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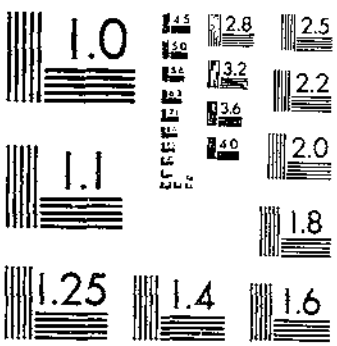

MICROCOPY RESOLUTION TEST CHART
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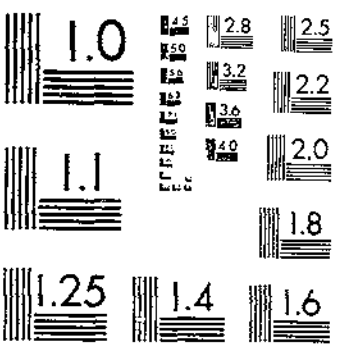

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GENETIC RELATIONS OF SOME COLOR FACTORS IN LETTUCE

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UNITED STATES DEPARTMENT OF AGRICULTURE, WASHINGTON, D. C.

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INTRODUCTION

The usual method of insuring against self-fertilization by emasculation in making crosses cannot be used in small-flowered compositae like *Lactuca*. In plants of this type, in which emasculation is not practicable, it is necessary to remove the pollen from the stigmas after the anthers have dehisced but before pollen germination. Depollination is generally effected by means of a small stream of water. The removal of pollen by means of water was first employed by Oliver (*19*)[4] in hybridizing a number of small-flowered species, including lettuce. Any method of pollen removal after the anthers have dehisced is open to error. Even with extreme care it is never a cer-

[1] Submitted for publication November 13, 1937.

[2] Adapted from a thesis submitted to the graduate school of the University of Maryland in partial fulfillment of the requirements for the degree of doctor of philosophy.

[3] The writer acknowledges his indebtedness to J. H. Beaumont and A. Lee Schrader, of the Department of Horticulture, University of Maryland, under whose sponsorship these studies were made, for guidance and helpful suggestions given throughout the course of the investigations; to V. R. Boswell, of the Bureau of Plant Industry, U. S. Department of Agriculture, for helpful suggestions in the arrangements and presentation of the results and for careful reading of the manuscript; and to W. B. Kemp, of the Department of Genetics and Statistics, University of Maryland, for reviewing the genetic data and for valuable assistance in computing the linkage values.

[4] Italic numbers in parentheses refer to Literature Cited, p. 36.

tainty that every grain of pollen has been removed from the stigmas and stylar bristles. Prolonged washing with water in removing the pollen often causes mutilation of the flower parts, resulting in failure to obtain seed. With 15 to 20 florets in a single head there is always the possibility that selfed and hybrid seed will be produced in the same head. It is obvious, then, that a knowledge of the expression of some dominant genetic factors is necessary in order to separate the selfed from the hybrid individuals in the population from a single head.

The studies herein reported were undertaken to determine the genetic behavior of certain color factors in the lettuce plant that might be used in separating selfs from hybrids in the progenies from lettuce flowers that have been artificially cross-pollinated. In addition to being of value in breeding and genetic studies with lettuce, a knowledge of the inheritance of these color characters, anthocyanin, chlorophyll shade, and seed color, is very important in classifying and standardizing the many lettuce varieties now in cultivation. The importance of color characters in variety classifications of crop plants is apparent in looking through the numerous variety classifications of cultivated plants that have been published. An example of such a classification is that on lettuce varieties by Tracy (*27*) in which anthocyanin, chlorophyll, and seed colors are among the important characters employed in identifying the hundred or more known varieties of cultivated lettuce (*Lactuca sativa* L.).

It is desirable that the characters used in separating the selfs from hybrids be recognizable early in the life of the plant. Much time and labor in the handling of plants can be eliminated if the undesired self can be recognized and discarded in the early stages. Chlorophyll and anthocyanin are characters that can be recognized while the plants are small. A knowledge of their mode of inheritance is a valuable aid in genetic studies in lettuce. Color of seed is of less value as an identifying character in separating selfs from hybrids, in that it is necessary to grow the plants to maturity in order to make use of it.

PREVIOUS STUDIES

The inheritance of pigments has been a fertile field for research in plant genetics. The inheritance of few plant characters has received more attention than that of the pigments, perhaps partly because the contrasts between the color types make them more easily classified than many other plant characters. The literature on the inheritance of the plant pigments is voluminous, and reference will be made here to only a few papers that are of particular interest in connection with the present studies.

A comprehensive bibliography on the chemistry and genetics of the anthocyanins was published by Onslow (*20*) in 1925. Since then many additional papers have appeared dealing with the genetic relations of the anthocyanins.

In the many studies reported on the inheritance of anthocyanin numerous gene relationships have been presented. The factor relationships vary from the simple single factor cases, of which there are many examples, to the very complex multiple-factor inheritance, of which the flower color in *Antirrhinum majus* L. is an illustration. Many of the known genetic ratios have been demonstrated in studies of the inheritance of anthocyanin.

Combes (*5, 6*), Everest (*11*), Keeble, Armstrong, and Jones (*17*), Onslow (*20, 21, 22*), Robertson and Robinson (*23*), Sando and Bartlett (*24, 25*), Willstätter and Mallison (*28*), and many others have studied the chemistry of the anthocyanins in its relation to Mendelian inheritance. The researches on the chemistry and genetics of color in plants indicate that in the inheritance of these pigments we are dealing with the inheritance of chemical compounds and chemical reactions.

Although there is a great volume of literature on the inheritance of anthocyanin in plants there is very little on the inheritance of these pigments in *Lactuca*. Durst (*10*) studied the genetics of anthocyanin in his inheritance studies on lettuce. In his study of the pigment in the leaves of the lettuce plant he recognized only one type or pattern of pigmentation, which he found to be due to a single dominant gene. In several crosses between pigmented and green varieties he obtained F_2 progenies which segregated 3 pigmented to 1 green. The three pigmented varieties used by Durst in his crosses were Big Boston, May King, and a pigmented plant from the wild species *Lactuca scariola* L., all belonging to the pigmented type classed by the writer as tinged. The green varieties used were Grand Rapids and Paris White Cos. The writer has found these two green varieties to belong to the same genotype, and when either of them is crossed with one of the pigmented varieties, Big Boston, May King, or the pigmented wild *L. scariola*, a ratio of 3 pigmented to 1 green is obtained in the F_2. If the pigmented plants used by Durst had been crossed with some other green variety such as Hanson, New York, or Deacon the F_2 phenotypes and distribution would still have been 3 pigmented to 1 green, but not all the pigmented plants would have been like the pigmented parent.

Lewis (*18*) states that the F_2 from a cross of the red variety Mignonette with the green variety Hanson consisted of 3 red plants to 1 green. Data on progenies from this same cross (No. 15), are presented in table 4 and agree with the results obtained by Lewis.

Chlorophyll deficiencies similar to the one herein reported which are non-Mendelian in inheritance have been observed in many plant species. Probably the first authentic case of this type of non-Mendelian chlorophyll deficiency in which variegation is inherited only through the variegated branches of the mother plant was that reported by Correns (*8*) in the four-o'clock, *Mirabilis japonica*. Such deficiencies have since been reported in some 40 different plant species. The species exhibiting this type of chlorophyll deficiency and the investigators reporting them have been summarized by De Haan (*14*). This type of deficiency is known to occur in a number of the important vegetable crop plants, including peas, beans, beets, corn, tomatoes, and peppers.

The term "status albomaculatus" has been applied by Correns (*8*) to chlorophyll deficiencies in which variegation is inherited only through the variegated portions of the mother plant and in which the inheritance is not affected by the pollen.

In the present investigations no attempt was made to account for the physiological basis of inheritance. Several theories have been advanced as possible explanations for the genetic behavior of this type of chlorophyll deficiency.

Anderson (*1*), Baur (*2*), Chittenden (*3*), Clausen (*4*), Correns (*7, 8*), Demerec (*9*), Gairdner and Haldane (*13*), Imai (*15*), Sturtevant (*26*),

and Yasui (*29*) give various interpretations to the genetic behavior of albomaculata plants.

Sturtevant (*26*) attributes the behavior of the chlorophyll in albomaculata plants to lethal genes to which the genes for variegation are coupled.

Demerec (*2*) explains this type of variegation on the basis of gene mutation. He assumes the white portion of the leaf to be due to a dominant mutable gene and that the green portions are due to mutation of the dominant gene for white to the recessive gene for green.

Baur (*2*) places special emphasis on the behavior of the plastids.

Correns (*8*) suggests that the plastids of the young cells in albomaculata plants occur in a labile state and may later change either to the normal condition having green plastids or to a state in which the plastids are colorless.

Yasui (*29*) assumes that the three colors green, variegated, and white characteristic of albomaculata plants are due to three different kinds of plastids.

The lack of agreement among those who have studied this type of inheritance indicates that much additional study must be made before the physiological basis of its inheritance can be satisfactorily explained.

Seed color in lettuce has been studied by Durst (*10*). He found that black seed in the variety Grand Rapids and the wild species *Lactuca scariola* is due to a single factor dominant to the factor for white seed in the varieties May King, Paris White Cos, and Big Boston. In each of the six crosses reported the F_2 segregated approximately 3 black-seeded to 1 white.

MATERIALS AND METHODS

SOURCES OF PARENT STOCKS

The plant materials used in the study of inheritance of color in the leaves and seed of lettuce were obtained from seedsmen's stocks of commercial varieties. Nine individual plants or progenies from them, each representing a different genetic type, were used in the crosses that supplied the materials for these studies. Each of these was selfed and tested for two generations, and each proved to be homozygous in respect to its anthocyanin phenotype. Each selection used in the crosses was identified by a capital letter which was used throughout as part of the pedigree number. The plant used as a representative of the red phenotype was selected from the variety Mignonette and was identified by the letter M. The parent stock of the spotted anthocyanin group was selected from the variety California Cream Butter and assigned the letter B. A selection from the variety Iceberg, identified by the letter I, was used as the parent in crosses involving the tinged anthocyanin phenotype.

In addition to the three stocks representative of the anthocyanin phenotypes, green plants selected from six commercial varieties were used as parents in crosses made to establish the validity of the hypothesis that a pair of complementary genes and a multiple allelomorphic series of three genes are involved in the genetics of the four color types, red, spotted, tinged, and green. Green plants were selected from the following varieties and given the identifying letter indicated: Hanson (H), New York (N), Grand Rapids (R), Transport (T), a hybrid selection from a cross of Mignonette×New York (MN), and a selection from a cross of New York×White Chavigne

(NC). The original seed of the variety Transport was obtained from Simon N. Groot, seed grower, of Enkhuizen, Netherlands. The variety White Chavigne was from Vilmorin-Andrieux & Cie., of Paris, France. The strain of Mignonette was from W. Atlee Burpee & Co., of Philadelphia, Pa. The stocks of New York, Hanson, Iceberg, and California Cream Butter were from the Ferry-Morse Seed Co., of San Francisco, Calif. The Grand Rapids was a selection from a stock of this variety that has been maintained by the Division of Fruit and Vegetable Crops and Diseases for several years.

The parent plants used in the crosses were all grown in 10-inch clay pots in a greenhouse. The ventilators and doors of the greenhouse were all covered with fine copper-wire screening to keep out as far as possible any insects that might be instrumental in cross-pollinating.

In most cases the seed of the parent stocks was planted in flats about the first of January. The seedlings were pricked off into other flats when they had reached a suitable size. The young plants were spaced 2 inches apart each way when transplanted and were permitted to remain in the flats until set permanently in the 10-inch clay pots.

In most cases the parent plants came into flower about the middle of May. Most of the hybridizing was done during the latter half of May and early June.

DEPOLLINATION AND HYBRIDIZATION

Normally, the lettuce flower opens in early morning and remains completely open for only a short period. The time of opening and the period the flower remains open depends largely on environmental conditions. When the night temperature is relatively high and the morning bright and warm the flowers open earlier and remain open for a shorter period than when the night temperature has been relatively low and the morning cloudy. Under optimum conditions for anthesis the flowers may remain open for less than an hour. Once the flower closes it never opens again. New buds open each morning during the flowering period. If the morning is cool and cloudy, lettuce flowers may open slowly and remain open for several hours. Flowers have been observed to remain open until after noon on cool, cloudy days. Under such unfavorable conditions of light and temperature the opening of the flowers is irregular and the emergence of the stigmas from the anther sheaths is quite irregular within a single head. Depollination and hybridization are very difficult when conditions are not favorable for rapid opening of the flowers. Pollen removal and hybridization were found to be most successful when done on bright, warm mornings. The short time that the flowers remain open under such conditions limits the number of flowers than can be worked in one day. The work of depollinating, crossing, tagging, and recording must be done rapidly if many crosses are to be made.

The anthers of the lettuce flower dehisce and the stigmas may be covered with pollen when they emerge from the anther sheath. Since the flowers are too small and delicate to permit emasculation, it is necessary to resort to some method of removing the pollen from the stigmas as soon as they emerge and before the pollen tubes have entered the stigmatic tissues. Jones (*16*) found that fertilization in lettuce takes place within a few hours after pollination. This emphasizes the necessity of prompt removal of the pollen from the

stigmas if self-pollination is to be avoided. The method of depollination with water first described by Oliver (*19*) was found to be the most satisfactory. This consists of washing the pollen from the stigmas by means of a small stream of water. The dentists' chip blowers illustrated in figure 1 were found to be very satisfactory for this purpose. Considerable water remains in the flower head after washing. This must be removed before the pollen to be used in the cross is applied. Small pieces of blotting paper were first used to remove the water from the heads. It was later found possible and more rapid to remove the water by a blast of air. A satisfactory method and the one used in much of this work consists in blowing the excess water from the heads by two or three puffs of air from the mouth. By this means it is possible to remove the water at once after washing and while the flower head is still held between the fingers as in the washing process.

