INHERITANCE OF CERTAIN CHARACTERS IN THE COWPEA (*VIGNA SINENSIS*). II.

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Introduction.

In the first part of this study (1919), the present writer has recorded the results of experiments on the inheritance in cowpeas of certain factors affecting flower colour, and colour and pattern of the seed coat. The present paper forms a further contribution towards our knowledge of the genetics of the cowpea.

During the progress of the work an insect pest, probably the cowpea weevil (*Chalcodermus annuus*), hitherto unrecorded in St Vincent, made its appearance. The ravages of this pest were so great that most of the seed from several of the F_1 and F_2 cultures was destroyed. For this reason the number of plants in some of the F_2 cultures is disappointingly small.

The experiments described below concern themselves with the following characters:

1. Anthocyanin in stem and leaf stalk.
2. Colour of seed coat pattern.
3. Pod colour.
4. Flower colour.

The Experimental Results.

1. *Anthocyanin in stem and leaf stalk.*

In many varieties of cowpea, the region near the junction of the main stem and petioles is dark red in colour, owing to the presence of anthocyanin pigment in the epidermal cells. The same pigment is also found at the junction of the pinnae with the leaf stalk. In other varieties pigmentation is totally absent. In crosses between pigmented and unpigmented kinds the following results were obtained :

The F_1. Fully pigmented.

The F_2. Five families were grown, and segregation occurred into the parental types in the following proportions:

Plants	Pigmented	Unpigmented
171	132	39
Expected ...	137	43

This ratio is close to the expected 3 : 1, characteristic of a single genetic difference. Several families were grown in F_3.

The F_3. Of fifteen families grown from F_2 pigmented plants, four families threw pigmented only. There were 91 plants in all. The remaining eleven families segregated into pigmented and unpigmented, the results being:

Plants	Pigmented	Unpigmented
369	276	93
Expected ...	277	92

The ratio of pigmented to unpigmented in segregating families is thus close to 3 : 1.

From F_2 unpigmented plants four families were grown in F_3. All bred true to the unpigmented condition, the number of plants being 140.

Conclusion.

The presence of anthocyanin colouration in the stem and leaf stalk of the cowpea is due to a single unit factor X, dominant to its absence.

2. *The Colour of the Seed Coat Pattern.*

In the first part of this study (1919) the present writer established the existence of three Mendelian factors affecting the colour of the seed coat pattern. These are respectively B (black), N (buff), and M (maroon). The genetic composition of the types previously worked with appears to be as follows :

Black	...	BNM
Brown	...	bNM
Buff	...	bNm
Maroon	...	bnM
Red	...	bnm

The two crosses previously studied were brown by red (bNM × bnm), giving in F_2, 12 brown and buff, 3 maroon, and 1 red, and black by brown (BNM × bNM), giving 3 black to 1 brown in F_2. The particular black used in this experiment must have contained the factor *M*, for when crossed with red, blacks, browns, maroons, and reds, appeared in F_2.

(*a*) *The cross Red by White.*

The red type used in the foregoing experiments was crossed with the white (cream) seeded variety Para. The following were the results:

The F_1. The colour of the seeds of the F_1 plants was exactly the same as the buff type bNm, alluded to above.

The F_2. Segregation occurred into buff, red, and white. Unfortunately only 18 plants produced seeds, but it will be seen that the ratio of the three colours was close to 9 : 3 : 4.

Plants	Buff	Red	White
18	11	3	4
Expected ...	10	3	5

The F_3. Sixteen families were grown in F_3. Five of the buffs segregated into buff, red and white in the 9 : 3 : 4 ratio:

Plants	Buff	Red	White
97	49	21	27
Expected ...	55	18	24

Four of the buffs threw buff and red approximately in the 3 : 1 ratio:

Plants	Buff	Red
80	62	18
Expected ...	60	20

Two of the buffs threw buff and white in approximately the 3 : 1 ratio:

Plants	Buff	White
112	78	25
Expected ...	84	28

Of the two reds grown in F_3, one segregated into 9 red and 3 white. The other gave a ratio of 15 buff, 13 red, and 12 white. Natural crossing probably caused this anomalous result.

