

**THE GENETIC RELATIONS OF SOME COLOR FACTORS IN  
LETTUCE (LACTUCA SATIVA AND L. SCARIOLA)**

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PART I

GENETIC RELATIONS OF FACTORS FOR ANTHOCYANIN

INTRODUCTION

The studies herein reported were undertaken to determine the genetic behavior of certain color factors in the lettuce plant. Aside from the value of such knowledge from the viewpoint of pure science, there are at least two very important practical applications of the results of this type of investigation. One of these is the use of known dominant factors in separating selfs from hybrids in the progenies from lettuce flowers which have been artificially cross-pollinated. A second application of such information to practical horticultural problems is in the standardization of varieties. If variety standardization is to be made a permanent working classification, it must be based on inheritable characters. The importance of anthocyanin pigment, chlorophyll shade and seed color in variety classifications of crop plants is apparent to one looking through the numerous variety classifications of cultivated plants which have been published. An example of such a classification is that on lettuce varieties by Tracy (18). Anthocyanin, chlorophyll and seed colors are among the important characters employed in identifying the one hundred or more known varieties of cultivated lettuce. A knowledge of the breeding behavior of a number of important characters would add greatly to their value in variety standardization. The ideal standard would be one based on genotypes.

It is in the first of these applications of genetic relations in lettuce that the writer is directly interested. In making crosses, the usual method of insuring against self-fertilization by emasculation is impossible in small flowered Compositae such as *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 by means of a small stream of water, first employed by Oliver (16), is now generally used. However, any method of pollen removal is open to error. Even with extreme care, it is almost impossible to remove every grain of pollen from the stigmas and stylar bristles. 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 of great value in a case of this kind where the development of seed cannot be relied upon as an indication that only cross-fertilization has taken place.

It is desirable that the characters used in separating the selfs from the hybrids be recognizable early in the life of the plant. Much time and labor can be saved if the undesired selfs can be recognized early, thereby saving the labor and expense of growing them to maturity. Chlorophyll and anthocyanin are color characters which develop early and the

various color types can be distinguished while the plants are quite small. A knowledge of their mode of inheritance is of considerable value in identifying hybrids and selfs in the same population. Seed color, a third character studied, is of less value than anthocyanin and chlorophyll in separating  $F_1$  plants in that it is necessary to grow plants to maturity in order to make use of it.

#### PREVIOUS STUDIES

The genetics of pigment inheritance has been reported for many plant species. The anthocyanins have been the subject of many genetic investigations. The inheritance of few plant characters has received more attention than the anthocyanin pigments. This is perhaps in part due to the contrasts between the color types which make them more easily classified than many other plant characters. Many papers have been published on the inheritance of anthocyanin in leaves, stems and flowers of plants. A comprehensive bibliography of the literature on the inheritance of these pigments was published by Wheldale (19) in 1916 listing 183 papers. During the twenty years which have elapsed since 1916, many additional papers have appeared dealing with the genetic relations of the anthocyanins.

In the 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 is an illustration. Many of the known genetic ratios have been demonstrated in studies of the inheritance of anthocyanin.

Keeble, Armstrong and Jones (14), Wheldale (19), Willstatter and Mallison (20) and many others have studied the chemistry of the anthocyanins. Wheldale (19) has made a study of the chemistry of these pigments in its relation to the Mendelian factors for flower color. From her chemical studies she concludes that anthocyanin in plants is either the result of an oxidation or a condensation of a flavone, or both.

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 inheritance 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 three pigmented to one 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. These all belong 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 Lactuca scariola, a ratio of three pigmented to one 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 three pigmented to one green, but all the pigmented plants would not have been like the pigmented parent.

Lewis (15) states that the  $F_2$  from a cross of the red variety Mignonette with the green variety Hanson consisted of three red plants to one green. Data on progenies from this same cross are presented in Table 8 of this paper and agree with the results obtained by Lewis.

## MATERIALS AND METHODS

### Sources of the Parent Stocks

The plant materials used in the study of inheritance of anthocyanin in the leaves 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 which 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 and 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 preceding the variety name. (H) Hanson, (N) New York, (R) Grand Rapids, (T) Transport, (MN) a hybrid selection from a cross of Mignonette x New York, and (NC) a

selection from a cross of New York x White Chavigne. The original seed of the variety Transport was obtained from Simon N. Groot Seed Grower of Enkhuizen, Holland. The variety White Chavigne was from Vilmorin-Andrieux & Co. of Paris, France. The strain of Mignonette was from the Burpee Seed 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 which has been maintained by the Division of Fruit and Vegetable Crops and Diseases of the United States Department of Agriculture 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 screened with fine copper wire screening to keep out as far as possible any insects which 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 two inches apart each way when transplanted and permitted to remain in the flats until set permanently in the 10-inch clay pots.

In most cases the parent plants came into flowering about the middle of May. Most of the hybridizing was done during

the latter half of May and early June.

#### Depollination and Hybridization

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 definitely 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 can never open again. A new lot of 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. The opening of the flowers is irregular and the emergence of the stigma from the anther sheaths is quite irregular within a single head under favorable conditions of light and temperature. 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 that 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 are 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 (13) found that fertilization in lettuce takes place within a few hours after pollination. This emphasizes the necessity of rapid removal of the pollen from the stigmas if self-pollination is to be avoided. The method of pollen removal first described by Oliver (16) 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 Plate I 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 blotter 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 of 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

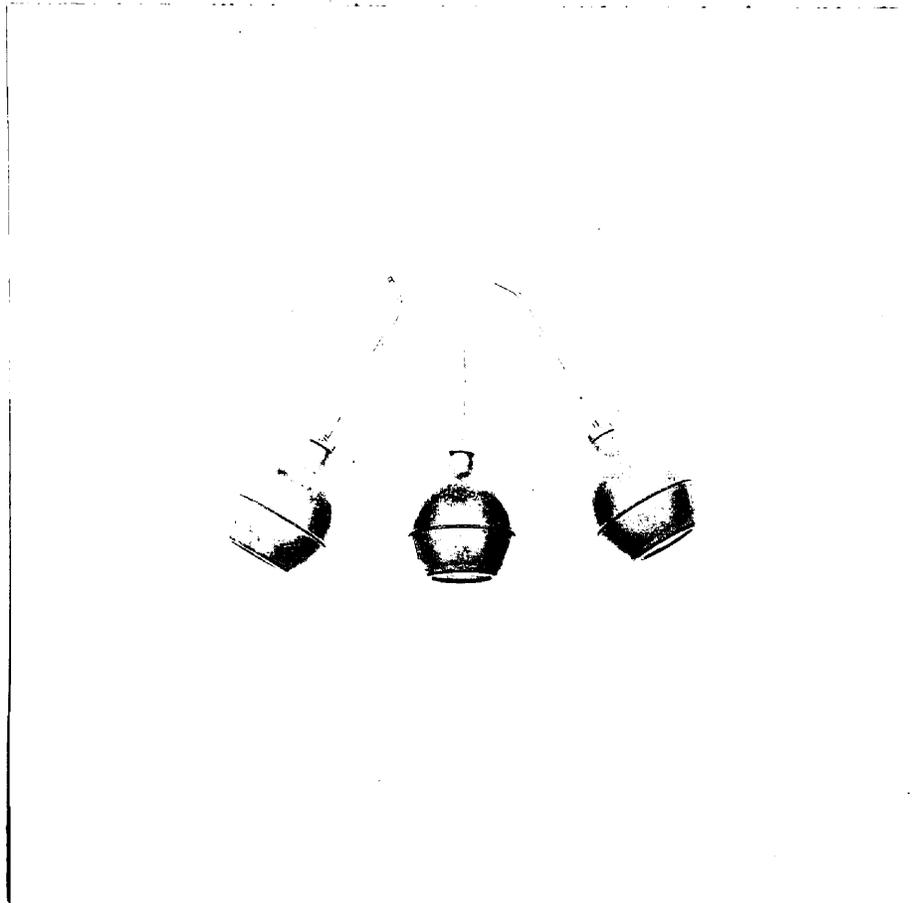


Plate I

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

once after washing and while the flower head is still held between the fingers as in the washing process.

Considerable time was spent in determining the best time to apply the water to the stigmas. It was early observed that the stigmas of the 15 to 20 individual flowers composing the lettuce flower head do not all emerge from the anther sheath at the same time. This irregularity of emergence of the stigmas is more pronounced when the temperature and light intensity are relatively low resulting in a slowing up of the activities associated with anthesis. This irregularity in emergence of the stigmas presents some difficulties in depollination. If the washing is begun as soon as the first stigmas appear beyond the anther sheath, it is necessary to continue the washing until the remaining stigmas have appeared, or to wash the heads again after the stigmas have all emerged. If the process of depollination is confined to a washing at the time the first stigmas have extended themselves beyond the sheath, the stigmas which have not yet appeared obviously will not be depollinated. As a result, when the late emerging stigmas are extended, sufficient pollen will be carried out of the sheath not only to fertilize the ovules of the flowers bearing these late appearing stigmas, but in all probability most of the stigmas which have been washed will receive pollen from them. The portion of the style below the stigma is covered with fine upturned bristles which carry pollen out of the sheath as the style elongates during anthesis (Figure 4). The stylar bristles continue to carry pollen out of the sheath after the stigmas have been washed if the washing is done at the time the first stigmas appear. Unless the pollen carried out of

the sheath by the stylar bristles is removed by a later washing, it may later reach the stigmas which have been washed and result in self-fertilization.

The pen and pencil drawings in Figures 1 to 4 show individual lettuce flowerets at different stages of anthesis. Figure 1 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 is shown in Figure 2. It is too early to start washing to remove the pollen when the first flowers in the head have reached this stage for, unless conditions are favorable for rapid opening of the flowers, a large percentage of the stigmas in the head may not yet have emerged from the sheath. The floweret shown in Figure 3 is at the stage found to be the most favorable for pollen removal. The highest percentage of successful crosses were obtained when the washing was done at this stage. If conditions are such that flower opening is slow, some of the stigmas may not have emerged and some may be beyond but, if pollen removal is to be limited to a single washing, best results are obtained when it is done at the time the majority of the styles have elongated to the point indicated in Figure 3. The stage illustrated in Figure 4 is too far advanced to obtain a high percentage of hybrid seed. At this stage the styles have extended well beyond the sheath. The styles are very limber and long enough to tangle with each other and with the corollas, making pollen removal difficult. The pollen tends to become sticky and hard to remove with water in the late stages.

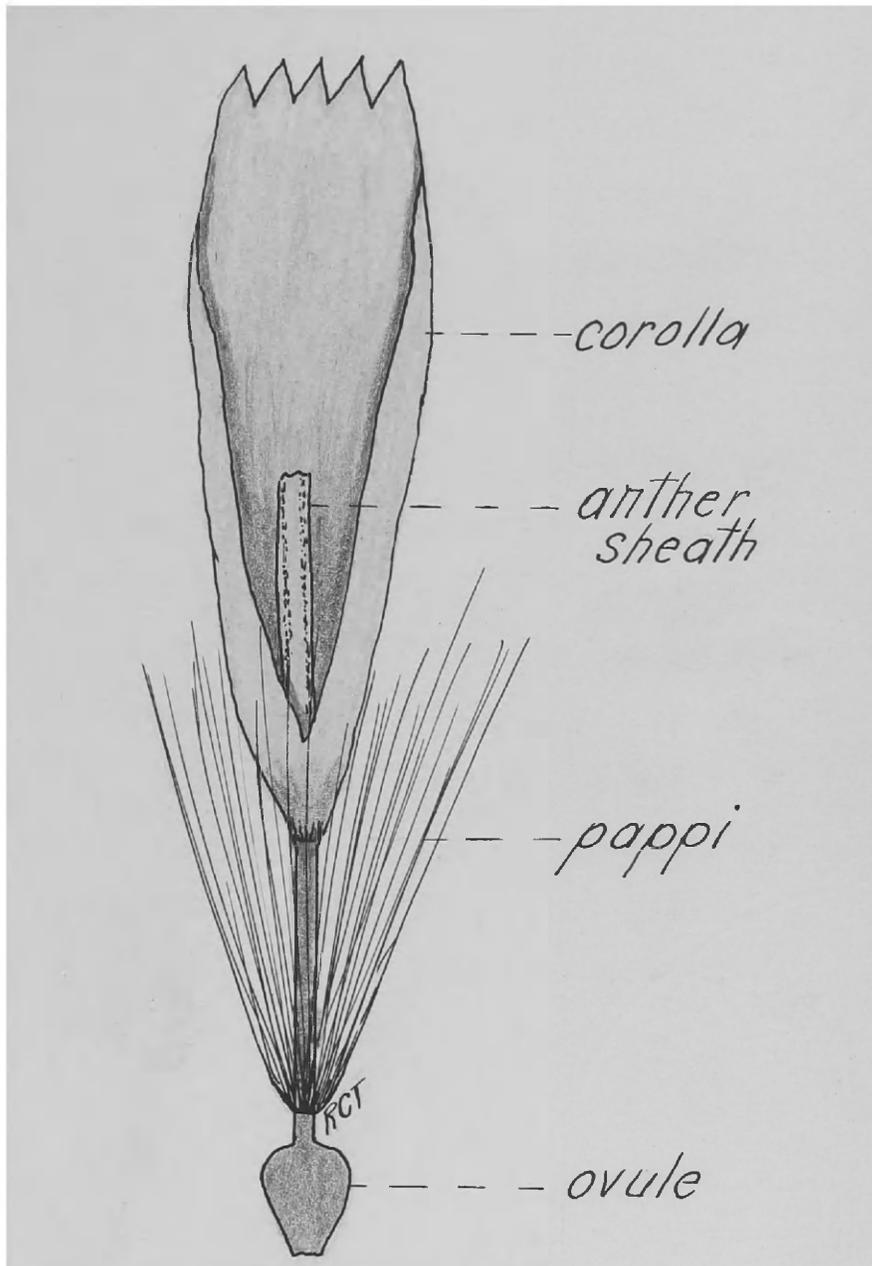


Figure 1. An individual lettuce floweret in an early stage of anthesis- just before the stigma emerges from the anther sheath.

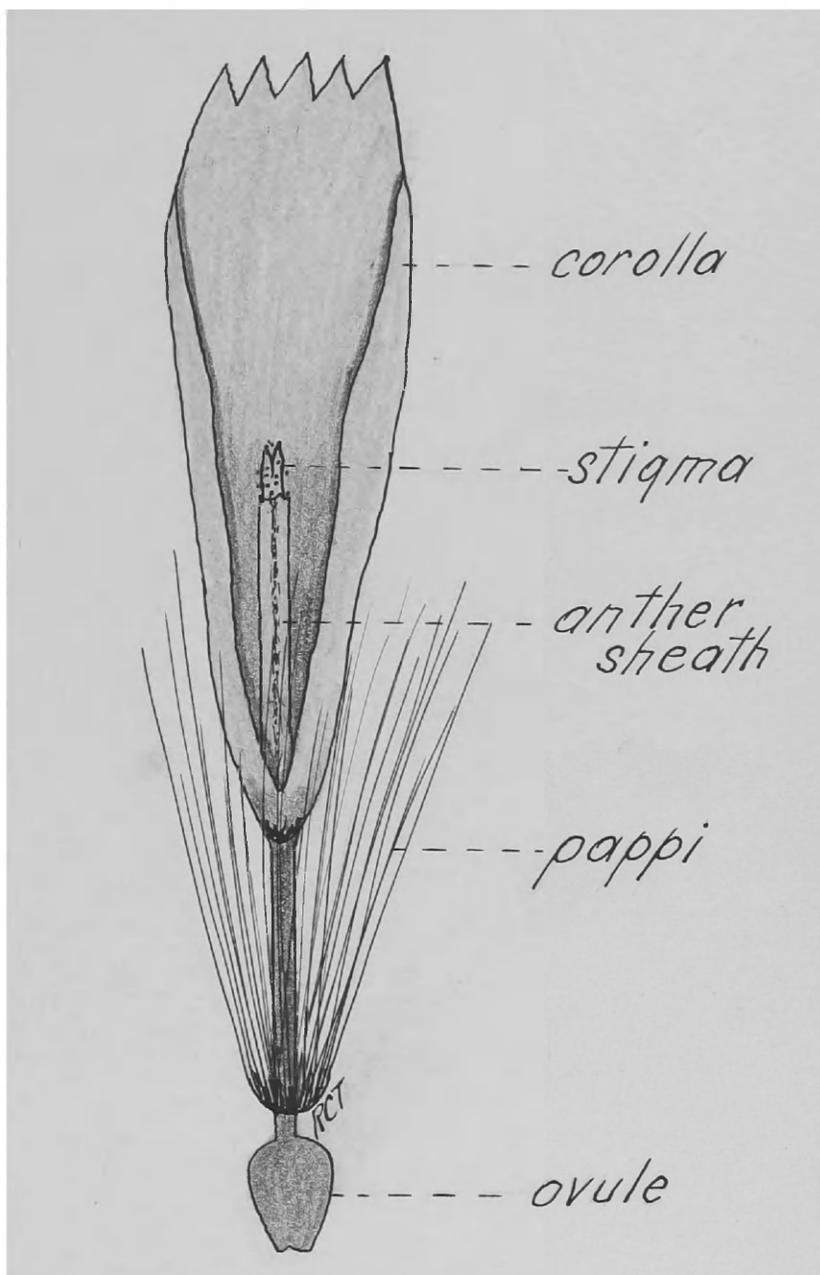


Figure 2. An individual lettuce floweret with the stigma emerging from the apex of the sheath. Most of the styles should be advanced beyond this stage before the washing is done.

