INHERITANCE OF

SEED COLOUR IN ALFALFA

"INHERITANCE OF SEED COLOUR IN ALFALFA"

by

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Inheritance of Seed Color in Alfalfa.

This paper presents the results of an investigation to determine the inheritance of black and white seed coat colors in alfalfa. The "black" character appeared to have arisen as a mutation in a third generation selfed line. Black x Yellow hybrid progenies in F_2 exhibited a wide range of seed coat color extending from normal yellow to dark mulatto. Inheritance proved to be fairly complex requiring the assumption of at least three factors. One gene, primarily responsible for pigmentation of the seed coat, and at least two modifying factors, were postulated as the most probable genetic explanation of the breeding behaviour. It was concluded that the character of black-seededness would be valueless from a utility standpoint.

Inheritance of the white seed coat character appeared to depend on a single factor difference, the white seeded parent being homozygous for a recessive factor which results in the absence of yellow pigment.

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INHERITANCE OF SEED COLOR IN ALFALFA.

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The extent to which normal well matured alfalfa seed may vary in color is not generally recognized. Close examination of a bulk sample or, better still, the seed from a population of individual plants, will show that while the dominant color is a bright greenish yellow, the density of color extends through a range of from very light yellow to orange or light brown. Of interest in this connection are certain exceptional plants which produced true black and true white seed. When the former were crossed with yellow seeded plants they gave F_1 hybrids which produced seeds of various shades of mulatto. It seemed worth while to investigate as far as possible the inheritance of the black and white seeded characters and to determine whether it might be feasible to use the former as a marker for identifying improved strains.

REVIEW OF LITERATURE.

The literature dealing with seed color studies in general is not very extensive and this is especially true with reference to leguminous plants. Seed color in several species of Leguminosae, however, has been investigated and there is considerable literature on seed color in some of the grain crops. Without attempting to cover all of the original papers, a brief review will be made of the more important inheritance studies relating to seed color in crop plants.

Nilsson - Ehle (10) reported the results which he obtained from crosses between red and white wheats. Ratios of 15:1 and 63:1 were obtained in F_2 families. In a cross between two red seeded varieties he obtained 15 red to 1 white. This result was explained on the basis of two independently inherited dominant factors for pigment, either of which could produce the red color. It was found also that the effect was cumulative, the shade of red being intensified when both factor pairs were present or when one or both occurred in the homozygous condition.

Harrington (6) concluded that Kitchener wheat contained two independently inherited factors for grain color, while some other varieties contained but one.

Summarizing the results of seed color studies with oats, Hayes and Garber (7) state, and quote other workers to the effect, that the color of the lemma of oats has been classified as black, brownish-red, gray, yellow and white. Different varieties exhibit different intensities in the development of a particular color. In some crosses between black and white a ratio of 15 blacks: 1 white was obtained in F_2,while the majority of crosses showed 3:1 ratios. The simplest explanation is that each color character is due to one or more factors, each factor when heterozygous causing partial or complete development of the charactor.

In maize, East and Hayes (2) and Emerson (3) found that

four factors,

C R A Pr produce the full purple-colored aleurone layer in the seed. The progeny of a plant heterozygous for three factors, C R A, which gives red seed, segregates in a ratio of 27 colored to 37 colorless, indicating that these three factors are complementary for color. The factor Pr, which converts the red color into purple, may suppress the characteristic expression of these non-allelomorphic factors. One factor may modify without completely suppressing the expression of another factor. Fraser (4) found that the intensity of color depended on a factor "In" the presence of which lightened the shade of color produced by the factors C R A Pr or C R A pr.

Conner and Karper (1) found the inheritance of seed color in sorghums in some cases to be monofactorial in character. In other cases they found intermediate forms giving distinct 1:2:1 ratios. Sieglinger (15) showed that in certain sorghum crosses the factors concerned in the inheritance of seed color included B, a factor for brown nucellar layer, which produces brown color in the epidermis in the presence of S; b, its allelomorph; S, a factor which causes the development of color in the epidermis of the seed; s, its allelomorph. R, a factor for dark red which causes the development of red color in the epidermis and r, its allelomorph.

The comparatively recent work on inheritance in soybeans by Woodworth, Owen, Stewart, and other investigators has been summarized by Woodworth (17). The efforts of these workers have shown that in the seed color inheritance of soybeans many factors are involved. The most important of these constitute two multiple allelomorphic series, one conditioning the expression of black and brown pigments in the seed coat and the other series acting as inhibiting factors.

Hallquist (5) postulates the following genetical formulae for *Lupinus angustifolium*:- R, a basic factor for pure red flower color and rust brown seed color, the double recessive having white flowers and white seeds. B, a factor, which together with R, gives bluish red flowers and earth brown seeds. In the presence of R, a factor V transforms the pure red color into violet. V does not influence the seed color.

The factors B and V give the full blue color when both are present. In the absence of R, no coloration is attained by any of them. Thus the formula R B V is required for blue color. Another factor F is necessary for the complete development of color. The color produced by the other factors becomes diluted when F is absent.

In *Vicia Faba*, Sirks (16) found that seed color depends primarily on a factor O. In the absence of O, whitish gray seeds are produced which are converted by Y into yellow white. O with P gives yellow, and with p purple seeds. Another factor Sc in the presence of O produces black seeds, and a factor M, causing mottling of seeds, is linked with O.

