FURTHER STUDIES ON THE INHERITANCE OF ANTHOCYANIN PIGMENTATION IN ASIATIC COTTON

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CONTENTS

I. Introduction

Many anthocyanin pigmentation patterns have been found in Asiatic cotton since the previous report (Silow & Yu, 1942) was written. The present paper covers three points. In the first place, the multiple allelic series is extended from the fourteen alleles, previously reported, to twenty; in other words, six more alleles are added. Secondly, in the previous paper the production of anomalies—namely, R_2^{AS} from $R_2^{WO} \times R_2^{OS}$, and R_2^{NO} from $R_2^{MS} \times R_2^{WO}$ and also from $R_2^{MS} \times R_2^{EO}$—could only be tentatively explained as being due either to (1) accidental contamination, (2) possible mutation, or (3) allelic interchange of inherited substance of the chromosome. The new facts make it possible to eliminate (1) and (2). Further, from the recent literature, it seems that another explanation may work quite as well. Thirdly, it appears from the new facts that the relationship of the different alleles in the multiple allelic series of anthocyanin pigmentation of Asiatic cotton requires further consideration, and also that the symbols of the series need further rearrangement in order that the relationship between them may be more easily understood.

The symbols of the newly obtained alleles have not been given, as their relationship is rather complicated; but a new scheme of nomenclature is suggested at the end of the paper. Silow also has obtained new alleles, eight in number (personal communication); and the fourteen new alleles cannot be symbolized adequately according to the system used in the previous paper. In order to avoid further change, those described in this paper are temporarily designated as new alleles *a*, *b*, *c*, *d*, *e* and *f*.

II. Description of the new types

Three of the six new alleles were used for hybridization. Two of the three were obtained from a collection of indigenous varieties, and the third was a segregate from material used in former studies. These three alleles were crossed with others, and from the offspring another three new alleles were obtained. Their characteristics are described in Table 1, in which some of the previously reported alleles, closely related to the new types, are also included for the purpose of comparison.

The new allele *a* (C.K. 7–27) was obtained from the late Prof. C. C. Feng. In the collection of indigenous varieties a similar type may be found, but it is not very common.

The new allele *e* (C.K. 6–27) was obtained in 1936 from our collection of varieties. It was crossed with R_2^{EO}, and a selection from the progeny of the hybrid was preserved. The original stock was lost during the war. The new allele *f* (C.K. 5–27) was a segregate from the cross $R_2^{FO} \times R_2^{OS}$; when first selected it was not recognized as a new allele, owing to its phenotypic resemblance to R_2^{FO}. In 1942 the new stock was used as the backcross parent with $R_2^{NO} \times R_2^{OS}$; and although it was predicted that half of the offspring would be red spotted, only parental types were obtained. Genetical studies of its characteristics were then made. This allele has also been obtained in India (1943), but the source is not known. The new alleles *b* (S.S. 6–32), *c* (S.S. 3–32), and *d* (S.S. 5–32) segregated out in culture, and true-breeding types of all of them have been obtained.

Table 1.* *Comparison of anthocyanin pigmentation in established types and new types*

Symbols or temporary designation	Petal spot	Petal lamina	Calyx intensity	Bracts	Leaf		Stem	Bolls
					Lamina	Veins		
R_2^{MS}	Red	Red margin	+	+ +	+	+	+ +	+ +
R_2^{VS}	Red	Not red	+	+	+ +	+ + +	+	+
New allele *a*	Red	Red margin	Tinged	Flush*	Flush	Flush	+	Sun red
New allele *b*	Tinged	Red margin	Tinged	Flush*	Flush	Flush	+	Sun red
New allele *c*†	Spotless	Red margin	Tinged	Flush*	Flush	Flush	+	Sun red
New allele *d*†	Spotless	Red margin	Tinged	Flush*	Flush	Flush	+	Sun red
R_2^{NO}	Spotless	Red margin	+ + +	+ + +	+ + + +	+ + +	+ +	+ +
R_2^{WO}	Spotless	Not red	+ + +	+ +	+ + + +	+ +	+ +	+ +
R_2^{AS}	Red	Not red	0	Flush	0	0	Sun red*	Flush
R_2^{TS}	Tinged ghost	Not red	0	0	0	0	0	0
New allele *e*	Tinged	Not red	0	0	0	0	0	0
R_2^{OS}	Ghost	Not red	0	0	0	0	0	0
R_2^{FO}‡	Spotless	Not red	0	0	0	0	0	0
New allele *f*‡	Spotless	Not red	0	0	0	0	0	0

* See Table 4 (Silow & Yu, 1942).
† Same in phenotype, but the genotype may be dissimilar; see later text.
‡ Same in phenotype, but dissimilar in genotype; see later text.