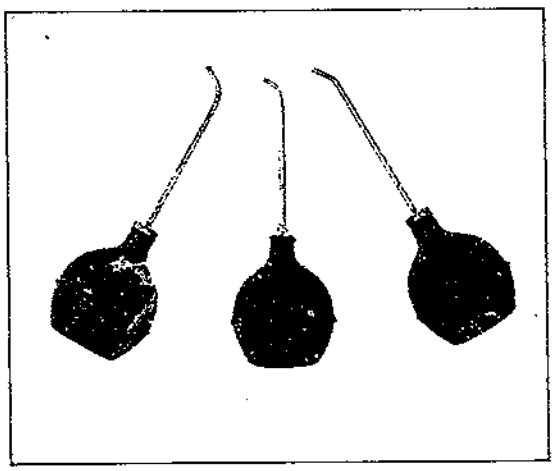


FIGURE 1.—Dentists' chip blowers found to be the most satisfactory means of applying water to lettuce heads for the removal of the pollen. The force with which the water is applied can be controlled by the pressure exerted on the bulb.

In order to speed the work involved in making the crosses a study was made to determine a satisfactory procedure for pollen removal.

Records were kept on a number of heads depollinated by washing once at different stages of anthesis. The pen drawings in figure 2 show individual lettuce florets at different stages of anthesis. Figure 2, *A*, shows a flower just before the stigma emerges from the anther sheath. The style is elongating rapidly at this time and the stigma is soon forced through the apex of the sheath, as shown in *B*. In *C* the flower is in an intermediate stage of development; the style is well extended beyond the sheath, and the stigmatic lobes are just beginning to separate and turn back on the style. A late stage of anthesis is shown in *D*. The flower head soon closes after the flowers reach this stage, and depollination and hybridization are then impossible.

In studying the procedure for pollen removal, records were kept on the number of hybrid and selfed seed produced in three groups of heads washed at different stages of anthesis. In the group recorded as washed at an early stage most of the stigmas had just emerged and were in the condition shown in figure 2, *B*. Those washed at the intermediate stage were heads in which the majority of the florets were at the stage indicated in *C*. The late group consisted of heads in which most of the florets were well advanced, having their styles fully extended and the stigmatic lobes turned back on the style.

A green plant of the variety White Chavigne was used as the maternal parent in this study. Pollen was obtained from a plant of the

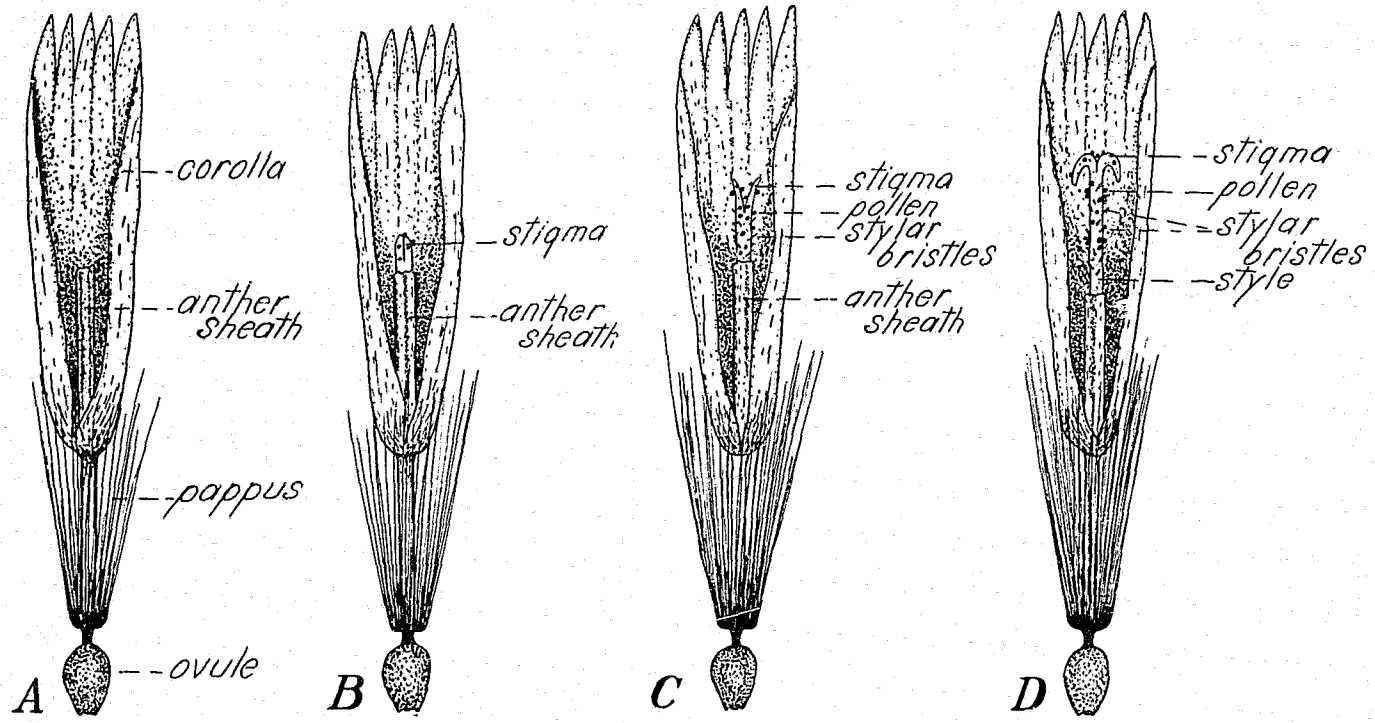


FIGURE 2.—*A*, An individual lettuce floret in an early stage of anthesis, just before the stigma emerges from the anther sheath. *B*, An individual lettuce floret with the stigma emerging from the apex of the sheath. Most of the styles should be extended beyond this stage before washing is begun. *C*, A lettuce floret at the stage found to be satisfactory for pollen removal by washing with water. *D*, A lettuce floret at a late stage of development. A flower at this stage is too far advanced for successful removal of pollen and cross-fertilization.

variety Mignonette. Some preliminary crosses had shown the anthocyanin pigment in the leaves and the black seed of Mignonette to be dominant characters. The F_1 plants resulting from this cross which had anthocyanin in the leaves and produced black seed were known to be successful crosses. It was then possible to separate the selfs from the hybrids in the progenies from the various heads. The results obtained are given in table 1.

Data were obtained from 23 flower heads depollinated at the early stage, 26 heads at the intermediate stage, and 28 heads in the late stage. Two flower heads in the early, three heads in the intermediate, and six heads in the late group failed to develop any seed. The early group averaged 13 seeds, the intermediate 10.6 seeds, and the late 8.3 seeds per head. Thirty heads taken at random from the same plant on which the crosses were made but which were not depollinated but permitted to self naturally averaged 16 seeds per head. Depollination and artificial cross-pollination greatly reduced the number of seeds per head.

In the cross-pollinated groups the intermediate gave the highest percentage of hybrid seed, 73.2 percent. The late group averaged only 27.6 percent hybrids, and the early group 33.8 percent.

The results indicate that a single washing at a stage of anthesis near that illustrated in figure 2, *C*, if done carefully, will result in a fairly high percentage of successful crosses. If some known dominant character is introduced into the cross from the pollen parent the undesired selfs can be isolated in the F_1 population.

TABLE 1.—*Data on progenies from lettuce flower heads depollinated at different stages of anthesis*

EARLY STAGE

Head No.	Total seeds per head	Selfed seeds	Hybrid seeds	Head No.	Total seeds per head	Selfed seeds	Hybrid seeds
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
1	15	12	3	14	20	8	12
2	16	9	7	15	17	13	4
3	18	8	10	16	21	18	3
4	7	7	0	17	16	11	5
5	10	6	4	18	0	0	0
6	17	9	8	19	17	9	8
7	14	8	6	20	15	12	3
8	16	14	2	21	13	8	5
9	19	12	7	22	9	4	5
10	13	8	5	23	11	10	1
11	5	5	0				
12	10	7	3	Total	299	198	101
13	0	0	0				

INTERMEDIATE STAGE

1	14	4	10	15	10	1	9
2	8	1	7	16	17	5	12
3	10	2	8	17	0	0	0
4	14	5	9	18	7	1	6
5	12	4	8	19	12	5	7
6	10	0	10	20	15	1	14
7	0	0	0	21	9	0	9
8	7	2	5	22	14	4	10
9	15	6	9	23	11	4	7
10	13	3	10	24	18	6	12
11	14	6	8	25	15	2	13
12	11	3	8	26	0	0	0
13	13	6	7				
14	8	3	5	Total	277	74	203

TABLE 1.—*Data on progenies from lettuce flower heads depollinated at different stages of anthesis*—Continued

LATE STAGE

Head No.	Total seeds per head	Selfed seeds	Hybrid seeds	Head No.	Total seeds per head	Selfed seeds	Hybrid seeds
	Number	Number	Number		Number	Number	Number
1	5	1	2	16	5	4	1
2	0	0	0	17	0	0	0
3	11	10	1	18	11	5	6
4	7	5	2	19	10	7	3
5	0	0	0	20	15	9	6
6	13	8	5	21	7	5	2
7	5	5	0	22	13	11	2
8	10	7	3	23	0	0	0
9	15	5	10	24	11	9	2
10	9	6	3	25	5	5	0
11	14	13	1	26	12	9	3
12	11	10	1	27	17	9	8
13	12	9	3	28	0	0	0
14	16	16	0				
15	0	0	0	Total	232	168	64

It is obvious that the less the floral parts are mutilated in depollination the better are the chances for a high percentage of successful crosses. The delicate floral parts are likely to be injured and a poor set of seed obtained if an attempt is made to remove every grain of pollen from the head. With 15 to 20 florets to a single head the chances of getting at least a few hybrid seeds are good even though some pollen remains after washing.

Depollination and successful crossing are more easily accomplished in some varieties than in others.

Varieties like Hanson, in which the central florets have very narrow, almost hairlike corollas, are more difficult to handle in depollinating and hybridizing than varieties like White Chavigne and California Cream Butter, which have broader and stiffer corollas. The narrow ligules of flowers such as Hanson have a tendency to tangle with the styles in the late stages of anthesis, making pollen removal difficult.

Heads from the pollen parent that had been protected from foreign pollen were used to pollinate the washed heads. The pollen was applied by inverting the head carrying the pollen and forcing its stigmas into the washed head. By a slight rotating movement of the pollen-bearing head a large quantity of pollen was transferred to the washed stigmas.

GENETIC RELATIONS OF FACTORS FOR ANTHOCYANIN

DESCRIPTION OF ANTHOCYANIN COLOR TYPES

Although the intensity and extent of the pigmented area in the leaves of lettuce vary greatly under different environmental conditions, three distinct types of intensity and pattern of the leaf pigment were readily distinguished in this work when the plants were all grown under the same conditions.

Green.—In addition to the three types—red, spotted, and tinged—carrying anthocyanin, there are numerous green varieties lacking anthocyanin in any portion of the plant (pl. 1, *A* and *B*). It will be shown in another section of this bulletin that most of these green plants carry some of the genetic factors for anthocyanin.

Tinged.—The most dilute anthocyanin phenotype includes such varieties as Iceberg, May King, and Big Boston. Only under conditions favorable for anthocyanin development is the pigment at all conspicuous in this type. In the seedling stage anthocyanin is confined almost entirely to a limited area around the margins of the leaves. At this stage the pigmentation of the tinged type can be easily observed only under conditions favorable for pigment development. Under very favorable conditions, such as low temperature with high light intensity, the entire exposed surface of the leaves of this type may show a tinge of anthocyanin. This type of coloration is referred to throughout this discussion as tinged. The colored illustrations (pl. 1, *C* and *D*) show this color type.

Spotted.—The second recognizable anthocyanin color type in lettuce is intermediate between the red and the tinged. The dense localized spots characteristic of the red are combined with the weak overcasting of pigment characteristic of the tinged. If the spots were removed from this type of plant it would be a typical tinged type. This type is referred to by the writer as spotted. The varieties California Cream Butter, Maximum, and Dutch Speckled Butter are among those belonging to the spotted group. This type is shown in plate 2, *A* and *B*.

Red.—The most heavily pigmented type includes such varieties as Mignonette, Prize Head, and Crisp As Ice. Under favorable conditions for the development of anthocyanin, plants of this phenotype appear to be heavily pigmented throughout the entire light-exposed portion of the plant. If conditions are unfavorable for anthocyanin the intensity of the pigment is much more dilute, and some exposed parts of the leaves may appear to be free of the pigment. In the seedling stage and under conditions unfavorable for anthocyanin, small localized areas of very dense pigment can be observed scattered at random over the exposed parts of the leaves. When the environment is favorable for anthocyanin these spots of dense pigment are submerged by the density of the pigment throughout the leaf and under such conditions are not easily observed. This anthocyanin phenotype has been designated as red by the writer and is so referred to throughout this bulletin. The red type is illustrated in plate 2, *C* and *D*.

