THE RELATION BETWEEN LIGHT VARIEGATED AND MEDIUM VARIEGATED PERICARP IN MAIZE[1]

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THE present report is based upon a restudy of variegated pericarp in maize, a mutable character which is well known through the important pioneer work of EMERSON (1914, 1917, 1929). The current investigation is concerned primarily with the relation between two distinct phenotypes, light variegated and medium variegated, both of which are unstable. The results obtained, however, bear directly on the allied problem of the kind of change which occurs when variegated is transformed to the stable self colored type.

Two facts emerging from EMERSON's experiments should be noted here. EMERSON (1917) found that in families obtained by selfing variegated plants heterozygous for the stable red cobbed white allele (*WR*),[3] variegated (*VV*) mutates somatically to self colored (*RR*) more than twice as frequently in heterozygotes (*VV/WR*), in which it is present once, as in homozygotes (*VV/VV*) which are duplex for the mutable gene. This observation, which will not be discussed further at present, has been amply confirmed in our work. The second fact was rather startling since, as EMERSON (1929) recognized, it might have been interpreted as evidence of gene contamination. The capacity of a *WR* allele of given origin seemingly to " stimulate " the mutation of variegated to self red in *VV/WR* heterozygotes was found to be reduced if, in the preceding generation, the gene passed through a *VV/WR* plant which showed a low grade of variegation. In a gross statistical sense, this relation also is confirmed in our experiments.

EMERSON (1929) considered it unlikely, however, that the *WR* allele itself was the element affecting the *VV/WR* heterozygote. He assumed, on the other hand, that the mutation modifier present in *WR* stocks was a gene (or genes) closely linked to, but distinct from, the *P* locus and subject to crossing over with alternative forms of the modifier occurring in the variegated races tested. Definitive evidence for the postulated second locus was not brought forward. The additional facts now at hand make it improbable that a satis-

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[3] The alleles occurring at the *P* locus are referred to throughout the paper by the superscripts of the symbols only. The first letter in the superscript indicates pericarp color and the second, cob color. The combined pericarp-cob characters are transmitted in unitary fashion. The full symbols are P^{VV}, variegated pericarp and cob; P^{RR}, red pericarp and cob; P^{WR}, white (colorless, or nearly colorless) pericarp, red cob; P^{WW}, colorless pericarp, white cob (ANDERSON 1924).