In studies of Indian Oil seeds, Shaw, et al (14) found a mode of inheritance of seed color which showed that color in the seed-coat was dependent on four separate factors, namely, D, M, G and X, one of which determines flower color as well. M, in the presence of D, converts the fundamental yellow color into fawn, and, if G is also present, fawn is changed into brown. If, however, D is absent, M has no effect and the fundamental yellow color is retained. Yellow is changed to grey by the addition of a factor G, and grey becomes brown when D is added. The factor X acts only as an intensifying factor converting yellow into dark yellow and fawn into dark fawn.

Tammes (17) and Kappert (8) found that color of seed in flax was due to three factors.

In Eleusine coracana, Rangaswamy, et al (12) found that two factors, designated B_1 and B_2, act singly or together to produce the brown pigment of the "ragi" grain. In the absence of both factors the grain is white. A factor D, that deepens the effect of the B factors, behaves as a simple dominant.

Rangaswamy and Narayanan (13) found that the six colors met with in grain of Setaria italica, were due to the interaction of three factors K, I and B.

MATERIAL AND METHODS.

Black Seeded Parent and Progeny.

The black seeded plant was discovered in a third generation selfed line of Grimm alfalfa. It was a good seed producer and appeared to be well developed although somewhat reduced in vigor, but not more so than other plants in the same progeny. Both selfed seed and open fertilized seed was obtained from this original black plant. It is believed that other plants in the same line were yellow seeded but definite information is lacking on this point.

In the next generation, 20 L_4 plants were produced from selfed seed and 20 plants also from open-fertilized seed taken from the original parent. The former were dwarf, being greatly reduced in vigor and the seed of all was either black or dark mulatto, except one which produced yellow seed. The progeny from open fertilized seed on the other hand was composed of plants so exceptionally vigorous that no doubt existed as to their hybrid origin. It was definitely known that the female parent was black seeded, while the male parents were presumed to be yellow, an altogether likely assumption. The seed produced by these plants exhibited various shades of mulatto but none of them were as black as the original parent or as light in color as the average for common alfalfa. All of the seed from each plant was remarkably uniform in color. The material available to the author at the beginning of this study consisted of selfed seed from 19 F_1 plants, 11 of the L_4

plants and open fertilized seed from the white seeded plant.

One of the most serious difficulties encountered in this study was the almost complete self-sterility of the L_4 blackseeded plants. Self-sterility was a characteristic of all plants in this selfed line, and it was more or less evident in some of the hybrid progeny when black seeded individuals were used as the female parent. Lack of fertility was evidently associated with loss of vigor due to inbreeding and there were indications that it may have been linked with factors which are responsible for pigment in the seed.

A study of the floral parts of these L_4 plants did not reveal anything out of the ordinary except a marked constriction of the pistil in the central region. Examination of numerous flowers of common alfalfa failed to disclose a similar constriction. It is not probable that this peculiarity affected fertilization, since F_1 seed was obtained with the L_4 plants as female parents, indicating that the ovules were functional. An examination of pollen from plants of this line showed that there was a considerable percentage of sub-normal grains and that the starch content of functional grains was less than normal, yet the amount of good pollen seemed ample to effect fertilization. Furthermore, the successful germination of these pollen grains on an articial medium and the fact that pollen from the L_4 plants was effective in fertilizing emasculated flowers of ordinary plants, indicates that a considerable proportion of the pollen was viable.

The root-tips of these plants were examined cytologically and in all cases the normal number of chromosomes ($2n = 32$) were observed, nor was there any evidence of chromosome abnormalities.

Since, as has been shown, the L_4 plants produced pollen grains and ovules which were functional and since there was no evidence of chromosome aberrations, it is evident that a case of self incompatability obtains in this line. It is also evident that whatever is the immediate cause of this self-incompatability, it may be regarded as one of the effects of continuous inbreeding.

Diagram I shows the various lots of breeding material which were grown and studied. It will be observed that seed was secured from Black x Yellow, Black x White, and White x Yellow hybrids. This, in addition to the F_2 families resulting from natural hybrids between the original Black parent and normal yellow seeded plants, provided the most important data which were obtained on the inheritance of seed color.

Most of the studies which have been made on inheritance have presented a problem in classification and the present one was no exception. It was soon apparent that we were dealing with a wide range of color variability due on the one hand to blending inheritance and on the other to pronounced differences in shades of color as a result of environmental conditions.

In view of the various shades of mulatto exhibited by F_1 natural crosses from near yellow to dark mulatto and

