	0	11	0
107	Dark	Dark	*CcBbVvriri*	*CcBbVvriri*	16	8	11
109	Dark	Dark	*CcBbVVRiri*	*CcBbVVRiri*	57	14	24
111	Dark	Bright	*C–BBVVRiri*	*C–bbVvriri*	84	0	0
152	Dark	Dark	*CcBbVVriri*	*CcBbVVRiri*	33	11	15
155	Cambodia	Bright	*ccbbVVriri*	*CCbbvvriri*	0	41	0
156	Bright	Bright	*C–bbVVRiRi*	*C–bbVvriri*	0	18	0
160	Bright	Dark	*CcbbVVriri*	*C–BbVvriri*	5	2	0
164	Bright	Cambodia	*CcbbVVriri*	*ccBBVVRiRi*	26	0	25
310	Bright	Dark	*C–bbVVRiri*	*C–BbVvRiri*	46	43	0

Some of the data from which the above genotypes were deduced were furnished by other matings.

mating #111, in which dark behaves as a homozygote, rules out the hypothesis necessary to account for such a ratio.

There is, therefore, evidence for a new locus with two alleles, the recessive of which when homozygous produces the bright red phenotype, provided *C* is present. Genotype *cc* is epistatic to the newly described alleles.

Within the bright red group, as well as within the dark red, there is a great variety of shades, but the two classes are quite distinct. Bright red has been used for want of a better term to designate the class, although some of the individuals covered by the term are not especially red; and the microscope reveals that some of the dark fish have as many erythrophores with as much pigment as the bright red fish. The difference between the two classes lies in the number of melanopores and/or the amount of melanin present. The new gene, therefore, reduces the amount of black and has been called *b*. *B* is the allele which produces the "normal" amount of melanin.

Within the deep zone of the dermis differences in the number of melanophores between *bb* and *B* may be very marked as shown by spawns #105 and 107 in Table 2. The size of the classes into which counts in this table are grouped increases as a geometric series. This was done to keep the relative errors in counting equal because absolute errors are a function of the size of the count.

In #109 the separation of bright and dark red by eye was easily made although counts in the deep zone come close to overlapping. It must be remembered that there are melanophores in the other zones which also influence the general color and these too are reduced in number in *bb* fish.

Examination of Table 2 shows that besides the alleles *B* and *b*, the residual

TABLE 2

Melanophore counts in the deep dermal zone of males

Number of melanophores	Spawn Number 100	101	103	104	105	106	107	109	110	111	112	113	152
0– 15							*1						
16– 18					*1	*1							
19– 22					*1		*3	*1					
23– 26								*1					
27– 31					*1	*1		*3					*1
32– 37					*3								*3
38– 45								*1					*1
46– 54								*1					*2
55– 64								*2					*1
65– 77								*2					
78– 93	2	1			1								
94–110		3			3		2	3	1				
111–132	5	2	6	1			1	1	1	2	1	5	2
133–159		1	2	2	1		2	5	2	2	3	4	2
160–191			6	3			1	12	1	2	2	1	
192–229			2	1				4			1		
230–265			1					1			1		
266–330								3					

* Belong to the bright red class; all others are dark.

genotype has a noticeable effect in governing the number of melanophores in the deep zone. There is the possibility that *Bb* causes a reduction as compared with *BB*, but #109 shows that if this is the case, the effect is not great enough to make a clear separation of the dark group into two classes.

UMRATH (1939) describes a red phenotype produced by a recessive gene, *m*. "Ein Zuruecktreten der Melanophoren" is caused by *mm*. The gene *m* may be the same as *b* but since UMRATH's description is fragmentary and his data equally so, there is no assurance that the two names apply to the same locus. If the color known to UMRATH in 1939 is the same as *bb*, it is strange that as late as 1949 fish fanciers in this country considered it rare, in view of the fact that new forms of aquarium fish are quickly imported by fanciers.

The median fins of *C–bb* Bettas have only a single line of melanophores around the free border. The remainder of the large fin areas is densely packed with large erythrophores but no melanophores. In *C–B–* fish a typical area of the caudal fin has about 40 melanophores per field under the microscope at high power (430×).

The overlying iridescent blues and greens for which the fighting fish is famous are traceable to two loci. One affects the density of the overlying iridocytes and the other the thickness of the guanine crystals and thus the particular color refracted.

UMRATH (1939) came to the correct conclusion that green and dull blue are traceable to the two homozygotes and blue to the heterozygote in spite of the very small number of spawns and specimens. WALLBRUNN (1948) corroborated

UMRATH's hypothesis and named the locus *G*. EBERHARDT (1941) had already named the locus *V* (viridens) but the war had kept his paper from reaching this country. *VV*(=*GG*) is the designation for steel blue fish, *vv*(=*gg*) for green and *Vv*(=*Gg*) for blue.

In some fish the iridocytes are so scarce that it is difficult to determine what color they produce. These fish are the homozygous recessives, *riri* (reduzierte Iridocyten) (EBERHARDT 1941). In some genetic backgrounds *riri* produces an iridescent spot on each scale making rows of green, blue or gunmetal dots. Never, however, does the homozygous recessive have extensive areas covered with iridocytes. EBERHARDT claimed to be able to distinguish *RiRi* from *Riri* by the amount of blue or green. He did not, however, separate them into two classes in his tables and the present author has had some heterozygotes as fully covered by iridocytes as any homozygotes. In the author's note (WALLBRUNN 1948) the dominant was designated *S* for sheen.