Three whites threw whites only in F_3, the number of plants in the three families being respectively 96, 16, and 20.

Interpretation of the cross Red by White.

The results of this cross may be explained by assuming that two factors are concerned:

R the factor for a red colour of the seed coat pattern.

N the factor for buff. This factor is without visible effect in the absence of R.

The cross red by white may be represented as nR × Nr, the types appearing in F_2 being:

9 NR	3 nR	3 Nr	1 nr
Buff	Red	White	White

(*b*) *The cross Black by White.*

A cross between a black and the white variety Para gave F_1 black. In F_2 segregation occurred into black, buff, and white in proportions approximating to a ratio of 9 : 3 : 4. The numerical results from three families were:

Plants	Black	Buff	White
95	50	21	24
Expected ...	53	18	24

No F_3 families were grown, but the F_2 data, together with the information acquired from the cross of red by white, enable a satisfactory explanation of the results to be made.

Two factors are concerned in the cross black by white.

R the factor for red.

B the factor for black, which is without visible effect except in presence of R.

The factor for buff, N, is common to both parents. The cross may be represented thus: black (BNR) by white (bNr), the types appearing in F_2 being:

9 BNR	3 BNr	3 bNR	1 bNr
Black	White	Buff	White

From the results of this experiment it will be seen that factor M is not necessary for the production of black.

(*c*) *The cross Black by Buff.*

The plants of the F_1 showed complete dominance of black. Some of the seeds were slightly mottled, and in these the ground colour was seen to be buff.

The F_2. The results of the F_2 are set forth below. Segregation occurred into black, buff, and red, in proportions suggesting the 12 : 3 : 1 ratio, though the fit is not very close.

Plants	Black	Buff	Red
841	644	129	68
Expected ...	632	158	53

The F_3.

1. 17 blacks bred true (480 plants).

2. 16 blacks segregated into black and buff in approximately the 3 : 1 ratio.

	Plants	Black	Buff
	440	348	92[1]
Expected ...		330	110

3. 14 blacks segregated into black and red in approximately the 3 : 1 ratio.

	Plants	Black	Red
	382	284	98
Expected ...		287	96

4. 21 blacks segregated into black, buff, and red in approximately the 12 : 3 : 1 ratio.

	Plants	Black	Buff	Red
	826	612	159	55
Expected ...		619	155	52

5. 8 buffs bred true (307 plants).

6. 19 buffs segregated into buff and red in the 3 : 1 ratio.

	Plants	Buff	Red
	636	462	174
Expected ...		477	159

7. 11 reds bred true (420 plants).

Interpretation of the cross Black by Buff.

Two factors are involved in this cross, B, the factor for black, and N the factor for buff. The factor R is common to both parents. The cross is of the nature black (BnR) by buff (bNR). The types appearing in F_2 are as follows:

9 BNR	3 BnR	3 bNR	1 bnR
Black	Black	Buff	Red

The results of this cross demonstrate that factors B and N are probably independently inherited, and that N is not essential for the full production of black.

It will be seen from the above summarised results that the ratios obtained go far to confirm the hypothesis that two independently inherited factors, B and N, are concerned in the cross black by buff.

[1] These numbers are somewhat far removed from expectation. In two families, Nos. 51 and 105, the number of buffs is abnormally small, being 2 out of 57, and 2 out of 47 respectively. It is possible that accidental mixture of seed has taken place. If these two families are not counted, the ratio of black to buff is 248 : 88, with expectation 252 : 84 on a 3 : 1 basis.

Significance of the Mottling.

It was pointed out above that some of the F_1 seeds showed a certain degree of mottling, and that the buff groundwork was visible in patches in these seeds. In the F_2 blacks, some of the plants showed no mottling at all, whilst of the others some showed red and others buff mottling. Eighteen blacks with no mottling were grown in F_3, and all bred true to black. Fifteen red-mottled blacks were grown in F_3, and all except one segregated into black and red. Many of the blacks were mottled red. Forty-one families were grown from buff mottled blacks. Of these sixteen segregated into black and buff, and twenty-two into black, buff, and red. Three bred true. It may be concluded that mottling indicates that factor B is present in a heterozygous condition, but that some plants showing slightly mottled seeds may be pure for this factor.