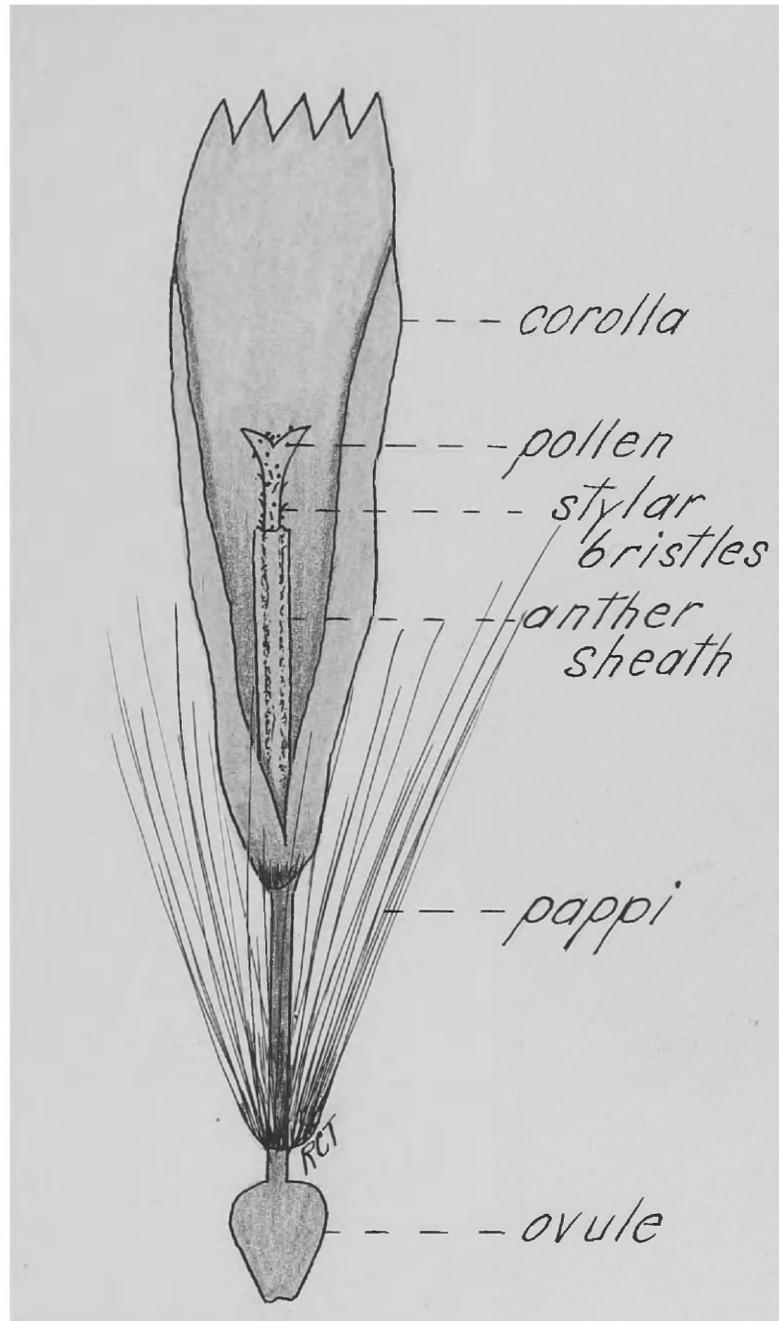


Figure 3. A lettuce flower at the stage found to be satisfactory for pollen removal by washing.

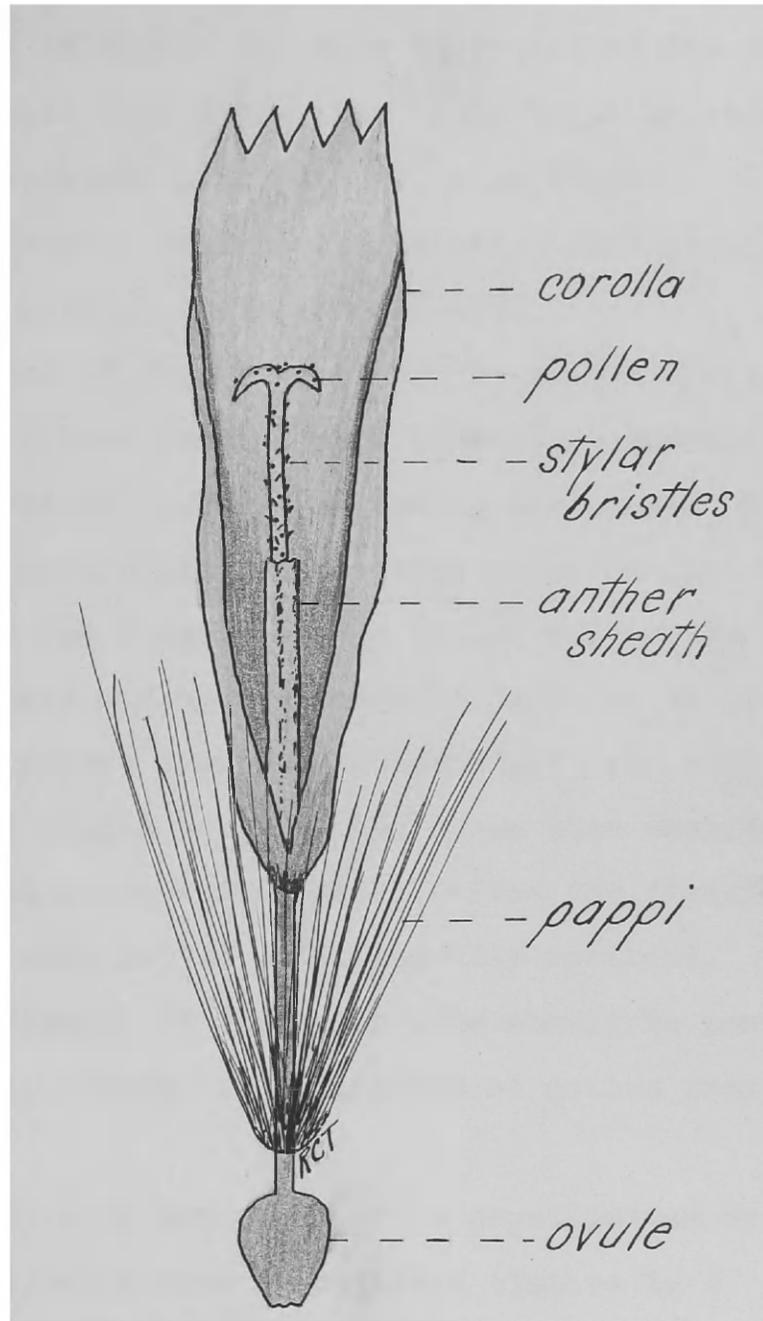


Figure 4. A lettuce floweret at a late stage of development. A flower at this stage is too far advanced for successful removal of pollen and cross fertilization.

It is likely that pollen tubes have entered the stigmatic lobes of some of the flowerets by the time they have reached the stage illustrated in Figure 4. Soon after all of the flowers of the head have reached this stage, the heads begin to close, the corollas become somewhat limp, and it is no longer possible to make successful cross-pollinations. This is especially true if the morning is bright and warm.

The limited number of flowers that can be worked during the short period the flower remains open necessitates economy in time in every operation involved in making the cross. Much time can be saved by economizing on the time required for pollen removal. Very few flowers can be worked during the short period the flowers are open if washing is begun as soon as the first stigmas appear from the anther sheath and continued until the last stigma has emerged. Even when washing was carefully done and every effort made to free the flowers of their own pollen, some selfed seed generally resulted. Consequently it is doubtful if very much time should be consumed in attempting to remove the last grain of pollen from the head.

Records were kept on a number of heads depollinated by washing once at different stages of anthesis (Tables 1, 2 and 3). In the heads recorded as washed at an early stage, most of the stigmas had just emerged and were in the condition shown in Figure 2. Those in the second or intermediate stage were heads in which the majority of the flowerets were at the stage indicated in Figure 3. The late group consisted of heads in which most of the flowerets were well advanced

Table 1. Data on progenies from lettuce flower heads  
depollinated at an early stage of anthesis.

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

Table 2. Data on progenies from lettuce flower heads  
depollinated at an intermediate stage of anthesis.

Head No.	Total Seed Per Head	Selfed Seed	Hybrid Seed
1	14	4	10
2	8	1	7
3	10	2	8
4	14	5	9
5	12	4	8
6	10	0	10
7	0	0	0
8	7	2	5
9	15	6	9
10	13	3	10
11	14	6	8
12	11	3	8
13	13	6	7
14	8	3	5
15	10	1	9
16	17	5	12
17	0	0	0
18	7	1	6
19	12	5	7
20	15	1	14
21	9	0	9
22	14	4	10
23	11	4	7
24	18	6	12
25	15	2	13
26	0	0	0
	277	74	203

Table 3. Data on progenies from lettuce flower heads depollinated at a late stage of anthesis.

Head No.	Total Seed Per Head	Selfed Seed	Hybrid Seed
1	3	1	2
2	0	0	0
3	11	10	1
4	7	5	2
5	0	0	0
6	13	8	5
7	5	5	0
8	10	7	3
9	15	5	10
10	9	6	3
11	14	13	1
12	11	10	1
13	12	9	3
14	16	16	0
15	0	0	0
16	5	4	1
17	0	0	0
18	11	5	6
19	10	7	3
20	15	9	6
21	7	5	2
22	13	11	2
23	0	0	0
24	11	9	2
25	5	5	0
26	12	9	3
27	17	9	8
28	0	0	0
	232	167	64

having their styles fully extended and the stigmatic lobes turned back on the style. This group was washed and pollinated just before the flower heads were ready to close, at a stage near that shown in Figure 4. A green plant of the variety White Chavigne was used as the maternal parent in this study. Some preliminary crosses had shown the red anthocyanin pigment in the leaves and the black seed of the variety Mignonette to be dominant characters. Pollen from a plant of Mignonette was used in pollinating the washed heads. The  $F_1$  plants resulting from the cross-pollination which had anthocyanin in the leaves and produced black seed were known to be successful crosses. It was possible then to separate the selfs from the hybrids in the progenies from the various heads.

Data were obtained from 26 flower heads depollinated at the early stage, 23 heads at the intermediate stage and 28 heads which were in the late stage. One flower head of the early, 2 heads of the intermediate and 6 heads of the late group failed to develop any seed. The early group averaged 13 seeds, the intermediate 10.6 seeds, and the late group 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 18 seeds per head. Depollination and artificial cross-pollination greatly reduced the number of seed set per head.

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

group 37.1 per cent.

The results indicate that a single washing at a stage of anthesis near that illustrated in Figure 3, if done carefully, will result in a fairly high percentage of successful crosses.

It is obvious that the less the floral organs are mutilated in pollen removal, the better the chances for a high percentage of successful crosses. The delicate floral organs 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. Some grains of pollen are difficult to remove and the flower parts are often injured if washing is continued in an effort to remove every grain of pollen from the head.

It was found to be a good practice to limit the removal of pollen to a single washing done when the majority of the styles have elongated to the point indicated in Figure 3.

Varieties differ considerably in the structure of the ligulate corollas. In varieties such as Hanson, the central flowerets have very narrow, almost hair-like, corollas while, in such varieties as White Chavigne and California Cream Butter, the corollas are broader and stiffer. This difference in corollas is a factor in depollination. Flower heads having the broader and stiffer corollas are more easily depollinated than those having the narrow type of corolla. The narrow ligules have a tendency to tangle with the styles, especially when the styles have become well extended. Flowers of this type are difficult to handle in washing and pollinating in the late stages. It is quite difficult to apply pollen to

the stigmas when styles and corollas are intertwined as is often the case after washing flowers of the Hanson type. In this type of flower it is particularly important that the washing and pollination be done before the style are fully extended.

Depollination was accomplished in the following manner: The flower head was clasped between the thumb and the first two fingers of the left hand in such a manner as to surround a portion of the pedicle and the involucre. The tips of the fingers and thumb supported the corollas of the flowerets. A dentists chip blower (Plate I) was grasped in the right hand after being filled with water. A stream of water was directed into the open flower. The pressure with which the water was applied was controlled by the pressure exerted on the bulb of the chip blower.

Approximately one-half bulb of water was used in washing each head.

The stigmas and styles were examined by means of a 20X hand magnifying lens after washing, as outlined above. If the stigmas and styles were free of pollen, the water was removed from the head by a puff or two of air from the mouth. If pollen still remained either on the stigmas or lodged in the stylar hairs, the head was washed again. When only one or two stray grain of pollen could be observed, washing was discontinued. Selfed seed resulting from a few grains of pollen remaining after washing were segregated in the F<sub>1</sub> as the crosses were made in such a way that the dominant character was introduced through the pollen parent. The dominant characters used had been determined by a few preliminary

crosses.

Heads from the pollen parents which 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 the pollen was transferred to the washed stigmas. An individual head was seldom used to pollinate more than one depollinated head.

Since the hybridization work was done in a screened greenhouse practically free of insects, the pollinated heads were frequently not covered after applying the pollen. In the earlier crosses, the heads were covered with glassine paper envelopes and clasped on by means of a small paper clip. This practice was later discontinued as the flower heads close very shortly after pollination and insect pollination was practically impossible. Any insect which could not find its way into most any kind of covering could not carry pollen into the head once it had closed.

#### DESCRIPTION OF THE 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 as regards 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, (Plates II and III). It will be shown in another section of this paper 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 favorable conditions 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 illustration Plates IV and V shows this color type.

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 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



Plate II. A leaf of the dark green phenotype.



Plate III. A leaf of the yellow green phenotype.



Plate IV. A leaf of the dark green,tinged phenotype.



Plate V. A leaf of the yellow green, tinged phenotype.



Plate VI. A leaf of the dark green, spotted phenotype.



Plate VII. A leaf of the yellow green, spotted phenotype.



Plate VIII. A leaf of the dark green, red phenotype.



Plate IX. A leaf of the yellow green, red phenotype.

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 paper. The red type is illustrated in the colored photographs (Plates VIII and IX).

Spotted: The third 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 which belong to the spotted group (Plates VI and VII).

#### FACTORS CAUSING VARIATION IN EXPRESSION OF THE COLOR CHARACTERS

##### Genotypic Factors

The intensity of pigment in each of the three anthocyanin types recognized in this discussion varies greatly under different environmental conditions. However, the differences among these three anthocyanin classes are quantitative under given conditions. Plants of the red group are much more intensely colored than plants of the spotted and tinged groups

under any combination of environmental conditions if all are subjected to the same treatment. The range in intensity of color in any group is very great. Under optimum conditions for the development of anthocyanin, the tinged and spotted groups may be more densely pigmented than even the red group would be under less favorable environment for the development of anthocyanin. There are other characteristics which in combination with the intensity of the pigment aid in differentiating the different types. The presence of the localized areas of dense pigment which give a spotted appearance to the foliage affords an easily recognizable difference by which the spotted and tinged groups can be identified. There is little difficulty in separating these two types except where conditions are extremely unfavorable for anthocyanin development. The intensity of pigmentation must be relied upon largely in separating the red from the spotted type since the localized spots of dense pigment are characteristic of both the red and the spotted types.

The variation in intensity of coloration resulting from the heterozygous condition of the genetic factors for anthocyanin add to the fluctuations in anthocyanin color. Plants homozygous for certain genetic factors are more strongly colored than those that are heterozygous for these characters. The several combinations of color genes which may occur result in variation in color intensity within an anthocyanin phenotype.

