

THE INHERITANCE OF CERTAIN BLACK AND BROWN PIGMENTS IN THE SOYBEAN

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IN the soybean (*Glycine max*), two main types of pigment in the seed coat have been described: green and yellow pigments, and black and brown pigments. In the latter group, strains of soybeans are known with black, imperfect black, brown, light brown, reddish brown, and buff pigments. Certain types are also known with combinations of black and brown pigments.

NAGAI (1921) and OWEN (1927) have shown that the black coats are really an intense purple, due to an anthocyanin pigment, and that the brown shades are due to phlobaphene pigments.

Two additional types of coloration are known. One is the brownish pigment found as a smudge on the coats of varieties having black pod color. This appears to be an infiltration of pigment from the pod. OWEN (1928) has identified it as an oxidation pigment.

Another colored condition is a purple blotch known as "purple spot" disease and is caused by the fungus *Cercosporina kikuchii*. MATSUMOTO and TOMOYASU (1925) found the pigment responsible for this coloration to have properties similar to anthocyanins.

REVIEW OF LITERATURE

These studies are concerned with the group of black and brown pigments due to anthocyanins and phlobaphenes. A number of investigators have studied the inheritance of these pigments but there has been some disagreement in their interpretations and there is considerable confusion in the genetic symbols assigned. The writer believes it will be well to attempt to coordinate these reports in the light of his experiments. This report is concerned with the *type* of pigment present in the seed coat rather than its distribution. However, to clarify the subject for the reader, it should be mentioned that the distribution of pigment is conditioned by an allelic series. *I* acts to prevent the normal development of pigment in the seed coat; *i*¹ restricts pigment to the hilum; *i*² permits the development of a saddle pattern extending from the hilum and *i* permits full pigmentation.

Table 1 is a list of the types previously reported, together with several not so far reported. To identify the colors more certainly, RIDGWAY'S (1912) designations have been included, together with the common names used by

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TABLE 1

Summary of symbols used by various authors to designate black and brown seed coat colors.

Nagai	Woodworth	Owen	Stewart	Williams	Ridgway's color	Common designation
C	H, (R ₂ T)	R ₂ T	R ₂ T	T
R	B, (R ₁)	R ₁	R ₁	R
RC	HB, (R ₁ R ₂ T)	R ₁ R ₂ T	R ₁ R ₂ T	RT	Black	Black
rCO	r ₁ R ₂ T	r ₁ R ₂ T	r ₁ R ₂ T	rT	Dresden brown	Brown
rCo	r ₁ ^o R ₂ T	r ^o T	Liver brown	Red-brown
c	R ₁ R ₂ t	R ₁ r ₂ tW	R ₁ R ₂ tW	RtW	Imperfect black
rc	r ₁ r ₂ ^t t	r ₁ r ₂ t	rtW	Hazel	Buff
....	R ₁ r ₂ t	r ₁ r ₂ tW	R ₁ r ₂ tW	RtW	Hazel	Buff
....	rtw	Hazel	Buff
....	r ^o tW	Hazel	Buff
....	r ^o tw	Hazel	Buff

the investigators. The genetic symbols used by NAGAI, WOODWORTH, OWEN, STEWART, and those proposed by the present author are also included.

PIPER and MORSE (1910) first reported the dominance of black over brown but did not assign any symbols to the genes involved. They reported a simple 3:1 ratio in F₂.

NAGAI (1921) also reported black dominant to brown and brown dominant to reddish brown. He assumed three factors to account for the colors he found. C was present in blacks and browns, c, in imperfect blacks and buffs. R and r distinguished between blacks and browns or between imperfect black and buff, when C and c respectively were present. To differentiate between brown and reddish brown, he assumed a factor pair O, o which in combination with Cr gave brown and reddish brown, respectively.

WOODWORTH (1921) postulated two complimentary factors, B and H, to account for black pigment. The types bbHH and BBhh were considered to be brown. No attempt was made to distinguish shades of brown. He also postulated that the double recessive would be yellow since both recessives seemed to reduce the pigment. Later (1932) he reported that crosses between light brown hilum and dark brown hilum gave black hilum in the F₁. He assumed complete linkage between H and the gene T, for pubescence color. Types with T had tawny pubescence and dark brown hilum and those with t had gray pubescence and light brown hilum.

OWEN (1928) used the symbols R₁ and R₂T to account for his results. Black was given the formula R₁R₂T, brown r₁R₂T or r₁r₂^tT, imperfect black R₁r₂tW. He found that in one cross, purple flower color (W) was necessary for the production of imperfect black.