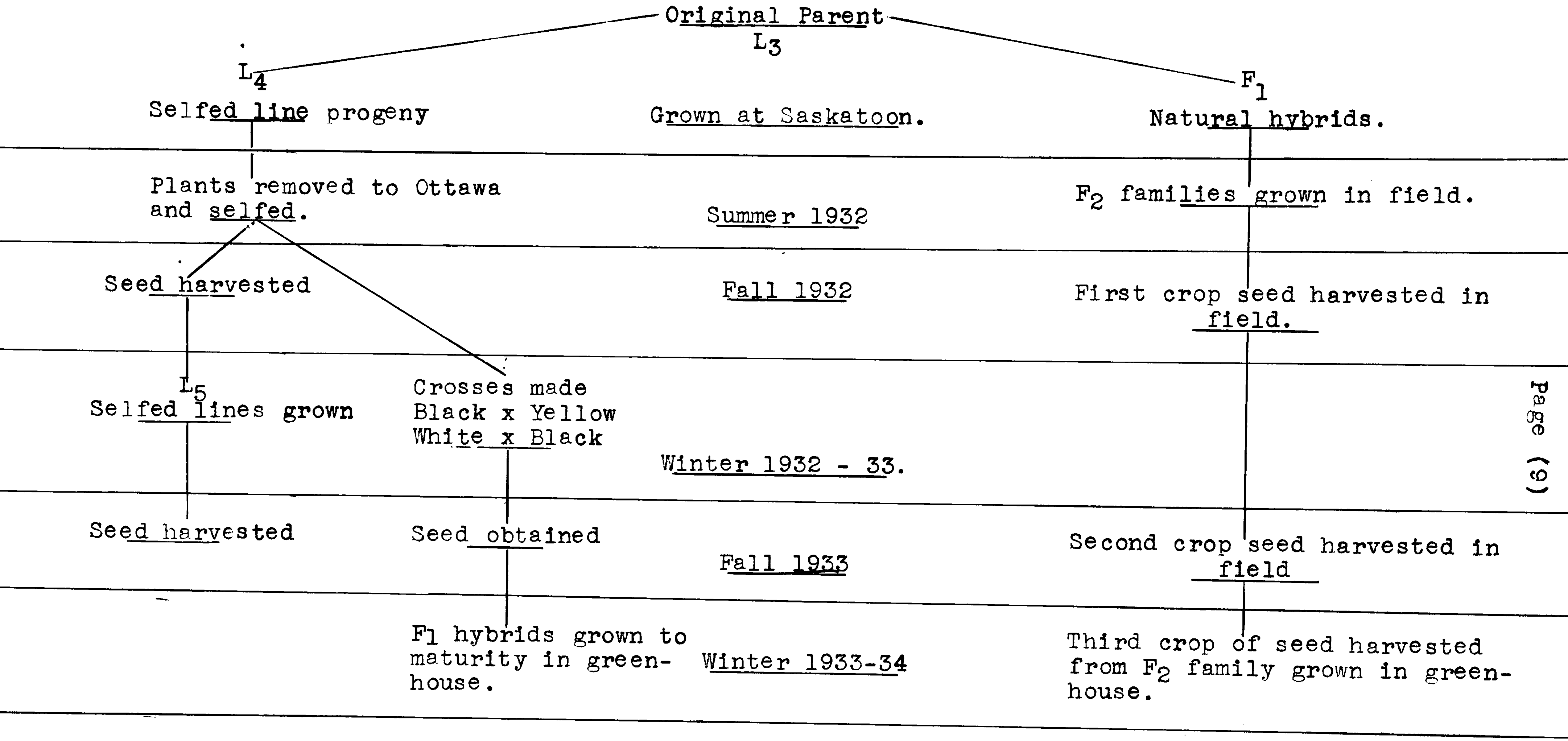

Diagram I. - Outline of experimental procedure showing the main lines of descent in the inheritance study of seed color in alfalfa.

in view of the wide range of variability due to segregation in the F_2 families produced from them (See Table 2), and furthermore the various differences in shades of color observed in a good sample of ordinary alfalfa seed, it was decided that a consistent analysis of the breeding material could be secured only by setting up an arbitrary scale of color values. Accordingly, seed samples were selected which could be used as type samples covering the entire range of color from light yellow on the one hand to dense black on the other. This resulted in a number of samples which were placed in series in as many compartments under transparent celluloid. The greatest degree of difference between any two adjacent samples was only sufficient to be recognized.

The procedure in classifying the individual plants consisted in placing the seed samples harvested from them in a sliding compartment by means of which it could be moved back and forth along the series of type samples until it was properly matched and its classification determined. It then received a number corresponding to the number of the type sample. Altogether 18 type samples were obtained, these being assigned numbers from 1 to 18, indicating the range in color from very light yellow to black. (See Plate I).

Very light yellow seed samples occurred quite frequently which appeared to be at least one distinct shade lighter in color than a bright, well matured sample of ordinary alfalfa. The so-called black seed was not a coal black but rather what might be described more correctly as a deep purplish black. The casual observer, however, would designate the color as black. The pigment was definitely located in the seed coat. Between yellow and black, as previously stated, were various shades of mulatto which graded into each other insensibly. That is to say, there were no distinct lines of demarcation which could be recognized.

To insure accuracy of classification all seed samples were examined twice by daylight and then under an electric fixture, which provided a uniform source of illumination. Finally all samples having the same color number were examined together and compared with one another. Any samples deviating significantly from the type sample of the group were reconsidered with respect to classification. When samples were available from the same plant representing seed produced by it in more than one season or in the greenhouse, the darkest color was considered to be closest to the correct classification for that particular plant.

The lack of color differences, sufficiently definite to form distinct color classes was the chief justification for establishing as large a series of color types as possible. It was recognized that these color classes should not be expected to correspond to as many phenotypes but it was thought that many rather than few sub-divisions of the color range would make for greater accuracy of classification. This was undoubtedly the case. Moreover, the procedure which was adopted permitted of any system of grouping found to be most appropriate in the light of the data as a whole.

In the first place, consideration had to be given to the degree of variability in color and its probable cause as between seed on individual plants matured in a single season, as between plants grown in the same season, and also as between the same plants grown in different seasons or in the greenhouse.