A third condition which further contributes to difference in the general appearance of the pigmented plants is the variation resulting from the combination of the anthocyanin

types with different shades of green in the chlorophyll. As will be pointed out in Part II of this paper, there are two distinct shades of green in lettuce varieties. One, a very dark shade of green, the other a yellowish green. The three anthocyanin types and the two chlorophyll groups may combine so that any one of the anthocyanin intensities may appear in combination with either of the shades of green, giving six possible combinations of the two colors red and green.

With all these factors tending to add to the variation in color of foliage, it might be wondered how distinct genetic types could be identified. It is fortunate that it is possible to control some of the environmental factors so as to emphasize the distinctions among the different genetic groups.

#### Environmental Factors

There are numerous conditions of environment which may cause variation in the intensity of the anthocyanin color in the leaves of lettuce. The differences in color are no doubt generally due to the interaction of several external factors. The individual effects of the separate factors are difficult to determine.

In this work a study was made of some of the external conditions influencing anthocyanin intensity for the purpose of determining the best method of handling lettuce plants to bring out or intensify the color. It was found that rapid growth at high temperature and low light intensity is accompanied by very weak development of anthocyanin. The identification of the different genetic types of anthocyanin intensity and

pattern was impossible under such conditions. Fortunately there are conditions under which the different types are readily distinguished and there is little difficulty in separating them in making a study of their genetic behavior.

Facilities for accurately controlling temperature, light and moisture were not available for studying the influence of these external conditions on the development of anthocyanin. However, it was possible to grow plants under wide extremes of light, moisture and temperature and from these cultures obtain some information on the development of the pigment under various environmental conditions.

Temperature. Temperature was found to be the most effective environmental factor in its influence on anthocyanin development. Plants were grown under a wide range of temperatures and the influence of temperature on pigment development noted. Some of the first studies were made of plants grown in the greenhouse during warm summer days when the temperature varied from 75° to 90° F. Pigment development under such conditions was found to be slow and the tinged type often developed little or no pigment. It was difficult to distinguish the tinged type from the green in plants grown at high temperatures. Even in the red and spotted types which normally develop considerable anthocyanin, the intensity of the pigment was much less than in plants grown at lower temperatures.

At the same time these genetic studies were being made, large plantings of breeding material were made each spring. The plants for the breeding work were started in the greenhouse and transplanted to the field generally the first week in April.

The pigmented types in the breeding plots always developed intense color while the temperatures were low. The color faded as the season advanced and the temperature increased.

The observation that pigment development was more intense in the plants in the field plots during the cool weather of spring suggested that it would save a great deal of time and labor if the material for the genetic studies could be grown in the greenhouse during the winter months when temperatures could be kept low. It was found that the color types could be easily distinguished in seedlings after four or five true leaves had developed when the plants were grown under conditions of low temperature. This method of handling was resorted to in most of the later studies. The seedlings were grown in flats on a greenhouse bench and the temperature held below 50° F. when outside temperatures would permit. No condition or combination of conditions was found to be as effective as low temperature in promoting the development of anthocyanin or in increasing its intensity.

Moisture. The influence of moisture on the development of anthocyanin is so closely correlated with other conditions that its specific influence is difficult to determine. Withholding moisture to the point of seriously interfering with growth activity seems to favor anthocyanin development, while rapid, succulent growth associated with abundant moisture supply is generally correlated with weak anthocyanin development. This is particularly true if the temperature is high. Even at low temperature, anthocyanin development seems to be favored by conditions approaching drouth. However, the moisture supply does not interfere with accurate determination

of phenotype at low temperatures.

The influence of moisture on the development and intensity of anthocyanin was studied in plants which were grown in flats in soil of different moisture content and under conditions of both high and low temperature. In some flats the moisture content was kept high---near the optimum for growth while the soil in other flats was permitted to become dry. The plants in the dry flats wilted severely during the warmer hours of the day. The actual moisture content of the soil was not determined in either case but was known to represent wide extremes of soil moisture. The pigmentation was more intense in plants grown at the lower moisture level when the temperature was high. There was less difficulty in identifying the different color types in plants grown under conditions of low moisture supply than in plants receiving near optimum moisture. The difference was most noticeable in the tinged type, many of which could be quite easily identified in the plants subjected to drouth, while in plants receiving nearly optimum moisture, it was often difficult to distinguish the tinged group from the green due to lack of pigment development in the tinged plants.

In making this study of the influence of moisture on pigment development, all the plants were grown at near optimum moisture conditions until they had developed eight to ten true leaves, then moisture was withheld from half of the flats and the other half continued at the higher moisture. The effect of moisture on anthocyanin development is no doubt closely associated with general growth processes. The fading of the

chlorophyll in the leaves of the plants at the lower moisture and high temperature was no doubt helpful in making it possible to separate the tinged plants from the green, since the anthocyanin type is more easily identified when the chlorophyll color is yellowish than when it is a dark green. Any environmental condition which interferes with normal growth causing a yellowing of the leaf seemed to cause the anthocyanin to stand out more distinctly and thereby aided in distinguishing the various types.

Low soil moisture content was found to be of less value as an aid in identifying the different color types than low temperature. The influence of low temperature in promoting and intensifying the anthocyanin is so much greater than any other environmental factor studied that it tended to mask the difference due to variation in soil moisture, light and soil fertility.

The effect of moisture was studied at both high and low temperature by growing plants in the greenhouse during the summer when the day temperature ranged from 75° to 95° F. and during the winter when the temperature was held below 50° F. when the outside temperatures would permit.

The fading of the chlorophyll in the leaves of low moisture, high temperature plants was probably the principal factor in aiding in separating the various color types in this series of cultures. At the lower temperatures there was little fading of the chlorophyll in the plants subjected to drouth and this, no doubt, accounted in a large measure for the failure of low moisture to aid in identifying the color types in plants grown at low temperature. When the temperature was kept below

50° F., intense pigment development resulted whether the moisture supply was adequate or not.

While extreme drouth at high temperature was an aid in classifying the color type by causing a yellowing of the chlorophyll less severe moisture deficiency tended to cause a darkening of the chlorophyll and to some extent made classification more difficult.

Light. Next to low temperature bright light seems to be the most effective agent in promoting anthocyanin development. As in the case of moisture, the action of light is of less importance at low, than at high, temperature. If the temperature is below 50° F., anthocyanin will develop at low light intensities. At high temperature, shading or other means of lowering the light intensity results in weak or no development of anthocyanin. Whether this is a direct light relationship or a condition associated with altered growth activity under low light intensity is a question not easily answered.

Color counts were made on two groups of plants grown in flats in the greenhouse, part of which were placed in full sunlight and part on a bench close to a partition wall which materially reduced the light intensity. The temperatures in the greenhouse varied between 75° and 90° F. during the day. It was found to be impossible to distinguish the various color groups with any degree of accuracy in the plants grown at the low light intensity. The shaded plants were very succulent and grew more rapidly than the plants in full sunlight. Some of the red and spotted plants developed considerable anthocyanin but most of the tinged type failed to develop enough pigment to distinguish them from the green type. In neither of these

lots of plants was it possible to accurately classify all of the plants, but many more could be classified in the plants subjected to full sunlight than in the lot grown in the shade.

Soil fertility. Conditions associated with soil fertility obviously influence plant color. Poor growth from almost any cause made the classification of the color types less difficult. The seeming increase in anthocyanin under conditions unfavorable for growth may be in part due to a fading of the green color of the chlorophyll which gives the impression of a stronger anthocyanin color. No detailed experiments were carried out to determine the influence of the various plant food elements on the development of anthocyanin, but some observations were made which were of value in determining the best conditions under which to grow plants for color study. Soils from various sources were used as media for growing the plants used in the color studies. Some of the soils used were known to be low in soil fertility. Plants grown in poor soil where growth was slow were observed to be more easily classified as to color type than plants in fertile soil that induced rapid succulent growth.

A light application of nitrate of soda was applied to some of the plants grown in poor soil resulting in a darkening of the chlorophyll, making it more difficult in most cases to classify the plants as to color type. A soil of rather low fertility was found to be preferable to extremely productive soil for the growing of plants for anthocyanin color studies.

Wilting. When conditions are not favorable for antho-

cyanin development, it is quite difficult to separate the plants belonging to the tinged group from the green group lacking anthocyanin. Under such conditions, many of the plants carrying the factor for "tinged" fail to develop the pigment. Consequently some plants are likely to be classed as green which genetically belong to the tinged group. Under such conditions slow wilting of the plants after removing them from the soil with their roots intact was found to induce anthocyanin development in the stem just below the last pair of leaves. If not dried too rapidly, this practice was found to be a very accurate means of separating the plants carrying the genetic factors for anthocyanin from plants lacking some of the genes necessary for anthocyanin to develop. This method of treatment 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 types.

It should be pointed out here that this treatment is distinctly different in its results from the low moisture treatment discussed above. The pigment development is more rapid and more localized in the wilting treatment than in the case of plants grown in soil of low moisture content. In the case of wilting of plants removed from the soil, the pigment development may be confined entirely to a band around the stem just below the last pair of leaves. The leaves of plants treated in this way dry and turn brown quite rapidly, hence the leaf color is of little value in classifying plants by this method. When the wilting progressed very rapidly, the stem

as well as the leaves turned brown before the pigment developed. Slow wilting permitted the pigment in the stem to develop before the tissues turned brown. While the genetic studies were made only on pigment in the leaves, it was possible to use the stem color in separating the pigmented from the green plants since all of the three leaf anthocyanin types also carry anthocyanin in the stems.

#### SEPARATION OF THE COLOR TYPES

Some of the first color studies were made on plants which had been transplanted to the field during the early part of April while the temperature was low and favorable for the development of anthocyanin. These plants developed intense pigment and the color classes were easily identified.

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 definitely classified. The red phenotype is the most easily distinguished. The spotted type is next. The most difficult distinctions are those of the tinged and green types. Under conditons not favorable for anthocyanin it is very difficult to separate the tinged and the green groups. Under such conditions it is necessary to resort to the wilting method mentioned in the discussion of environmental 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 the temperatures were low little difficulty was experienced in classifying the plants into the four phenotypes without resorting to wilting.

#### DISCUSSION

Temperature, light, moisture and nutrition are among the environmental factors influencing the presence and intensity of anthocyanin in the lettuce plant. Low temperature, high light intensity, low available moisture and low soil fertility were indicated to have a favorable influence on the development of the pigment. Pigment development was always weak in plants grown at high temperature. Shading tended to reduce the amount and made classification of the color types very difficult if not impossible, especially if the temperature was high. Light as well as the moisture and nutritional conditions was unimportant at low temperatures because the pigment developed at low temperature regardless of the effects of these other factors.

It can be stated with certainty that, of the external factors mentioned as influencing the development of anthocyanin, that low temperature is the most effective agent in stimulating pigment development and in bringing out clearly the characteristics of the red, spotted and tinged types to permit easy classification of individuals. Temperatures below 60° F. during the night are effective but

lower temperatures (40° to 50° F.) are more desirable. Day temperatures may be relatively high if the night temperature is low.

It is doubtful if accurate classification of the color classes can be made at high temperatures to which the lettuce plant is not adapted.

The slow wilting of the plants after they have been removed from the soil is a valuable aid in separating the pigmented plants from the green but is of little value in separating the colored types from each other.

## 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 the 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, gives tinged.

The genes R,  $r'$  and  $r$  form a multiple allelomorphic series which 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 twelve homozygous genotypes are possible and have the phenotypic expression here indicated.

	<u>Genotype</u>	<u>Phenotype</u>
1.	CCRRTT	Red
2.	CC $r'$ $r'$ TT	Spotted
3.	CCrrTT	Tinged
4.	CCRRtt	Green
5.	CC $r'$ $r'$ tt	"
6.	CCrrtt	"
7.	ccRRTT	"
8.	cc $r'$ $r'$ TT	"
9.	ccrrTT	"
10.	ccRRtt	"
11.	cc $r'$ $r'$ tt	"
12.	ccrrtt	"

Of the possible homozygous genotypes, nine have been isolated and their genetic constitution tested. Only three of the possible genotypes, cc $r'$  $r'$ TT, ccRRtt, and cc $r'$  $r'$ tt,

Table 4. F<sub>2</sub> and F<sub>3</sub> data from cross No. 45 of red B (CCRRTT) x spotted B (CCr'r'TT)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants				
F <sub>1</sub> Plant No.	F <sub>1</sub> Plant Color	F <sub>2</sub> Progenies		
		Red	Spotted	
MB-1	Red	113	33	
MB-2	do	107	32	
MB-3	do	115	29	
MB-5	do	117	41	
MB-6	do	97	33	
MB-7	do	108	39	
MB-9	do	113	35	
MB-11	do	110	40	
Observed		879	282	
Calculated 3:1		870	291	
Deviation		9	9	
Dev.		0.9		
P. E.				

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants				
F <sub>2</sub> Plant No.	F <sub>2</sub> Plant Color	F <sub>3</sub> Progenies		
		Red	Spotted	
MB-1-4	Red	149	0	
MB-1-6	do	125	0	
MB-2-5	do	169	0	
MB-3-3	do	51	0	
MB-3-4	do	162	0	
MB-7-2	do	165	0	
MB-11-3	do	93	0	
MB-11-4	do	186	0	
		1100	0	
MB-1-1	Red	124	38	
MB-1-3	do	131	39	
MB-2-2	do	127	40	
MB-2-4	do	132	37	
MB-3-1	do	122	26	
MB-5-2	do	122	45	
MB-5-4	do	97	27	
MB-5-5	do	135	39	
MB-6-7	do	121	44	
MB-6-8	do	133	51	
MB-7-2	do	137	49	
MB-7-3	do	123	45	
MB-9-2	do	131	43	
MB-9-4	do	135	35	
MB-11-1	do	140	51	
Observed		1910	605	
Calculated 3:1		1896	629	
Deviation		24	24	
Dev.		1.63		
P. E.				
MB-1-7	Spotted	0	51	
MB-2-1	do	0	47	
MB-2-3	do	0	101	
MB-3-2	do	0	35	
MB-5-1	do	0	93	
MB-6-3	do	0	41	
MB-7-1	do	0	37	
MB-11-3	do	0	52	
MB-11-5	do	0	49	
MB-11-6	do	0	43	
MB-11-7	do	0	55	
		0	604	

have not been isolated.

Numerous hypothesis were formulated during the course of these investigations but the one outlined above is the only one which accounts for all of the segregation ratios appearing among the F<sub>2</sub> and F<sub>3</sub> progenies.

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.

Grosses Involving Allelomorphic Series Rr'r

Red (CCRRTT) x Spotted (CCr'r'TT)

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<sub>1</sub> plants were obtained from this cross 25 of which were red. The remaining eight 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<sub>2</sub> 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  $F_1$ 's 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 Table 4. 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 three red to one spotted in the ratio of approximately two segregating to one true breeding.

Red (CCRRTT) x Tinged (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 the 24 plants, 19 were red and obviously hybrids. The remaining five plants were tinged and typical plants for the variety Iceberg.  $F_2$  progenies from seven of the 19 red  $F_1$  plants segregated red and tinged in the ratio of three red to one tinged.

The tinged  $F_2$  plants produced only tinged  $F_3$  progenies. The  $F_3$  progenies showed that the  $F_2$  reds consisted of true

Table 5. F<sub>2</sub> and F<sub>3</sub> data from cross No. 132 of Red M (CCRRTT) x tinged I (CCrrTT)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants				
F <sub>1</sub> Plant No.	F <sub>1</sub> Color	F <sub>2</sub> Progenies		
		Red	Tinged	
MI-2	Red	95	30	
MI-4	do	78	23	
MI-5	do	85	20	
MI-7	do	75	22	
MI-8	do	83	21	
MI-11	do	71	25	
MI-13	do	94	35	
Observed		581	176	
Calculated 3:1		568	189	
Deviation		13	13	
Dev.				
P. E.		1.65		

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants				
F <sub>2</sub> Plant No.	F <sub>2</sub> Color	F <sub>3</sub> Progenies		
		Red	Tinged	
MI-2-2	Red	110	0	
MI-2-3	do	107	0	
MI-2-7	do	53	0	
MI-2-14	do	51	0	
MI-4-2	do	104	0	
MI-5-1	do	49	0	
MI-7-2	do	103	0	
MI-11-1	do	50	0	
MI-13-4	do	47	0	
		674	0	
MI-2-1	Red	83	38	
MI-2-4	do	75	27	
MI-2-5	do	43	9	
MI-2-8	do	50	10	
MI-2-9	do	79	20	
MI-2-11	do	49	14	
MI-2-13	do	43	11	
MI-4-3	do	41	12	
MI-4-5	do	76	21	
MI-5-2	do	77	13	
MI-5-3	do	39	15	
MI-7-3	do	51	21	
MI-8-1	do	102	38	
MI-13-3	do	81	22	
Observed		889	271	
Calculated 3:1		870	290	
Deviation		19	19	
Dev.				
P. E.		1.91		
MI-2-6	Tinged	0	53	
MI-2-10	do	0	47	
MI-2-12	do	0	51	
MI-4-1	do	0	93	
		0	244	

Table 6. F<sub>2</sub> and F<sub>3</sub> data from cross No. 39 of spotted  
B (CCr'r'TT) x tinged I (CCrrTT)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants				
F <sub>1</sub>	:	F <sub>1</sub>	:	F <sub>2</sub> Progenies
Plant No.	:	Color	:	Spotted : Tinged
:	:	:	:	:
BI-1	:	Spotted	:	38 : 15
BI-2	:	do	:	83 : 26
BI-3	:	do	:	35 : 10
BI-5	:	do	:	41 : 17
BI-6	:	do	:	39 : 9
BI-7	:	do	:	40 : 18
BI-8	:	do	:	43 : 11
BI-10	:	do	:	45 : 12
BI-11	:	do	:	39 : 6
BI-13	:	do	:	42 : 8
Observed				445 : 132
Calculated 3:1				433 : 144
Deviation				12 : 12
Dev.				1.82
P. E.				