III. MATERIAL

Pedigree no.	Symbols or temporary designation	Sources
C.K. 8–27	R_2^{AS}	Selfed indigenous variety
N.K. 2–26	R_2^{OS}	Selfed segregate
N.K. 1–26	R_2^{NO}	Selfed cross-over
C.K. 1–27	R_2^{WO}	Selfed segregate
C.K. 2–27	R_2^{BO}	Selfed indigenous variety
C.K. 4–27	R_2^{FO}	Selfed segregate
C.K. 7–27	New allele *a*	Selfed indigenous variety
S.S. 6–32	New allele *b*	Segregate
S.S. 3–32	New allele *c*	Segregate
S.S. 5–32	New allele *d*	Segregate
C.K. 6–27	New allele *e*	Selfed indigenous variety
C.K. 5–27	New allele *f*	Selfed segregate

All the material, except the new allele *e*, belongs to the same species, *Gossypium arboreum* L. The genetical backgrounds are similar, and the growth habits normal. The characters studied are expressed very clearly, so that classification of the progeny incurs no difficulty. The new allele *e* was also originally in *G. arboreum*, but the material carrying it which was used in this investigation was derived from a segregate of an interspecific cross involving *G. herbaceum* L.; special attention was paid to fertility and growth habit during selection.

IV. Experimental records

A. *Crosses involving new allele e* (C.K. 6–27)

All the data concerning the new allele *e* are presented in Table 2. The F_1 plant from the cross between R_2^{OS} and the new allele *e* is similar to R_2^{AS} in phenotype. The cross between R_2^{OS} and R_2^{FO} gives a similar result. Such complementary interaction between alleles

Table 2

Combinations	Generations	Season	Progeny
*a** (C.K. 7–27) × *e**	B.C. to *f*	1943	298 *a* : 321 *e*
	B.C. to *f*	1944	62 *a* : 40 *e*
	Total		360 *a* : 361 *e*
	F_2	1943	140 *a* : 62 *e*
	F_2	1944	6 *a* : 2 *e*
	Total		146 *a* : 64 *e*
R_2^{AS} (C.K. 8–27) × *e*	B.C. to *f*	1943	45 R_2^{AS} : 46 *e*
R_2^{OS} (N.K. 2–26) × *e*	B.C. to *f*	1944	16 R_2^{AS}† : 21 *e*
	B.C. to *e*	1944	16 R_2^{AS}† : 16 *e*
	B.C. to R_2^{OS}	1944	24 R_2^{AS}† : 27 R_2^{OS}
	F_2	1943	46 R_2^{AS}† : 33 R_2^{OS} : 28 *e*
R_2^{NO} (N.K. 1–26) × *e*	B.C. to *f*	1943	3 R_2^{NO} : 3 *e*
	B.C. to *f*	1944	5 R_2^{NO} : 7 *e*
	Total		8 R_2^{NO} : 10 *e*
	F_2	1944	6 R_2^{NO} (with tinged spot) : 2 *e*
R_2^{WO} (C.K. 1–27) × *e*	B.C. to *f*	1943	9 R_2^{WO} : 7 *e*
	B.C. to *f*	1944	32 R_2^{WO} : 27 *e*
	Total		41 R_2^{WO} : 34 *e*
	F_2	1943	10 R_2^{WO} : 14 R_2^{WO} (with tinged spot) : 8 *e*
	F_2	1944	6 R_2^{WO} : 8 R_2^{WO} (with tinged spot) : 5 *e*
	Total		16 R_2^{WO} : 22 R_2^{WO} (with tinged spot) : 13 *e*
R_2^{BO} (C.K. 2–27) × *e*	B.C. to *f*	1943	2 R_2^{BO} : 2 *e*
	B.C. to *f*	1944	52 R_2^{BO} : 44 *e*
	Total		54 R_2^{BO} : 46 *e*
	F_2	1944	2 R_2^{BO} : 12 R_2^{BO} (with tinged spot) : 4 *e*

* *a* and *e* = new alleles *a* and *e*, respectively).
† R_2^{AS} phenotype by complementary reaction between alleles (see text).

with respect to petal-spot expression has been described before in this series (Hutchinson, 1932; Silow & Yu, 1942). Evidently the new allele *e* and R_2^{FO} have a general similarity, and morphological comparison shows no difference between them except for the tinged spot. The hybrids from the new allele *e* and the spotless alleles all show tinged spot like the new allele *e* and have the body coloration of the other (spotless) parent. In Table 2 there are six combinations of crosses in all; three of them between the new allele *e* and spotted, and the other three between the new allele *e* and spotless. Some of the F_2 families are small, but it is safe to conclude that the new allele *e* is a member of the anthocyanin multiple allelic series.