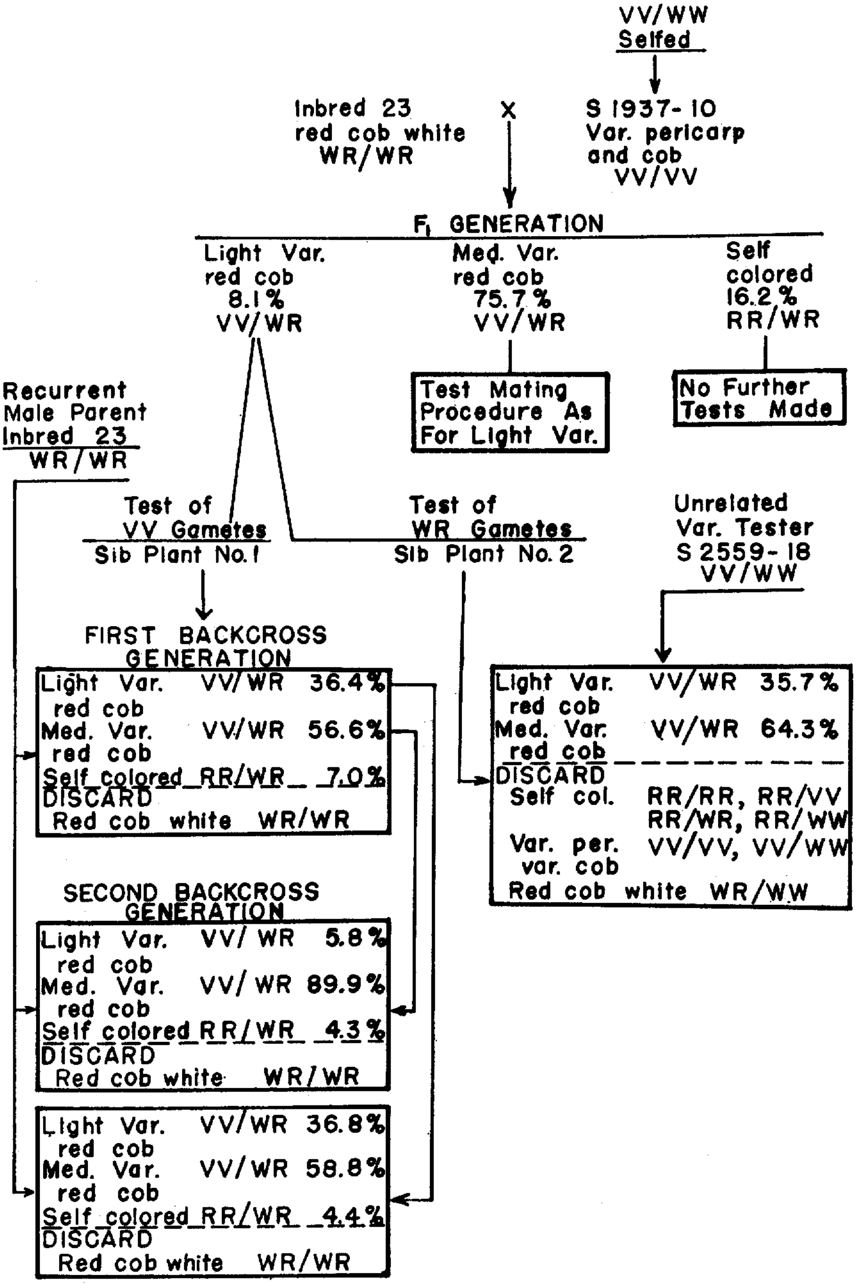


FIGURE 1.—The mating plan followed in testing the composition of the *VV* and *WR* gametes from *VV*/*WR* heterozygotes with reference to factors conditioning light and medium variegation. The numerical results presented for illustrative purposes relate to certain families involving inbred line 23.

factory explanation of the phenomenon can be formulated in these classical genetic terms.

Two further points should be mentioned bearing on the relation between the current work and that on variegated pericarp which preceded it. Stocks were employed in the present study in which two classes of variegated plants, light and medium, could be distinguished with much greater regularity than appears to have been possible with EMERSON'S material. Variegated pericarp in maize is subject to wide variation in phenotypic expression, and the use of families comprising sharply distinct classes of variegated plants is an important methodological advantage. The second consideration is the weight to be given the occurrence of twin spots on variegated ears. Twin spots (e.g., a light variegated area adjacent to a self colored patch on an otherwise medium variegated ear) of one or another kind have been recognized since the beginning of genetic studies on maize pericarp. They occur sporadically, however, and, when present, are often of insufficient size and contrast to be scored with certainty. It has not been clear from the previous work whether twin spots are of basic significance for variegation or are merely an interesting minor class of pericarp mutations. EMERSON did not make a point of them. We regard the evidence from twin spots as fundamental to an understanding of the central problem.

MATERIALS AND EXPERIMENTAL PROCEDURE

EMERSON'S work suggested that the relationship most in need of clarification was the apparent transmission by both *VV* and *WR* gametes from *VV/WR* heterozygotes of major genetic elements affecting the variegated phenotype. Of particular interest was his observation that the capacity of the *WR* gamete to condition the expression of variegated seemed to be influenced by the variegation grade of the parent plant. A two-fold body of evidence was needed, one bearing on the composition of the *VV* gametes and the other on the properties of the *WR* gametes. It appeared important also that the two sets of facts be obtained under conditions in which they could be related to each other directly. The rather simple mating system used in the present experiments, illustrated diagrammatically in figure 1, met these requirements.

A series of *VV/WR* and *VV/WW* hybrids were established by pollinating a number of inbred field corn lines with a single collection of pollen from a homozygous variegated plant both of whose *VV* genes had been derived from a selfed *VV/WW* parent. It was a very helpful circumstance that each of the resulting groups of hybrids comprised two discrete classes of individuals, light variegated and medium variegated, rather than a population varying continuously in variegation grade, as had been expected. These light and medium variegated F_1 hybrids were then tested in two ways.

One set of plants in each hybrid series was mated recurrently with the appropriate *WR/WR* or *WW/WW* inbred line through two generations. This procedure was designed to disclose the properties of the *VV* gametes emerging from the hybrids.