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants				
F <sub>2</sub>	:	F <sub>2</sub>	:	F <sub>3</sub> Progenies
Plant No.	:	Color	:	Spotted : Tinged
:	:	:	:	:
BI-1-1	:	Spotted	:	43 : 0
BI-1-7	:	do	:	37 : 0
BI-2-3	:	do	:	51 : 0
BI-3-5	:	do	:	55 : 0
BI-6-6	:	do	:	49 : 0
BI-8-1	:	do	:	45 : 0
BI-11-2	:	do	:	53 : 0
BI-11-5	:	do	:	47 : 0
				380 : 0
:	:	:	:	:
BI-1-3	:	do	:	41 : 9
BI-1-6	:	do	:	32 : 15
BI-2-4	:	do	:	45 : 9
BI-2-6	:	do	:	47 : 12
BI-3-1	:	do	:	36 : 11
BI-3-3	:	do	:	45 : 7
BI-5-2	:	do	:	47 : 15
BI-5-4	:	do	:	38 : 11
BI-5-5	:	do	:	43 : 8
BI-6-1	:	do	:	35 : 15
BI-6-5	:	do	:	46 : 17
BI-7-1	:	do	:	37 : 14
BI-7-3	:	do	:	31 : 20
BI-7-5	:	do	:	39 : 16
BI-8-3	:	do	:	42 : 12
BI-8-4	:	do	:	38 : 15
BI-10-2	:	do	:	45 : 16
BI-10-4	:	do	:	43 : 12
BI-11-7	:	do	:	48 : 7
Observed				778 : 241
Calculated 3:1				764 : 255
Deviation				14 : 14
Dev.				1.50
P. E.				
:	:	:	:	:
BI-1-4	:	Tinged	:	0 : 48
BI-1-5	:	do	:	0 : 45
BI-2-2	:	do	:	0 : 55
BI-3-2	:	do	:	0 : 53
BI-5-3	:	do	:	0 : 51
BI-6-2	:	do	:	0 : 47
BI-7-4	:	do	:	0 : 41
BI-10-1	:	do	:	0 : 45
BI-13-2	:	do	:	0 : 49
				0 : 444

breeding reds and reds segregating three red to one tinged, in ratio of approximately two heterozygous to one homozygous.

The data from the  $F_2$  and  $F_3$  progenies from the cross No. 132 red M by tinged I are presented in Table 5.

Spotted (CCr'r'TT) x Tinged (CCrrTT)

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 four tinged plants. The four tinged plants were obviously selfed plants of Iceberg. Five hundred seventy-seven  $F_2$  individuals from 10 spotted  $F_1$  plants consisted of 445 spotted and 132 tinged, a very close fit for a 3:1 ratio.

The results of  $F_2$  and  $F_3$  progeny tests from cross No. 39 are presented in Table 6.

The tinged  $F_2$  plants were all homozygous for tinged. Four hundred forty-four plants from eight tinged  $F_2$  families were all tinged. Eight of the 27 spotted  $F_2$  families tested in the  $F_3$  were homozygous for spotted. The remaining 19 spotted  $F_2$  families tested in the  $F_3$  all segregated spotted and tinged. Of the 1019  $F_3$  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 Table 4, 5 and 6 show that the difference in intensity and pattern of pigment between the three types is in each case due to a single factor.

Table 7. F<sub>2</sub> and F<sub>3</sub> data from cross No. 161 of green  
H (CCRRtt) x green MN (ccRRTT)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants				
F <sub>1</sub> Plant No.	F <sub>1</sub> Color	F <sub>2</sub> Progenies		
		Red	Green	
H-MN-1	Red	29	25	
H-MN-3	do	23	31	
H-MN-4	do	32	23	
H-MN-6	do	35	18	
H-MN-7	do	30	24	
H-MN-8	do	22	31	
Observed		171	152	
Calculated 9:7		182	141	
Deviation		11	11	
Dev.		1.83		
P. E.				

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants				
F <sub>2</sub> Plant No.	F <sub>2</sub> Color	F <sub>3</sub> Progenies		
		Red	Green	
H-MN-1-3	Red	55	0	
H-MN-6-2	do	55	0	
		110	0	
H-MN-1-1	Red	35	20	
H-MN-3-2	do	31	22	
H-MN-4-3	do	27	21	
H-MN-7-2	do	26	29	
H-MN-7-5	do	25	30	
Observed		144	128	
Calculated 9:7		153	119	
Deviation		9	9	
Dev.		1.9		
P. E.				
H-MN-1-2	Red	43	11	
H-MN-1-6	do	38	16	
H-MN-3-3	do	46	9	
Observed		127	36	
Calculated 3:1		122	41	
Deviation		5	5	
Dev.		1.34		
P. E.				
H-MN-3-1	Green	0	54	
H-MN-4-2	do	0	55	
H-MN-4-4	do	0	55	
H-MN-6-1	do	0	49	
H-MN-7-3	do	0	51	
H-MN-7-4	do	0	53	
H-MN-8-2	do	0	55	
H-MN-8-3	do	0	55	
H-MN-8-5	do	0	52	
		0	479	

Since the factor for red is dominant to the factors for spotted and tinged and 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 a pair of complementary allelomorphs control the presence or absence of the pigment is afforded by the following data from a cross between two green plants which gave only red plants in the  $F_1$ .

Crosses Involving the Complementary Factor Pairs (Cc) and (Tt)

Green H (CCRRtt) x Green MN (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 variety New York and the variety Mignonette. Fifteen red plants were obtained in the  $F_1$  from this cross. Three hundred and twenty-three  $F_2$  plants grown from six  $F_1$  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 three red to one green, and reds segregating nine red to seven green. The breeding behavior of progenies from this cross is given in Table 7. The segregations obtained are what would be expected if the presence or absence of pigment were controlled by complementary factors. In this

Table 8. F<sub>1</sub> and F<sub>2</sub> data from cross No. 15 of red M  
(CCRRTT) x green H (ccRRtt)

F <sub>1</sub> Plant No.	F <sub>1</sub> Color	F <sub>2</sub> Progenies	
		Red	Green
MH-2	Red	54	23
MH-5	do	57	17
MH-6	do	115	38
MH-7	do	953	325
MH-12	do	582	170
MH-14	do	441	150
Observed		2202	722
Calculated 3:1		2193	731
Deviation		9	9
Dev.		.57	
P. E.			

Table 9. F<sub>1</sub> and F<sub>2</sub> data from cross No. 47 of red M  
(CCRRTT) x green MN (ccRRTT)

F <sub>1</sub> Plant No.	F <sub>1</sub> Color	F <sub>2</sub> Progenies	
		Red	Green
M-MN-740	Red	82	28
M-MN-742	do	79	18
M-MN-745	do	74	26
M-MN-747	do	91	19
M-MN-749	do	85	26
M-MN-751	do	93	36
M-MN-752	do	73	21
M-MN-768	do	88	31
Observed		664	205
Calculated 3:1		652	217
Deviation		12	12
Dev.		1.4	
P. E.			

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 Tables 8 and 9. Each of these crosses gave F<sub>2</sub> progenies consisting of approximately three red and one 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). No F<sub>3</sub> progenies were grown from either cross No. 15 or cross No. 47.

The F<sub>2</sub> distribution of three red to one green obtained from cross No. 15, Table 8, agrees with the results obtained by Lewis (15) from this same cross.

According to the hypothesis there are only two genotypes that will give a red F<sub>1</sub> when crossed with each other and an F<sub>2</sub> of three red to one 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 heterozygous 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.

Table 10. F<sub>2</sub> and F<sub>3</sub> data from a cross No. 1 of red M (CCRRTT) x green N (ccrrtt)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants					
F <sub>1</sub> Plant No.	F <sub>1</sub> Color	F <sub>2</sub> Progenies			
		Red	Tinged	Green	
MN-2	Red	35	9	15	
MN-4	do	32	7	10	
MN-5	do	67	18	30	
MN-7	do	38	10	29	
MN-8	do	67	15	31	
MN-9	do	93	19	39	
MN-12	do	66	20	30	
MN-13	do	39	10	19	
Observed		427	104	203	
Calculated 9:3:4		413	138	183	
Deviation		14	34	20	
$\chi^2$		-----			11.03
P		-----			Less than .01
F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants					
F <sub>2</sub> Plant No.	F <sub>2</sub> Color	F <sub>3</sub> Progenies			
		Red	Tinged	Green	
MN-2-5	Red	55	0	0	
MN-4-2	do	52	0	0	
MN-4-11	do	49	0	0	
MN-7-1	do	55	0	0	
		211	0	0	
MN-2-1	Red	44	10	0	
MN-2-6	do	38	17	0	
MN-4-7	do	45	9	0	
MN-5-2	do	44	11	0	
MN-5-5	do	45	10	0	
MN-7-6	do	48	7	0	
MN-8-2	do	39	15	0	
MN-8-8	do	42	13	0	
MN-9-5	do	43	12	0	
Observed		388	104	0	
Calculated		369	123	0	
Deviation		19	19	0	
Dev.		-----			2.93
P. E.		-----			
MN-2-3	Red	85	0	23	
MN-2-9	do	45	0	10	
MN-4-4	do	48	0	16	
MN-4-10	do	41	0	12	
MN-5-3	do	44	0	12	
MN-8-6	do	48	0	7	
MN-8-7	do	42	0	12	
Observed		353	0	91	
Calculated 3:1		333	0	111	
Deviation		20	0	20	
Dev.		-----			3.25
P. E.		-----			
MN-2-2	Red	65	20	25	
MN-2-4	do	69	9	28	
MN-4-3	do	30	9	16	
MN-5-1	do	30	7	18	
MN-7-2	do	36	11	8	
MN-7-3	do	31	9	15	
MN-7-7	do	35	5	14	
MN-8-5	do	24	6	23	
MN-9-2	do	30	10	15	
MN-9-5	do	31	9	14	
MN-12-2	do	26	8	17	
MN-12-7	do	32	11	11	
MN-13-1	do	33	7	13	
MN-13-2	do	29	10	14	
Observed		537	141	240	
Calculated 9:3:4		516	172	230	
Deviation		21	31	10	
$\chi^2$		-----			6.88
P		-----			Less than .05
MN-2-6	Tinged	1	55	0	
MN-4-1	do	0	53	0	
MN-12-3	do	0	55	0	
		1*	163	0	
MN-1-8	Tinged	0	40	15	
MN-4-6	do	0	40	15	
MN-7-5	do	0	54	19	
MN-8-1	do	0	37	20	
MN-9-3	do	0	38	16	
MN-9-7	do	0	37	17	
Observed		0	226	102	
Calculated 3:1		0	246	182	
Deviation		0	20	20	
Dev.		-----			3.78
P. E.		-----			
MN-4-5	Green	0	0	54	
MN-5-3	do	0	0	49	
MN-5-4	do	0	0	49	
MN-7-4	do	0	0	55	
MN-8-6	do	0	0	55	
MN-9-1	do	0	0	55	
MN-9-8	do	0	0	48	
MN-12-4	do	0	0	51	
MN-12-6	do	0	0	55	
MN-13-5	do	0	0	54	
MN-13-7	do	0	0	55	
MN-13-9	do	0	0	55	
		0	0	635	

The red plant probably a contamination.

Interactions between the Multiple Allelomorphic Series (Rr'r) and the Complementary Pairs (CcTt)

The proposed hypothesis assumes nine possible genotypes which have 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 factor pairs Cc and Tt. Two of these genotypes have been demonstrated by the above crosses involving (H) and (MN).

The green plant (N) selected from the variety New York in a cross No. 1 with the red plant (M) as the pollen parent gave red F<sub>1</sub> plants. The F<sub>2</sub> segregated red, tinged and green plants. Of the 733 F<sub>2</sub> plants, 427 were red, 104 were tinged, and 203 were green. As indicated in Table 10, the  $\chi^2$  value 11.030 with a P value of less than .01 show this distribution to be 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 733 plants. Although the deviation is significant the observed distribution is nearer a 9:3:4 ratio 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.

The one red plant in the F<sub>3</sub> from the tinged plant MN-12-3 was considered as a mixture.

F<sub>3</sub> progenies were grown from 35 red, nine tinged and 12 green F<sub>2</sub> plants. All of the expected segregation ratios appeared in the F<sub>3</sub>. The behavior of the F<sub>2</sub> and the F<sub>3</sub> progenies is given in Table 10.

The F<sub>2</sub> and F<sub>3</sub> progeny tests from cross No. 1 show that the green plant (N) may have either of the genetic formulae (CCr<sub>1</sub>rtt) 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<sub>1</sub> from cross No. 156 between the green plant (N) and the tester (MN) was lacking in anthocyanin. Two hundred nine F<sub>2</sub> plants from four selfed F<sub>1</sub> plants were all green (Table 11). The (N) plant then must carry the dominant (TT) and the recessive (cc). The tinged plants in the F<sub>2</sub> progenies from cross No. 1 given in Table 10 show that the plant (N) carries the recessive (r) for pigment intensity and pattern. Its genetic formula therefore must be (ccrrTT).