B. *Crosses involving new allele f* (C.K. 5–27)

Combinations	Generations	Seasons	Progenies
*e** (C.K. 6–27) × *f**	B.C. to *f*	1943	52 *e* : 45 *f*
	F_2	1943	78 *e* : 20 *f*
f × R_2^{OS} (N.K. 2–26)	B.C. to *f*	1944	8 R_2^{OS} : 11 *f*
	B.C. to R_2^{OS}	1944	12 R_2^{OS}

* *e* and *f* = new alleles *e* and *f*, respectively.

The F_1 plant of the first cross listed above is similar to the new allele *e*, and the second similar to R_2^{OS}. This shows that the new allele *f* is completely recessive. Crosses involving the new allele *f* gave only a few plants, but from the records it is reasonable to conclude that the new allele *f* gives simple Mendelian ratios with the new allele *e* and R_2^{OS}. In other words, the new allele *f* is also one of the members of the anthocyanin multiple allelic series.

In phenotype R_2^{FO} and the new allele *f* are identical, but the latter does not give a coloured spot when interacting with R_2^{OS}, while the former does. It is evident that these two are analogous.

R_2^{OS} and R_2^{FO} are the lowest members previously reported in the anthocyanin series of Asiatic cotton. In this paper it is pointed out that the new allele *f* is recessive to these alleles, and is probably the basic member of the anthocyanin multiple allelic series.

C. *Crosses giving new types in addition to extracted parental forms*

Combinations	Generations	Seasons	Progeny
*a** (C.K. 7–27) × *e* (C.K. 6–27)	B.C. to *f*	1943	298 *a* : 321 *e* : 1 *b*
a (C.K. 7–27) × R_2^{BO} (C.K. 2–27)	F_2	1943	91 *a* : 19 R_2^{BO} : 1 *c*
a (C.K. 7–27) × *f* (C.K. 5–27)	B.C. to *f*	1943	3 R_2^{AS} : 106 *a* : 130 *f* : 1 *d*

* *a* ... *f* = new alleles *a* ... *f*, respectively.

The characteristics of the new allele *b* resemble those of the new allele *a*, but the former has a tinged spot, which is no doubt received from the other parent, C.K. 6–27, instead of the deep-red spot of the latter. The new allele *b* has the characteristics of both parents; it is a cross-over in nature. In this cross, more than 700 plants from backcross populations and more than 200 from F_2 populations have been classified over a period of 2 years, and only one cross-over-type plant has been found.

The new allele *c* also possesses characteristics of both parents; that is, most parts of the plant body are pigmented as in the new allele *a*, and the basal part of the petal is like that of the R_2^{BO} parent. In a segregating F_2 population of not less than 100 plants, only one cross-over occurred.

The new allele *d* has petals that are margined like the one parent and spotless like the other. The segregating population contained 240 plants, which included three R_2^{AS} plants in addition to the parental types and the new allele *d*. These three plants are evidently cross-over in nature just like the new allele *d*, but their expression is identical with that of the existing allele, characteristic of the variety in general cultivation. Furthermore, it seems worthy of mention that the new allele *d* and the three R_2^{AS} plants occurred in the same boll row.

The anomalous plants were selfed and progeny observations were made. All of them proved heterozygotes. Five of the six anomalies segregated out from a group of backcrosses in which narrow leaf was used as a marker character. All of them had intermediate leaves, and therefore their offspring showed segregation in leaf shape. The design of the backcrosses, and the fact that a leaf-shape marker was used, eliminates the possibility that these newly obtained R_2^{AS} plants may have been the result of contamination. Moreover, in respect to anthocyanin pattern they are not distinguishable from the 'wild' type (i.e. the type in general cultivation), so that a new designation is obviously not needed.