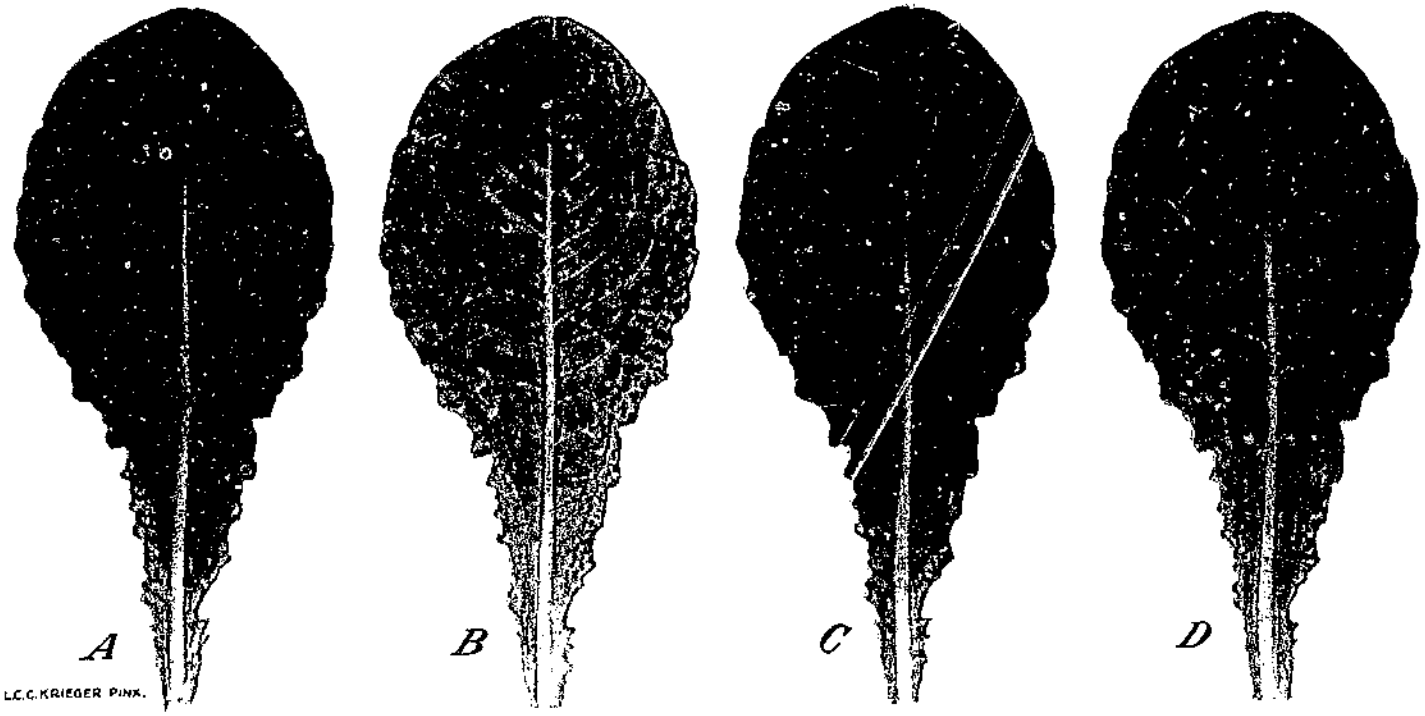
FACTORS CAUSING VARIATION IN EXPRESSION OF COLOR CHARACTERS

There may be considerable variation in color within a single phenotype, due to differences in the genetic constitution of the individuals composing it. Plants homozygous for certain genetic factors for anthocyanin are more strongly colored than those that are heterozygous for these characters. The several combinations of color genes that may occur result in variation in the color intensity within an anthocyanin phenotype.

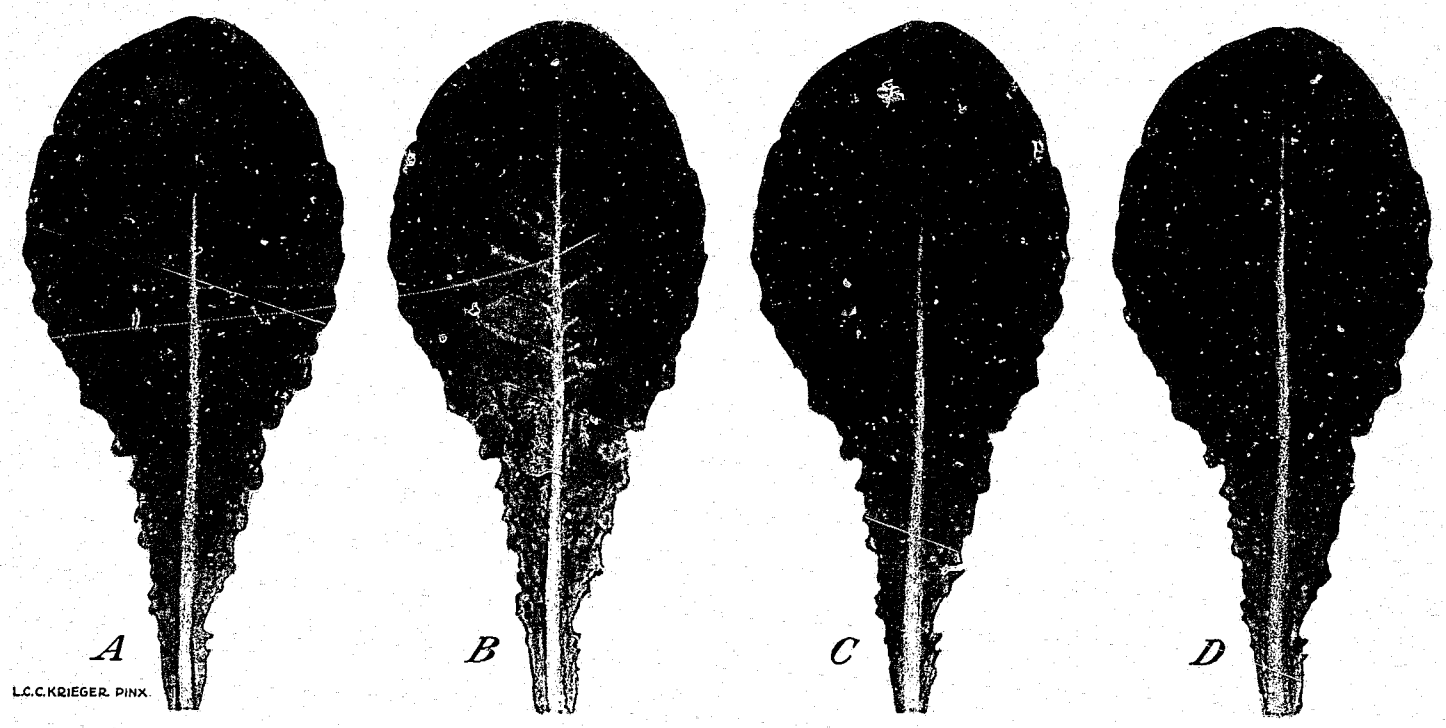
Variations in the shade of green in the leaves may affect the general appearance of pigmented plants. Since there are at least two shades of green that may occur in combination with any of the three anthocyanin color types, six combinations of the two colors red and green are possible. The intensity of color in both the anthocyanin and the chlorophyll may be influenced by such environmental factors as light, temperature, nutrition, and moisture supply.

With so many factors capable of causing variation in color of the foliage, it might be wondered how distinct genetic types can be identified. It is fortunate that by controlling some of the environmental factors it is possible to emphasize the distinctions among the different genetic groups.

Temperature was found to be the most effective environmental factor in its influence on anthocyanin development. Strong anthocyanin development can be induced by growing lettuce plants at relatively low temperature. At temperatures below 50° F. intense anthocyanin pigmentation develops and little difficulty is experienced in classifying plants into the different color types. Although temperature, light, moisture, and nutritional conditions were all found to



A, A leaf of the dark-green phenotype; *B*, a leaf of the yellow-green phenotype; *C*, a leaf of the dark-green-tinged phenotype; *D*, a leaf of the yellow-green-tinged phenotype.



A, A leaf of the dark-green-spotted phenotype; *B*, a leaf of the yellow-green-spotted phenotype; *C*, a leaf of the dark-green-red phenotype; *D*, a leaf of the yellow-green-red phenotype.

influence the development of anthocyanin in the lettuce plant, it can be stated with certainty that low temperature is more effective than any of the other agents mentioned in stimulating pigment development.

When it was necessary to grow populations during periods when it was not possible to maintain very low temperatures it was found to be difficult to distinguish the tinged from the green phenotype, because of the weak development of anthocyanin in plants belonging to the tinged group. Little difficulty was experienced in identifying plants to the red and spotted phenotypes.

When conditions were not favorable for the development of anthocyanin it was found possible to separate the tinged from the green phenotype by slowly wilting the plants after removing them from the soil with most of their roots intact. By this method of wilting anthocyanin development was induced in the tinged type. Tinged plants so treated developed a band of pigment around the stem just below the last pair of leaves by which they could be distinguished from plants belonging to the green phenotype. This method is not satisfactory for the separation of pigmented plants into the three types—red, spotted, and tinged—but is a very accurate means of separating these groups from the green type.

SEPARATION OF COLOR TYPES

Since it had been found that low temperature was effective in bringing out the color characters of the color types, many of the later progenies were grown in a greenhouse during the winter months, when it was possible to take advantage of the influence of low temperture on the development of the pigment. Most of the material used in the study of the segregation of the color types was grown in flats on a greenhouse bench, as this was found to be the most economical method of handling the large number of plants required.

In making the counts of the four types—red, spotted, tinged, and green—as they occurred in the various progenies, the most easily recognizable types were counted first and removed from the flats. As soon as the phenotype of the plant could be definitely determined it was removed, leaving, each time the plants were examined, those that could not be classified. The red phenotype is the most easily distinguished, the spotted next. The most difficult distinctions are those of the tinged and green types. Under conditions not favorable for anthocyanin development it is very difficult to separate the tinged and green groups. Under such conditions it is necessary to resort to the wilting method mentioned in the discussion of factors influencing anthocyanin development. By first removing the red and spotted phenotypes and then slowly wilting the remaining plants the tinged and green types could be very accurately classified. By growing the progenies during the winter when temperatures were low, little difficulty was experienced in classifying the plants into the four phenotypes without resorting to wilting.

GENETIC ANALYSIS OF COLOR TYPES

WORKING HYPOTHESIS

Seven different genes are necessary to account for the behavior of the four phenotypic color classes—"red," "spotted," "tinged," and

"green"—studied in this investigation. The seven genes with the symbols assigned are as follows:

(1) *C*, A gene for a chromogen base. *C* is necessary for the production of any anthocyanin pigment.
(2) *c*, The recessive allelomorph of *C* which constantly gives green.
(3) *T*, A gene for pigmentation which must be present with *C* for any anthocyanin development.
(4) *t*, The recessive allelomorph of *T* which constantly gives green.
(5) *R*, A gene for intensity of pigment which when present with *C* and *T* gives red.
(6) *r'*, A gene for color intensity recessive to *R* which when present with *C* and *T* gives spotted.
(7) *r*, A gene recessive to both *R* and *r'* which when present with *C* and *T* give tinged.

The genes *R*, *r'*, and *r* form a multiple allelomorphic series that controls the intensity and pattern of anthocyanin pigment. The multiple allelomorphic series *R*, *r'*, and *r* is independent of the complementary factor pairs *Cc* and *Tt*, which control the presence or absence of pigment.

From this series of genes the following 12 homozygous genotypes are possible and have the phenotypic expression here indicated.

	Genotype	Phenotype
1.	*CCRRTT*	Red.
2.	*CCr'r'TT*	Spotted.
3.	*CCrrTT*	Tinged.
4.	*CCRRtt*	Green.
5.	*CCr'r'tt*	Do.
6.	*CCrrtt*	Do.
7.	*ccRRTT*	Do.
8.	*ccr'r'TT*	Do.
9.	*ccrrTT*	Do.
10.	*ccRRtt*	Do.
11.	*ccr'r'tt*	Do.
12.	*ccrrtt*	Do.

Of the 12 possible homozygous genotypes, 9 have been isolated and their genetic constitution has been tested. Only three of the possible genotypes, *ccr'r'TT*, *ccRRtt*, and *ccr'r'tt*, have not been isolated.

According to the hypothesis there can be but one homozygous genotype for each of the three anthocyanin groups, red, spotted, and tinged. The pigment difference between these three groups depends upon which member of the multiple allelomorphic series *R*, *r'*, and *r* is present. Each group carrying anthocyanin differs genetically from the other anthocyanin groups by only a single factor.

Dark-green chlorophyll color in the leaves typical of the varieties New York and Mignonette was found to be dominant to the yellow green typical of the varieties Hanson and Iceberg. The dominant dark-green gene was represented by the symbol G and the recessive yellow-green gene by *g*.

Black seed coats were found to be inherited as a single gene dominant to the recessive gene for white seed coats. The dominant gene for black was given the symbol *W* and the recessive gene for white *w*.

In studying the breeding behavior in the F_2, populations were grown from several (5 to 13) F_1 plants and the progeny behavior of each recorded. The F_2 progenies from F_1 plants were tested for goodness of fit to the calculated ratio. In most cases the deviation from the expected ratio was small. For the sake of brevity in presenting the

results, only the totals for all of the F_2 individuals from a particular cross are given in the tables. In presenting the data for the F_3 progenies only the totals for all of the F_2 families giving the same F_3 segregation ratio are given in the tables. The number of families entering into the F_3 totals is given. The number of individuals in the F_2 and F_3 populations studied ranges from 45 to 1,278 with an average of about 75 plants.