STEWART (1930) agreed with OWEN in the formula for black, but showed that OWEN's assumption of an allelic series at the R₂ locus was not necessary, and further showed that OWEN's use of formulae was inconsistent and not in accord with the facts. STEWART assumed that both R₁r₂tw and r₁r₂tW would give buff coats and R₁r₂tW, imperfect black. He gave evidence of an allelic series at the R₁ locus, R₁T being black, r₁T brown, and r₁^oT reddish brown.

EXPERIMENTAL DATA

Black × Brown

The writer is inclined to agree with STEWART's interpretation, but wishes to stress several points not brought out by former investigators. In the inheritance of black, brown, and reddish brown, all the writer's results have agreed with those of STEWART and support the idea of an allelic series at the R_1 locus. The following summaries of the F_2 3:1 ratios obtained by the writer serve to substantiate STEWART's allelic series.

		Black	Brown	Red-brown	chi ²
Black (R) × Brown (r)	Actual	576	2002474
	Expected	582	194
Black (R) × Red-brown (r^o)	Actual	298	98	.0135
	Expected	297	99
Brown (r) × Red-brown (r^o)	Actual	335	111	.0030
	Expected	334.5	111.5

As will be seen in the above table, the actual ratios are very close to the calculated ratios. Only a multiple allelic series can explain these results. NAGAI had used O , o for brown and reddish brown, so STEWART used the superscript "°" to tie his r_1^o to NAGAI's results. Plants producing black, brown, or reddish brown seed may have either purple (W) or white (w) flowers.

Closely linked genes (R_2T) vs. a single gene, T

As mentioned above, NAGAI, WOODWORTH, OWEN, and STEWART have all postulated at least two factors as necessary for the full development of black and brown color. NAGAI used R and C , WOODWORTH suggested B and H , but OWEN and STEWART have postulated a third gene (R_2), closely or completely linked with T and complementary to R_1 . It has been observed that in general, full colored blacks and browns have tawny pubescence as opposed to gray pubescence on imperfect black and buff types. There are, however, some blacks with gray pubescence, and for this reason OWEN and STEWART proposed the factor R_2 as complementary to R_1 and very closely linked with T , the factor for tawny pubescence. The black-seeded, gray pubescent types were then considered to be crossovers (R_1R_2t).

The writer prefers to drop R_2 and use only T , which has been assumed to be completely linked with R_2 . WOODWORTH (1921) at first was inclined to this usage. He says, "As the linkage is complete between H (now R_2) and T , the combined effects of these two factors may be thought of as due to a single factor which has more than one effect. Thus, T , the factor for tawny pubescence, may be thought of as not only being able to change the pubescence color from gray to tawny, but also as acting with B (now R_1) to change the hilum color from brown to black. This is the most convenient way of regarding the situation."

In all the crosses studied in the soybean no instance of crossing over between R_2 and T has been reported. This fact casts considerable doubt on the existence of more than one gene.

The imperfect black type

OWEN and STEWART have mentioned an "imperfect black" type with black pigment and gray pubescence. In types where only the hilum is pigmented, the black pigment is confined more to the center of the hilum and is usually surrounded by a narrow buff band. Where the whole coat is pigmented, varying mixtures of buff and black pigment appear. Sometimes there is so little black that the coat appears to be almost buff, while in other strains, the black is so intense that the buff pigment is not noticeable. However, the black pigment is always much duller than the normal RT black. WOODWORTH (1921) had thought of this as a crossover type, R_1R_2t , but OWEN (1928) and STEWART (1930) give R_1r_2t as the formula for imperfect black. In the simplified system suggested above, imperfect black would have the formula RtW . That imperfect black is not a crossover type is indicated by the fact that it is distinct phenotypically from true black and that the imperfect black type is only developed in the presence of W . That imperfect black is really R can be proven by crossing it with black (R), and brown (r).

In crosses between imperfect black (RtW) and blacks of the composition RTW , black is dominant and only the parental types segregate in F_2 . In crosses between imperfect black and brown (rTW), the F_1 is always pure black, indicating that the imperfect black must be R . The results of the writer all agree with previous work that imperfect blacks must have gray pubescence (t) and purple flowers (W).

The black-coated, gray pubescent types mentioned above are introductions from the Orient. If these are really crossover types of the formula R_1R_2t , then in crosses with ordinary gray pubescent types, only gray pubescent types should be produced in F_1 and F_2 .