The phenotypic expressions of the new alleles *c* and *d* are really the same, though their genotypes may be different. The spotless character of the new allele *c* is obtained from

R_2^{BO}, which has a complementary effect, giving a deep-red spot, with R_2^{OS}. The spotless character of the new allele *d* is possibly the same as that of the new allele *f*, which is recessive to ghost spot, R_2^{OS}. If the suggested sources of the spotless character in *c* and *d* are correct, then the genetic constitution of these alleles should be different. In other words, the new alleles *c* and *d* may be analogous, a relation similar to that existing between the new allele *f* and R_2^{FO}.

It seems to be necessary to point out, too, that new alleles *b*, *c* and *d* have a common parent, new allele *a*. Although the other parents of these three new alleles are different in shade of pigmentation—namely, green tinged, sun-red spotless, and green recessive spotless—yet the plant-body colour and red petal margin of the three new alleles are indistinguishable, as shown in Table 1.

V. Discussion

The variations of anthocyanin pigmentation in Asiatic cottons are very remarkable. The senior writer collected the materials during 1934–6. Samples were obtained from the cotton-producing counties of China, and from each sample a row of about twenty plants was sown. The collection did not cover the whole country, for only a portion of the counties sent samples. More than half of the new types described previously and in the present paper were found in the collection, and the others were obtained through genetic studies. It is reasonable to hope that more materials will be obtained if a thorough study is made.

In the previous paper, the occurrence of R_2^{NO} and R_2^{AS} from allelic crosses that did not include them was considered under three categories, namely, possible contamination by natural crossing, mutation, and interchange of substance between chromosomes. The data in the foregoing section show clearly that interchange of substance between chromosomes is the only satisfactory explanation.

According to Hutchinson's original hypothesis (Hutchinson, 1934), there are two centres in the anthocyanin gene, one of which determines basal anthocyanin, the other its distribution. When an episome is present on the former centre, spotted petal is produced; in its absence the petal is spotless. Several episomes may be attached to the latter (distribution) centre, varying from four in the allele R_2^{RS} (full red, spotted) to one in R_2^{OS} (green, ghost spot). Hutchinson used these two gene centres and their attached episomes to interpret the constitution of the six alleles of the anthocyanin multiple allelic series, and he also predicted which of various combinations should be possible, which impossible. His hypothesis is not adequate to interpret the present situation because numerous new facts have accumulated; but it can still be used as a basis for discussion, assuming temporarily that gene centres control the mechanism of the problem. For convenience of discussion, the basal anthocyanin and distribution centres may be termed centres I and II, respectively.

Anomalies obtained from allelic crosses of anthocyanin multiple allelic series are listed below:

(1) $R_2^{MS} \times R_2^{WO} \rightarrow R_2^{NO}$.
(2) $R_2^{MS} \times R_2^{EO} \rightarrow R_2^{NO}$ (coloration of plant body is not so deep as that obtained in (1)).
(3) $R_2^{WO} \times R_2^{OS} \rightarrow R_2^{AS}$.
(4) New allele $a \times$ new allele $e \rightarrow$ new allele *b*.
(5) New allele $a \times R_2^{BO} \rightarrow$ new allele *c*.
(6) New allele $a \times$ new allele $f \rightarrow$ new allele $d + R_2^{AS}$.
(7) $R_2^{FO} \times R_2^{OS} \rightarrow$ new allele *f*.

Their sources and characteristics provide good material for testing the correctness of Hutchinson's hypothesis.

In the first place, the possibility of the occurrence of a basic recessive member like the new allele *f* was predicted by Hutchinson as a corollary of his scheme. Assuming that the types R_2^{FO} and R_2^{OS} each carry one episome, on gene centres I and II respectively, the new recombinations R_2^{AS} and new allele *f* should segregate out from a cross between these types, if interchange of genetic substance occurred between chromosomes. The new allele *f* was, in fact, obtained in such a cross, as shown in (7).

Secondly, the new allele *c* (5) occurs in the same way as R_2^{NO} ((1) and (2)). It is a new recombination from a coupling phase, if the terms of linkage studies may be used here. This fact shows that the Hutchinson hypothesis must be somewhat modified. According to the hypothesis, the episomes that affect the coloration of the petal margin, leaf, and calyx are attached to centre II. R_2^{NO} and the new allele *c* have no episome on this centre, but do have coloration of petal, leaf lamina, and calyx. It is reasonable to assume that the episomes affecting the coloration of plant body in spotless alleles may be attached to centre I, or, possibly, to a third gene centre.