CROSSES INVOLVING THE ALLELOMORPHIC SERIES Rr'r

Spotted (*CCr'r'TT*) × Red (*CCRRTT*)

The red plant M selected from the variety Mignonette was used as the pollen parent in a cross No. 45, with the spotted plant designated as B selected from the variety California Cream Butter. Thirty-three F_1 plants were obtained from this cross, 25 of which were red; the remaining 8 plants were spotted. The 25 red plants were distinctly different from either parent, indicating a heterozygous condition. Careful observation of the eight spotted plants indicated that they were true to type for California Cream Butter. These eight spotted plants were permitted to produce seed, from which an F_2 population of each was grown. None of the eight populations gave any segregation as regards anthocyanin type. All plants grown were typical of California Cream Butter. F_2 populations were grown from eight of the red F_1 plants.

The F_2 progenies from the red F_1 plants segregated red and spotted in a 3 : 1 ratio. F_3 progenies were grown from both the red and spotted F_2 plants.

The behavior of the F_2 and F_3 progenies from cross No. 45 is shown in tables 2 and 3. The progenies from the F_2 spotted plants gave nothing but spotted progenies. The F_2 reds consisted of true-breeding reds and reds segregating 3 red to 1 spotted in the ratio of approximately 2 segregating to 1 true-breeding.

Tinged (*CCrrTT*) × Red (*CCRRTT*)

The red plant M was used as the pollen parent in a cross with the tinged plant I. Twenty-four plants were grown from the seed from this cross No. 132. Of these 24 plants, 19 were red and obviously hybrids; the remaining 5 were tinged and typical plants for the variety Iceberg. F_2 progenies from 7 of the 19 red F_1 plants segregated red and tinged in the ratio of 3 red to 1 tinged (table 2).

TABLE 2.—*Records of F_2 progenies from selfed F_1 plants from crosses involving the multiple allelomorphic genes Rr'r*

Cross No.	Parental genotypes	F_1 plants	F_1 plant color	F_2 progenies				Segregation ratio	Deviation, 3 : 1 ratio	Diff. / P. E.
				Red	Spotted	Tinged	Total			
		No.		No.	No.	No.	No.			
MB-45	*CCr'r'TT* × *CCRRTT*	8	Red	879	282		1,161	3 : 1	8.25	0.83
MI-132	*CCrrTT* × *CCRRTT*	7	do	581		176	757	3 : 1	13.25	1.65
BI-39	*CCrrTT* × *CCr'r'TT*	10	Spotted		445	132	577	3 : 1	12.25	1.75

TABLE 3.—*Records of F_3 progenies from selfed F_2 plants from crosses involving the multiple allelomorphic series Rr'r*

Cross No.	Parental genotypes	F_2 families	F_2 plant color	F_3 progenies				Deviation, 3 : 1 ratio	Dev. / P. E.
				Red	Spotted	Tinged	Total		
		No.		*No.*	*No.*	*No.*	*No.*		
M-6-45	*CCrr'TT* × *CCRRTT*	8	Red	1,100			1,100		
		15	do	1,910	605		2,515	28.75	1.62
MI-132	*CCrr'TT* × *CCRRTT*	11	Spotted		604		604		
		9	Red	674			674		
		11	do	889		271	1,160	19.0	1.01
		4	Tinged			244	244		
BI-39	*CCrr'TT* × *CCr'r'TT*	6	Spotted		380		380		
		19	do		778	241	1,019	13.75	1.47
		9	Tinged			444	444		

TABLE 4.—*Records of F_2 progenies from selfed F_1 plants from crosses involving the complementary genes Cc and Tt*

Cross No.	Parental genotypes	F_1 plants	F_1 plant color	F_2 progenies			Segregation ratio	Deviation	Dev. / P. E.
				Red	Green	Total			
		No.		*No.*	*No.*	*No.*			
H-MN-161	*ccRRTT* × *CCRRtt*	6	Red	171	152	323	9 : 7	10.7	1.78
MH-15	*CCRRtt* × *CCRRTT*	6	do	2,202	722	2,924	3 : 1	9.0	.57
M-MN-47	*ccRRTT* × *CCRRTT*	8	do	664	205	869	3 : 1	12.25	1.42

The tinged F_2 plants produced only tinged F_3 progenies. The F_3 progenies showed that the F_2 reds consisted of true-breeding reds and reds segregating 3 red to 1 tinged in the ratio of approximately 2 heterozygous to 1 homozygous.

The data for the F_2 and F_3 progenies from the cross No. 132 red M by tinged I are presented in tables 2 and 3.

Tinged (*CCrrTT*) × Spotted (*CCr'r'TT*)

The spotted plant B was used as the pollen parent in a cross with the tinged plant I. The F_1 from this cross No. 39 consisted of 41 spotted and 4 tinged plants. The four tinged plants were obviously selfed plants of Iceberg. Five hundred and seventy-seven F_2 individuals from 10 spotted F_1 plants consisted of 445 spotted and 132 tinged, a close fit for a 3 : 1 ratio.

The results of F_2 and F_3 progeny tests from cross No. 39 are presented in tables 2 and 3.

The tinged F_2 plants were all homozygous for tinged. Four hundred and forty-four plants from nine tinged F_2 families were all tinged. Eight of the twenty-seven spotted F_2 families tested in the F_3 were homozygous for spotted; the remaining 19 tested in the F_3 all segregated spotted and tinged. Of the 1,019 F_3 plants from the 19 segregating F_2 families, 778 were spotted and 241 tinged. The deviation of 14 from the calculated values is a close fit for a 3 : 1 ratio.

The F_2 and F_3 progenies from crosses between the three anthocyanin color types given in tables 2 and 3 show that the difference between

the three types in intensity and pattern of pigment is, in each case, due to a single factor.

Since the factor for red is dominant to the factors for spotted and tinged, the factor for spotted is dominant to the factor for tinged; and never more than two of the anthocyanin types ever appeared in any population, it is assumed that the genes controlling the intensity and pattern of anthocyanin form a multiple allelomorphic series.

Evidence in support of the hypothesis that two pairs of complementary genes control the presence or absence of the pigment is afforded by the following data from a cross between two green plants that gave only red plants in the F_1.

CROSSES INVOLVING THE COMPLEMENTARY GENES Cc AND Tt

Green MN (*ccRRTT*) × Green H (*CCRRtt*)

The green plant H selected from the variety Hanson was used as the pollen parent in cross No. 161, with the green plant MN selected from a lot of hybrids from a cross between the varieties New York and Mignonette. Fifteen red plants were obtained in the F_1 of this cross. Three hundred and twenty-three F_2 plants grown from 6 F_1 plants consisted of 171 red and 152 green. This is a close fit for a 9:7 ratio.

The green F_2 plants all gave only green progenies in the F_3. The red F_2's were found to consist of true-breeding reds, reds segregating 3 red to 1 green, and reds segregating 9 red to 7 green. The F_2 and F_3 breeding behavior of progenies from this cross is given in tables 4 and 5. The segregations obtained are what would be expected if the presence or absence of pigment were controlled by complementary factors. In this case both parents carried the factor (*RR*) for intensity and pattern, as is shown by the progenies from crosses No. 15 and No. 47 between each of these green plants and the homozygous red plant M given in table 4. Each of these crosses gave F_2 progenies consisting of approximately 3 red and 1 green. Since no spotted or tinged plants appeared in the progenies from either cross, both of the green plants H and MN must carry the dominant *RR*. The F_3 progenies grown from cross No. 15 are given in table 15. No F_3 progenies were grown from cross No. 47.

TABLE 5.—*Records of F_2 progenies from selfed F_2 plants from cross involving complementary genes Cc and Tt*

Cross No.	Parental genotypes	F_2 plant color	F_2 families	F_3 progenies			Segregation ratio	Deviation	Dev./P. E.
				Red	Green	Total			
			No.	No.	No.	No.			
H-MN-161	*ccRRTT* × *CCRRtt*	Red	2	110		110			
		do	5	144	128	272	9:7	9.00	1.63
		do	3	127	36	163	3:1	4.75	1.27
		Green	9		479	479			

The F_2 distribution of 3 red to 1 green obtained from cross No. 15 (table 4) agrees with the results reported by Lewis (*18*) from this same cross.

According to the hypothesis, there are only two genotypes that will give a red F_1 when crossed with each other and an F_2 of 3 red to 1 green when crossed with a homozygous red. These two green genotypes have the genetic formulae *ccRRTT* and *CCRRtt*. It is assumed that the plant H is represented by one and the plant MN by the other. There is no means by which it can be determined which of these has the homozygous dominant *CC* and which the homozygous dominant *TT*. Numerous crosses involving the H type indicate that the factor carried by H gives a more intense pigmentation in the heterozygous condition than the same condition of the factor carried by MN. The factor *CC* has been arbitrarily assigned to the type represented by the plant H.

This assumes that the plant H has the genetic formula *CCRRtt* and the plant MN is of the *ccRRTT* type.

INTERACTIONS BETWEEN THE MULTIPLE ALLELOMORPHIC SERIES Rr'r AND THE COMPLEMENTARY PAIRS Cc AND Tt

The proposed hypothesis assumes nine possible genotypes having a green phenotypic expression. Six of these green genotypes have been demonstrated by crosses involving the interaction of the multiple allelomorphic series *Rr'r* and the complementary factory pairs *Cc* and *Tt*. Two of these genotypes have been demonstrated by the above crosses involving H and MN.

Green N (*ccrrTT*) × Red M (*CCRRTT*)

The green plant N selected from the variety New York in cross No. 1 with the red plant M as the pollen parent gave red F_1 plants. The F_2 segregated red, tinged, and green plants. Of the 734 F_2 plants 427 were red, 104 were tinged, and 203 were green. As indicated in table 6, the X^2 value 11.030 with a *P* value of 0.004 for the F_2 distribution is a poor fit for a 9:3:4 ratio.

The red plants were in excess and the tinged fell short of the calculated values for a 9:3:4 ratio for 734 plants. Although the deviation is significant, the observed distribution is nearer a 9:3:4 than a 27:9:28 ratio. That this deviation from the calculated values is likely due to linkage between the factors *CR* and *cr* will be discussed later.

F_3 progenies were grown from 35 red, 9 tinged, and 12 green F_2 plants. All of the expected segregation ratios appeared in the F_3. The behavior of the F_2 and the F_3 progenies is given in tables 6 and 7.

The F_2 and F_3 progeny tests from cross No. 1 show that the green plant N may have either of the genetic formulae *CCrrtt* or *ccrrTT*. The green plant MN, shown to be of the type *ccRRTT*, was used as a tester in determining the condition of the complementary factor pairs *Cc* and *Tt*. The F_1 from cross No. 156 between the green plant N and the tester MN was lacking in anthocyanin. Two hundred and nine F_2 plants from four selfed F_1 plants were all green (table 8). The N plant then must carry the dominant *TT* and the recessive *cc*. The tinged plants in the F_2 progenies from cross No. 1 given in table 6 show that the plant N carries the recessive *r* for pigment intensity and pattern. Its genetic formula therefore must be *ccrrTT*.

TABLE 6.—*Records of color in F_2 progenies from selfed F_1 plants from crosses involving interaction between the multiple allelomorphic series of genes Rr'r and the complementary genes Cc and Tt*

Cross No.	Parental genotypes	F_1 plant color	F_1 plants	F_2 progenies					Segregation ratio	Deviation	Dev./P. E.	χ^2	P
				Red	Spotted	Tinged	Green	Total					
			Number	*Number*	*Number*	*Number*	*Number*	*Number*					
MN-1	*ccrrTT* × *CCRRTT*	Red	8	427		104	203	734	9:3:4			11.03	0.004
MT-38	*CCr'r'tt* × *CCRRTT*	do	13	658	214		318	1,190	9:3:4			1.98	.30
MN-T-117	*CCr'r'tt* × *ccRRTT*	do	10	213	108		250	571	27:9:28			12.76	.003
M-NC-43	*ccrrtt* × *CCRRTT*	do	13	572		144	684	1,300	27:9:28			9.66	.011
MR-33	*CCrrtt* × *CCRRTT*	do	7	382		113	177	672	9:3:4			1.87	.40
NR-28	*CCrrtt* × *ccrrTT*	Tinged	6			261	223	484	9:7	11.25	1.53		
MW-40	*CCrrTT* × *CCRRTT*	Red	6	252		75		327	3:1	6.75	1.45		
WT-51	*CCr'r'tt* × *CCrrTT*	Spotted	5		152	42	78	272	9:3:4			3.07	.20

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TABLE 7.—*Records of color in F_3 progenies from selfed F_1 plants from crosses involving interaction between the multiple allelomorphic series of genes Rr'r and the complementary genes Cc and Tt*