(*d*) *The cross New Era by White.*

New Era is one of the best known varieties of cowpea, being highly valued by the farmers of the Southern States as a forage crop. The seeds are thickly and uniformly dotted with a dark blue anthocyanin pigment, on a buff background. The New Era pattern is always associated with purple colouration of the tip of the young pod, calyx, and peduncle.

The F_1 of the cross between New Era and the white variety Para had seeds with the characteristic New Era pattern but with a less intense dotting. In F_2, segregation occurred into New Era, buff, and white approximately in the 9 : 3 : 4 ratio.

Plants	New Era	Buff	White
17	10	2	5
Expected ...	10	3	4

In F_3 three families were grown. One of these was from a white and bred true (23 plants). The other two were from plants of the New Era type; both segregated into New Era, buff, and white in the 9 : 3 : 4 ratio, as follows:

	Plants	New Era	Buff	White
	30	14	6	10
	24	11	4	9
Totals ...	54	25	10	19
Expected ...	—	30	10	14

Interpretation of the results of the cross New Era by White.

The results obtained in the cross New Era by white indicate that two factors are involved:

R the factor for red.

E the factor for New Era pattern on the seed coat, also producing purple colouration of the tip of the young pod, and in the calyx and peduncle. This factor is without effect in the absence of R.

The factor for buff, N, is common to both parents. The cross is thus New Era (ENR) by white (eNr), and the types appearing in F_2 are:

9 ENR	3 eNR	3 ENr	1 eNr
New Era	Buff	White	White

It is recognised that the number of plants on which these conclusions are based is very small, and it is intended to repeat the cross in order to obtain a larger series of numbers.

(*e*) *The cross New Era by Black.*

The factors B (black), and E (New Era), both produce as one of their effects anthocyanin colouration of the tip of the young pod, calyx, and peduncle, though B produces a more intense colouration than E. It has been shown that both these factors are allelomorphic to their absences. Thus the cross black (BNR) by buff (bNR) will give in F_2 the ratio 3 black to 1 buff, while the cross New Era (ENR) by buff (eNR) will give in F_2, 3 New Era to 1 buff. If the factors B and E are inherited independently, a cross between black and New Era should produce the double recessive, buff, once in sixteen times in F_2, thus:

9 BENR	3 BeNR	3 bENR	1 beNR
?	Black	New Era	Buff

The actual results obtained in the cross black by New Era may be set forth as follows:

The F_1. Black.

The F_2. In F_2 were obtained 73 black and 24 New Era. These results approximate to the 3 : 1 ratio.

Another cross between black and New Era gave the following results:

The F_1. Black.

The F_2. Segregation took place into (*a*) black, (*b*) buff New Era, (*c*) red New Era.

	Plants	Black	Buff New Era	Red New Era
	841	644	129	68
			197	
Ratio ...		3·3 :	1·0	

The presence of both red and buff New Era need not concern us at this point. The important point to note is that the double recessive, be, does not occur.

The F_3.

1. Black bred true in 17 families containing 480 plants.

2. In 51 families black segregated into black and New Era in the 3 : 1 ratio.

Plants		Black		New Era
1648		1244		404
Expected	...	1236		412
Ratio	...	3·1	:	1·0

3. New Era bred true in 38 families containing 1363 plants.

From the results of these experiments it may be concluded that:

(*a*) B and E are not inherited independently.

(*b*) B and E behave as though they were allelomorphic to each other, but it need only be assumed at present that these two factors show repulsion probably on a basis higher than 1 : 15.

(*f*) *The cross Para (albino) by Black-eye.*

This experiment was designed to show whether either or both of the two pattern factors D and H are present in Para. Since the seed coat of Para is unpigmented no pattern factor will have visible expression.

The F_1. Black solid.

The F_2. Segregation took place in the coloured types into Solid, Watson, Holstein, and Small-eye in approximately the 9 : 3 : 3 : 1 ratio, thus:

	Solid	Watson	Holstein	Small-eye
	DH	Dh	dH	dh
	24	8	3	1
Expected	22	7	7	2

It is clear that Para contains both D and H. These factors have no visible expression except in presence of the pigment factor R, brought in by the black parent.