Even disregarding the successive occurrence of R_2^{NO} and the new allele *c* in these studies, Hutchinson's hypothesis still requires modification. Ghost spot was supposed to be due to the presence of a single basic episome on centre II, to which, in the case of types with plant-body coloration, additional episomes were supposedly attached. Neither R_2^{WO} nor R_2^{BO} has the ghost-spot character, but their plant bodies have essential pigment, the episomes for which do not seem to be attached to the episome for ghost spot. This shows that Hutchinson's hypothesis requires modification from still another point of view.

Thirdly, the new allele *d* from (6) is a recombination of the new allele *f* and the red margin, red leaf, and red stem of the new allele *a*. In this new allele there is no episome on centre II as in R_2^{OS}; but it cannot be determined whether or not an episome is present on centre I, because even if present it might not have been transferred when the recombination of genetic substances took place. It is not distinguishable phenotypically. However, it seems noteworthy that the three R_2^{AS} plants and the new allele *d* were raised from the same boll. It is evident that all four plants were recombinations. According to Hutchinson's supposition, R_2^{AS} has an episome on each centre, and these interact with each other to produce the characteristics of sun-red stem and red spot. If the recombination R_2^{AS} has episomes on both centres, then the other recombination in the new allele *d* should not have an episome on centre I. In other words, the new allele *d* has the episomes producing coloration in petal margin, leaf, and stem, but has no episomes on either centre I or centre II. Therefore, it seems clear that there must be a third centre, concerned with plant-body coloration.

Fourthly, occurrence of the new allele *b*, as shown in (4), is quite comparable with that of *c* in (5). Both of them recombine the plant-body coloration and red-petal margin of one common parent with the petal-spot character of the other. As seen in the data, the new allele *e*, the parent having the same spot coloration as the new allele *b*, has the characteristic of all spotless alleles; namely, it gives a dark-red spot when interacting with ghost spot. From this similarity, one is inclined to infer that the new allele *e* and other spotless alleles have a common genetic substance. This situation may be comparable with that of plant-body coloration, in which a high-grade member, besides having its own special character, commonly has the characteristic of a lower-grade one, from

which it differs, as Hutchinson considered, only in carrying an additional episome. In this case, both new allele *e* and its derived type, new allele *b*, should have an episome on the same gene centre as that of R_2^{FO}. The lightly tinged red-petal spot is similar in R_2^{TS} and the new allele *e*, though in the former it overlies ghost spot and in the latter, spotless. The similarity may not be due to their having the same genetic substance; from the genetic analysis it appears that R_2^{TS} is closely related to R_2^{OS}, and new allele *e* to R_2^{FO}. As mentioned before, R_2^{OS} and R_2^{FO} have episomes on different gene centres.

Fifthly, as was reported previously, many R_2^{AS} plants were found among the offspring of crosses between R_2^{WO} and R_2^{OS}, as shown in (3). As no reciprocal combination was found, and since R_2^{AS} is the most common type in general cultivation, such anomalies were suspected to be the outcome of accidental contamination by natural crossing, even though controlled selfing is practised in genetic study. From the present evidence it may be concluded that the reciprocal recombination from such a cross would be a type analogous to R_2^{WO} and indistinguishable from it phenotypically. The relation between the analogous type and R_2^{WO} is just like that between the new allele *c* and the new allele *d*. In this case, even if such a recombination occurred along with R_2^{AS}, it could not be detected phenotypically from its analogue, R_2^{WO}.

To sum up, it may be concluded that the anthocyanin alleles in Asiatic cotton involve at least three gene centres. They are:

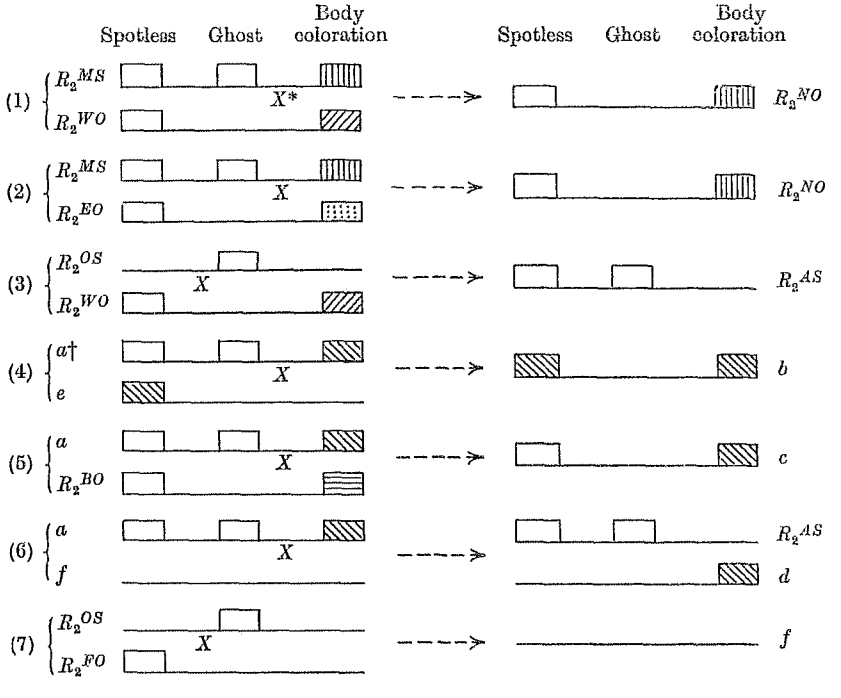
(I) Centre of genetic substance for spotless,
(II) Centre of genetic substance for ghost spot, and
(III) Centre of genetic substance for coloration of other parts of plant body.

In the terminology of Hutchinson's hypothesis, each of these centres carries several episomes.

Although Hutchinson's original hypothesis and the modifications of it that have been suggested here are based entirely on genetic studies, the facts do not seem inconsistent with McClintock's recent cytological findings in corn (McClintock, 1944). Her observations showed that the pale-yellow deficiency is due to the loss of the heterochromatic knob of chromosome 9; an additional loss of a part of the adjacent chromomere causes the white deficiency, and the yellow-green locus is situated on the chromomere just beside the heterochromatic knob. Genetical facts show that normal green, pale-yellow deficiency, and white deficiency form one allelic series, while normal green, yellow-green mutant, and white deficiency form another allelic series, but that pale-yellow deficiency and yellow-green mutant give a green hybrid in combination. This situation is just like that which occurs in the anthocyanin pigmentation of Asiatic cotton. All the spotted and ghost-spot types (R_2^{RS}, R_2^{MS}, ..., R_2^{DS}; R_2^{OS}) form one allelic series, and the spotted and spotless types (R_2^{RS}, R_2^{MS}, ..., R_2^{DS}; R_2^{NO}, ..., R_2^{FO}) form another; but when R_2^{OS} is combined with any spotless type, then the resulting plant has a petal spot different from both parents, and when any one spotted allele is crossed with any one spotless allele, cross-over types may be found in the progeny. Cytological data concerning anthocyanin pigmentation in Asiatic cotton are completely lacking; yet although there is no evidence of chromosome deficiencies as in corn, the genetical observations are quite comparable. It is therefore not unreasonable to suppose that the so-called anthocyanin allelic series in Asiatic cotton may involve several loci, and that the anomalous 'cross-over' types reported here are the results of intergenic interchange, i.e. normal cytological crossing-over.

One may ask why genes affecting the same character are crowded together on the chromosome. Dunn & Caspari (1945) recently reported their work on the mutations of the tail and axial skeleton in the mouse. They pointed out that mutations affecting the characteristics of the same part of the body tissue may occur in adjacent regions of the chromosome. The reason suggested is that a change in a certain segment of chromosome is likely to be extensive and to cause abnormalities in early developmental processes, the effects of which converge on the axial structures of the mouse. From this it would appear that genes having similar effects, or segments of chromatin determining similar characters, may sometimes be located adjacently on the chromosome.

The question arises whether the present data provide critical evidence to distinguish between a situation involving *intergenic* recombinations, as in the maize and mouse cases cited, and *intragenic* rearrangements, as postulated by Hutchinson. If the separable units are really adjacent loci, it should be possible not only to predict new cross-over types to be expected from crosses between parents differing in more than two units, but also to obtain some evidence of the linear order of the separable units on the chromosome. On the other hand, if the recombinations are a result of intragenic rearrangement they need show no evidence of linear order. But bearing the former possibility in mind, it may be of interest to reconsider the crosses in which the anomalous types occurred. If the linear order of the loci on the chromosome is as shown in the following diagram, then all of the so-called anomalies would represent expected cross-overs.



* Crossing-over has taken place. † *a*...*f*=new alleles *a*...*f*.