If a homozygous green of the type (CCr'r'tt) were crossed with a homozygous red (CCRRTT) the resulting F<sub>1</sub> should be red and the F<sub>2</sub> should segregate red, spotted and green in the ratio of nine red, three spotted and four green. The data presented in Table 13 of progenies from a 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 12, 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 appear in the progenies from the cross No. 117. The progenies from

Table 11. F<sub>1</sub> and F<sub>2</sub> data from cross No. 156 of green N  
(ccrTT) x green MN (ccRRTT)

F <sub>1</sub> Plant No.	F <sub>1</sub> Color	F <sub>2</sub> Progenies Green
N-MN-1	Green	55
N-MN-3	do	51
N-MN-5	do	49
N-MN-6	do	54
		A209

Table 12. F<sub>2</sub> and F<sub>3</sub> data from cross no. 117 of green MN  
(ccRRTT) x green T (Cr'r'tt)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants						
F <sub>1</sub> Plant No.	F <sub>1</sub> Color	Red	Spotted	Green		
MN-T-1	Red	21	8	20		
MN-T-3	do	18	12	24		
MN-T-4	do	15	8	22		
MN-T-7	do	31	9	24		
MN-T-8	do	33	12	20		
MN-T-10	do	20	11	33		
MN-T-11	do	22	18	25		
MN-T-12	do	18	11	30		
MN-T-13	do	13	10	27		
MN-T-15	do	22	9	25		
Observed		213	108	250		
Calculated 27:9:28		231	77	240		
Deviation		18	31	10		

X<sup>2</sup> - - - - - √14.300  
P - - - - - Less than .01

Table 13. F<sub>2</sub> and F<sub>3</sub> data from a cross No. 38 of red M  
(CCRRTT) x green T (CCr'r'TT)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants					
F <sub>1</sub> Plant No.	F <sub>1</sub> Color	F <sub>2</sub> Progenies			
		Red	Spotted	Green	
MT-1	Red	38	13	21	
MT-2	do	66	24	35	
MT-4	do	35	17	24	
MT-5	do	64	30	25	
MT-6	do	42	17	21	
MT-7	do	74	13	27	
MT-9	do	50	13	23	
MT-10	do	38	11	15	
MT-13	do	63	18	30	
MT-14	do	33	7	19	
MT-15	do	91	28	45	
MT-16	do	31	9	15	
MT-19	do	33	10	18	
Observed		658	214	318	
Calculated 9:3:4		669	223	298	
Deviation		11	9	20	
	$\chi^2$	= 1.928			
	P	= .30			

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants					
F <sub>2</sub> Plant No.	F <sub>2</sub> Color	F <sub>3</sub> Progenies			
		Red	Spotted	Green	
MT-4-1	Red	165	0	0	
MT-7-3	do	77	0	0	
		242	0	0	
MT-1-3	Red	80	30	0	
MT-6-1	do	68	30	0	
MT-9-5	do	40	15	0	
MT-10-2	do	44	11	0	
MT-13-4	do	42	13	0	
Observed		274	99	0	
Calculated 3:1		280	93	0	
Deviation		6	6	0	
Dev.		= 1.06			
P. E.					
MT-2-2	Red	74	0	22	
MT-2-7	do	71	0	23	
MT-5-2	do	43	0	12	
MT-6-4	do	44	0	10	
Observed		232	0	67	
Calculated 3:1		224	0	75	
Deviation		8	0	8	
Dev.		= 1.57			
P. E.					
MT-1-1	Red	63	19	25	
MT-2-5	do	61	22	30	
MT-4-2	do	32	11	12	
MT-4-4	do	27	6	16	
MT-9-3	do	31	9	15	
MT-10-4	do	65	21	29	
MT-13-1	do	29	11	15	
Observed		308	99	142	
Calculated 9:3:4		309	103	137	
Deviation		1	4	5	
	$\chi^2$	= .341			
	P	= .8			
MT-4-3	Spotted	0	32	15	
MT-7-2	do	0	36	19	
MT-7-4	do	0	42	13	
MT-10-1	do	0	43	12	
Observed		0	153	59	
Calculated		0	159	53	
Deviation		0	6	6	
Dev.		= 1.41			
P. E.					
MT-1-5	Green	0	0	65	
MT-6-3	do	0	0	77	
MT-7-1	do	0	0	54	
MT-9-1	do	0	0	53	
MT-9-2	do	0	0	49	
MT-10-3	do	0	0	55	
MT-13-2	do	0	0	55	
		0	0	408	

Table 14. F<sub>2</sub> and F<sub>3</sub> data from cross No. 43 of red M (CORRTT) green NC (corrtt)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants				
F <sub>1</sub> Plant No.	F <sub>1</sub> Color	F <sub>2</sub> Progenies		
		Red	Tinged	Green
M-NC-669	Red	36	15	39
M-NC-671	do	48	11	52
M-NC-672	do	40	12	43
M-NC-678	do	47	11	48
M-NC-680	do	41	11	47
M-NC-681	do	46	9	35
M-NC-682	do	35	13	52
M-NC-687	do	51	10	44
M-NC-690	do	45	15	49
M-NC-700	do	57	12	42
M-NC-702	do	36	8	48
M-NC-703	do	39	8	44
M-NC-704	do	51	9	41
Observed		572	144	564
Calculated		548	183	569
Deviation		24	39	15
X <sup>2</sup>		9.670		
P		Less than .01		
F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants				
F <sub>2</sub> Plant No.	F <sub>2</sub> Color	F <sub>3</sub> Progenies		
		Red	Tinged	Green
MNC-672-9	Red	54	0	0
		54	0	0
MNC-669-3	Red	45	10	0
MNC-680-4	do	44	11	0
MNC-700-2	do	39	12	0
Observed		128	33	0
Calculated		121	40	0
Deviation		7	7	0
Dev.		1.89		
P. E.				
MNC-669-1	Red	38	0	16
MNC-669-9	do	42	0	13
MNC-671-3	do	35	0	18
MNC-672-7	do	38	0	15
MNC-680-2	do	36	0	17
Observed		208	0	79
Calculated 3:1		215	0	72
Deviation		7	0	7
Dev.		1.41		
P. E.				
MNC-678-3	Red	33	0	22
MNC-680-7	do	34	0	21
MNC-702-1	do	37	0	23
Observed		104	0	68
Calculated 9:7		96	0	74
Deviation		8	0	8
Dev.		1.83		
P. E.				
MNC-680-3	Red	27	5	20
MNC-680-9	do	32	9	15
MNC-682-4	do	30	15	10
MNC-682-7	do	34	8	13
MNC-690-9	do	33	10	11
MNC-702-3	do	31	11	12
MNC-702-5	do	35	9	10
MNC-703-6	do	30	7	16
MNC-703-8	do	29	6	17
MNC-704-1	do	35	8	11
MNC-704-4	do	36	9	10
Observed		352	97	145
Calculated 9:3:4		334	111	149
Deviation		18	14	4
X <sup>2</sup>		2.918		
P		Less than .02		
MNC-669-7	Red	23	7	26
MNC-672-2	do	21	7	25
MNC-672-4	do	28	3	23
MNC-678-1	do	16	5	30
MNC-680-5	do	30	8	21
MNC-690-2	do	24	7	24
MNC-703-5	do	30	3	21
Observed		172	40	170
Calculated 27:9:28		158	53	165
Deviation		14	13	5
X <sup>2</sup>		4.581		
P		Between .01 and .02		
MNC-671-5	Tinged	0	55	0
MNC-682-1	do	0	53	0
		0	108	0
MNC-669-4	Tinged	0	35	15
MNC-672-6	do	0	36	18
MNC-678-2	do	0	41	14
MNC-690-1	do	0	42	12
MNC-690-5	do	0	39	16
MNC-700-6	do	0	39	15
Observed		0	232	90
Calculated 3:1		0	242	80
Deviation		0	10	10
Dev.		1.91		
P. E.				
MNC-671-1	Tinged	0	28	26
MNC-678-5	do	0	22	23
Observed		0	50	49
Calculated 9:7		0	56	43
Deviation		0	6	6
Dev.		2.06		
P. E.				
MNC-669-3	Green	0	0	55
MNC-669-8	do	0	0	47
MNC-671-4	do	1	0	49
MNC-671-6	do	0	0	55
MNC-672-3	do	0	0	55
MNC-680-1	do	0	0	52
MNC-680-6	do	0	0	48
MNC-682-5	do	0	0	50
MNC-682-9	do	0	0	55
MNC-690-4	do	0	0	55
MNC-690-6	do	1	0	55
MNC-700-4	do	0	0	46
MNC-700-5	do	0	0	51
MNC-700-7	do	0	0	53
MNC-702-2	do	0	0	55
MNC-702-7	do	0	0	55
MNC-703-1	do	0	0	55
MNC-703-3	do	0	0	52
MNC-704-2	do	0	0	55
2*		0	0	998

\* Two red plants probably contaminations.

cross No. 117 gave 213 red, 108 spotted and 250 green. The  $\chi^2$  of 14.300 with a P value of less than .01 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 paper.

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). The  $F_2$  gave 572 red, 144 tinged and 584 green (Table 14). The  $\chi^2$  value of 9.670 with a P value of less than .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, Table 10, and cross No. 117, Table 12, 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 14. 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 which would give an  $F_2$  distribution of 27 red, nine tinged and 28 green in a cross with a homozygous red.

The green plant R selected from the variety Grand Rapids proved to have the genetic formula (CCrrtt). In a cross No. 33 with the homozygous red M, the F<sub>1</sub> plants were all red (Table 15). The F<sub>2</sub> gave 382 red, 103 tinged and 177 green plants. The X<sup>2</sup> 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 nine red, three tinged and four green segregates in the F<sub>2</sub>. The data presented in Table 10 from cross No. 1 shows that one of these genotypes (ccrrTT) is represented by the green plant N. The data given in Table 16 from cross No. 28 between plants N and R show that these two plants do not have the same genetic constitution. The F<sub>1</sub> plants from this cross were tinged. The F<sub>2</sub> progenies segregated tinged and green in the ratio of approximately nine tinged to seven green. Four hundred eighty-four F<sub>2</sub> plants from six F<sub>1</sub> 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<sub>3</sub> progenies were not grown from cross No. 28. The behavior of the F<sub>1</sub> and F<sub>2</sub> 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 10 and 11, have shown the plant N to be of the type (ccrrTT), R must have the formula (CCrrtt).

The data in Table 17 from cross No. 37 between the green plants H and R is further evidence that R carries the dominant

Table 15. F<sub>2</sub> and F<sub>3</sub> data from cross No. 33 of red M (CCRRTT) x green R (CCrrtt)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants					
F <sub>1</sub> Plant No.	F <sub>1</sub> Color	F <sub>2</sub> Progenies			
		Red	Tinged	Green	
MR-1	Red	70	25	34	
MR-3	do	41	9	20	
MR-4	do	69	20	20	
MR-6	do	43	10	23	
MR-7	do	67	15	37	
MR-9	do	43	13	23	
MR-10	do	50	11	21	
Observed		382	103	177	
Calculated 9:3:4		373	124	165	
Deviation		9	21	12	
X <sup>2</sup>		----- 4.65			
P		----- 0.1			

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants					
F <sub>2</sub> Plant No.	F <sub>2</sub> Color	F <sub>3</sub> Progenies			
		Red	Tinged	Green	
MR-3-6	Red	77	0	0	
MR-4-3	do	55	0	0	
		132	0	0	
MR-1-3	Red	44	11	0	
MR-1-6	do	39	16	0	
MR-4-5	do	45	10	0	
MR-6-2	do	47	8	0	
MR-10-9	do	44	11	0	
Observed		219	56	0	
Calculated 3:1		206	69	0	
Deviation		13	13	0	
Dev.		----- 2.68			
P. E.					
MR-3-3	Red	38	0	17	
MR-4-1	do	46	0	9	
MR-6-7	do	41	0	14	
Observed		125	0	40	
Calculated 3:1		124	0	41	
Deviation		1	0	1	
Dev.		-----			
P. E.					
MR-1-1	Red	30	9	16	
MR-1-4	do	35	8	13	
MR-3-5	do	28	14	13	
MR-6-6	do	30	10	15	
MR-9-3	do	27	11	17	
MR-9-7	do	34	15	6	
Observed		184	67	79	
Calculated 9:3:4		185	62	83	
		1	5	4	
X <sup>2</sup>		----- 0.60			
P		----- 0.7			
MR-3-1	Tinged	0	75	0	
MR-6-4	do	0	55	0	
MR-9-1	do	0	49	0	
		0	179	0	
MR-1-5	Tinged	0	38	17	
MR-4-2	do	0	45	10	
MR-6-3	do	0	34	21	
MR-7-1	do	0	40	15	
MR-7-7	do	0	39	16	
Observed		0	196	79	
Calculated 3:1		0	206	69	
Deviation		0	10	10	
Dev.		----- 2.06			
P. E.					
MR-3-4	Green	0	0	75	
MR-4-4	do	0	0	77	
MR-7-2	do	0	0	54	
MR-10-1	do	0	0	49	
MR-10-5	do	0	0	55	
		0	0	310	

Table 16. F<sub>2</sub> data from cross No. 28 of green N (ccrrTT)  
x green R(CCrrtt)

F <sub>1</sub> Plant No.	F <sub>1</sub> Plant Color	F <sub>2</sub> Progenies	
		Tinged	Green
NR-5	Tinged	55	51
NR-6	do	27	25
NR-7	do	60	56
NR-11	do	59	43
NR-13	do	29	23
NR-15	do	31	25
Observed		261	223
Calculated 9:7		272	212
Deviation		11	11
Dev.	----- 1.49		
P. E.			

Table 17. F<sub>2</sub> data from cross no. 37 of green H(CCRRtt)  
x green R(CCrrtt)

F <sub>1</sub> Plant No.	F <sub>1</sub> Plant Color	F <sub>2</sub> Progenies
		Green
HR-1	Green	39
HR-3	do	43
HR-5	do	38
HR-6	do	45
HR-7	do	54
HR-8	do	51
HR-9	do	55
HR-10	do	55
HR-11	do	49
HR-13	do	52
		485

(CC) and the dominant (TT). No pigmented plants appeared in the F<sub>1</sub> of this cross as would have been the case if (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).

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), (ccr'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 (Lactuca 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 near 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<sub>2</sub> and F<sub>3</sub> progenies from these crosses indicate that the wild plant belonged to the tinged type (CCrrTT) since it exhibited the

Table 18. F<sub>2</sub> and F<sub>3</sub> data from cross No. 49 of red M  
(CCRR<sup>TT</sup>) x tinged wild (CCrr<sup>TT</sup>)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants					
F <sub>1</sub>	:	F <sub>1</sub>	:	F <sub>2</sub> Progenies	
Plant No.	:	Color	:	Red	Tinged
MW-1	:	Red	:	45	10
MW-3	:	do	:	43	12
MW-4	:	do	:	37	17
MW-5	:	do	:	39	14
MW-6	:	do	:	41	13
MW-7	:	do	:	46	9
Observed				252	75
Calculated 3:1				245	82
Deviation				7	7
Dev.	----- 1.33				
P. E.					

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants					
F <sub>2</sub>	:	F <sub>2</sub>	:	F <sub>3</sub>	
Plant No.	:	Color	:	Red	Tinged
MW-1-3	:	Red	:	54	0
MW-1-5	:	do	:	51	0
	:		:	105	0
MW-1-2	:	Red	:	47	8
MW-2-3	:	do	:	41	13
MW-2-4	:	do	:	39	16
MW-3-3	:	do	:	44	11
MW-5-1	:	do	:	40	15
MW-5-2	:	do	:	45	9
Observed				256	72
Calculated				246	82
Deviation				10	10
Dev.	----- 1.89				
P. E.					
MW-1-1	:	Tinged	:	0	49
MW-2-2	:	do	:	0	55
MW-3-1	:	do	:	0	55
MW-4-3	:	do	:	0	51
MW-4-5	:	do	:	0	53
				0	263

same breeding behavior as the tinged plants of cultivated lettuce.

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 twenty-seven  $F_2$  individuals from six  $F_1$  plants gave 252 red and 75 tinged. The deviation of seven from the calculated values for a 3:1 ratio for 327 plants is not significant. (Table 18).

All of the  $F_3$  progenies from five 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 three red to one tinged. The deviation from the calculated for a 3:1 ratio was 10, which is 1.89 times the probable error.

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 five selfed  $F_1$  plants and, out of 272 plants, 152 were red, 42 tinged and 78 green. The  $\chi^2$  value of 3.065 with a P value between .20 and .30 shows this to be a good fit for a 9:3:4 ratio. (Table 19)

$F_3$  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 three tinged to one green. Selfed spotted  $F_2$  plants gave progenies breeding true for spotting

Table 19. F<sub>2</sub> and F<sub>3</sub> data from cross No. 51 of tinged wild W (CCrrTT) x green T (CCr'r'tt)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants					
F <sub>1</sub> Plant No.	F <sub>1</sub> Color	F <sub>2</sub> Progenies			
		Spotted	Tinged	Green	
WT-1	Spotted	31	3	19	
WT-2	do	33	7	15	
WT-3	do	31	8	16	
WT-5	do	30	11	13	
WT-7	do	27	13	15	
Observed		152	42	78	
Calculated 9:3:4		153	51	68	
Deviation		1	9	10	
		χ <sup>2</sup> - - - - - 3.065			
		P - - - - - Between .20 and .30			

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants					
F <sub>2</sub> Plant No.	F <sub>2</sub> Color	F <sub>3</sub> Progenies			
		Spotted	Tinged	Green	
WT-1-2	Spotted	54	0	0	
		54	0	0	
WT-3-2	Spotted	36	17	0	
WT-5-1	do	43	12	0	
WT-5-4	do	39	14	0	
Observed		118	43	9	0
Calculated		121	40	0	0
Deviation		3	3	0	
Dev.		- - - - - 0.81			
P. E.					
WT-1-1	Spotted	45	0	8	
WT-1-4	do	39	0	15	
WT-2-3	do	40	0	13	
WT-5-2	do	36	0	19	
WT-7-1	do	37	0	18	
WT-7-3	do	38	0	14	
Observed		235	0	87	
Calculated 3:1		242	0	80	
Deviation		7	0	7	
Dev.		- - - - - 1.35			
P. E.					
WT-2-1	Spotted	29	8	18	
WT-2-2	do	31	11	13	
WT-3-4	do	27	9	19	
WT-5-3	do	33	7	14	
WT-5-4	do	30	9	15	
Observed		150	44	79	
Calculated		154	51	68	
Deviation		4	7	11	
χ <sup>2</sup>		- - - - - 2.844			
P		- - - - - Between .20 and .30			
WT-1-3	Tinged	0	49	0	
WT-7-2	do	0	53	0	
		0	102	0	
WT-2-4	Tinged	0	38	16	
WT-2-5	do	0	39	15	
WT-5-5	do	0	40	15	
Observed		0	117	46	
Calculated 3:1		0	122	41	
Deviation		0	5	5	
Dev.		- - - - - 1.34			
P. E.					
WT-1-4	Green	0	0	55	
WT-1-5	do	0	0	53	
WT-1-7	do	0	0	55	
WT-3-1	do	0	0	55	
WT-3-3	do	0	0	54	
WT-7-3	do	0	0	51	
WT-7-5	do	0	0	53	
		0	0	376	

progenies segregating spotted, tinged and green in the ratio of nine spotted, three tinged and four green, progenies segregating three spotted to one tinged and progenies giving three spotted to one green (Table 19).