Cross No.	Parental genotype	F_2 families	F_2 plant color	F_3 progenies					Segregation ratio	Deviation	Dev./P. E.	χ^2	P
				Red	Spotted	Tinged	Green	Total					
		Number		*Number*	*Number*	*Number*	*Number*	*Number*					
MN-1	*ccrrTT* × *CCRRTT*	4	Red	211				211					
		9	do	388		104		492	3:1	19.00	2.93		
		7	do	353			91	444	3:1	20.00	3.25		
		15	do	537		141	240	918	9:3:4			6.92	0.04
		3	Tinged			163		163					
		6	do			226	102	328	3:1	20.00	3.78		
		12	Green				635	635					
MT-38	*CCr'r'tt* × *CCRRTT*	2	Red	242				242					
		5	do	274	99			373	3:1	5.75	1.02		
		4	do	232			67	299	3:1	7.75	1.53		
		7	do	308	99		142	549	9:3:4			.31	.85
		2	Spotted		129			129					
		4	do		153		59	212	3:1	6.00	1.41		
		7	Green				408	408					
M-NC-43	*ccrrtt* × *CCRRTT*	1	Red	54				54					
		3	do	128		33		161	3:1	7.25	1.96		
		5	do	208			79	287	3:1	7.25	1.46		
		3	do	104			66	170	9:7	8.37	2.21		
		11	do	352		97	145	594	9:3:4			2.90	.25
		7	do	172		40	170	382	27:9:28			4.27	.15
		2	Tinged			108		108					
		6	do			232	90	322	3:1	9.50	1.81		
		2	do			50	49	99	9:7	5.70	1.72		
		19	Green				998	998					
MR-33	*CCrrtt* × *CCRRTT*	2	Red	132				132					
		5	do	219		56		275	3:1	12.75	2.63		
		3	do	125			40	165	3:1	1.25	.33		
		6	do	184		67	79	330	9:3:4			.20	.9
		3	Tinged			179		179					
		5	do			196	79	275	3:1	10.25	2.12		
		5	Green				310	310					
MW-49	*CCrrTT* × *CCRRTT*	2	Red	105				105					
		6	do	256		72		328	3:1	10.00	1.89		
		5	Tinged			263		263					
WT-51	*CCr'r'tt* × *CCrrTT*	1	Spotted		54			54					
		3	do		118	43		161	3:1	2.75	.74		
		6	do		235		87	322	3:1	6.50	1.24		
		5	do		150	44	79	273	9:3:4			2.77	.25
		2	Tinged			102		102					
		3	do			117	46	163	3:1	5.25	1.41		
		7	Green				376	376					

Green T (*CCr'r'tt*) × Red M (*CCRRTT*)

If a homozygous green of the type *CCr'r'tt* were crossed with a homozygous red *CCRRTT* the resulting F_1 should be red and the F_2 should segregate red, spotted, and green in the ratio of 9 red, 3 spotted, and 4 green. The data presented in tables 6 and 7 of progenies from cross No. 38 between a green plant T selected from the variety Transport and the red plant M agrees with the above factorial analysis. A homozygous green of the type *ccr'r'TT* would also give this same distribution in a cross with a homozygous red. That the plant T was of the type *CCr'r'tt* and not of the type *ccr'r'TT* is demonstrated by the data given in table 6, from cross No. 117 between the green plant T and the *Cc* tester MN. If the plant T were of the genotype *ccr'r'TT* no pigmented plants would have appeared in the progenies from this cross. The F_2 progenies from cross No. 117 gave 213 red, 108 spotted, and 250 green. The χ^2 of 12.76 with a *P* value of 0.003 shows this to be a poor fit for a 27:9:28 ratio. The deviation from a 9:3:4 ratio is greater than for the 27:9:28 ratio. The plant T then is indicated to have the formula *CCr'r'tt*. The deviation from the calculated values may be due to a linkage relation discussed in another part of this bulletin.

TABLE 8.—*F_1 and F_2 data from cross No. 156 of green MN (ccRRTT) × green N (ccrrTT)*

F_1 plant No.	F_1 plant color	F_2 progenies; green
		Number
N-MN-1	Green	55
N-MN-3		51
N-MN-5		49
N-MN-6		54
Total		209

Green NC (*ccrrtt*) × Red M (*CCRRTT*)

The green plant NC selected from a lot of hybrid material from a cross between the varieties New York and White Chavigne proved to be the triple recessive *ccrrtt*. This plant gave a red F_1 in a cross with the homozygous triple dominant red M *CCRRTT* cross No. 43. The F_2 gave 572 red, 144 tinged, and 584 green (table 6). The χ^2 value of 9.66 with a *P* value of 0.01 shows this to be a poor fit for a 27:9:28 ratio. While the deviation is significant, the observed distribution is nearer a 27:9:28 than a 9:3:4 ratio. As in the case of cross No. 1 and cross No. 117 (table 6), the deviation from the calculated distribution may be due to linkage, to be discussed later. F_3 progenies were grown from 30 red, 10 tinged, and 19 green F_2 plants, with the results given in table 7. The deviations from the calculated values in some of the F_3 progenies indicate that some condition operated to prevent the expected distribution, although all of the ratios expected in the F_3 were obtained. According to the hypothesis the genotype *ccrrtt* is the only one that would give an F_2 distribution of 27 red, 9 tinged, and 28 green in a cross with a homozygous red.

Green R (*CCrrtt*) × Red M (*CCRRTT*)

The green plant R selected from the variety Grand Rapids proved to have the genetic formula *CCrrtt*. In cross No. 33 with the homozygous red M the F_1 plants were all red. The F_2 gave 382 red, 113 tinged, and 177 green plants (table 6). The χ^2 and P values show this to be a close fit for the 9:3:4 ratio expected if R was of either genetic formula *CCrrtt* or *ccrrTT*. According to the hypothesis, these are the only genotypes that will give a ratio of 9 red, 3 tinged, and 4 green segregates in the F_2 when crossed with the homozygous red M. The data presented in tables 6 and 7 from cross No. 1 show that one of these genotypes *ccrrTT* is represented by the green plant N. The data given in table 6 from cross No. 28 between plants N and R show that these two plants do not have the same genetic constitution. The F_1 plants from this cross were tinged. The F_2 progenies segregated tinged and green in the ratio of approximately 9 tinged to 7 green. Four hundred and eighty-four F_2 plants from 6 F_1 families gave 261 tinged and 223 green. This is a deviation of 11 from the calculated 9:7 distribution. A deviation of 11 is 1.49 times its probable error and is not significant. F_3 progenies were not grown from cross No. 28. The behavior of the F_1 and F_2 progenies is what would be expected if one of the parents was of the formula *ccrrTT* and the other *CCrrtt*. Since cross No. 1 and cross No. 156 (tables 6, 7, and 8) have shown the plant N to be of the type *ccrrTT*, R must have the constitution *CCrrtt*.

The data in table 9 from cross No. 37 between the green plants H and R is further evidence that R carries the dominant *CC* and not the dominant *TT*. No pigmented plants appeared in the F_1 of this cross, as would have been the case if the plant R carried the dominant *TT*, for crosses No. 15 and No. 161 show the green plant H to be of the *CCRRtt* type and would have given pigmented plants when crossed with R if this plant carried the dominant *TT*.

TABLE 9.—*F_2 data from cross No. 37 of green R (CCrrtt) × green H (CCRRtt)*

F_1 plant No.	F_1 plant color	F_2 progenies; green
		Number
HR-1	Green	30
HR-3		43
HR-5		38
HR-6		46
HR-7		54
HR-8		51
HR-9		55
HR-10		56
HR-11		49
HR-13		52
Total		482

In the course of these studies crosses were made which involved 25 varieties and many hybrid selections, but none of these proved to belong to any of the genotypes (*ccRRtt*, *cc′r′tt*, or *ccr′r′TT*).

CROSSES INVOLVING WILD LETTUCE (LACTUCA SCARIOLA)

Since cultivated lettuce (*Lactuca sativa*) is considered to have arisen from the wild species (*L. scariola*), the question naturally arose as to whether the anthocyanin in the wild species is inherited in the same manner as the pigment in the cultivated varieties. A

wild plant was selected from 54 plants grown from seed harvested from a plant growing in the wild in Rock Creek Park, Washington, D. C. The intensity and pattern of the pigment in this plant was in general similar to the type designated as tinged in the cultivated varieties. Later progenies from this plant showed it to be homozygous for the pigmented phenotype.

The wild species was found to be genetically compatible with cultivated varieties. Crosses were made between the wild plant and two cultivated varieties. The F_2 and F_3 progenies from these crosses indicate that the wild plant belonged to the tinged type *CCrrTT*, since it exhibited the same breeding behavior as the tinged plants of cultivated lettuce.

Tinged W (*CCrrTT*) × Red M (*CCRRTT*)

The wild plant was identified by the letter W. This plant was used as the maternal parent in cross No. 49 with the homozygous red plant M. The F_1 plants from this cross were all red. Three hundred and twenty-seven F_2 individuals from 6 F_1 plants gave 252 red and 75 tinged. The deviation of 6.75 from the calculated values for a 3:1 ratio for 327 plants is not significant (table 6).

All of the F_3 progenies from 5 tinged F_2 plants were tinged. Two red F_2 plants when self-pollinated gave only red plants in the next generation. Six red F_2 plants when selfed gave progenies segregating red and tinged in the ratio of approximately 3 red to 1 tinged. The deviation from the calculated for a 3:1 ratio was 10, which is 1.89 times the probable error.

Green T (*CCr'r'tt*) × Tinged W (*CCrrTT*)

In cross No. 51 pollen from the wild plant was applied to flowers on a green plant of the variety Transport (T), known to be of the type *CCr'r'tt*. The F_1 plants were all spotted. F_2 progenies were grown from 5 selfed F_1 plants, and out of 272 plants 152 were spotted, 42 tinged and 78 green. The χ^2 value of 3.065 with P value between 0.20 and 0.30 shows this to be a good fit for a 9:3:4 ratio (table 6).

F_3 progenies from F_2 green plants gave only green. F_3 progenies from F_2 tinged plants gave true-breeding tinged progenies and progenies segregating tinged and green in the ratio of approximately 3 tinged to 1 green. Selfed spotted F_2 plants gave progenies breeding true for spotting, progenies segregating spotted, tinged, and green in the ratio of 9 spotted, 3 tinged, and 4 green, progenies segregating 3 spotted to 1 tinged, and progenies giving 3 spotted to 1 green (table 7).

EVIDENCE OF LINKAGE

The data presented in table 6 of progenies from crosses Nos. 1, 117, and 43 between parents carrying the allelomorphs *Cc* and *Rr'r* in different conditions indicate that linkage exists between these two allelomorphs. In each of these three crosses the discrepancy in the F_2 populations is beyond the limits of random sampling. In cross No. 1 between the homozygous red M *CCRRTT* and the green N *ccrrTT* and in cross No. 43 between the homozygous red M *CCRRTT* and the green NC *ccrrtt* the number of tinged plants in the F_2 fell short of the calculated expectancy and the number of red plants was in excess of the calculated. In cross No. 117 between the green plant MN *ccRRTT* and the green plant T *CCr'r'tt* the number of spotted

plants in the F_2 exceeded and the number of red fell short of the calculated number. The deviation from the calculated expectancy is in each case much greater than in any of the other 13 crosses studied. The deviations are in the direction expected if the allelomorphs *Cc* and *Rr'r* are carried by the same chromosome.

Since emasculation is not practicable, it is almost impossible to obtain controlled backcrosses in lettuce. It is necessary to resort to F_2 and F_3 progeny distributions for the determination of linkage values.

Linkage between *C* and *R* was calculated from the F_2 distribution from cross No. 1 (table 6). By assuming *R*– and *rr* to be in the ratio of 3:1, *C* and *R* were found to have a linkage value of 0.64, or 36 percent of recombinations. After determining the linkage value from the F_2 distribution from cross No. 1, the F_2 distributions from crosses No. 43 and No. 117 were tested for goodness of fit on the basis of calculated values determined for linkage between *C* and *R*. In both cases the observed values for the three types were within the limits of error. It is assumed then that the allelomorphs *Cc* and *Rr'r* have a linkage value of about 0.64.

The χ^2 of 11.03 for the deviation from the calculated frequencies in the F_2 from cross No. 1 (table 6) is beyond the reasonable limits of chance with two degrees of freedom. It is possible with one degree of freedom to determine the ratio of *C*– v. *cc* with an assumed 3:1 ratio between *R*– and *rr*, $(a+b)-3d=0$. In the F_2 population the discrepancy is 78 beyond the limits of random sampling; $531-609=-78$.

The squared standard error for random sampling in the expected 9:3:4 ratio is equal to $3n$, or 2,202. $(3\times734)=2,202$ The squared deviation is 6,084 $(-78^2=6,084)$. $\chi^2=\frac{6,084}{2,202}=2.76$

With one degree of freedom, 1.96 standard error shows odds of 19:1. $1.96^2=3.84=\chi^2$ for odds of 19:1. A χ^2 of 2.76 (odds of about 10:1) shows the discrepancy for *C*– v. *cc* to be within the limits of error.

This leaves one degree of freedom for the estimation of linkage between *C* and *R*. It is necessary in this case to assume a 3:1 ratio for *R*– v. *rr*. On this assumption class *d* will be divided into two groups, d_1 and d_2, equivalent to *ccR–TT* and *ccrrTT*. If the ratio of *C*– and *cc* had been exactly 3:1, the classes *b* and d_1 would have been equal. Class d_2 contains 203 plants instead of the calculated 177 $\left(\frac{427+104}{3}=177\right)$. Class d_1 then should contain 119 plants (177:-203::104:*x*), and class d_2, 84 plants $(203-119=84)$.

The distribution of the four classes would then be as shown in table 10.

TABLE 10.—*Distribution of classes in F_2 of cross No. 1 according to linkage values*

Class	Observed	Calculated
	Number	
a	427	¼(2+*y*)
b	104	¼(1−*y*)
d_1	119	¼(1−*y*)
d_2	84	¼(*y*)
Total	734	

Let $\sqrt{y}$=linkage between C and R. With C and R independent, y should equal 0.25 and the classes would be: $a=\frac{1}{4}(2.25)$; $b=\frac{1}{4}(0.75)$; $d_1=\frac{1}{4}(0.75)$ and $d_2=\frac{1}{4}(0.25)$, and the ratio would be 9:3:3:1. The ratio would change with any change in y.