Examination has shown that with no alternative arrangement of the units is it possible to account for the anomalies, without involving double cross-overs. The chance of obtaining double cross-overs between such closely linked units would be infinitely small, even if it

were mechanically possible. Thus, though the validity of the scheme suggested above requires further testing, it clearly provides a satisfactory explanation for the data at present available.

Another interesting fact, which may be more or less related to the three-loci interpretation, is the apparent stability of plant-body coloration, including character of petal margin, as compared with character of petal-spot coloration. It has been mentioned in the case of new alleles *b*, *c* and *d*, which have one parent in common (new allele *a*), that although their spot colour has changed, apparently through crossing-over, yet in shade of plant-body pigmentation they are quite similar to new allele *a*. In other cases of recombination, alterations in plant-body colour occur in addition to changes in petal character, but there is no good reason for supposing that the former is due to crossing-over between units controlling plant-body pigmentation. Thus the R_2^{NO} recombination type obtained from $R_2^{WO} \times R_2^{MS}$ has a more intense plant-body pigmentation than the same type extracted from $R_2^{MS} \times R_2^{EO}$, but the colour dilution in the latter cross is expected, since the R_2^{EO} parent is a strain of *G. herbaceum* in which suppressors of anthocyanin are known to be present.* In general, interspecific crosses involving anthocyanin alleles, in the combination dark-coloured *arboreum* × light-coloured *herbaceum*, show a very complex segregation in plant-body colour, and the dark-coloured parental type is rarely recovered. Again, the cross-over type R_2^{AS}, obtained from $R_2^{WO} \times R_2^{OS}$, has a sun-red plant-body colour, intermediate between those of the parents. But the hybrid resulting from a parallel cross, $R_2^{FO} \times R_2^{OS}$, has sun-red pigmentation, too, even though both its parents have green plant bodies. It is more natural to assume that the sun-red colour resulting from both crosses is due to the interaction of a spotless component with ghost spot than to postulate two different mechanisms. So far, no cases of recombination in plant-body pigmentation, comparable to the cross-over types affecting petal coloration, have been observed.

If three loci really control the anthocyanin pigmentation of the Asiatic cottons, then what was formerly considered a single allelic series may more logically be separated into three. The present members of each series are as follows:

(1) Coloration of plant-body series. Full red—red margin—red margin (dilute plant body)—red leaf—red vein—red calyx—sun red—thumbnail red—green plant body.

(2) Ghost-spot series. Tinged on ghost—ghost—basic recessive.

(3) Spotless series. Tinged on spotless—spotless—basic recessive.

If the allelic members in the three series can combine with and separate from each other through crossing-over purely by chance, then there would be many more types expected than the present twenty. Some of the combinations, however, seem likely to give the same expression; for instance, the tinged on ghost spot gives practically the same phenotypical red spot when combined with either tinged on spotless, or spotless. This would cut down the number of phenotypically distinguishable members. In the left-hand portion of Table 3 the existing types are located with respect to their body coloration and spot characters, and the expected types are left blank.

A possible complication that may occur in the further analysis of the anthocyanin inheritance in Asiatic cottons needs to be mentioned; that is, the possibility of unequal crossing-over (Lewis, 1941). In a group of at least twenty—and probably many more—

* Hutchinson (1932) considered that anthocyanin intensifiers are present in *G. arboreum*, which is a different way of describing what in the present instance may be considered the same phenomenon.

variables, it might be suspected that some are the result of unequal exchange of genetic substance. Furthermore, unequal crossing-over can occur even in apparently homozygous stocks (Sturtevant, 1926; Bridges, 1936). As far as the data reported in this paper are concerned there would appear to be no reason to suspect this complication, as among seven cross-combinations from which new types other than the extracted parental forms were obtained, one gave both of the types expected on a basis of reciprocal interchange, and in the six remaining cases the constitution of the new types was not inconsistent with the same hypothesis. There are, however, fifteen members which, since they were obtained from natural populations, provide no critical information of their mode of origin.