The results of a cross between T24A,² of the formula rtW , and T69 (P.I. 64698),³ a black bean with a clear gray pubescence, as good a gray as T24A, indicate that T69 is not a crossover type. The formula of T24A has been proven in a number of crosses. The F_1 was black-seeded with gray pubescence, but the F_2 progeny consisted of 46 gray to 12 tawny plants. If this is really a 13:3 ratio, as it may well be, the results can be explained by assuming a dominant gene T_2 for gray pubescence, epistatic to T_1 for tawny pubescence. Since it is well established that T24A is rt , any gene for tawny pubescence must come from T69 which accordingly must be T_1 . Then T69 would be T_1T_2 and T24A would be t_1t_2 . If such is the case, it is evident that T_2 has no effect on seed coat color. At any rate, T69 can not be considered as a crossover type.

In view of these facts, and because it is the simpler explanation, the writer prefers to use only T as complementary to R . Possibly NAGAR's C , which is apparently identical with T , should be used because of prior usage, but since T indicates the effect on the pubescence color (from *tawny*) and

² The prefix "T" refers to type numbers in the file of the Plant Breeding Division of the Agronomy Department of the University of Illinois.

³ The prefix P.I. indicates introductions of the Office of Foreign Plant Exploration and Introduction of the United States Department of Agriculture.

since NAGAI has made no mention of the effect of *C* on pubescence color (which is strange indeed, since he must have had the two pubescence colors segregating in some of his crosses where the effect of *T*, *t* on seed coat color is quite pronounced), the writer prefers to retain the present symbol *T*, simply dropping *R*₂ and assigning its properties to *T*. *R*₁ then becomes simply *R*.

Imperfect Black × Black

As mentioned above, crosses between purple-flowered black and imperfect black give 3 black to 1 imperfect black in F₂, since only *T* is segregating. However, in crosses between white-flowered black and imperfect black, both *W* and *T* are segregating. In the cross between Peking (*RTw*) and T24 (*RtW*) the following F₂ ratio was observed:

	Actual	Expected (9:3:3:1)	chi ²
Purple-flowered black (<i>RTW</i>)	109	108	.4815
White-flowered black (<i>RTw</i>)	38	36	
Imperfect black (purple flowers) (<i>RTW</i>)	35	36	
Buff (white flowers) (<i>Rtw</i>)	14	12	

Flower color has no effect on seed coat color on RT plants but it does on Rt plants.

Imperfect Black × Brown

As mentioned above, the cross between imperfect black and purple-flowered brown (*rT*) gives black in the F₁. The cross between T116, a purple-flowered brown (*rTW*) and T24, an imperfect black, gave the following ratio in F₂:

	Actual	Expected (9:3:3:1)	chi ²
Black (<i>RTW</i>)	78	76.5	.1569
Brown (<i>rTW</i>)	25	25.5	
Imperfect black (<i>RtW</i>)	24	25.5	
Buff (<i>rtW</i>)	9	8.5	

In the cross between imperfect black and white-flowered brown (*rTw*), we have all three genes segregating. The F₁ is black with tawny pubescence and purple flowers. The F₂ of the cross between T24 (*RtW*) and Harbinsoy (*rTw*) gave the following results:

	Actual	Expected (27:9:9:9:3:3:4)	chi ²
Black pigment, purple flowers (<i>RTW</i>)	66	67.5	.221
Black pigment, white flowers (<i>RTw</i>)	23	22.5	
Imperfect black pigment, purple flowers (<i>RtW</i>)	24	22.5	
Brown pigment, purple flowers (<i>rTW</i>)	22	22.5	
Brown pigment, white flowers (<i>rTw</i>)	8	7.5	
Buff pigment, purple flowers (<i>rtW</i>)	7	7.5	
Buff pigment, white flowers (<i>Rtw</i> and <i>rtw</i>)	10	10.0	

The buff types

The three crosses just mentioned have each produced some buff-colored types. It will be noted that in the first of these, the gene *W* is responsible for the difference between imperfect black and buff and in the second cross *R* is responsible. In the third cross, both genes are segregating. It is evident that there are two genetic types of buff, indistinguishable phenotypically.

In table 1 it will be seen that these two buff types, *rtW* and *Rtw*, have been reported before. It is interesting to note that apparently *R* and *W* can be substituted in this case and give the same effect on the seed coat. This is not strange when it is recalled that the gene *H* produces anthocyanin pigment in both the flower and the stem. The buff hilum and buff types of STEWART were all of the *rtW* type, and those of OWEN also were *rtW*, except C3 and its segregates. Most of WOODWORTH's buff (light brown) hilum types were of the *Rtw* type. Illini, Wea, Dunfield, Mukden, Blackhawk, and many white-flowered, gray pubescent grain varieties are of this type. Mandarin and Habaro are of the *rtW* type.