Seed color of F_1 natural hybrids in 1931 exhibited wide differences as between plants, a fact that will merit further discussion, but examination of these showed remarkable uniformity within each sample. These plants matured under ideal weather conditions in Western Canada. Uniformity was less striking in single plant samples harvested at Ottawa in 1932 and 1933. There remained the question, therefore, as to whether or not the density of color might not be influenced to some extent by weather conditions which preceded maturity of the seed. There was also the possibility that seed color might become intensified between the time that the pods first appeared to be ripe and the time that the seed was harvested. This is a matter of some importance since pods taken from plants in the field will not all have matured at the same time. An attempt was made to settle this point by growing a number of plants in the greenhouse under controlled temperature and illumination. Each pod was labelled when it first appeared to be ripe and seed samples having the same date labels were harvested thereafter at intervals of two or three days. Comparison of these samples of seed demonstrated that the color had reached its full expression when the pods had turned brown and that it did not change materially after that time. Seed produced on single plants in the field, however, matures over a considerable period of time during which atmospheric conditions may be anything but uniform. There is the probability therefore that atmospheric conditions in the field may be responsible for more or less variability in seed color produced by individual plants matured at different dates in the same season.

It was equally difficult to determine the degree to which color intensity may vary as between plants of the same genetic constitution grown in the same season, for the reason that environmental effects on seed color could not be divorced from genetic effects. It would seem that the influence of climatic conditions in these cases should not be much greater than in the case of seed from pods taken from the same plants at different times as they reach maturity. What evidence there was indicated that such effects were not very marked but probably sufficient to increase very materially the difficulty of making an accurate genetic classification of the material.

The greatest degree of variation in color was found to occur between seed samples taken from the same plants in different seasons. Seed was harvested in both 1932 and 1933 from the same plants and in the case of one large F_2 family they were then taken from the field and propagated in pots in the greenhouse with artificial illumination during the winter of 1933-1934. The seed produced under all three environmental conditions by each plant was then classified according to the arbitrary scale of color values and each was assigned the number corresponding to the type sample which it resembled most closely. Of 102 plants tested in this way, the seed samples from 18 plants were classified under a single color type, 47 under two, 25 under three, while 12 plants varied in color by as many as four shades in the color series.

White Seeded Parent and Progeny.

The white seeded plant was found in the alfalfa breeding nursery at the University of Saskatchewan. It was one among many plants of the Grimm variety which were being harvested individually for seed. This plant had white flowers and the seed was wholly lacking in yellow pigment. Lack of color in both flowers and seeds indicated that in all probability the plant was homozygous recessive with respect to pigmentation. Open-fertilized seed was secured but the plant itself has not been available for this study.

In the spring of 1932, a progeny of 80 seedlings were grown in the greenhouse at Ottawa from this white alfalfa seed. These were transplanted into the field early in the spring. The plants flowered and produced seed the first season. Among these were found two white flowered and white seeded plants.

It was assumed that these came from selfed seed and that the remaining 78 plants represented F_1 hybrids, the result of natural crossing between the original white seeded plant and normal yellow seeded individuals. All of the plants with colored flowers produced yellow seed, a fact which argues for the theory that the original parent was a double recessive.

In the fall of the year the two white-flowered and white seeded plants grown at Ottawa in 1932 were transplanted into pots and removed from the field into the greenhouse. One of these (W.S.1) proved to be completely male sterile. Examination of the pollen showed that it was non-functional. The other plant (W.S.2) gave a few selfed seed from which eight plants were obtained, all of which produced colorless flowers and seeds, a further indication of the homozygous recessive character of the colorless condition.

Although W.S.1 was male sterile it produced seed readily when fertilized with the pollen from other plants, and because it was unnecessary to emasculate the flowers, this plant was used,rather than W.S.2, in most of the crosses with yellow seeded and black seeded plants as male parents.

In 1932 a large number of the plants were selfed which had been grown from open-fertilized seed taken from the original white seeded parent. It was assumed, with considerable justification, that those which had produced colored flowers and seeds were White x Yellow natural F_1 hybrids. In the spring of 1933 about 900 seedlings comprising 10 L_2 families were transplanted into the breeding nursery. Under normal conditions this material would have flowered and produced some seed the first season, thus providing definite information on the genetics of the white seeded character. Unfortunately this alfalfa nursery was severely attacked by leaf hoppers. Although the plants were saved by repeated treatments with insecticide, the growth was retarded to such an extent that the plants failed to set seed and a large proportion of them failed to bloom. Of those which blossomed a considerable number had white flowers but it was impossible to secure reliable ratios.

BREEDING BEHAVIOUR.

Diagram 2 shows the results obtained in two successive generations from selfing the parent plant. Figures in brackets indicate the color type. In the first generation from selfed seed (L_4) 20 plants were obtained of which 11 produced seed. Ten of these varied in color from 14 to 18 and one produced yellow seed. This is evidence for the heterozygous character of the parent.

Repeated attempts were made to obtain selfed seed from the L_4 plants both in the field and in the greenhouse. In all, approximately 8000 flowers were tripped artificially. Seed was obtained from only four plants and the resulting progenies contained a total of only 47 individuals. The color classification of seed produced by the L_5 plants in relation to the L_4 progeny and the original parent is shown in Table I.