3. *Pod Colour.*

In certain varieties of cowpea the pod is green in the early stages but shortly before maturity it develops anthocyanin pigment throughout, turning through various shades of colour until when almost ripe it is of a deep purplish-red colour. In purple podded plants there is also colouration of the tip of the young pod, calyx, and peduncle, similar to that caused by the factors B (black), and E (New Era).

In crosses of purple pod with non-purple pod, the F_1 is purple podded, though less intensely than the parent type. In F_2 segregation occurs into purple and non-purple as follows:

Family	Purple		Non-purple
5*g*	89		30
5—1	76		22
5—3	19		5
5—5	48		11
5—6	14		4
5—8	111		21
5—9	30		5
5—9 *a*	42		11
5—9 *b*	20		6
Totals ...	449		115
Expected ...	423		141
Ratio ...	3·9	:	1·0

This ratio is somewhat far removed from the simple 3 : 1 type, and for this reason the F_2 families have been presented separately.

The F_3.

1. 7 purples segregated into purple and non-purple.

Family	Purple		Non-purple
1	31		8
2	27		6
3	28		8
4	23		5
5	23		4
6	22		4
7	19		8
Totals ...	173		43
Expected...	162		54
Ratio ...	5·0	:	1·0

2. 2 purples bred true (119 plants).

3. 4 non-purples bred true (89 plants).

The peculiar feature of these results is the ratio 3·9 : 1·0 of purple to non-purple in F_2, and the very similar ratio of 4·0 : 1·0 in heterozygous F_3 families. It is evident that a much larger series of numbers is required before the exact mode of inheritance of purple pod is elucidated. It may be assumed meanwhile that one main factor, P, is responsible for purple pod.

Relation between the factors B (*black*), E (*New Era*), *and* P (*purple pod*).

The interesting feature of the three factors B, E, and P, is that any of them may produce anthocyanin pigmentation of the young pod, calyx, and peduncle. E causes a lesser development of colour than B, and B slightly less than P.

The relation of B and E has already been dealt with, and it was shown that these two factors repel each other in gametogenesis. The relation between the factors B and P will now be discussed.

The cross Black (Bp) *by Purple Pod* (bP).

The F_1. The F_1 possessed black seeds and purple pods, though the colour of the pod was less intense than that of the purple podded parent.

The F_2. In the F_2 three types appeared, viz. BP, Bp, and bP. Classifying the F_2 the results were:

Plants	BP	Bp	bP
273	168	40	65

Taking the ratio of B to b we obtain 208 : 65 (expectation 205 : 68), while the ratio of P to p is 233 : 40 (expectation 205 : 68).

The F_3.

1. Progeny of black, purple-podded F_2 plants.

Families	Plants	BP	Bp	bP
14	556	305	129	122

Here the ratio of B to b is 434 : 122, and P to p is 427 : 129. The expectation in both cases on a 3 : 1 basis is 417 : 139.

2. Progeny of non-black, purple podded F_2 plants.

Families	Plants	BP	Bp	bP
23	1036	0	0	1036

3. Progeny of black, non-purple podded F_2 plants.

Families	Plants	BP	Bp	bP
5	181	0	181	0

The main conclusions which may be arrived at from a survey of these results are:

1. The ratio of black to non-black in both F_2 and F_3 cultures is close to 3 : 1, confirming the conclusion previously arrived at that black is allelomorphic to its absence.

2. The ratio of purple pod to non-purple pod in F_2 is somewhat far removed from 3 : 1, being 5·9 : 1·0, although in the segregating F_3 families the 3 : 1 ratio is more closely followed. Thus it is not certain that purple pod is due entirely to a single factor P. This conclusion was arrived at from a study of other crosses.

3. The non-appearance of the double recessive non-black, non-purple pod in the F_2 and F_3 cultures of the cross Bp by bP, indicates that these two factors are repelled on a basis probably higher than 7 : 1.