Fisher's (*12*) maximum likelihood formula, $ny^2-(a-2b-2d_1-d_2)y-2d_2=0$, was used in solving for y. $734\ y^2-(427-208-238-84)y-168=0$.

$$y=0.4136$$
$$\sqrt{y}=0.64$$

This indicates a linkage between C and R with 36 percent of recombinations. A recombination value of 0.50 would be expected if they were not in the same chromosome. By substituting the class frequencies expected from the value for y and solving for goodness of fit we obtain a χ^2 of 2.76, which is the value obtained for the deviation of $C-$ v. *cc* from the expected 3:1 ratio (table 11). The calculated linkage between C and R is then correct if $R-$ v. *rr* is a 3:1 ratio.

TABLE 11.—*Calculation of goodness of fit for the class frequencies expected from the value of y*

Class	Frequency		$C-O$	$\frac{(C-O)^2}{C}$
	Observed	Calculated		
	Number	*Number*		
a	427	442.9	15.9	0.57
b	104	107.6	3.6	.12
d_1	119	183.5	19.5	2.07
d_2	84			
Total	734	734		$2.76=\chi^2$

The F_2 distribution from cross No. 43 (table 6) with a χ^2 of 9.66 is also beyond the limits of reasonable chance.

If we apply the linkage value obtained in the above case to the F_2 distribution from cross No. 43 we obtain the following values for the four classes of gametes.

The gametes of the type *CRT* will constitute 45.18 percent $[(3\times032^2)+(4\times0.32\times0.18)+(2\times0.18^2)]\ 0.75=0.4518$. There will be 11.07 percent of gametes of the type *C-rrTT* $[(2\times0.32\times0.18)+(0.18^2)]\ 0.75=0.1107$. The remaining 43.75 percent consists of the types that give green plants.

The goodness-of-fit test with the class frequencies calculated from the above proportions gives a χ^2 of 0.81 (table 12). On the basis of 36 percent of recombinations between C and R there is a close fit between the observed and calculated numbers for the different classes.

TABLE 12.—*Goodness-of-fit test of F_2 populations from cross No. 48 assuming 36 percent of cross-overs between C and R*

Class	Frequency		$C-O$	$\frac{(C-O)^2}{C}$
	Observed	Calculated		
	Number	*Number*		
a	572	587.3	15.3	0.40
b	144	143.9	.1	.00
d	584	568.8	15.2	.41
Total	1,300	1,300.0		$0.81=\chi^2$

When the calculated linkage between C and R was applied to the F_2 population from cross No. 117 (table 6), the observed numbers for the different classes were found to be within limits of error.

On the basis of 36 percent of cross-overs between C and R each parent should produce gametes in the following proportions: CR, 0.18; Cr, 0.32; cR, 0.32; cr, 0.18. The type CRT will be produced in the following proportion: $[(3\times0.18^2)+(4\times0.18\times0.32)+(2\times0.32^2)]$ $0.75=0.3993$. The $C\text{–}r'r'T\text{–}$ type will be produced in the proportion $[(2\times0.18\times0.32)+(0.32^2)]0.75=0.1632$. The remaining 0.4375 will consist of the types that produce green plants. Calculating goodness of fit for the observed frequencies, using the above proportions in determining the calculated values, a χ^2 of 3.337 is obtained. With one degree of freedom the deviation of the observed class values from the values calculated on the basis of linkage is within the limits of error. Goodness of fit of the observed class frequencies on the basis of 36 percent of cross-overs between C and R is shown in table 13.

TABLE 13.—*Goodness of fit test of F_2 populations from cross No. 117 assuming 36 percent of cross-overs between C and R*

Class	Frequency		$C-O$	$\frac{(C-O)^2}{C}$
	Observed	Calculated		
	Number	*Number*		
a	213	228	15.0	0.987
b	108	93.2	14.8	2.350
d	250	249.8	.2	.000
Total	571	571.0		$3.337=\chi^2$

DISCUSSION

The data presented in tables 4, 5, and 6 of progenies from crosses Nos. 161, 117, and 28, in which two green parents gave pigmented plants in the F_1, show that the presence or absence of anthocyanin is in each case the result of complementary genes. Each green parent carries some gene for pigment not carried by the other parent, and when they are brought together in the zygote pigment develops.

The F_2 progenies from cross No. 28 (table 6) between the two green plants N and R gave only tinged and green plants in the ratio of approximately 9 tinged to 7 green. This is the characteristic segregation when complementary factors are involved. The typical complementary factor ratio of 9:7 obtained in the F_2 shows that any other factors that influence the development of anthocyanin must be alike and in the homozygous condition in each parent. The behavior of progenies from this cross demonstrates that the presence or absence of the pigment is controlled by complementary factors but gives no information as to the condition of the gene or genes controlling the expression of the intensity and pattern.

The breeding behavior of the progenies from cross No. 45 (tables 2 and 3), in which the red plant M was crossed with the spotted plant B, shows that the red behaves as a simple dominant over the spotted type, since the F_1 was red and the F_2 segregated approximately 3 red to 1 spotted.

That the red also behaves as a simple dominant over the tinged type is shown in the progenies from cross No. 132 (tables 2 and 3), in which

a red was crossed with a tinged. The F_1 was red and the F_2 segregated red and tinged in the ratio of approximately 3 red to 1 tinged.

The spotted type that was shown in cross No. 45 (tables 2 and 3) to be recessive to the red is shown to be dominant to tinged by the progenies from cross No. 39 (tables 2 and 3) between the spotted and tinged types. The F_1 was spotted and the F_2 distribution was approximately 3 spotted to 1 tinged.

The tests of progenies from crosses Nos. 45, 132, and 39 (tables 2 and 3), which involve crosses between each of the three pigmented types, red, spotted, and tinged, show each type to differ from the others by a single factor. Each of the three possible combinations, spotted × red, tinged × red, and tinged × spotted, gave 3:1 F_2 ratios. In none of the three cases did more than two of the three types appear. The dominance of the red over the spotted and tinged and the dominance of the spotted over the tinged and the failure of more than two of the three types to appear in any one population demonstrates that the genes for red, spotted, and tinged are located in the same locus of the chromosome and constitute a multiple allelomorphic series.

Since only tinged and green plants appeared in the progenies from cross No. 28 (table 6) between two green parents, N and R, these plants must have both carried the gene for intensity and pattern in the recessive condition. If we assign the symbol *Cc* to one of the complementary allelomorphs and *Tt* to the other, and *R r'r* to the multiple allelomorphic series controlling intensity and pattern, then one parent in cross No. 28 must have the formula *ccrrTT* and the other *CCrrtt*. The tinged plant then would have the formula *CCrrTT*.

As pointed out above, the red, spotted, and tinged types differ from each other by only a single Mendelian factor, and the genes controlling the appearance of these three types form a multiple allelomorphic series. The tinged type has been shown to be of genetic constitution, *CCrrTT*. The spotted type differs from the tinged only in the allelomorphic series of which *r* is the recessive member. Since *r'* has been assigned as the symbol for the spotted character in the *Rr'r* allelomorph, the spotted plant has the formula *CCr'r'TT*. This leaves the gene *R* representing the red type in the allelomorphic series, and its formula then is *CCRRTT*.

Since the allelomorphs *Cc* and *Tt* must be present and at least one member of each must be in the dominant condition for pigment to develop, according to this hypothesis there can be only one homozygous red genotype, *CCRRTT*, one homozygous spotted genotype, *CCr'r'TT*, and one homozygous tinged genotype, *CCrrTT*.

Any plant, then, having either the *Cc* or *Tt* allelomorphs in the recessive condition (*cc* or *tt*) must be lacking in pigment, regardless of the condition of the multiple allelomorphic series *Rr'r*. This makes possible nine different genotypes, *ccRRTT*, *ccr'r'TT*, *ccRRtt*, *ccrrTT*, *CCRRtt*, *CCr'r'tt*, *CCrrtt*, *ccr'r'tt*, and *ccrrtt*, which have the green phenotypic expression.

The departure from the calculated segregations in the F_2 progenies from crosses in which the allelomorphs *Cc* and *Rr'r* were in a different condition in each parent indicates that there is a linkage between these two allelomorphs. In one such case a cross-over value of 36 percent was computed from the data. When this value was applied to the other similar cases the observed values were found to be within

the limits of random sampling. The allelomorphs *Cc* and *Rr'r* are in the same chromosome and show about 36 percent of recombinations.

In the 17 crosses from which progenies were studied, 14 of them involved at least 1 green parent. The segergation ratios obtained in these progenies indicate that six of the green parents were of different genotypes having the following genetic constitutions: *CCRRtt*, *CCr'r'tt*, *CCrrtt*, *ccrrTT*, *ccRRTT*, and *ccrrtt*. This leaves the genotypes *ccRRtt*, *ccr'r'TT*, and *ccr'r'tt* not represented by any of the varieties studied.

Data are available from many other crosses not presented here which indicate the anthocyanin genotypes of a number of other varieties of lettuce. The probable genotype of the lettuce varieties studied, including those presented here, are as follows:

New York No. 5084	*ccrrTT*.	Big Boston	*CCrrTT*.
Hanson	*CCRRtt*.	May King	
Grand Rapids	*CCrrtt*.	Iceberg	
Paris White Cos		Density	
Dark Green Cos		Wild (*Lactuca scariola*)	
Unrivaled		Mignonette	*CCRRTT*.
White Chavigne		Prize Head	
Malta		Crisp As Ice	
Early Curled Simpson		California Cream Butter	*CCr'r'TT*.
Mammoth Black-Seeded Butter		Maximum	
Deacon	*CCr'r'tt*.	Dutch Speckled Butter	
Transport			
Salamander			
Hubbard's Market			

While no genetic studies were made of the factors controlling anthocyanin in parts of the lettuce plant other than the leaves, some observations were made that are of interest in this connection.

It was observed that the intensity of pigment in the stem of the lettuce plant varied somewhat in different varieties but did not have the patterns characteristic of the leaf-color types. No spotting characteristic of the spotted-leaf type was ever observed in the stem. All plants carrying pigment in the leaves also had pigment in the stem. Pigment was often discernible in the stems under conditions unfavorable for leaf pigment. The correlation between leaf and stem pigmentation under certain conditions could be used to separate plants having pigmented leaves from those having no leaf pigment. Stem pigment could not be used in classifying the pigmented types, red, spotted, and tinged, since the stem pigment does not follow these types.

No plant was observed to have pigmented leaves or stem which did not also have pigmented ray flowers in the inflorescence. But as in the case of stem pigment, the pigmentation in the ray flowers does not follow the leaf-pigment patterns. It was obvious that there is more than one color type in the ray flowers. Some study was made of the pigment in the flowers, but sufficient data are not available to determine the genetic relations of pigment inheritance in the ray flowers.

The involucre bracts may also carry anthocyanin. The pigment pattern in the involucral bracts more nearly corresponds to the leaf pattern than in any other of the plant parts studied. The spots of dense pigment characteristic of the spotted-leaf type were also observed in the involucral bracts of some varieties and in some hybrid progenies.

It seems quite certain that at least some of the factors controlling anthocyanin in the leaves are also involved in pigmentation of the stems, ray flowers, and involucral bracts, since the presence of pigment in any one of these organs is always accompanied by pigment in all of the other parts mentioned. It is also quite obvious that pigmentation in the ray flowers, involucral bracts, and stem are not controlled by exactly the same gene combination that controls leaf coloration.

INHERITANCE OF SOME CHLOROPHYLL CHARACTERS

CHLOROPHYLL CHARACTERS EXHIBITING MENDELIAN INHERITANCE

Two types of chlorophyll coloration are readily identified among the varieties of lettuce lacking anthocyanin in their leaves. It is a little more difficult to distinguish the difference in chlorophyll color in the presence of the anthocyanin, although under favorable conditions, that is, where the chlorophyll has not faded or become yellowish as the result of unfavorable growing conditions, the two shades can be easily identified when the red pigment is present. The variety New York is typical of the dark-green type; the lighter or yellow-green type is well represented by Hanson. Environmental conditions may cause considerable variation in the green color of both types. High nitrogen supply tends to darken the color of the leaf. Limiting moisture, but not to the point of seriously checking growth, tends to produce a darker chlorophyll than an abundance of moisture; on the other hand, severe drought causes a yellowing of the chlorophyll. Conditions that may cause a yellowing of the chlorophyll are many. Any condition that seriously interferes with growth may cause a yellowing. Extreme drought, excessive moisture, abnormally high temperature, nutritional deficiencies, and toxic soil constituents are among the common causes of yellowing in plants. In spite of the variations in shade of green resulting from external factors, it is not difficult to identify the two types, dark green and yellow green, under normal conditions. The dark-green type is shown in plate 1, *A*, while *B* is from a typical plant of the yellow-green type.

Any one of the three anthocyanin colorations, red, spotted, or tinged, may accompany either of the two chlorophyll types. In the variety Iceberg the yellow green is accompanied by the tinged type of anthocyanin (pl. 1, *D*). In Density the tinged anthocyanin is on a dark-green chlorophyll. This type of coloration is shown in plate 1, *C*. The spotted anthocyanin and the dark green occur together in the variety California Cream Butter plate 2, *A*. In plate 2, *B*, is shown the combination in which the spotted anthocyanin is on a yellow green. This type has been observed in a number of hybrid lines. The variety Mignonette is a red anthocyanin on a dark-green chlorophyll. Plate 2, *C*, is typical of a red anthocyanin on a dark-green chlorophyll. The variety Prize Head is a red anthocyanin on a yellow green. This combination of anthocyanin and chlorophyll is illustrated in plate 2, *D*. The colored plates are not of the varieties mentioned as typical of the various anthocyanin and chlorophyll types; only the color characters illustrated are typical of the varieties mentioned. Many of the other plant characters in the illustrations are distinctly different from those of the varieties mentioned as typical of the type.