The *WR* gametes from the two classes of variegated F_1 hybrids were separately assayed for their influence on variegation. Single collections of pollen from unrelated *VV/WW* individuals were spread widely over the silks of a parallel set of the hybrid plants in question. A series of *VV/WR* offspring, recognized by their red cob, was thus obtained in which the *WR* gene marked a sample of gametes of known origin in terms of the variegation class, light or medium, of the parent plants. This kind of test mating served not only to extract *WR* from the two types of hybrids but also to reestablish directly *VV/WR* combinations in which the possible effects on variegation of the inheritance accompanying the *WR* allele could be observed.

A single collection of pollen from plant S1937-10, which bore a light variegated ear, was the source of the variegated allele introduced into all the F_1 hybrids with one unimportant exception. The ancestry of S1937 traced to a previously unpedigreed variegated pericarp plant whose pollen was used on a white cobbed white inbred line known as 4Co63. One of the resulting hybrids was then backcrossed to 4Co63, giving rise to family S1707. Plant 1 in the latter family was selfed, and from the seed obtained duplicate families, S1930 and S1755, were grown in different years. The immediate parent of S1937 was plant S1755-3, selfed. The single exception to the use of S1937-10 as the foundation variegated plant was the use of seed from a cross with S1930-1 as the pollen source in obtaining the line 8 hybrids referred to in table 5. S1930 and S1937 had a common ancestor in plant S1707-1, selfed. There was no difference in the results obtained with the two close relatives.

The inbred field corn lines used were: 8, 22, 23, 28, 70, M14, 40B, and the aforementioned 4Co63. The first six of these are red cobbed whites; the last two are white cobbed whites. All had been selfed 10 generations or more, and were highly uniform. The lines are unrelated to each other, and are representative of the inbred stocks currently in use in the production of commercial hybrid field corns adapted to southern Wisconsin. Strains 8, 22, 23, 28, and 70, being of Wisconsin origin, are commonly referred to in the hybrid corn literature as W8, W22, etc. The W prefix is omitted here in order to avoid possible confusion with the same symbol used in designating certain *P* alleles. The expanded symbols *WR*-8, *WR*-22, etc., appearing occasionally in the paper, merely indicate the inbred line from which the *WR* allele in question was derived. Generally speaking, the red cobbed whites of different origins are not distinguishable phenotypically from each other.

Variegated plants descended from an open pollinated stock obtained from a Colorado farmer and quite unrelated to S1937 were employed as pollen parents in the test crosses referred to in table 5. Families S2559 and S2560 were obtained by selfing two plants grown from seeds on the same ear. Family 7-5 was similarly obtained from another ear.

A definitive classification of the mutations of variegated to self color would require an accounting of each red area appearing on a kernel. Such a classification is not possible. Only the larger stripes and patches can be certainly identified and counted. Many small colored areas are discrete, but they grade downward in size until the mutant phenotype is represented by rather weakly pigmented tissue within which stripes cannot be distinguished even with magnification. Size of the well-defined colored areas is an index of the number of cell divisions which have intervened between the initial mutated cell and maturity of the tissue. Apparently mutations to red pericarp may occur at any stage of ontogeny although, as ANDERSON and EYSTER (1928), have shown, they are much more frequent, relative to cell number, at the later stages of development. These characteristics of variegated pericarp make it necessary to use purely empirical methods in estimating the frequency of mutations over the entire spectrum as, for example, throughout a single ear. What is scored in such a case is a generalized phenotype whose characteristics are determined by the number and distribution within a complex cell lineage of numerous mutations of the same kind.

After experimenting with other methods, a procedure was adopted for classifying entire variegated ears similar to that used by EMERSON (1929). Ears were selected as standards representing seven classes varying in intensity of striping from very light, i.e., one stripe or less per kernel, on the average (class 1), to very heavy striping (class 7). Each test ear was compared with the standards and then assigned the variegation grade of the class to which it corresponded. The method is rapid and, as EMERSON found, gives repeatable results for which, however, high precision cannot be claimed. Occasionally families are encountered which cannot be classified satisfactorily by this procedure due to nonconformity of the ears in one or another respect to the standards chosen. This shortcoming is disconcerting in particular instances, but it was found not to detract seriously from the general usefulness of the method.

A much simpler method of classifying our material came to hand with recognition of the fact that the variegated plants in the F_1 and backcross families usually fell into two discrete classes. These classes were termed light variegated and medium variegated, respectively. They are illustrated in figure 2, together with the stable self colored type to which both mutate. This dichotomy within families segregating variegated was not perceived until the group involving inbred 4Co63, on which figure 2 is based, was classified. Once the difference was recognized in the 4Co63 families, in which it was sharply expressed, it was looked for and found at once in the others.