On the chromosome hypothesis of Morgan *et al.* (1915), the factors B, P, and E, are located in the same chromosome. The evidence is not strong enough to say that complete repulsion occurs between B and E, and between B and P; the factors are, however, so near together that crossing over seldom occurs.

If these three factors could be shown to occupy identical loci, they would then constitute a triple series of multiple allelomorphs. In nearly all cases in which multiple allelomorphs have been discovered, the factor-differences produce similar effects. The fact that B, P, and E, all produce colouration of young pod, calyx, and peduncle as one of their effects is an argument in favour of the multiple allelomorph theory.

4. *Flower Colour.*

In the F_2 of a cross between red (dark flower), and Para (white flower), it was expected that segregation would occur into 3 dark and 1 white. Some of the apparently white flowers were found on close examination to possess a minute degree of anthocyanin pigmentation in the wings. This cannot usually be seen with the naked eye, and is best detected by dipping the flower in dilute hydrochloric acid, when a pink flush appears if pigmentation be present. This type of flower colour may conveniently be termed "tinged." The proportions in which dark, tinged, and white, occurred in the cross red by Para were:

Plants	Dark	Tinged	White
18	14	3	1

The F_3 results were as follows:

1. 3 darks segregated into dark, tinged, and white.

	Plants	Dark	Tinged	White
	118	95	19	4
	46	35	10	1
	46	34	10	2
Totals	210	164	39	7
Expectation on 12 : 3 : 1 basis	—	157	39	13

2. 7 darks segregated into dark and tinged in the 3 : 1 ratio.

Plants	Dark	Tinged
94	73	21
Expected ...	71	23

3. 3 darks bred true (76 plants).

4. 2 tinged segregated into tinged and white in the 3 : 1 ratio.

Plants	Tinged	White
116	90	26
Expected ...	87	29

5. 1 tinged bred true (16 plants).

Interpretation of the Results.

The evidence, although incomplete, indicates that the tinged type of flower is due to a factor which may be called G. This factor is recessive to D, the factor for dark flower, and tinged and white form a pair of allelomorphic characters.

It was thought at first that the cross was of the nature DGR × Dgr giving in F_2:

9 DGR	3 DgR	3 dGr	1 dgr
Dark	Dark	Tinged	White

or altogether 12 dark, 3 tinged, and 1 white. According to this hypothesis the number of darks segregating into dark and tinged, and dark and white respectively in F_3 should be equal in number. Actually no darks segregated into dark and white, whereas 7 segregated into dark and tinged. The F_3 families which segregate into all three types should exhibit the 12 : 3 : 1 ratio. The actual figures show considerable deficiency in the number of whites. It may be noted that it is not easy to distinguish between tinged and white in some plants, and possibly some plants have been classified as tinged which are really white.

On the whole the evidence is in favour of the view that the cross is DGR by Dgr, but a much larger series of numbers is necessary to substantiate the theory.

The two factors D and L have no visible effect except in presence of the factor R (red). The factor G produces the tinged type of flower in plants with white seeds.

General Summary.

1. The presence of anthocyanin colouration in stem and leaf stalk is due to a factor X, dominant to its absence.

2. The colours of the seed coat pattern, black, brown, buff, maroon, red and white may be regarded as due to various combinations of four factors, B (black), N (buff), M (maroon), and R (red), thus:

Black	...	BNMR
,,	...	BNmR
,,	...	BnmR
,,	...	BnMR
Brown	...	bNMR
Buff	...	bNmR
Maroon	...	bnMR
Red	...	bnmR
White	...	due to absence of R

3. The New Era pattern of the seed coat is due to a factor E, which has no visible effect in absence of R.

4. The albino type Para carries the two seed coat pattern factors D and H (either H_1 or H_2). They produce no visible effect except in presence of R.

5. The purple colour of the pod is due to one main factor P. The marked deficiency in the expected number of recessives leads to the view that more than one factor may be involved.

6. The factors B (black), and E (New Era), show repulsion on a basis probably higher than 1 : 15.

7. The factors B (black), and P (purple pod), show repulsion, probably on a basis higher than 1 : 7.

8. The type of flower colour known as tinged is due to a factor G, dominant to its absence, but recessive to D, the factor for dark flower.

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