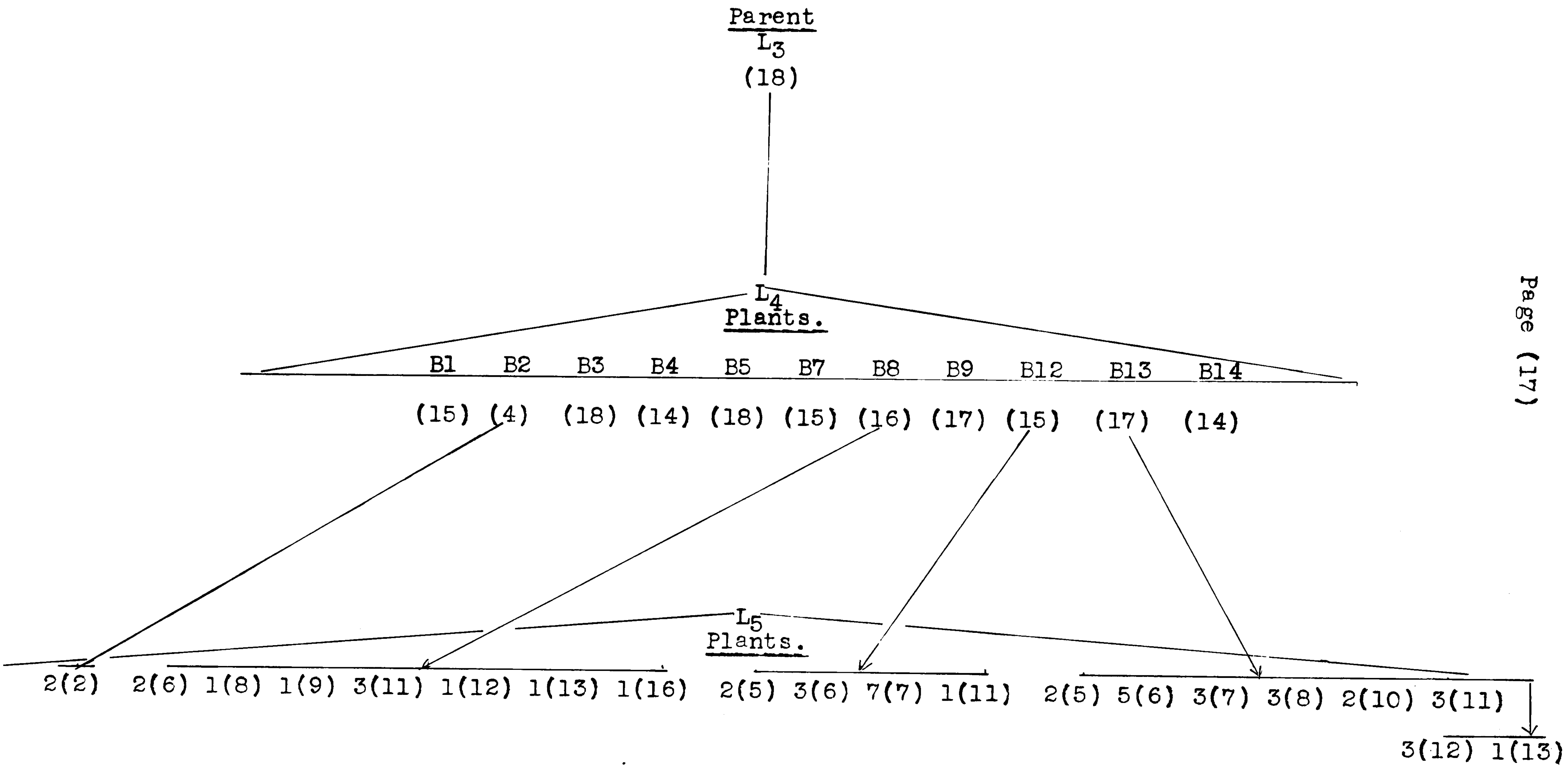

Diagram 2: - Distribution with respect to seed color of fourth and fifth generation inbred progenies from the original black-seeded parent plant. (Seed color classes in brackets).

TABLE I. - Classification of the original L_3 black seeded parent and the fourth and fifth generation selfed lines, according to type samples.

Designation of plants.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
	Seed Color Classes																		
L_3																		1	1
L_4				1										2	3	1	2	2	11
L_5 (B2)		2																	2
L_5 (B8)						2		1	1		3	1	1			1			10
L_5 (B12)					2	3	7				1								13
L_5 (B13)					2	5	3	3		2	3	3	1						22

With reference to Table I it is worthy of note that selfed seed was easily obtained from the original L_3 parent while the L_4 plants were almost or completely self-sterile. The difference in this respect can be accounted for by the fact that the L_3 plant was reasonably vigorous and well developed, whereas all of the L_4 plants were much reduced in size and decidedly lacking in vigor of growth. Kirk (9) has shown that inbreeding has a very pronounced effect on seed production, so much so that the majority of selfed lines produce very little seed in the fourth generation and many of them are completely sterile. He has shown also that pronounced loss of vigor may take place in any generation. In this case it occurred in the fourth.

In view of the high degree of self-sterility exhibited by the L_4 plants, it seems altogether probable that the 47 plants reported in L_5 may have resulted from accidental fertilization with foreign pollen. Their position in the color series (see Table I) strongly suggests that they are F_1 Black x Yellow hybrids rather than fifth generation selfed lines. This number of accidental hybrids from some 8000 flowers which were tripped might occur even with the best of technique. At any rate it is safer to disregard these plants when considering the data.