As all the puzzles are cleared up, we can turn to the problem of nomenclature of this complicated group involving numerous members. The R_2 symbol with a superscription system (Hutchinson & Silow, 1939) promises to be very satisfactory and needs no radical change; but since the analysis indicates that three genetic units are probably involved in controlling the anthocyanin characters, a three-letter superscription would be pre-

Table 3. *Suggested nomenclature of the existing types of anthocyanin pigmentation of Asiatic cottons*

	Present symbols						Suggested symbols					
Spot intensity ... ... / Extent of anthocyanin on plant body	Dark-red spot	Tinged on ghost	Ghost spot	Tinged on spotless	Spotless	Basic spotless	Dark-red spot	Tinged on ghost	Ghost spot	Tinged on spotless	Spotless	Basic spotless
Full red	R_2^{RS}	—	—	—	—	—	R_2^{RGS}	—	—	—	—	—
Red margin	R_2^{MS}	—	—	—	R_2^{NO}*	—	R_2^{MGS}	—	—	—	R_2^{MOS}	—
Red margin, dilute plant body	a†	—	—	b*†	c*†	d*†‡	R_2^{NGS}	—	—	R_2^{NOF}	R_2^{NOS}	R_2^{NOO}
Red leaf	R_2^{LS}	—	—	—	—	—	R_2^{LGS}	—	—	—	—	—
Red vein	R_2^{VS}	—	—	—	R_2^{VO}	—	R_2^{VGS}	—	—	—	R_2^{VOS}	—
Red calyx	R_2^{CS}	—	—	—	—	—	R_2^{CGS}	—	—	—	—	—
Sun red	R_2^{AS}	—	—	—	R_2^{BO}	—	R_2^{AGS}§	—	—	—	R_2^{AOS}	—
Thumbnail red	R_2^{DS}	—	—	—	R_2^{EO}	—	R_2^{DGS}	—	—	—	R_2^{DOS}	—
Green plant body	R_2^{AS}*	R_2^{TS}	R_2^{OS}	e†	R_2^{FO}	f*†	R_2^{OGS}§	R_2^{OTO}	R_2^{OGO}	R_2^{OOF}	R_2^{OOS}	R_2^{OOO}

* Synthetic (in addition, an R_2^{AS} type with sun-red plant body was synthesized).
† $a \ldots f$=new alleles $a \ldots f$ respectively.
‡ Genetic constitution not tested. § Analogous.

ferable to the two-letter one. The writers suggest using the first letter of the superscription to denote the coloration of plant body as a whole, excluding petal spot, with which the second and third letters are solely concerned. In other words, each letter would represent one of the three possible multiple allelic series. For the purpose of illustrating the possible order on the chromosome of these three units, the second letter would denote ghost spot and the last spotless. If such a system is adopted, the notation of the existing types will be altered as shown in the right-hand portion of Table 3. In the table, red-spot types are tentatively designated as *GS*, since there is no evidence at present to indicate to which combination out of the four possibilities (viz. *TF*, *TS*, *GF* and *GS*) the spot is due, for all of the three last named give the same result, as reported previously and in the present paper. Though direct evidence from the *TF* combination is not available, it would be likely to give red spot, too, and be indistinguishable from the other three.

VI. SUMMARY

The so-called multiple allelic series of anthocyanin pigmentation of Asiatic cotton is extended from fourteen to twenty members. Two of the additional members were found in indigenous material, and the remaining four were synthesized from existing types.

The genetic substance affecting the characters in this so-called multiple allelic series seems to be separable. Sometimes, in certain crosses, two members of the series may be combined to form another one, or, on the other hand, one member may be split into two others. But such combining and splitting take place only between petal characters, or between plant-body coloration and petal characters; and plant-body coloration, itself, seems to be very stable. At least in one case, the crossing-over phenomenon is very clearly established, in that both cross-overs and parental types are recovered in the F_2 progeny. The so-called multiple allelic series, therefore, may be subdivided into several series. In addition to the facts presented in this paper, evidence of closely linked genes affecting the same part of the body tissue has been reported recently both in corn and in the mouse.

Hutchinson's explanation of the inheritance of anthocyanin pigmentation in Asiatic cotton is considered in detail in this paper. It is found that some modification is necessary to make it fit the new facts. Probably a three-unit mechanism will prove more acceptable than his original two-gene-centre hypothesis. In spite of requiring minor modifications, however, his hypothesis agrees reasonably well with the cytological findings recently reported in corn.

That the three units may really be three closely linked but separable loci is in accordance with the genetic facts. On this basis, there is a multiple allelic series corresponding to each locus, namely, plant-body coloration, ghost spot, and spotless series. These consist of nine, three, and three alleles, respectively; and the number of possible combinations among the series is sufficient to account for the existing types.

A new system of nomenclature of anthocyanin characters in Asiatic cottons is suggested, in order to illustrate the genetic behaviour of the members and to clarify the relationship of the different types.

Although this paper adds some new facts and interpretations, there is still a long way to go before a solution of the entire problem is reached.

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