That the types identified as *rtW* and *Rtw* are really such can be proven by crossing them. If the assumption is correct, the F_1 should have imperfect black pigmentation (*RrttWw*). In the cross between T122 (*Rtw*) and T24A (*rtW*), the F_1 was imperfect black as expected.

The F_2 of this cross gave the following results:

	Actual	Expected (9:3:4 basis)	chi ²
Imperfect black (<i>RtW</i>)	206	202.5	.1642
Purple-flowered buff (<i>rtW</i>)	65	67.5	
White-flowered buff (<i>Rtw</i>) and (<i>rtw</i>)?	89	90.0	

This ratio is evidently a 9:3:4 ratio, indicating that the triple recessive (*rtw*) is a buff indistinguishable from *Rtw*.

The identification of the *rtw* type was of special interest. Since the recessive alleles of all three genes seem to act to reduce pigmentation, it was wondered if the triple recessive might be devoid of black and brown pigment, *i.e.*, a recessive yellow.

In this cross we should expect 22 plants of the triple recessive (*rtw*). If these were yellow they would have been easily distinguished, but no yellow-seeded plants occurred.

WOODWORTH (1921) postulated that the combination $r_1(r_2)t$ would be yellow or colorless due to the fact that both recessives reduce the pigment. In a cross between P.I. 20406, a colorless hilum yellow, and P.I. 20854, black, he considered the F_2 ratio to be a 9 black : 6 brown : 1 colorless (r_1r_2t) but since environmental causes made all the seed coats mottle with streaks of pigment, he was unable to distinguish the latter classes and recorded instead a 9:7 ratio.

The writer would prefer to ascribe the colorless hilum of P.I. 20406 to the gene *I* for inhibition of pigment since in all crosses at the Illinois Station, this variety (named Elton) has behaved as if of the constitution *RtI*.

In order to positively identify the triple recessive, another cross was made. In this, T91 ($rTtw$) was crossed with T24A (rtW). In this cross only T and W were segregating. Since both parents were homozygous r , any white-flowered, gray pubescent plant would be of the type rtw . Actually, there were three such in a population of 95 plants. All of these showed the buff color and were indistinguishable from T24A (rtW), in color of coat. These experiments would indicate then that the dominant alleles of the genes T , W simply add something to a base color of buff rather than that the recessive allelomorphs remove color, or else that the chemical effect of these genes on seed coat color is almost identical.

The writer has added several other new types. Previous writers had made no mention of what the r^o combinations with t might be. In the cross between P.I. 82235, a reddish brown, tawny pubescent type (r^oTW), and P.I. 82273, a buff with purple flowers and gray pubescence, the F_1 was phenotypically r^oTW and the F_2 segregated in a simple ratio of 3 reddish brown : 1 buff, showing that P.I. 82273 is really an r^otW buff. The actual figures were 29 r^oTW : 10 r^otW .

The triple recessive type r^otw has not previously been mentioned. This type was obtained from a cross between Ogema (r^oTtw) and P.I. 82273 (r^otW). The F_1 was reddish brown like the Ogema parent but had purple flowers. The F_2 segregated as follows:

	Actual	Expected (9 : 3 : 3 : 1) basis	chi ²
Purple-flowered, red brown (r^oTW)	123	121.5	.0988
White-flowered, red brown (r^oTtw)	39	40.5	
Purple-flowered, buff (r^otW)	41	40.5	
White-flowered, buff (r^otw)	13	13.5	

Since all T plants were red brown (r^o) it is evident that there was no segregation at the R locus, so all the buff-seeded plants must be r^ot . The white-flowered, buff-seeded plants must then be r^otw . These were indistinguishable in color from r^otW buffs.

Crosses between buff and other colors

Since there are five genetic types of buff and the combinations of these with black, brown, reddish brown, and imperfect black are too numerous to report in detail, no attempt will be made to illustrate these crosses.

It has been mentioned above that crosses between Rtw buff and rtW or r^otW buffs will give imperfect black in F_1 but other crosses between buffs will give only buff in F_1 and F_2 .

SUMMARY

The somewhat confused literature on the inheritance of black and brown pigments in the seed coat of the soybean has been summarized and coordinated. Three new genetic types of buff are reported. Black, brown, and reddish brown are due to an allelic series, R , r , r^o . These colors are developed

only when combined with *T*, for tawny pubescence. In combination with *t* (gray pubescence), they give imperfect black, buff, and buff, respectively. The imperfect black type only develops in combinations with *W* for purple flower color. All white-flowered, gray pubescent types have buff pigment in the coat unless inhibited by some member of the *I* series. There are five genetic types with buff pigment, indistinguishable phenotypically.

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