#### DISCUSSION

The data presented in Table 7, 12 and 16 of progenies from crosses, numbers 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 16), between the two green plants (N) and (R) gave only tinged and green plants in the ratio of approximately nine tinged to seven 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 which influence the development of anthocyanin must be alike and in the homozygous condition in each parent. The behavior of progenies from this cross demonstrate 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, (Table 1) 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 three red to one spotted.

That the red also behaves as a simple dominant over the tinged type is shown in the progenies from cross No. 132 (Table 5), 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 three red to one tinged.

The spotted type which was shown in cross No. 45, (Table 1) to be recessive to the red, is shown to be dominant to tinged by the progenies from cross No. 39, (Table 6) between the spotted and tinged types. The  $F_1$  was spotted and the  $F_2$  distribution was approximately three spotted to one tinged.

The tests of progenies from crosses, numbers 45, 132 and 39, (Tables 4, 5 and 6), 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, red x spotted, red x tinged, and spotted x tinged gave 3:1 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 16) 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 (Rr'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 there can be according to this hypothesis 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.

In the seventeen crosses from which progenies were studied, fourteen of them involved at least one green parent. The segregation 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) which were not represented by any of the varieties studied.

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

New York No. 5084	ccrrTT	
Hanson	CCRrtt	
Grand Rapids	}	
Paris White Cos		
Dark Green Cos		
Unrivalled		
White Chavigne		CCrrtt
Malta		
Early Curled Simpson		
Mammoth Black Seeded Butter		

Deacon	)	
	)	
Transport	)	
	)	CCr'r'tt
Salamander	)	
	)	
Hubbard's Market	)	
	)	
Big Boston	)	
	)	
May King	)	
	)	
Iceberg	)	CCrrTT
	)	
Density	)	
	)	
Wild ( <u>L. scariola</u> )	)	
	)	
Mignonette	)	
	)	
Prize Head	)	CCRRTT
	)	
Crisp as Ice	)	
	)	
California Cream Butter)	)	
	)	
Maximum	)	CCr'r'TT
	)	
Dutch Speckled Butter	)	

While no genetic studies were made of the factors controlling anthocyanin in parts of the lettuce plant other than in the leaves, some observations were made which are of interest and worth mentioning 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 discernable in the stems under conditions unfavorable for leaf pigment. The correlation between leaf and stem pigmentation could under certain conditions 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 is obvious that there is more than one color type in the ray flowers. Some study was made of the pigment in the flowers but insufficient data are 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 involucre bracts more nearly corresponds to the leaf pattern than in any other of the plant parts studied. The localized spots of dense pigment characteristic of the spotted leaf type were also

observed in the involucre 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 involucre bracts since the presence of pigment in any 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, involucre bracts and the stem are not controlled by exactly the same gene combination as controls leaf coloration.

#### EVIDENCE OF LINKAGE

The data presented in Tables 10, 12 and 14 of progenies from crosses 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 discrepancies in the  $F_2$  populations is beyond the limits of random sampling. In cross No. 1 (Table 10) between the homozygous red M ( $CCRRTT$ ) and the green N ( $ccrrTT$ ) and in cross No. 43 (Table 14) 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 (Table 12) 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 thirteen 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 back-crosses 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 10). By assuming R- and rr to be in the ratio of 3:1, C and R were found to have a linkage value of .643. 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 .64 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 .64.

The  $\chi^2$  of 11.03 for the deviation from the calculated frequencies in the  $F_2$  from cross No. 1, Table 10, 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- vs. cc with a 3:1 ratio  $(a+b)-3d=0$ . In the  $F_2$  population the discrepancy is 78 beyond the limits of random. sampling.  $531-609 = -78$ .

V	f	d	fd	fd <sup>2</sup>
a	9	1	9	9
f	4	1	3	4
d	3	-3	-12	36
	16		0	$\frac{48}{16} = 3$

The squared standard error for random sampling in the expected 9:3:4 ratio is equal to  $3n$  or 2202 ( $3 \times 734$ ) = 2202. The squared deviation is 6084 ( $78^2 = 6084$ ).  $\frac{6084}{2202} = 2.76 = \chi^2$ .

With one degree of freedom, 1.96 standard errors shows odds of 19:1.  $1.96^2 = 3.84 = \chi^2$  for odds of 19:1. A  $\chi^2$  of 2.76 (odds of about 10:1) shows the discrepancy for C- vs. 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- vs. 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<sub>1</sub> would have been equal.

Class d contains 203 plants instead of the calculated 177

$\left(\frac{427 + 104}{3} = 177\right)$ . Class d<sub>1</sub> then should contain 119 plants (177:203:104:X) and class d<sub>2</sub>, 84 plants (203-119 = 84.)

The distribution of the four classes would then be:

observed	calculated
a - 427	$\frac{1}{4} (2+y)$
b - 104	$\frac{1}{4} (1-y)$
d <sub>1</sub> - 119	$\frac{1}{4} (1-y)$
d <sub>2</sub> - 84	$\frac{1}{4} (y)$
734	

Let  $\sqrt{y}$  linkage between C and R. With C and R independent y should equal .25 and the classes would be: - a =  $\frac{1}{4}(2.25)$ ; b =  $\frac{1}{4} (.75)$ ; d<sub>1</sub> =  $\frac{1}{4}(.75)$  and d<sub>2</sub> =  $\frac{1}{4}(.25)$  and the ratio would be 9:3:3:1. The ratio would change with any change in y.

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

$$y = .4136$$

$$\sqrt{y} = .643$$

The calculated linkage between C and R is .643 when the value expected, if they were not in the same chromosome, would be .5. By substituting the class frequencies expected

from the value for  $y$  and solving for goodness of fit we obtain  $\chi^2$  of 2.76 which is the value obtained for the deviation of C- vs. cc from the expected 3:1 ration (Table 20). The calculated linkage between C and R is then correct if R- vs. rr is a 3:1 ratio.

Table 20. Calculation of goodness of fit for the class frequencies expected from the value of  $y$ .

Classes	Frequencies		C=O	$\frac{(O - O)^2}{O}$
	Observed	Calculated		
a	427	442.9	15.9	.57
b	104	107.6	3.6	.12
d <sub>1</sub>	119)	183.5	19.5	2.07
d <sub>2</sub>	84)			
	734	734		2.76 = $\chi^2$

The  $F_2$  distribution from cross No. 43 (Table 14) with an  $\chi^2$  of 9.67 is also beyond the limits of reasonable chance.

If we apply the linkage value .64 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:

CR, .32; Cr, .18; cR, .18 and cr, .32. .4518 of the gametes will be of the type CRT  $[(3 \times .32^2) + (4 \times .32 \times .18) + (2 \times .18^2)] .75 = .4518$ . .1107 of the gametes will be of the type C - rrTt  $[(2 \times .32 \times .18) + (.18^2)] .75 = .1107$ . The remaining .4375 will consist of the types which give green plants.

The goodness of fit test with the class frequencies calculated from the above proportions gives an  $\chi^2$  of .81 (Table 21).

With one degree of freedom the deviation from the calculated is not significant. On the basis of .64 linkage between C and R there is a close fit between the observed and calculated numbers for the different classes.

Table 21. Goodness of fit test of  $F_2$  populations from cross No. 43 assuming a .64 linkage between C and R.

Classes	Frequencies		(C - O)	$\frac{(C - O)^2}{O}$
	Observed	Calculated		
a	572	587.3	15.3	.40
b	144	143.9	.1	.00
d	584	568.8	15.2	.41
	1300	1300.0		.81 = $\chi^2$

When the calculated linkage of .64 between C and R was applied to the  $F_2$  population from cross No. 117 (Table 12), the observed numbers for the different classes were found to be within limits of error.

On the basis of .64 linkage between C and R each parent should produce gametes in the following proportions:- CR, .18; Cr, .32; cR, .32 and cr, .18. The type CRT will be produced in the following proportion:-  $\left[ (3 \times 18^2) + (4 \times .18 \times .32) + (2 \times 32^2) \right] .75 = .3993$ . The C-r't-T- type will be produced in the following proportion:-  $\left[ (2 \times .18 \times .32) + (.32^2) \right] .75 = .1632$ . The remaining .4375 will consist of the types which produce green plants. Calculating goodness of fit for observed frequencies using the above proportions in determining the calculated values we obtain a  $\chi^2$  of 3.337. With one degree

of freedom the deviation of the observed class values from the values calculated on the basis of .64 linkage is within the limits of error. Goodness of fit of the observed class frequencies on the basis of .64 linkage between C and R is shown in Table 22.

Table 22. Goodness of fit test of F<sub>2</sub> population from cross No. 117 assuming a .64 linkage between C and R.

Classes	Frequencies		C - O	$\frac{(O - O)^2}{O}$
	Observed	Calculated		
a	213	228	15.0	.987
b	108	93.2	14.8	2.350
d	250	249.8	.2	.000
	571	571.0		3.337 = $\chi^2$

PART II

THE INHERITANCE OF SOME CHLOROPHYLL CHARACTERS  
IN LETTUCE

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 the variety 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. Severe drouth on the other hand causes a yellowing of the chlorophyll. Conditions which may cause a yellowing of the chlorophyll are many. Any condition which seriously interferes with growth may cause a yellowing. Extreme drouth, 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 and yellow green under normal conditions. The dark green type is shown in the colored illustration Plate II. The colored illustration in Plate III is of a typical plant of the yellow green type.

Any one of the three anthocyanin colorations, red, spotted or tinged described in Part I may accompany either of the two chlorophyll types. The variety Mignonette is a red anthocyanin on a dark green chlorophyll. The colored illustration in Plate VIII 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 colored Plate IX. In the variety Iceberg the yellow green is accompanied by the tinged type of anthocyanin. Colored Plate V. In Density the tinged anthocyanin is on a dark green chlorophyll. This type of coloration is shown in colored Plate IV. The spotted anthocyanin and the dark green occur together in the variety California Cream Butter. Colored Plate VI. Colored Plate VII is an illustration of the combination in which the spotted anthocyanin is on a yellow green. This type has been observed in a number of hybrid lines. 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.

Table 23. F<sub>2</sub> data from cross No. 15 of dark green red M  
(GGCORRTT x yellow green H (ggCRRtt))

Plant No.:	F <sub>1</sub> Color	F <sub>2</sub> Progenies			
		Dark green:red	Dark green:green	Yellow green:red	Yellow green:green
MH-1	Dark green	59	18	13	4
MH-7	red	61	20	17	7
MH-12	"	56	22	13	2
MH-14	"	93	27	32	8
MH-15	"	85	30	31	5
Observed		354	117	106	26
Calculated	9:3:3:1	339	113	113	38
Deviation		15	4	7	12

X<sup>2</sup> - - - - - 5.03  
P - - - - - 0.1

	Dark green	Yellow green
Observed	471	132
Calculated	452	151
Deviation	19	19
Dev.	- - - - - 2.5	
P. E.		

It is possible that there exists a third chlorophyll type intermediate between the dark green and the yellow green. Such varieties as Deacon and Unrivalled cannot be definitely classed as either a dark 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 difficult determinations as to whether an intermediate between dark and yellow green exists or not.

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.

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 Table 23.

The  $F_1$  plants were dark green, red. Six hundred three individuals were grown from 5  $F_2$  plants. Of these, 354 were dark green, red, 106 yellow green, red, 117 dark green without anthocyanin and 26 were yellow green lacking anthocyanin. The P value of 0.1 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 green with and without

Table 24. F<sub>3</sub> data from cross No. 15 of dark green red M (GGCCRRTT)  
x yellow green H(ggCRRtt)

F <sub>2</sub> Plant No.	F <sub>2</sub> Color	F <sub>3</sub> Progenies			
		Dark Green: Red	Dark: Green	Yellow Green: Red	Yellow: Green
MH-7-11	: Dark green:	129	: 0	: 0	: 0
	: red				
		129	: 0	: 0	: 0
MH-1-4	: Dark	91	: 0	: 29	: 0
MH-1-7	: green	104	: 0	: 27	: 0
MH-7-2	: red	93	: 0	: 25	: 0
MH-12-1	: "	89	: 0	: 32	: 0
MH-15-5	: "	102	: 0	: 27	: 0
Observed		479	: 0	: 140	: 0
Calculated 3:1		464	: 0	: 155	: 0
Deviation		15	: 0	: 15	: 0
Dev.		----- 2.07			
P. E.					
MH-1-1	: Dark	107	: 23	: 0	: 0
MH-1-3	: green	98	: 31	: 0	: 0
MH-7-4	: red	87	: 30	: 0	: 0
MH-12-2	: "	94	: 35	: 0	: 0
Observed		386	: 119	: 0	: 0
Calculated		378	: 127	: 0	: 0
Deviation		8	: 8	: 0	: 0
Dev.		----- 1.22			
P. E.					
MH-1-2	: Dark	78	: 30	: 24	: 8
MH-1-5	: green	81	: 27	: 22	: 5
MH-1-6	: red	47	: 13	: 17	: 7
MH-1-9	: "	62	: 25	: 19	: 3
MH-7-1	: "	70	: 22	: 26	: 5
MH-7-7	: "	89	: 31	: 25	: 11
MH-14-2	: "	70	: 22	: 18	: 5
MH-15-1	: "	67	: 19	: 23	: 7
Observed		564	: 189	: 174	: 51
Calculated		550	: 183	: 183	: 61
Deviation		14	: 6	: 9	: 10
X <sup>2</sup>		----- 2.64			
P		----- 0.2			
MH-1-11	: Dark green:	0	: 127	: 0	: 0
MH-12-2	: " "	1	: 131	: 0	: 0
		1	: 258	: 0	: 0
MH-7-8	: Dark green:	0	: 122	: 0	: 43
MH-14-1	: " "	0	: 135	: 0	: 30
MH-14-4	: " "	0	: 127	: 0	: 40
Observed		0	: 384	: 0	: 113
Calculated 3:1		0	: 373	: 0	: 124
Deviation			11	: 0	: 11
Dev.		----- 1.69			
P. E.					
MH-1-12	: Yellow	0	: 0	: 157	: 0
MH-7-9	: green red	0	: 0	: 161	: 0
		0	: 0	: 318	: 0
MH-1-13	: Yellow	0	: 0	: 85	: 26
MH-7-3	: green	0	: 0	: 123	: 33
MH-12-7	: red	0	: 0	: 43	: 12
MH-15-1	: "	0	: 0	: 119	: 45
MH-15-4	: "	0	: 0	: 92	: 27
Observed		0	: 0	: 462	: 143
Calculated		0	: 0	: 454	: 151
Deviation		0	: 0	: 8	: 8
Dev.		----- 1.11			
P. E.					
MH-1-15	: Yellow	0	: 0	: 0	: 132
MH-7-5	: green	0	: 0	: 0	: 123
MH-14-1	: "	0	: 0	: 0	: 117
		0	: 0	: 0	: 372

anthocyanin were grouped together as were also all of the yellow green. 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 2.5 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 presented in Table 24. 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 probably 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.

The data given in Table 25 from cross No. 132 of a dark green red M and a yellow green,tinged I indicates 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 seven  $F_2$  families gave 225 dark green,red, 63 yellow green, red, 72 dark green,tinged and 17 yellow green,tinged.

Table 25. F<sub>1</sub> and F<sub>2</sub> data from cross No. 132 of dark green red M(GGCCRRTT) x yellow green tinged I (ggOCrrTT)

Plant No.	F <sub>1</sub> Color	F <sub>2</sub> Progenies			
		Dark green:red	Dark green:tinged	Yellow green:red	Yellow green:tinged
MI-1	Dark:green	36	5	9	3
MI-3	red	27	15	9	4
MI-6	"	36	9	8	2
MI-9	"	31	13	10	3
MI-10	"	37	6	8	1
MI-14	"	28	12	9	3
MI-15	"	30	12	10	1
Observed		225	72	63	17
Calculated	9:3:3:1	212	71	71	24
Deviation		13	1	8	7

$\chi^2$  - - - - - 3.7

P - - - - - 0.2

Dark green:Yellow green

Observed	297	:	80
Calculated 3:1	<u>283</u>	:	<u>94</u>
Deviation	14	:	14
Dev.	- - - - - 2.47		
P. E.			