It is possible that there exists a third chlorophyll type intermediate between the dark green and the yellow green. Such varieties as Deacon and Unrivaled cannot be definitely classed as either a dark green or a yellow green if New York and Hanson are considered as typical representatives of these two green types. As already mentioned, the shade of chlorophyll coloring is influenced to some degree by the environment. While it is not difficult to identify plants of the color of the dark-green New York and the yellow-green Hanson, variations due to environmental conditions are sufficient to make it difficult to determine whether or not an intermediate exists between the dark and yellow greens.

The dark-green chlorophyll type, characteristic of the varieties New York and Mignonette, was found to be inherited as a single factor difference dominant to the yellow-green type and independent of the genes for anthocyanin.

(ggCCRRu)×(GGCCRRTT)

A dark-green-red plant from Mignonette was used as the pollen parent in cross No. 15 with a yellow-green plant lacking anthocyanin from the variety Hanson. The behavior of the progenies from this cross is indicated in tables 14 and 15.

(ggCCrrTT)×(GGCCRRTT)

The F_2 data given in table 14 from cross No. 132 of a dark-green-red M and a yellow-green tinged I indicate the independence of the factors for anthocyanin and those for chlorophyll. As in cross No. 15, there was a tendency for the dark-green type to exceed and the yellow-green to fall short of the expected values, although the deviation was not significant. Progeny tests of 377 F_3 individuals from 7 F_2 families gave 225 dark-green red, 63 yellow-green red, 72 dark-green tinged, and 17 yellow-green tinged. The deviation from the calculated values for a 9:3:3:1 ratio was found to have a P value of 0.3, indicating a close fit. When the 377 plants were divided into groups of dark green and yellow green without regard for the anthocyanin, 297 were dark green and 80 were yellow green, or a deviation of 14 from a calculated 3:1 ratio. A deviation of 14 for 377 plants is 2.47 times its probable error, hence hardly significant.

(ggCCRRu)×(GGccrrTT)

The inheritance of chlorophyll color was studied in the progenies from a third cross No. 14 between the dark-green N and the yellow-green H plants. The F_1 of this cross was dark-green red, as would be expected from the breeding behavior of these two parents as shown in tables 4, 5, 6, and 7. The F_2 segregated dark-green red, yellow-green red, dark-green tinged, yellow-green tinged, dark green, and yellow green. The F_2 segregation for the anthocyanin pigment types should be in the ratio of 27 red, 9 tinged, and 28 green. Assuming a 3:1 segregation for the dark-green and yellow-green types, the F_2 segregation for the two pigments should give a ratio of 81 dark-green red, 27 dark-green tinged, 84 dark green, 27 yellow-green red, 9 yellow-green tinged, and 28 yellow green. The segregations of the F_2 and F_3 progenies studied are given in tables 14 and 15.

TABLE 14.—*Record of color in F_2 progenies from selfed F_1 plants from crosses involving dark-green (GG) and yellow-green (gg) parents*

Cross No.	Parental genotypes	F_1 plant color	F_1 plants	F_2 progenies							Segregation ratio	χ^2	P
				Dark-green red	Dark green tinged	Dark green	Yellow-green red	Yellow-green tinged	Yellow green	Total			
			Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*			
MH–15	*ggCCRRtt* × *GGCCRRTT*	Dark-green red	5	354		117	106		26	603	9:3:3:1	4.85	0.20
MH–132	*ggCCrrTT* × *GGCCRRTT*	do	7	225	72		63	17		377	9:3:3:1	3.50	.30
NH–14	*ggCCRRtt* × *GGccrrTT*	do	11	601	132	561	196	40	147	1,677	84:28:28:27:27:9	36.77	.01

TABLE 15.—*Record of color in F_3 progenies from selfed F_2 plants from crosses involving dark-green and yellow-green parents*

Cross No.	Parental genotypes	F_2 families	F_2 plant color	F_3 progenies					Segregation ratio	Deviation	Dev. / P. E.	χ^2	P
				Dark-green red	Dark green	Yellow-green red	Yellow green	Total					
		Number		*Number*	*Number*	*Number*	*Number*	*Number*					
MH–15	*ggCCRRtt* × *GGCCRRTT*	1	Dark-green red	129				129					
		5	do	479		140		619	3:1	14.75	2.06		
		4	do	386	119			505	3:1	7.25	1.07		
		8	do	564	189	174	51	978	9:3:3:1			2.68	0.40
		2	Dark green		258			258					
		3	do		384		113	497	3:1	11.25	1.69		
		2	Yellow-green red			318		318					
		5	do			462	143	605	3:1	8.25	1.11		
		3	Yellow green				372	372					
NH–14	*ggCCRRtt* × *GGccrrTT*	7	Dark green		739			739					
		23	do		1,961		578	2,539	3:1	56.75	3.87		
		10	Yellow green				1,054	1,054					

The large χ^2 of 36.77 with five degrees of freedom for the F_2 segregation (table 14) indicates a very poor fit for the expected 84:81:28:27:27:9 ratio. There was an excess of red and a shortage of tinged in both the dark-green and yellow-green groups. This deviation from the calculated values may in part be accounted for by the linkage between *Cc* and *Rr'r*, discussed earlier. As in the previous crosses Nos. 15 and 132, the number of yellow-green plants was short of the calculated for a 3:1 ratio when the dark-green and yellow-green plants were grouped disregarding the anthocyanin pigment. The shortage of yellow-green plants also contributed to the large value for χ^2 in the F_2. Of the 1,677 plants grown in the F_2 from 11 selfed F_1 plants, 1,294 were dark green and 383 were yellow green. This is a deviation of 36 from the calculated for a 3:1 ratio A deviation of 36 from a 3:1 ratio in a population of 1,677 is 3.01 times its probable error, indicating a poor fit.

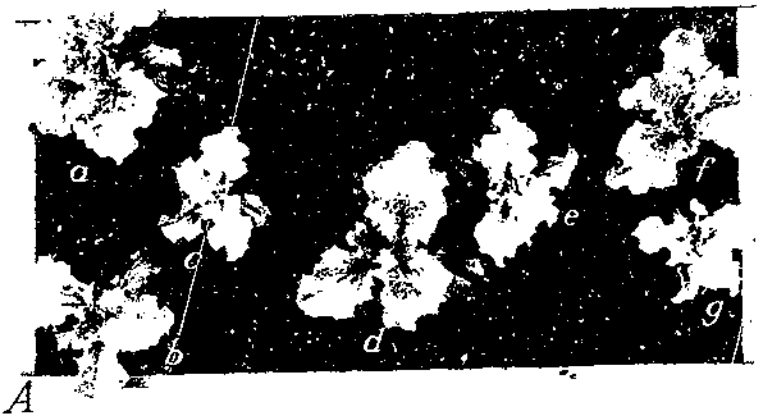
F_3 populations were grown from 30 dark-green F_1 plants. Twenty-three of the 30 plants segregated dark green and yellow green in the F_3. Out of 2,539 F_3 plants from the 23 segregating F_2 plants, 1,961 were dark green and 578 were yellow green, a deviation of 57 from a 3:1 ratio. A deviation of 57 in a population of 2,539 is 3.87 times its probable error. While in each of the three crosses, Nos. 15, 132, and 14, the dark-green and yellow-green types appeared in approximately a 3:1 ratio, there was a consistent shortage of yellow green.

The F_1 plants were dark-green red. Six hundred and three individuals were grown from five F_1 plants; of these, 354 were dark-green red, 106 yellow-green red, 117 dark green without anthocyanin, and 26 were yellow green lacking anthocyanin (table 14). The *P* value of 0.20 for the deviation of the observed values from the calculated expectancy for a 9:3:3:1 ratio indicates a fairly close fit. All of the dark greens with and without anthocyanin were grouped together, as were also all of the yellow greens. The dark-green group contained 471 plants and the yellow-green 132, or a deviation of 19 from the calculated values for a 3:1 ratio. For 603 individuals a deviation of 19 is 2.65 times its probable error and hardly significant.

Further evidence in support of the assumption that the dark-green chlorophyll type is inherited as a single Mendelian factor dominant to the yellow-green type and independent of the factors for anthocyanin is offered by the F_3 progenies from cross No. 15, presented in table 15. All of the segregations expected from the behavior of the F_2 progenies appeared in the F_3. While the deviations from the calculated values are not significant, there was a general tendency for the dark-green type to exceed and the yellow-green to fall short of the calculated values.

A careful study of many of the green plants lacking anthocyanin revealed no evidence of a third type of green in the progenies from either cross No. 14, No. 15, or No. 132.

The deficiency of yellow-green plants may be due to error in classification of the plants into the two groups, but the consistency of the shortage in the numerous progenies points to some other cause. It seems likely the low number of yellow-green plants may be due to some weakness of this genotype. This is suggested by the weakness of the yellow-green plants as compared with the dark-green. In practically all of the progenies studied the dark-green plants were observed to be more vigorous than the yellow-green plants in the same



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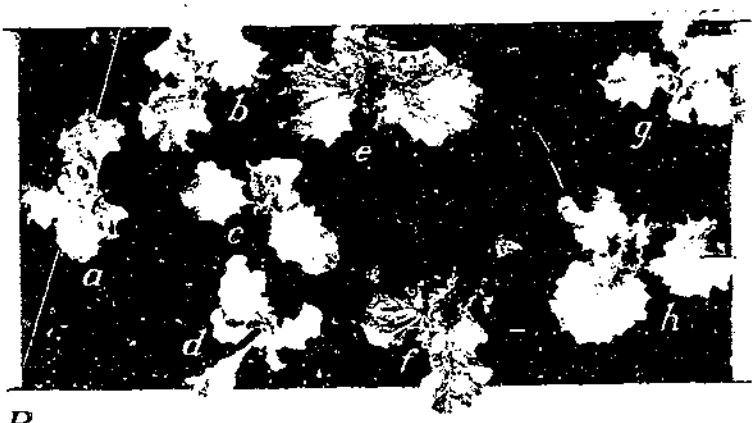

B

A, Difference in size of dark-green and yellow-green plants in the same F_2 population from cross No. 14 between the dark-green New York and the yellow-green Hanson. Plants *a*, *b*, *d*, and *f* are dark green, and plants *c*, *e*, and *g* are yellow green. *B*, Difference in size of dark-green and yellow-green plants in the same F_2 population from cross No. 15 between the dark-green-red Marquette and the yellow-green Hanson. Plants *b*, *e*, *f*, and *h* are dark green. Plants *a*, *c*, *d*, and *g* are yellow green.

A, A chlorophyll-deficient plant in which one side of the plant is entirely green and the opposite side highly deficient in chlorophyll. *B*, A lettuce plant lacking chlorophyll in a very large part of its leaf area. Most plants as deficient in chlorophyll as the one shown die before reaching maturity and seed production.

progeny. It has not been possible to explain the lack of vigor in the light-green plants, since some of the largest and apparently vigorous varieties of lettuce are of the yellow-green type. The difference observed may be largely a matter of growth rate. None of the yellow-green plants observed to be smaller and less vigorous than the dark-green plants in the same progeny were grown to maturity in order to compare their size at maturity with the dark-green type. It is possible that the observed difference in size of the two types would have been lost as the plants reached maturity.

Plate 3 shows progenies from crosses involving dark-green and yellow-green parents. *A* shows a flat of F_2 plants from a cross of the dark-green plant (N) with the yellow-green-tinged plant (I). Plants *a*, *b*, *d*, and *f* were dark green, and plants *c*, *e*, and *g* were yellow green. The dark-green plants averaged more than twice the size of the lighter green type. Plate 3, *B*, shows a group of F_2 plants from a cross of the dark green-red plant (M) with the yellow-green plant (H). Plants *b*, *e*, *f*, and *h* were dark green, and plants *a*, *c*, *d*, and *g* were yellow green. These two lots of plants are typical of the variation observed in the size of the two types of plants in respect to their chlorophyll color. Whether this is a difference in growth rate or a genetic weakness in the yellow-green genotype is a matter of speculation. If it indicates a genetic weakness it may help to explain the shortage of yellow-green plants in the progenies from crosses between the two types.

CHLOROPHYLL DEFICIENCY EXHIBITING NON-MENDELIAN INHERITANCE

Among 52 F_1 plants grown in 1930 from a cross between the varieties Transport and New York, 1 was observed to have peculiarly blotched leaves (pl. 4, *A*). Portions of the leaves were entirely devoid of chlorophyll. The extent of the whitish area varied in different leaves from 50 percent or more to leaves that were entirely green. Seed was saved from this plant, and a large F_2 population was grown in 1931. The variation in color noted in the leaves of the F_1 plants was observed in the cotyledons of the F_2. Some of the F_2 seedlings were pure albinos. These died within a few days after emergence. Many of the F_2 individuals were entirely green and appeared to be normal plants. A considerable proportion of the F_2 seedlings showed the blotching in the cotyledons characteristic of the leaves of the original F_1 plant. The proportion of green and white areas in the cotyledons of these blotched plants varied from almost entirely green to almost entirely white. Many of those that had a high percentage of chlorophyll-deficient tissue died in the seedling stage. The vigor of different individuals appeared to be proportional to the extent of green tissue.