Tests for linkage

EBERHARDT (1941) found the three loci *C*, *Ri*, and *V* to be assorted randomly.

To test for linkage of *b* with *C* (Table 3) only spawns #107 and 109 can be used since these are the only cases in which the combinations of genes entering the parental zygotes are known. We may write the genotypes of the parents as

TABLE 3

Test for random assortment of b *with* C

	No. 107	No. 109	$\frac{Cb}{cB} \times \frac{Cb}{cB}$ Total	Calculated	
C–B–	16	57	73	73.1	
C–bb	8	14	22	24.4	
cc–	11	24	35	32.5	
	35	95	130	130.0	χ^2=.428 P ≃.80

fractions with the meaning that the combinations of genes in the numerator or denominator came from the same gamete. Calculations based on random assortment fit so well that no linkage is indicated although the number is small.

Spawns #105 and 310 can be used to test for random assortment of *b* with *V* (Table 4). The combined data are embarrassingly close to the theoretical values calculated for random assortment.

Spawn #310 and the *C–* fish from #109 can be used to test for random assortment of *b* with *Ri* (Table 5). The two matings are different and are therefore treated separately. The #109 fish obviously fit random assortment very well. Although the *Bbriri* class in #310 is four larger and the *bbRi–* class is five smaller than calculated on the basis of random assortment, these should both be smaller if the loci are linked; hence together they deviate only one from the calculated

TABLE 4

Test for random assortment of b *with* V

	No. 105	No. 310	Total	$\frac{Vb}{vB}$ ♀ × $\frac{Vb}{vB}$ ♂ Calc.		Obs.	Calc.
VVbb	11	21	32	31			
					original classes	63	62
VvBb	8	23	31	31			
Vvbb	5	22	27	31			
					new classes	61	62
VVBb	11	23	34	31			
	35	89	124	124	$\chi^2 = 0.0262$	.80<P< .90	

TABLE 5

Test for random assortment of b *with* Ri

	No. 109 $\frac{b\ ri}{B\ ri} \times \frac{b\ ri}{B\ Ri}$					No. 310 $\frac{b\ ri}{b\ Ri} \times \frac{b\ ri}{B\ Ri}$		
	obs.	calc.		obs.	calc.	obs.	calc.	$\frac{(o-c)^2}{c}$
B–Ri–	41	40.0				30	30	0.0
			original classes	44	44.4			
bbriri	3	4.4				11	10	.10
bbRi–	11	13.3				25	30	1.60
			new classes	27	26.6			
B–riri	16	13.3				14	10	.83
	71	71.0				*80	80	χ^2=2.53
								.30<P<.50

* Nine fish were too small to classify with respect to *Ri*.

value. If the data from the two spawns are combined and also the first two and last two classes of the table combined since the first two should be larger and the last two smaller than calculated if *B* and *Ri* are linked, observed values are 85 and 66 whereas the calculated are 84.4 and 66.6.

SUMMARY

The wide range of colors in the Betta is traceable to a few major and perhaps many minor loci of which only a few have been thoroughly investigated. Melanin production and distribution is largely controlled by two loci, *C* and *B*, both dominants of which must be present for the production of the common red-brown background body color which is presumably the wild type. Homozygosity for *b* very greatly reduces the black on the body and eliminates it from all but the peripheral single layer of cells on the median fins. Cambodia (*cc*) is a more drastic

reduction of black on the body and the same in the fins as *bb*. The gene *c* is epistatic to *b*.

The *bb* fish have been called bright red because they often look very reddish. This, however, is not always the case and the amount of red depends upon other genes which have yet to be investigated.

Overlying iridocytes where present give a metallic luster, the color of which depends upon alleles at the *V* locus. Green = *vv*, blue = *Vv*, and gunmetal or steel blue = *VV*.

The iridocytes may be so sparse as to make their color almost impossible to detect or they may form a complete covering over the body and fins. All grades between these two extremes are found and often the covering is not uniform. One major locus (*Ri*) controls the density with *riri* having little or no sheen and *Ri*– a considerable amount. *Ri* is either completely dominant or nearly so.

So far, no linkage has been detected in the Betta.

Table 6 relates the major phenotypes to genotypes.

TABLE 6

Relationship of major phenotypes to genotypes

	riri–	*Ri–vv*	*Ri–Vv*	*Ri–VV*
C–B–	Body: Red brown to black Fins: Dark red to black	Body: Green (over red brown to black) Fins: Green (and often red)	Body: Blue (over red brown to black) Fins: Blue (and often red)	Body: Gunmetal (over red brown to black) Fins: Gunmetal (and often red)
C–bb	Body: Pale amber to bright red Fins: Red	Body: Red with overlying green Fins: Red and green	Body: Blue over red= purple Fins: Purple	Body: Gunmetal over red= mauve Fins: Mauve
cc B– or *ccbb*	Body: Light pink to red Fins: Colorless to red	Body: Light pink to red with overlying green Fins: Red and green to complete green	Body: Light pink to red with overlying blue Fins: Red and blue to complete blue	Body: Light pink to red with overlying silver Fins: Red and silver to complete silver

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