The deviation from the calculated values for a 9:3:3:1 ratio was found to have a P value of 0.2 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 3.47 times its probable error, hence hardly significant.

The inheritance of chlorophyll color was studied in progenies from a third cross No. 14 between the dark green (N) and the yellow green plant (H).  $F_2$  and  $F_3$  progenies from cross No. 14 are given in Table 26. As in the two previous crosses the yellow green type was short of the calculated number. The  $F_1$  plants were all dark green and the  $F_2$  segregated dark green and yellow green. Of the 1675 plants recorded in the  $F_2$ , 1285 were dark green and 390 were yellow green. The deviation was 27 from the calculated 3:1 ratio. Progenies from seven selfed dark green  $F_2$  plants produced only dark green plants in the next generation.  $F_3$  progenies from 23 selfed dark green  $F_2$  plants gave dark green and yellow green. One thousand nine hundred forty-seven of these were dark green and 580 were yellow green, a deviation of 52 from the 3:1 ratio. The deviation in this case is significant being 3.52 times

its probable error.

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, 15 or 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 genetic 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 plants in the same 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 small and lacking in vigor were grown to maturity, hence it was not possible 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. Plates X and XI show photographs of progenies from crosses.

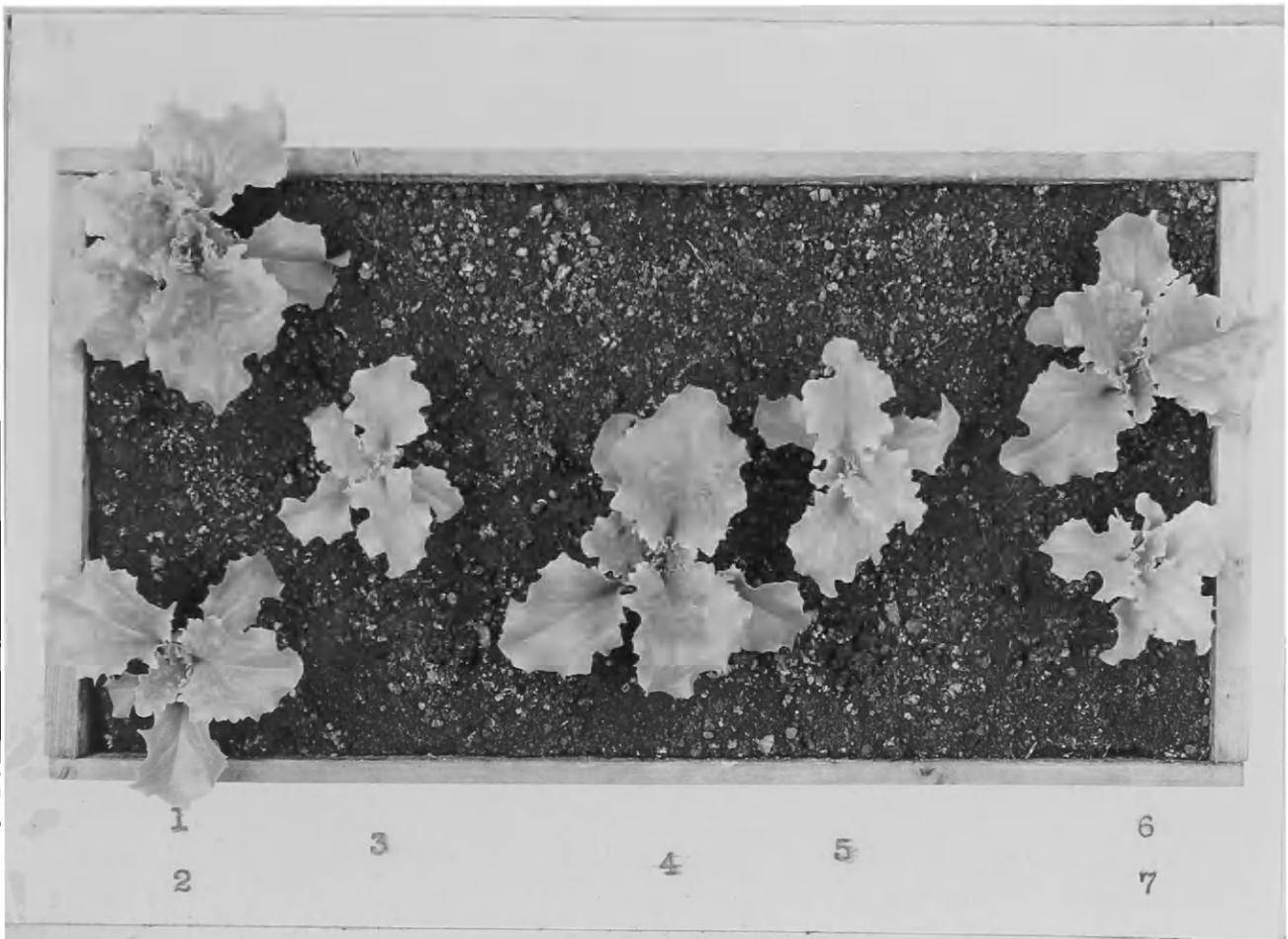
involving dark and yellow green parents. Plate X shows a flat of  $F_2$  plants from a cross of the dark green plant (N) with the yellow green tinged plant (I). Plants 1, 2, 4, and 6 were dark green and plants 3, 5 and 7 were yellow green. The dark green plants averaged more than twice the size of the lighter green type. Plate XI shows a group of  $F_2$  plants from a cross of the dark green red plant (M) with the yellow green plant (H). Plants 2, 5, 6 and 7 were dark green and plants 1, 3, 4 and 8 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

##### A Chlorophyll Character Exhibiting

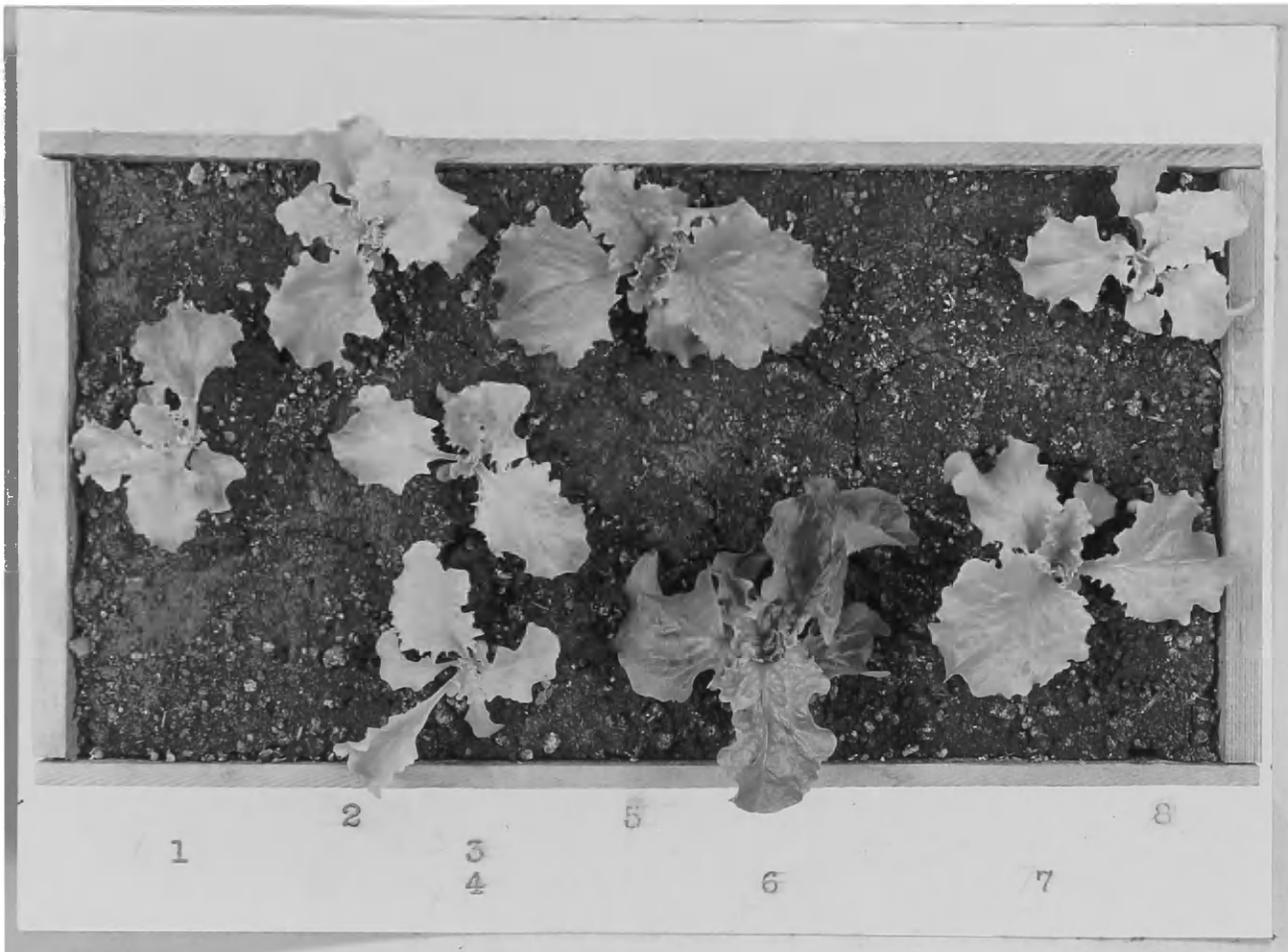
##### Non-Mendelian Inheritance

Among 52  $F_1$  plants grown in 1930 from a cross between the varieties Transport and New York, one plant was observed to have peculiarly blotched leaves (Plate XII). Portions of the leaves were entirely devoid of chlorophyll. The



**Plate X**

Showing the difference in size of dark and yellow green plants in the same  $F_2$  population. Plants 1, 2, 4 and 6 were dark green and plants 3, 5 and 7 were yellow green.



**Plate XI**

Showing size difference between dark and yellow green plants in an  $F_2$  population from a cross of a dark green, red plant of Mignonette and a yellow green, nonpigmented plant of Hanson. Plants 2, 5, 6 and 7 were dark green and plants 1, 3, 4, and 8 were yellow green.

extent of the whitish area varied in different leaves from 50 per cent or more to leaves that were entirely green. Seed was saved from this plant and a large F<sub>2</sub> population grown in 1931. The variation in color noted in the leaves of the F<sub>1</sub> plants was observed in the cotyledons of the F<sub>2</sub>. Some of the F<sub>2</sub> seedlings were pure albinos. These died within a few days after emergence. Many of the F<sub>2</sub> individuals were entirely green and appeared to be normal plants. A considerable proportion of the F<sub>2</sub> seedlings showed the blotching in the cotyledons characteristic of the leaves of the original F<sub>1</sub> 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 per cent of chlorophyll-deficient tissue died in the seedling stage. The vigor of different individuals appeared to be proportional to the extent of green tissues.

F<sub>2</sub> plants showing various amounts of white tissue were selected and grown to maturity. The seed of each was harvested separately and an F<sub>3</sub> population grown from each. The proportion of green, blotched and albino plants was different in each population as shown by the  $X^2$  and P values for the deviations from the average for the ten populations (Table 27). All but two of the ten distributions deviate



**Plate XII**

**A photograph of the original chlorophyll-deficient plant from which all of the material studied was obtained.**



**Plate XIII**

**A photograph of a plant lacking chlorophyll  
in a very large part of its leaf area.**



**Plate XIV**

**A photograph of a chlorophyll-deficient plant showing a type of deficiency in which the portion of the leaf on one side of the midrib is entirely albino and the other half of the leaf may be mottled or all green.**



**Plate XV**

**A photograph of an individual leaf from a chlorophyll-deficient plant showing in more detail the albino and mottled portions.**

significantly from the average (Table 27).

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 which were wholly green gave only green progenies. Nine hundred and twenty-six seedlings were grown from these five plants and all were entirely green.

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 was selected which bore nothing but green bracts, a second group bearing blotched bracts varying in the extent of chlorophyll deficient tissue and a third group was selected from portions of the plant which were very deficient in chlorophyll. Only heads which bore bracts entirely free of chlorophyll were included in this later group. The seed from each head was harvested and kept in separate envelopes. The seed from the individual heads were planted separately. The resulting progenies are given in Table 28.

Some of the variations in the pattern of the chlorophyll

Table 27. F<sub>3</sub> progenies from chlorophyll-deficient plant.

F <sub>2</sub> Plant:		F <sub>3</sub> Segregation				Total	χ <sup>2</sup>	P
Number	F <sub>2</sub> Pheno- type	Green	White	Blotched				
3	Green	251	0	0	251			
5	do	147	0	0	147			
8	do	176	0	0	176			
10	do	193	0	0	193			
12	do	159	0	0	159			
<b>Total</b>		<b>926</b>	<b>0</b>	<b>0</b>	<b>926</b>			
1	Blotched	137	15	53	205	10.405	.01	
2	do	136	10	61	207	31.355	.01	
4	do	103	31	54	188	4.391	.10	
6	do	155	43	47	245	7.042	.05	
7	do	167	5	23	195	122.06	.01	
9	do	148	11	75	234	37.00	.01	
11	do	161	27	34	222	17.67	.01	
13	do	112	75	99	286	64.34	.01	
14	do	185	10	41	236	60.22	.01	
15	do	101	58	79	238	39.01	.01	
<b>Total</b>		<b>1405</b>	<b>285</b>	<b>566</b>	<b>2256</b>			

Table 28. Progenies from flower heads having green, blotched and white bracts.

Flower head Number	Bract Color	Color of Progenies		
		Green	White	Blotched
G 1	Green	10	0	0
2	"	17	0	0
4	"	21	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
		<b>210</b>	<b>0</b>	<b>0</b>
W 2	White	0	9	0
3	"	00	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
14	"	0	7	0
15	"	0	7	0
17	"	0	11	0
19	"	0	9	0
20	"	0	6	0
23	"	0	13	0
		<b>0</b>	<b>133</b>	<b>0</b>
B 1	Blotched	9	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	"	9	5	4
14	"	15	2	1
19	"	17	0	3
23	"	13	3	4

deficient areas which occur in progenies from deficient plants are shown in Plates XIII, XIV, and XV. The plant shown in Plate XIII has near the maximum of deficient area in order for the plant to maintain itself to maturity. As plants approach the albino type the sooner they die from starvation due to low synthetic activities.

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 with pollen from heads bearing blotched bracts. Flower heads bearing only albino bracts were pollinated with pollen from heads having only green bracts and others with pollen from heads having blotched bracts. In order to make certain that the resulting seed were the result of cross-fertilization and no self-fertilized ovules, maternal parents were selected which were free of anthocyanin pigment and pollen was

Table 29. Data on effect of pollen on color of F<sub>1</sub> plants.

Mother: Number:	Color of Bracts:		F <sub>1</sub> Plant Color		
	Color of Head of Pollen	Color of Head of Maternal	Green	White	Blotched
W 3	Green	White	0	5	0
4	"	"	0	7	0
7	"	"	0	4	0
8	"	"	0	9	0
11	"	"	0	11	0
13	"	"	0	3	0
14	"	"	0	5	0
15	"	"	0	8	0
17	"	"	0	6	0
21	"	"	0	10	0
27	"	"	0	15	0
28	"	"	0	11	0
31	"	"	0	7	0
			0	101	0
W51	Blotched	White	0	2	0
52	"	"	0	3	0
55	"	"	0	4	0
57	"	"	0	7	0
58	"	"	0	5	0
59	"	"	0	3	0
61	"	"	0	8	0
63	"	"	0	11	0
64	"	"	0	5	0
65	"	"	0	7	0
			0	55	0
G 2	White	Green	13	0	0
3	"	"	5	0	0
4	"	"	4	0	0
5	"	"	7	0	0
7	"	"	17	0	0
8	"	"	11	0	0
9	"	"	15	0	0
11	"	"	3	0	0
13	"	"	10	0	0
14	"	"	8	0	0
17	"	"	129	0	0
20	"	"	2	0	0
21	"	"	9	0	0
			116	0	0
G43	Blotched	Green	11	0	0
44	"	"	3	0	0
47	"	"	9	0	0
48	"	"	7	0	0
53	"	"	11	0	0
54	"	"	9	0	0
57	"	"	10	0	0
58	"	"	6	0	0
60	"	"	8	0	0
61	"	"	7	0	0
62	"	"	13	0	0
			94	0	0

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 which carried anthocyanin were hybrids as all selfed flowers would produce seed which would be free of the factors for anthocyanin. The progenies which resulted from the various crosses are given in Table 29.