One other feature of Table I that is of interest, is the occurrence of the single yellow seeded plant classified under 4. There is little doubt that this individual properly belongs in the L_4 selfed line since it was dwarf and otherwise similar to the other plants in appearance. As will be seen, it is not so difficult to account for this plant as for the absence of individuals in the color series from 5 to 13 inclusive. The smallness of the population, however, must be taken into consideration.

Table 2 shows the distribution of F_2 plants in relation to the color series. These families are the progenies of F_1 hybrids obtained from crosses between the original black seeded parent and normal yellow seeded plants. It is important to note that the male parents are not necessarily the same for any two families.

TABLE 2 - Classification of F_2 families, according to type samples.

F_2 families	Seed Color Classes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
1		1		2	2	1	2		4	4	$\underline{4}$	2							22
2	1	8		13	7	$\underline{6}$	6	9	12	3	4	1							70
3		1		4	$\underline{13}$	15	12	9	16		1								71
4			1			1	$\underline{2}$		2	2									8
5	2	1	1	16	6	$\underline{1}$	12	3	11	4									57
6				3		3	$\underline{2}$	7	8	4		1							28
7	16	4	3	12	9	22	27	$\underline{5}$	43	47	17	5	4	2					216
8	4		1	6		3	4	3	$\underline{23}$	16	5	3							68
9	1			2		10	8	12	$\underline{15}$	11	3	5	1			1			69
10			1	4	1	1	3	3	3	2	3	$\underline{8}$	6	3	2	2	3	4	49
11				1	1	1	2	1	1	1	4	$\underline{2}$	1	3	1				19
12	10	2		7	6	4	19	$\underline{8}$	20	6	2	2	2		1				89
13	4	1			1	7	8	4	10	$\underline{8}$	8	7	7	5	2	2		3	77
14	5	2	1	2	2	4	12	3	$\underline{12}$	11	3	2	2						61
15	3		4	19	12	25	47	17	127	$\underline{117}$	61	49	13	2	1	1			498
16	1		1	5	2	9	$\underline{16}$	5	9	5	2	1							56
17			2	2	1	$\underline{14}$	30	3	4	2	1								59
18				1	2	3	5		$\underline{3}$	2	4		1		1				22
19	5	1			2	16	17	$\underline{4}$	10	4	1	1							61
																			1598

Note: - The heavy underline indicates the color classification of the F_1 parent of each family.

A heavy underline indicates the color classification of the F_1 parent of each family. The F_1 hybrids obviously must have been essentially different with respect to genetic constitution in order to account for the pronounced dissimilarity of F_2 progenies in the distribution of individuals. These peculiarities of family behaviour in breeding must be attributed to the heterozygosity of the female parent, and probably also to differences in the genetic constitution of the male parents.

An interesting and very significant feature of Table 2 is the deficiency of black and dark mulatto seeded plants. Only two families produced black seed and only 24 plants out of approximately 1600 are classified in the four darkest groups. Even in the color range above 12, at which point there appeared to be a transition from medium to dark mulatto, there are only 77 plants. This fact strongly suggests that most of the plants in the range of darkest seed color have been eliminated by a factor or combination of factors which produce gametic or zygotic lethal effects.

Table 3 shows the distribution of F_1 hybrid plants with respect to seed color from Black x Yellow crosses. The first horizontal row contains the F_1 hybrids obtained by fertilizing the original black seeded plant with pollen from yellow seeded parents, while B_1, B_2 --- B_{14} are the L_4 plants shown in Table 1. As might be expected, many of the hybrids are located in the intermediate color classes from 9 to 12 inclusive, indicating that there is a blending of color inheritance, but on the other hand it is difficult to account for the disproportionate numbers which are classified as 5, since this color class corresponds closely to what may be regarded as normal yellow. The distribution resulting from L_3 Black x Yellow F_1 plants approaches much more closely to what might be expected from Mendelian segregation than does that of any of the L_4 Black x Yellow hybrid groups.

TABLE 3. - Classification of F_1 hybrids from Black x Yellow crosses according to type samples.

	Seed Color Classes																		
Crosses	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
L_3 Pl x Y					1	3	3	3	4	2	1	2							19
L_4 BI x Y				4		5	1	4	3	1	4	1							23
L_4 B2 x Y		1			12	6													19
L_4 B4 x Y					3	1	2		1										7
L_4 B5 x Y					3	2	2		3										10
L_4 B8 x Y					6	3		3	7										19
L_4 B9 x Y					1			4	10	2	4								21
L_4 B12 x Y					18		2		4		2	3							29
L_4 B13 x Y					3		3	1	2		10	3	1	1					24
L_4 B14 x Y								1	2		1	3							7

Table 4 gives the distribution of F_1 hybrids from crosses between the white seeded plants previously referred to (W.S.1 and W.S.2) and the same black seeded L_4 plants which were used as female parents in Black x Yellow crosses (see Table 3). The crosses listed in Table 4, however, had the white seeded plants as female parents. Reciprocal crosses were not possible since the latter were male sterile.