Generally speaking, light variegated ears were found to fall into classes 1 and 2 and medium variegated ears into classes 3 and 4, rarely 5, as these categories were delimited by the standard ears referred to earlier. The respective light and medium variegated phenotypes were found to be fairly constant for related families, but they varied somewhat from one inbred group to another, depending presumably on the residual inheritance.

The validity of the classification into light variegated and medium variegated depends upon the regularity of occurrence of a discontinuity between

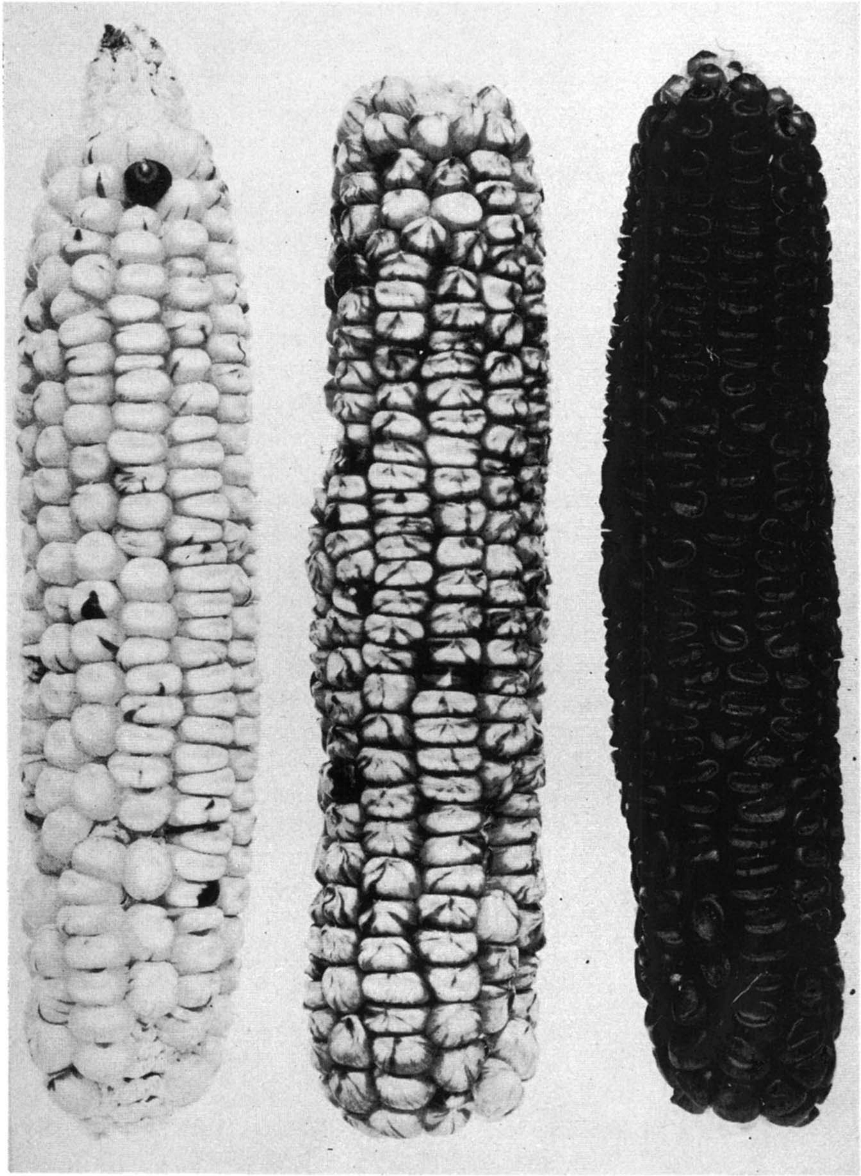


FIGURE 2.—Light variegated (left), medium variegated (middle), and self colored (right) ears in the 4Co63 backcross series.

these classes in segregating families. A discontinuity was clearly apparent, with only a few exceptions, in both the first and second backcross generation families involving inbreds 8, 22, 23, and 4Co63. The same statement applies to the test cross families listed in table 5, involving inbreds 8, 22, 23, 28, and 70. When present at all, intermediate ears were few in numbers, and the remaining ears corresponded closely to the light and medium variegateds, respectively, in sib families in which the distribution definitely was discontinuous. A few " bizarre " ears were encountered in which there appeared to be an irregular mixture of more or less well defined light and medium variegated areas. Such ears were placed according to the class of tissue which predominated. Some of the various exceptional ears among the several thousand scored may have represented genetically different variegation types. They were not hand-pollinated and so could not be tested further. Others were probably merely variant members of the two prevailing classes.