F_2 plants showing various amounts of white tissue were selected and grown to maturity. The seed of each was harvested separately and an F_3 population grown from each. The proportion of green, blotched, and albino plants was different in each population, as shown by segregations recorded in table 16.

The number of blotched and albino plants in each population varied with the amount of white tissue in the leaves of the mother plants. The more white and blotched area in the leaves of the F_2 plants the greater the proportion of blotched and albinos in the F_3. The five F_2 plants that were wholly green gave only green progenies. From these 5 plants 926 seedlings were grown, and all were entirely green.

TABLE 16.—*F_3 progenies from chlorophyll-deficient plants*

F_2 plant No.	F_3 phenotype	F_3 segregation			
		Green	White	Blotched	Total
		No.	No.	No.	No.
3	Green	251	0	0	251
5		147	0	0	147
8		176	0	0	176
10		193	0	0	193
12		159	0	0	159
Total		926	0	0	926

F_2 plant No.	F_3 phenotype	F_3 segregation			
		Green	White	Blotched	Total
		No.	No.	No.	No.
1	Blotched	137	15	53	205
2		136	10	61	207
4		103	31	54	188
6		185	43	47	245
7		167	5	23	195
9		148	11	75	234
11		161	27	34	222
13		172	75	39	286
14		185	10	41	236
15		101	58	79	238
Total		1,403	285	506	2,256

In 1933 and again in 1934 individual flower heads on blotched plants were tagged and records kept as to the color of the bracts forming the involucre of the head. Three types as regards color of bracts were selected—one lot of heads that bore nothing but green bracts, a second group bearing blotched bracts varying in the extent of chlorophyll-deficient tissue, and a third group in which the bracts were entirely free of chlorophyll. The seed from each head was harvested, kept in a separate envelope, and planted separately. The resulting progenies are given in table 17.

Some variations in the pattern of the chlorophyll-deficient areas are shown in plate 4. The nearer the plant approaches the albino type the sooner it dies from starvation, due to low synthetic activity. Most plants as near albino as the one shown in plate 4, *B*, never reach maturity and seed production.

TABLE 17.—*Progenies from flower heads having green, white, and blotched bracts*

Flower head No.	Bract color	Progenies of color indicated		
		Green	White	Blotched
		Number	Number	Number
1	Green	10	0	0
2		17	0	0
4		23	0	0
5		11	0	0
7		14	0	0
8		14	0	0
10		18	0	0
11		9	0	0
13		12	0	0
15		13	0	0
16		15	0	0
18		17	0	0
19		11	0	0
20		13	0	0
21		15	0	0
Total		210	0	0
2	White	0	9	0
3		0	4	0
5		0	10	0
6		0	11	0
7		0	10	0
9		0	3	0
11		0	21	0
13		0	12	0

Flower head No.	Bract color	Progenies of color indicated		
		Green	White	Blotched
		Number	Number	Number
14	White	0	7	0
15		0	7	0
17		0	11	0
19		0	9	0
20		0	6	0
23		0	13	0
Total		0	133	0
1	Blotched	0	3	5
2		5	3	4
3		2	5	3
5		0	2	7
6		5	2	5
8		12	1	4
9		5	4	8
10		2	2	11
12		11	3	5
13		0	5	4
14		15	2	1
19		17	0	3
23		13	3	4
24		15	3	1
26		3	2	11
Total		123	40	76

Seed from heads bearing only green bracts produced only entirely green plants. The seed from heads bearing only albino bracts gave only albino seedlings, which soon died. The progenies from heads bearing blotched bracts segregated albino, green, and blotched in various ratios, depending upon the extent of albino and green areas in the bracts of the involucre.

A study was made as to the mode of inheritance of chlorophyll deficiency in this material. Flowers bearing only green involucre bracts were pollinated with pollen from flowers bearing albino bracts only and from heads bearing blotched bracts; flower heads bearing only albino bracts were pollinated with pollen from heads having only green bracts and others from heads having blotched bracts. In order to make certain that the resulting seeds were the result of cross-fertilization and not self-fertilized ovules, maternal parents were selected that were free of anthocyanin pigment and pollen was obtained only from plants carrying anthocyanin. By making the crosses in this manner it was certain that all of the plants grown from these crosses that carried anthocyanin were hybrids, as all selfed flowers would produce seed free of the factors for anthocyanin. The progenies that resulted from the various crosses are given in table 18.

In no case did the pollen have any effect on the color of the resulting progenies. Seed from heads bearing only green bracts produced only green seedlings whether the flowers were fertilized with pollen from heads bearing albino, green, or blotched bracts. Seed from heads bearing only albino bracts produced only albino progenies whether fertilized with pollen from heads bearing green, blotched, or albino involucres.

The deficiency in chlorophyll seems to be affected only through the maternal parent and is non-Mendelian.

TABLE 18.—*Data on effect of pollen on color of F_1 plants*

Mother head No.	Color of bracts of pollen head	Color of bracts of maternal head	F_1 plant color			Mother head No.	Color of bracts of pollen head	Color of bracts of maternal head	F_1 plant color		
			Green	White	Blotched				Green	White	Blotched
			No.	*No.*	*No.*				*No.*	*No.*	*No.*
3	Green	White	0	5	0	2	White	Green	13	0	0
4			0	7	0	3			5	0	0
7			0	4	0	4			4	0	0
8			0	9	0	5			7	0	0
11			0	11	0	7			17	0	0
13			0	3	0	8			11	0	0
14			0	5	0	9			15	0	0
15			0	8	0	11			3	0	0
17			0	6	0	13			10	0	0
21			0	10	0	11			8	0	0
27			0	12	0	17			12	0	0
28			0	11	0	21			2	0	0
31			0	7	0	24			9	0	0
Total			0	101	0	Total			116	0	0
51	Blotched	White	0	2	0	43	Blotched	Green	11	0	0
52			0	3	0	44			3	0	0
55			0	4	0	47			9	0	0
57			0	7	0	48			7	0	0
58			0	5	0	53			11	0	0
59			0	3	0	54			9	0	0
61			0	8	0	57			10	0	0
63			0	11	0	58			6	0	0
64			0	5	0	60			8	0	0
65			0	7	0	61			7	0	0
Total			0	55	0	62			13	0	0
						Total			94	0	0

INHERITANCE OF SEED COLOR

COLOR TYPES IN LETTUCE SEED

Three colors are generally recognized in the seed of lettuce. According to Jones (*16*), seed color in lettuce is due to pigment carried in the seed coat. Most of the cultivated varieties have either black or white seed. A few varieties, however, are known as yellow-seeded, although the color is more nearly buff than yellow. Yellow Seeded Butter and Giant Summer are among the varieties having yellow seed. Durst (*8*) found black to be dominant to white in progenies from crosses involving the black-seeded variety Grand Rapids and wild species *Lactuca scariola* and the white-seeded varieties May King, Paris White Cos, and Big Boston. F_2 data presented from five crosses involving the above varieties indicated a single factor difference. In each case the distribution in the F_2 was approximately 3 black to 1 white.

GENETICS OF SEED COLOR

The progenies grown for the studies on the inheritance of anthocyanin supplied material for a study of the inheritance of seed-coat color. Records were kept of the seed color in numerous progenies from crosses involving black- and white-seeded parents. The black-seeded varieties included, Mignonette (M), California Cream Butter (B), Grand Rapids (R), and Transport (T). The white-seeded parents were Hanson (H), New York (N), Unrivaled (U), and Iceberg (I).

The shade of color varies somewhat in the black-seeded varieties, not only between varieties, but different lots of seed of the same variety may differ slightly if grown and harvested under different conditions. Seed of some varieties appears jet black; others have a brownish cast. Whether this is a quantitative genetic difference has not been determined. These various shades of black all show the same genetic relation when crossed with a white-seeded type. In the progenies from each of the six crosses between black-seeded and white-seeded varieties studied, the black acted as a simple dominant over the white, giving ratios of 3 black to 1 white in the F_2.

Of 128 individuals from 9 selfed F_1 plants from cross No. 67 (table 19) between the black-seeded variety Transport and the white-seeded Unrivaled, 101 produced black seed and 27 white. This is a deviation of five from the calculated values for a 3:1 ratio. The deviation was 1.52 times its probable error and not significant. The F_3 from selfed white F_2 plants produced only white-seeded progenies. The selfed black-seeded F_2 plants segregated in the F_3 as true-breeding black progenies and progenies consisting of 3 black to 1 white. Of 27 selfed black F_2 plants from which the F_3 was studied, 11 were homozygous for black and 16 were heterozygous. The behavior of the F_3 progenies of cross No. 67 is given in table 20.

The black-seeded Grand Rapids (R) was crossed with the white-seeded Iceberg (I) in cross No. 135 (tables 19 and 20). The F_1 plants all produced black seed. Progenies were grown from 10 selfed F_1 plants. Of the 244 plants in the F_2, 186 developed black seed and 58 white. This is a deviation of only 3 from a perfect 3:1 ratio. Thirteen black-seeded F_2 plants proved by their progenies in the next generation to be homozygous for black. Sixteen F_2 blacks segregated 3 black to 1 white in the next generation. F_3 progenies were grown from 13 white-seeded F_2 plants. All 13 were white seeded.

TABLE 19.—*Records of seed-coat color in F_2 progenies from selfed F_1 plants from crosses between black-seeded (WW) and white-seeded (ww) parents*

Cross No.	Parental genotypes	F_1 plants	F_1 seed color	F_2 progenies			Deviation, 3 : 1 ratio	Dev. / P. E.
				Black	White	Total		
		Number		*Number*	*Number*	*Number*		
UP–67	ww × WW	9	Black	101	27	128	5.0	1.52
IR–135		10		186	58	244	3.0	.66
MI–132		7		129	39	168	3.0	.79
MN–1		8		209	63	272	5.0	1.04
BI–39		8		112	40	152	2.0	.56
MH–15		6		149	43	192	5.0	1.23
Total				886	270	1,156	19.0	1.91

TABLE 20.—*Records of seed-coat color in F_3 progenies from selfed F_2 plants from crosses of black-seeded (WW) and white-seeded (ww) parents*

Cross No.	Parental genotypes	F_2 seed color	F_2 families	F-progenies		Total	Deviation, 3 : 1 ratio	Dev. / P. E.
				Black	White			
			Number	*Number*	*Number*	*Number*		
UP–67	ww × WW	Black	11	124		135		
Do		do	16	280	85	365	0.25	1.12
Do		White	8		114	114		
IR–135		Black	13	139		139		
Do		do	16	238	72	310	5.5	1.07
Do		White	13		149	149		
MI–132		Black	9	156		156		
Do		do	14	214	74	288	2.0	.40
Do		White	7		94	94		
MN–1		Black	3	62		62		
Do		do	13	217	82	299	7.25	1.44
Do		White	8		124	124		
BI–39		Black	4	70		70		
Do		do	14	249	78	327	3.75	.71
Do		White	8		154	154		
MH–15		Black	8	142		142		
Do		do	12	183	66	249	3.75	.81
Do		White	5		106	106		

Color of seed was studied in the progenies from cross No. 132 between the black-seeded Mignonette (M) and the white-seeded Iceberg (I) (tables 19 and 20). The seed of all F_1 plants was black. One hundred and sixty-eight plants were grown from seven selfed F_1 plants. Of these the seed of 129 were black and 39 were white. Selfed white-seeded F_2 plants produced only white seed in the next generation. Nine self-pollinated black-seeded F_2 plants proved to be homozygous for black, producing only black seed in the F_3. Fourteen selfed black-seeded F_2 plants segregated in the next generation as blacks and whites in the ratio of approximately 3 black to 1 white. The seeds of 214 were black and of 74 were white, a deviation of 2 from a 3 : 1 ratio.

Data on seed-coat color in progenies from cross No. 1 between the black-seeded Mignonette (M) and the white-seeded New York (N), cross No. 39 between black-seeded California Cream Butter (B) and white-seeded Iceberg (I), and from cross No. 15 between black-seeded Mignonette (M) and white-seeded Hanson (H) are given in tables 19 and 20. In each of these three crosses the F_1 produced only black seed and the F_2 segregated black- and white-seeded in the ratio of approximately 3 black to 1 white.

The progenies from the six crosses of black-seeded with white-seeded varieties studied confirm the results obtained by Durst (*10*)

showing that seed-coat color in lettuce is inherited on a simple Mendelian basis and that black seed is a simple dominant over white seed.

Crosses were made between black-seeded and yellow-seeded and between white-seeded and yellow-seeded varieties, but through some error most of the lots of seed were mixed and sufficient material is not now available to determine the breeding behavior of the yellow-seeded type when crossed with the black and white types.

SUMMARY

The genetics of the inheritance of anthocyanin pigment in the leaves of lettuce was studied in progenies from 16 crosses involving 9 different homozygous genotypes. Seven genes were found to be necessary to account for all of the segregations obtained in the F_2 and F_3 progenies.

Three genes, *Rr'r*, form a multiple allelomorphic series controlling the intensity and pattern. The presence or absence of anthocyanin was found to be controlled by two complementary factor pairs, *Cc* and *Tt*.

The multiple allelomorphic series *Rr'r* and the allelomorphs *Cc* showed linkage with 36 percent of recombinations.

The dark-green chlorophyll color (*GG*) in lettuce leaves was found to act as a monogenic dominant to yellow-green (*gg*).

A chlorophyll deficiency in the leaves proved to be non-Mendelian in its inheritance. A single plant may bear seed that will produce green, blotched, or albino plants. Chlorophyll deficiency is inherited only through the maternal parent.

Black seed (*WW*) behaved as a single factor dominant to white seed (*ww*).

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