The distribution of F_1 individuals in Table 4 is similar to that in Table 3, except that in the former a large group of plants occur in the color series under 1 and 2, whereas they are wholly absent in Table 3. These color types correspond to a very light shade of yellow which is not commonly found in a well matured sample of alfalfa. Since there is little doubt that the female parents were homozygous recessives with respect to pigmented seeds, it is reasonable to assume that those plants which fall in groups 1 and 2 are heterozygous for the factor or factors which condition the normal yellow seed color. The sharp distinction between light yellow, corresponding to types 1 and 2 in the color range, and typical yellow corresponding to types 4 and 5 is very conspicuous.

In Table 4, as in Table 3, the complete absence of black and the darker shades of mulatto in the color series from 13 to 18 inclusive, as well as the decided tendency toward skewed distributions toward the yellow end of the series, are considerations worthy of note. It would appear also that in Table 4 the largest frequencies from White x Black crosses occur in color types 9, 10 and 11, while those in Table 3 from Black x Yellow crosses are found under color number 5, which is yellow.

There is one other observation in Table 4 that baffles explanation on any theory of Mendelian inheritance. This is the fact that W.S.$_1$ x B$_2$ gave two F$_1$ hybrids which were classified under 11 in the color series. B$_2$ is the yellow seeded L$_4$ segregate from the original black seeded plant, and W.S.1 lacks both the black and yellow pigment. It is difficult to understand how this union could possibly result in offspring with mulatto colored seed coats. It is instructive to compare the distribution of F$_1$ plants from this cross with that of the B$_2$ x Yellow cross in Table 3, since the same L$_4$ yellow seeded segregate was used in both.

TABLE 4. - Classification of F$_1$ hybrids from White x Black crosses according to type samples.

Crosses	Seed Color Classes																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
WS1xL$_4$B$_2$	12	1				2	1				2								18
WS1xL$_4$B$_4$	7	3			1		3		1	6	6								27
WS1xL$_4$B$_5$					1	2		2		1	4								10
WS1xL$_4$B$_8$	1	3			4			3	9	1									21
WS2xL$_4$B$_8$	3			2	1		1		4										11
WS1xL$_4$B$_9$	7	2		2	3	1		3	4	16	13	3							54
WS1xL$_4$B12	8	3		3	4	1	6	2	4	5	10	4							50
WS1xL$_4$B13	2				1	1	3	1	3	7	4								22
WS2xL$_4$B13					3						2								5

DISCUSSION OF RESULTS.

There are two ways in which the original black seeded plant may have originated. Black-seededness may have appeared as a result of repeated selfing since it was found in a third generation selfed line; or it may have arisen as a mutation in the parent plant one generation before. Under the first assumption it is necessary to postulate that the black color was carried along in the heterozygous condition and that it found expression only when the factors which conditioned this character had reached homozygosity. It is apparent from the data that the original plant did not breed true for the character in question.

If black-seededness appeared as the result of mutation it is unlikely that the mutation occurred at more than one locus. It is reasonable to expect, therefore, that the plant would be heterozygous for the factor responsible for this character. This was found to be the case, which argues for the theory of origin by mutation. It presupposes that black-seededness was due primarily to a single factor difference.

It is obvious from the distribution of plants in F_2 families that if a monofactorial mutation was responsible for the appearance of black or dark purple pigmentation in the seed of the original parent plant, this change in the genetic constitution does not provide an adequate explanation of the type of segregation which was obtained. The distribution of individuals is such as to suggest a fairly complex type of inheritance requiring the assumption of two or more modifying factors to account for the variability in seed color. Thus at least three genetic factors would be involved, and quite possibly more, in the inheritance of black-seededness. In addition there is certainly at least one factor for yellow in the homozygous condition which is responsible for the yellow pigment of normal alfalfa seed. The absence of this factor or factors results in white seed.

According to this analysis of seed color inheritance the factorial constitution of the original black seeded parent may be written YY C_1c_1 C_2c_2 Bb where the genes are represented by Y for yellow pigment, B for black pigment, and C_1 C_2 for factors which modify the expression of the black color. It is necessary to assume heterozygosity for the three factors responsible for black and mulatto because the presence of factors in the homozygous condition would not alter the picture. The number of modifying factors, however, could be postulated as three instead of two with equal justification.

It is not possible with the available data to arrive at a genetic formula which is entirely acceptable. The single factor mutation for black seems to be a logical assumption. It is not unreasonable to expect that modifying factors should affect the expression of this character. A seed sample of ordinary alfalfa exhibits a wide range of color variation which in all probability has a genetic basis. In most of the seed color studies with leguminous plants, modifying factors have been found to play a prominent part. On the other hand, while the three factor hypothesis fits the facts reasonably well there are certain features of the data which are difficult to harmonize. Some of these are doubtless due to variation as the result of environmental effects which influence the physiology of the plant. Assuming, however, that the genes are complementary and their effects cumulative, the suggested genetic complex provides a fairly satisfactory hypothesis as a possible explanation. The most serious discrepencies in the data are the deficiency of black and dark mulatto seeded plants in F_2 families and the occurrence and breeding behaviour of the yellow seeded L_4 segregate previously mentioned. The deficiencies can only be accounted for by assuming that these plants have been eliminated by gametic or zygotic lethals.

There is considerable evidence to indicate that pigmentation of the seed coat may be profoundly influenced by physiological factors.