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 seedling 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 involucre.

Similar non-Mendelian chlorophyll-deficiencies have been observed in many different species. The numerous species exhibiting this type of inheritance of chlorophyll-deficiency and the investigators reporting them have been summarized by de Haan (8).

The term status albomaculatus has been applied by Correns (5) to this type of chlorophyll inheritance in

which variegation is inherited only through the variegated portions of the mother plant and without effect of the pollen.

Several theories have been advanced as possible explanation for the genetic behavior of this type of chlorophyll-deficiency.

Anderson (1), Baur (2), Chittenden (3), Clausen (4), Correns (5) and (6), Demerec (9), Gairdner and Haldane (11), Imai (12), Sturtevant (17) and Yasui (22) give various interpretations to the genetic behavior of albomaculata plants.

Sturtevant (17) attributes the variation in chlorophyll in albomaculata plants to lethal genes to which the genes for variegation are coupled.

Demerec (9) explains this type of non-Mendelian inheritance on the basis of gene mutation.

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

Correns (5) suggests that the plasma of the young cells in the variegated portions of the plant 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.

The lack of agreement among those who have studied

this particular type of inheritance indicates that much additional study must be made before this type of inheritance can be satisfactorily explained.

## INHERITANCE OF SEED COAT COLOR

### Color Types in Lettuce Seed

Three colors are generally recognized in the seed of lettuce. According to Jones (13), seed color in lettuce is due to pigment carried in the seed coat. Most of the cultivated varieties have either black or white seed. There are a few varieties however that 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 (10) 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<sub>2</sub> data presented from five crosses involving the above varieties indicated a single factor difference.

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 appear jet black while others have a brownish coat. 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 and white seeded varieties studied the black acted as a simple dominant over the white giving ratios of three black to one white in the F<sub>2</sub>.

#### Genetics of Seed Color

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

Of 228 individuals from 10 selfed F<sub>1</sub> plants from cross No. 67 (Table 30) between black seeded variety Transport and white seeded Unrivalled, 101 produced black and 27 white seed. 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<sub>3</sub> selfed white F<sub>2</sub> plants produced only white seeded progenies. The selfed black seeded F<sub>2</sub> plants segregated

Table 30. F<sub>2</sub> and F<sub>3</sub> data on seed coat color in progenies from cross No. 67 of white seeded Unrivalled (ww) x black seeded Transport (WW)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants				
F <sub>1</sub> Plant No.	F <sub>1</sub> Seed Color	F <sub>2</sub> Progenies		
		Black	White	
UT-1	Black	5	2	
UT-2	do	10	3	
UT-3	do	7	2	
UT-4	do	11	4	
UT-5	do	10	2	
UT-7	do	19	4	
UT-8	do	13	3	
UT-9	do	11	3	
UT-10	do	15	4	
Observed		101	27	
Calculated 3:1		96	32	
Deviation		5	5	
Dev.		1.52		
P. E.				

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants				
F <sub>2</sub> Plant No.	F <sub>2</sub> Seed Color	F <sub>3</sub> Progenies		
		Black	White	
UT-1-3	Black	13	0	
UT-1-6	do	6	0	
UT-2-4	do	7	0	
UT-2-5	do	21	0	
UT-4-2	do	13	0	
UT-4-3	do	14	1	
UT-4-6	do	9	0	
UT-5-7	do	11	0	
UT-7-1	do	5	0	
UT-7-6	do	10	0	
UT-7-7	do	15	1	
		124	2*	
UT-1-1	Black	31	7	
UT-1-2	do	24	7	
UT-2-2	do	16	5	
UT-2-3	do	9	2	
UT-2-6	do	11	5	
UT-3-1	do	8	3	
UT-3-3	do	15	4	
UT-5-2	do	17	7	
UT-5-6	do	25	9	
UT-5-8	do	24	6	
UT-7-2	do	29	8	
UT-7-3	do	17	3	
UT-7-5	do	13	5	
UT-8-1	do	11	4	
UT-8-3	do	17	8	
UT-8-6	do	13	2	
Observed		280	85	
Calculated 3:1		274	91	
Deviation		6	6	
Dev.		1.08		
P. E.				
UT-3-4	White	0	25	
UT-3-5	do	0	9	
UT-4-1	do	0	11	
UT-5-1	do	0	11	
UT-5-3	do	0	17	
UT-7-4	do	0	8	
UT-8-4	do	0	10	
UT-8-5	do	0	23	
		0	114	

\*Two white probably a contamination

in the  $F_3$  as true breeding black progenies and progenies consisting of three black to one 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 black seeded Grand Rapids (R) was crossed with the white seeded Iceberg (I) in cross No. 135 (Table 31). 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 and 58 white seed. This is only a deviation of three 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 three black to one white in the next generation.  $F_3$  progenies were grown from 13 white seeded  $F_2$  plants. All were white seeded except one plant from IR-7-2 which produced black seed. This one plant was disregarded as it is believed that it was an accidental mixture.

Color of seed was studied in the progenies from the cross No. 132 between the black seeded Mignonette (M) and the white seeded Iceberg (I) (Table 32). The  $F_1$  was black. One hundred sixty-eight plants were grown from seven selfed  $F_1$  plants. Of these 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 three black to one white. Two hundred fourteen were black and 74 were white, a deviation of two 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) are given in Table 33. Progenies from cross No. 39 between black seeded California Cream Butter (B) and white seeded Iceberg (I). (Table 34). And data in Table 35 from a cross No. 15 between black seeded Mignonette (M) and white seeded Hanson (H). 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 three black to one white. The one black seeded plant in the  $F_3$  from a self-pollinated white  $F_2$  plant MN-2 was disregarded as it was likely an accidental mixture.

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.

Table 31. F<sub>2</sub> and F<sub>3</sub> data on seed coat color in progenies from cross No. 135 of white seeded Iceberg (ww) x black seeded Grand Rapids (WW)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants				
F <sub>1</sub> Plant No.	F <sub>1</sub> Seed Color	F <sub>2</sub> Progenies		
		Black	White	
IR-1	Black	30	5	
IR-2	do	24	7	
IR-3	do	22	4	
IR-5	do	17	7	
IR-6	do	19	7	
IR-7	do	13	5	
IR-8	do	14	6	
IR-9	do	16	10	
IR-10	do	19	4	
IR-11	do	12	3	
Observed		186	58	
Calculated 3:1		183	61	
Deviation		3	3	
Dev.		0.65		
P. E.				

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants				
F <sub>2</sub> Plant No.	F <sub>2</sub> Plant Color	F <sub>3</sub> Progenies		
		Black	White	
IR-1-3	Black	8	0	
IR-1-6	do	17	0	
IR-2-2	do	7	0	
IR-2-3	do	30	0	
IR-2-7	do	19	0	
IR-3-5	do	23	0	
IR-5-1	do	14	0	
IR-6-2	do	5	0	
IR-6-3	do	13	0	
IR-7-1	do	7	0	
IR-9-3	do	4	0	
IR-10-2	do	8	0	
IR-11-2	do	11	0	
		139	0	
IR-1-4	Black	15	6	
IR-1-5	do	17	4	
IR-2-1	do	21	7	
IR-2-8	do	8	3	
IR-3-4	do	11	5	
IR-5-3	do	22	6	
IR-5-5	do	28	6	
IR-6-4	do	13	4	
IR-8-3	do	20	5	
IR-9-1	do	5	1	
IR-9-4	do	8	3	
IR-10-1	do	13	4	
IR-10-5	do	17	7	
IR-11-1	do	14	4	
IR-11-2	do	17	4	
IR-11-4	do	9	3	
Observed		238	72	
Calculated		233	77	
Deviation		5	5	
Dev.		0.90		
P. E.				
IR-1-1	White	0	5	
IR-1-2	do	0	11	
IR-2-5	do	0	7	
IR-2-6	do	0	14	
IR-3-1	do	0	3	
IR-3-6	do	0	11	
IR-3-7	do	0	5	
IR-5-2	do	0	9	
IR-5-7	do	0	21	
IR-7-2	do	1	15	
IR-7-4	do	0	29	
IR-9-2	do	0	7	
IR-10-4	do	0	12	
		1*	149	

\*1 Black seeded probably a contamination.

Table 32. F<sub>2</sub> and F<sub>3</sub> data on seed coat color in progenies from cross no. 132 of black seeded Mignonette (WW) x white seeded Iceberg (ww)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants				
F <sub>1</sub> Plant No.	F <sub>1</sub> Seed Color	F <sub>2</sub> Progenies		
		Black	:	White
MI-2	Black	26	:	10
MI-3	do	23	:	7
MI-5	do	18	:	4
MI-7	do	21	:	4
MI-8	do	12	:	7
MI-11	do	14	:	3
MI-13	do	15	:	4
Observed		129	:	39
Calculated 3:1		126	:	42
Deviation 3:1		3	:	3
Dev.		0.79		
P. E.			:	

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants				
F <sub>2</sub> Plant No.	F <sub>2</sub> Seed Color	F <sub>3</sub> Progenies		
		Black	:	White
MI-2-3	Black	11	:	0
MI-4-1	do	19	:	0
MI-4-3	do	13	:	0
MI-4-4	do	27	:	0
MI-5-3	do	17	:	0
MI-5-6	do	19	:	0
MI-8-1	do	9	:	0
MI-8-5	do	15	:	0
MI-8-6	do	26	:	0
		156	:	0
MI-2-1	Black	23	:	9
MI-2-4	do	16	:	7
MI-2-5	do	9	:	2
MI-4-2	do	11	:	5
MI-5-1	do	21	:	6
MI-5-2	do	19	:	7
MI-7-2	do	14	:	6
MI-7-3	do	13	:	2
MI-11-3	do	12	:	6
MI-11-4	do	18	:	4
MI-11-5	do	15	:	5
MI-13-2	do	20	:	8
MI-13-3	do	11	:	3
MI-13-5	do	12	:	4
Observed		214	:	74
Calculated		216	:	72
Deviation		2	:	2
Dev.		0.40		
P. E.			:	
MI-4-6	White	0	:	7
MI-5-4	do	0	:	13
MI-7-1	do	0	:	9
MI-8-2	do	0	:	23
MI-8-3	do	0	:	11
MI-11-1	do	0	:	19
MI-13-4	do	0	:	12
		0	:	94

Table 33. F<sub>2</sub> and F<sub>3</sub> data on seed coat in progenies from cross no. 1 of black seeded Mignonette (WW) x white seeded New York (ww)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants				
F <sub>1</sub> Plant No.	F <sub>1</sub> Seed Color	F <sub>2</sub> Progenies		
		Black	:	White
MN-2	Black	42	:	10
MN-4	do	29	:	7
MN-5	do	31	:	10
MN-7	do	17	:	5
MN-8	do	18	:	9
MN-9	do	14	:	5
MN-12	do	26	:	8
MN-13	do	32	:	9
Observed		209	:	63
Calculated		204	:	68
Deviation		5	:	5
Dev.		----- 1.0		
P. E.			:	

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants				
F <sub>2</sub> Plant No.	F <sub>2</sub> Seed Color	F <sub>3</sub> Progenies		
		Black	:	White
MN-2-1	Black	33	:	0
MN-4-3	do	21	:	0
MN-8-2	do	8	:	0
		62	:	0
MN-2-2	Black	5	:	3
MN-2-4	do	16	:	7
MN-2-5	do	32	:	8
MN-4-1	do	23	:	9
MN-5-4	do	11	:	3
MN-7-1	do	17	:	7
MN-7-2	do	11	:	5
MN-8-3	do	21	:	6
MN-8-5	do	15	:	7
MN-9-2	do	17	:	8
MN-12-1	do	12	:	5
MN-12-3	do	20	:	7
MN-12-4	do	17	:	7
Observed		217	:	82
Calculated 3:1		224	:	75
Deviation		7	:	7
Dev.		----- 1.37		
P. E.			:	
MN-2-1	White	0	:	19
MN-2-6	do	1	:	23
MN-4-2	do	0	:	13
MN-5-1	do	0	:	16
MN-5-2	do	0	:	7
MN-7-1	do	0	:	11
MN-8-1	do	0	:	21
MN-8-4	do	0	:	14
		1*	:	124

\*1 black seeded plant probably a contamination.

Table 34. F<sub>2</sub> and F<sub>3</sub> data on seed coat color in progenies from cross No. 39 of black seeded California Cream Butter (WW) x white seeded Iceberg (ww)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants				
F <sub>1</sub> Plant No.	F <sub>1</sub> Seed Color	F <sub>2</sub> Progenies		
		Black	White	
BI-1	Black	11	2	
BI-2	do	17	5	
BI-3	do	9	2	
BI-5	do	13	4	
BI-6	do	14	6	
BI-7	do	22	9	
BI-8	do	11	5	
BI-10	do	15	6	
Observed		112	40	
Calculated 3:1		114	38	
Deviation		2	2	
Dev.		0.55		
P. E.				

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants				
F <sub>2</sub> Plant No.	F <sub>2</sub> Seed Color	F <sub>2</sub> Progenies		
		Black	White	
BI-1-2	Black	13	0	
BI-2-4	do	19	0	
BI-5-2	do	22	0	
BI-7-3	do	16	0	
		70	0	
BI-1-3	Black	27	10	
BI-1-5	do	19	5	
BI-1-6	do	5	2	
BI-1-8	do	13	6	
BI-2-2	do	21	8	
BI-3-2	do	25	7	
BI-3-3	do	23	6	
BI-5-5	do	9	2	
BI-6-1	do	14	7	
BI-6-2	do	30	9	
BI-7-4	do	19	5	
BI-8-1	do	11	2	
BI-8-3	do	11	3	
BI-10-2	do	22	6	
Observed		249	78	
Calculated 3:1		245	82	
Deviation		4	4	
Dev.		0.76		
P. E.				
BI-1-1	White	0	15	
BI-1-4	do	0	19	
BI-1-7	do	0	27	
BI-2-3	do	0	17	
BI-3-5	do	0	31	
BI-5-4	do	0	22	
BI-7-1	do	0	13	
BI-10-4	do	0	10	
		0	154	

Table 35. F<sub>2</sub> and F<sub>3</sub> data on seed coat color in progenies from cross No. 15 of black seeded Mignonette (WW) x white seeded Hanson (ww)

F <sub>2</sub> Progenies from Selfed F <sub>1</sub> Plants				
F <sub>1</sub> Plant No.	F <sub>1</sub> Seed Color	F <sub>2</sub> Progenies		
		Black	White	
MH-2	Black	33	12	
MH-5	do	23	6	
MH-6	do	17	7	
MH-7	do	26	8	
MH-12	do	31	6	
MH-14	do	19	4	
Observed		149	43	
Calculated 3:1		144	48	
Deviation		5	5	
Dev.		1.23		
P. E.				

F <sub>3</sub> Progenies from Selfed F <sub>2</sub> Plants				
F <sub>2</sub> Plant No.	F <sub>2</sub> Seed Color	F <sub>3</sub> Progenies		
		Black	White	
MH-2-2	Black	17	0	
MH-2-7	do	23	0	
MH-5-1	do	11	0	
MH-7-2	do	15	0	
MH-7-3	do	9	0	
MH-12-4	do	27	0	
MH-12-6	do	19	0	
MH-14-1	do	21	0	
		142	0	
MH-2-1	Black	11	5	
MH-2-3	do	9	4	
MH-2-5	do	15	4	
MH-5-3	do	23	10	
MH-5-5	do	12	3	
MH-5-6	do	8	3	
MH-6-1	do	22	6	
MH-6-2	do	17	5	
MH-7-1	do	21	7	
MH-7-4	do	14	5	
MH-7-5	do	19	7	
MH-12-2	do	12	7	
Observed		183	66	
Calculated		187	62	
Deviation		4	4	
Dev.		0.87		
P. E.				
MH-2-4	Black	0	13	
MH-5-3	do	0	28	
MH-6-5	do	0	19	
MH-12-1	do	0	31	
MH-12-3	do	0	15	
		0	106	

Crosses were made between black seeded and yellow seeded and between white 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 nine 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 ( $Cc$ ) were found to have a linkage value of .64.

The dark green chlorophyll color in lettuce leaves was found to act as a single Mendelian factor dominant to the gene for the yellow green.

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

Black seed was found to behave as a single factor dominant to the gene for white seed.

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