An interesting case in point is that of seed-coat mottling in soybeans which has been studied superficially by many workers. Enough has been done to show that plant physiology assumes the major role in this phenomena. Inheritance undoubtedly plays a part but most yellow or green seeded varieties are subject to mottling under certain environmental conditions. Heredity is undoubtedly the ultimate controlling factor, but mottling is apparently inhibited except under those peculiar conditions which make the expression of this character possible.

Owen (11) discusses this question rather fully and refers to the work of Onslow who found, among other things, that some species formed starch and others did not. To quote; "The interesting point in this connection is the high negative correlation that was found between starch and pigment formation. In species that were not able to synthesize starch, sugars accumulated; and since sugars are used in the formation of anthocyanins, a logical reason can be given for pigmentation. Plants that were able to form starch, she assumes, made use of their sugars in that process, and the quantity of sugar left was insufficient for the production of pigments." Since the black and brown pigments which are primarily responsible for mottling were found to be glucosides, an explanation of their production by means of an accumulation of sugars has been proposed by Owen. He found the most striking evidence for this theory in his observations that mottling was greatly increased by reviving the growth of plants after the seeds were practically mature. Also it was found that mottling varied on different parts of the same plant.

In the case of pigmentation of seed in alfalfa there is no doubt as to the dominant part played by hereditary factors. At the same time there is reason to believe that physiological factors influenced the development of pigment to a greater or less extent. If this were the case, a satisfactory explanation of the data on breeding behaviour would become extremely difficult. At the same time it would provide a partial answer for some of the discrepancies which were found in an attempt to compare the results with genetic expectations.

If, for instance, we accept Owen's (11) suggestion and assume that the original black seeded parent plant not only carried a factor for black-seededness but also that it lacked the normal power of synthesizing starch, its behaviour in breeding would depend as much on the type of inheritance exhibited by the latter as by the former. Assuming segregation in both cases to be regular, normal Mendelian ratios would still be expected, the factor or factors for "starch formation" simply acting as color determiners or modifiers. But if the inability to form starch was also conditioned by vigor of growth, as appears to be the case with seed production, then the least vigorous plants would tend to have pigmented seed provided they also possessed the necessary inheritance. Conversely, the most vigorous plants would tend to produce seed with the minimum amount of pigment. The segregation obtained in F_2 would then be influenced by the degree of heterozygosity of individual plants in each family as well as by the random assortment of factors which condition seed coat color. This hypothesis could be used quite effectively to explain the distributions obtained in F_2 families, the marked deficiency of dark seeded plants in Table 2 and the unexpected types of F_1 plants secured from the W.S.1 x L4,B2 crosses in Table 4. It would also help to explain why such a large proportion of the F_1 plants in Tables 3 and 4 occur at the yellow end of the color series.

Once it is established that physiological causes are operative in modifying the expression of a character, the difficulty of a satisfactory solution to the problem is greatly increased and the opportunities for speculation are numerous. Most of the seed color studies with leguminous plants have run into this difficulty and the present study is no exception in this respect.

One of the objectives in this investigation was to determine whether it might be possible to utilize seed coat pigmentation in alfalfa as a character for identifying an improved strain. Such a distinguishing feature might sometime prove very useful because of the fact that varieties of alfalfa are so similar in appearance. Its utilization for this purpose, however, could be practical only if the character was inherited in a simple manner and preferably as a double recessive. Inheritance in this case has been shown to be fairly complex with the further probability that physiological factors affect the expression of the character. Furthermore it was not possible to secure fertile homozygous black seeded plants because of self-incompatability which characterized all of the inbred plants in this line. It was concluded therefore that the character in question was valueless from a utility standpoint.

SUMMARY.

1. This paper presents the results of an investigation to determine the inheritance of black and white seed coat characters in alfalfa and whether or not the former could be utilized in breeding as a marker for identifying improved strains.

2. The black seeded plant was discovered in a third generation selfed line of Grimm alfalfa and the white seeded plant occurred in a population of Grimm alfalfa plants which had not been inbred.

3. The fourth generation selfed line from the original black seeded parent consisted of 20 plants all of which, except one which had yellow seeds, produced either black or very dark mulatto seeds. A similar number of F_1 natural hybrids, between normal yellow seeded male parents and the original black seeded plant, produced seed which varied in color from light to dark mulatto. The results obtained from numerous Black x Yellow and White x Black crosses were reported.

4. Each of 19 F_2 families of the Black x Yellow cross

exhibited a wide range of variability with respect to seed coat color extending from yellow on the one hand to fairly dark mulatto on the other. With the exception of two families there was a marked deficiency of plants in the very dark color classes.

5. The available evidence indicated that the white seeded parent was homozygous for a recessive factor which results in the absence of yellow pigment, and that the inheritance of this character was comparatively simple. Inheritance of the black seeded character, on the other hand, was fairly complex, requiring the assumption of at least three factor pairs. The original black seeded plant was thought to have arisen as a single gene mutation. This gene, primarily responsible for pigmentation of the seed coat, together with at least two modifying factors, were postulated as the most probable genetic factorial basis to account for the breeding behaviour of the original black seeded parent.

6. The data strongly suggests that the expression of seed coat color was influenced to some extent by physiological factors which were conditioned by the environment.

7. It was concluded that the character of black-seededness would be valueless from a utility standpoint.

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Plate I - Typical specimens of alfalfa seeds selected from the seed color classes showing the range of segregation in progenies of crosses between black-seeded and yellow-seeded parent plants.

A. Kellett del. ad nat.