In contrast to the above groups, several of the backcross families based upon inbreds M14 and 40B were found difficult or impossible to classify in these terms. This was especially the case in the second backcross generation which was grown in 1951, an unusually cool season throughout, in which a killing frost occurred before the ears in these relatively late maturing families were fully ripe. Since pericarp color attains its full development late in growth of the plant, immaturity is a handicap in classification. The ears in more than half these families, however, could be separated clearly into the two phenotypes. This fact suggests that the M14 and 40B series do not differ basically from the others. The residual inheritance carried by these two inbreds, including that for late maturity, appears to be relatively unfavorable, however, for sharp expression of the difference between light and medium variegated. The available data on the M14 and 40B series are not reported because of their varying dependability from family to family. These stocks are being further studied.

EXPERIMENTAL DATA

The data bearing on the distribution of variegated and self colored in the first and second backcross families have been tabulated according to the respective inbred lines which served as the recurrent parents. Since a common variegated parent, S1937-10, was involved in all the initial matings, the four series of families, corresponding to inbreds 8, 22, 23, and 4Co63, respectively, might be expected to be similar. It will be seen that, in fact, the same general relations are in evidence in each backcross group. There are significant variations in detail, however, both between and within groups.

F_1 *generation*

Three kinds of offspring, light variegated, medium variegated, and self colored, occurred in approximately the same proportions in each of the F_1 families resulting from application of a single collection of pollen from the homozygous variegated plant, S1937-10, to inbred 8, 22, 23, 28, and 4Co63. The distribution among the three phenotypes of 279 individuals classified in the five families was 10.7 percent, 72.4 percent, and 16.8 percent, respectively.

Backcrosses to inbred 8

The data relating to the first and second backcross generations of the appropriate F_1 plants to inbred 8 are brought together in table 1. The variegation

TABLE 1

Distribution of offspring of VV/WR-8 light and medium variegated plants backcrossed once and twice to inbred line 8.

Family	Var. grade seed parent	Offspring		% among colored		
		Total	Colored	Light var.	Med var.	Self
		First backcross generation				
6-70	1	116	51	41.2	52.9	5.9
6-71	2	96	48	52.1	45.8	2.1
6-72	2	188	98	52.0	44.9	3.1
6-73	2	185	89	42.7	53.9	3.4
6-74	3	336	175	4.0	91.4	4.6
6-75	3	283	159	5.7	88.6	5.7
6-77	4	272	141	1.4	95.1	3.5
		Second backcross generation				
62-1	1	187	85	65.9	31.7	2.4
62-2	2	186	105	54.3	41.9	3.8
62-3	1	119	59	49.2	45.7	5.1
62-4	1	94	45	57.8	37.8	4.4
62-5	2	117	55	49.1	45.4	5.5
62-6	2	158	84	51.2	45.2	3.6
62-7	2	127	59	47.5	50.8	1.7
62-8	1	90	45	42.2	51.1	6.7
62-9	3	95	45	11.2	86.6	2.2
62-10	3	113	71	5.6	93.0	1.4
62-11	3	153	78	5.1	83.4	11.5
62-12	3	111	54	3.7	90.7	5.6
62-13	3	158	70	5.7	85.7	8.6
62-14	3	198	107	6.5	87.0	6.5
62-15	3	128	73	1.4	90.4	8.2
62-16	3	114	57	1.8	96.4	1.8
62-17	3	215	107	1.9	93.4	4.7
62-18	3	158	81	2.5	93.8	3.7
62-19	3	216	109	9.2	88.0	2.8
62-20	3	170	78	5.1	88.5	6.4
62-21	3	142	78	1.3	94.9	3.8

grade of the parent ear from which each family was derived is shown in column 2. The pistillate parents in both generations were heterozygous for colorless pericarp, so that about one-half the offspring are of the latter type. These individuals are of no immediate interest except as they confirm the fact that the distribution of colored (variegated plus self red) and colorless pericarp is Mendelian.

Five characteristics of the data in table 1 are noteworthy.

1. The colored pericarp plants in each family include the three phenotypes which appeared in F_1, namely, light variegated, medium variegated, and self colored.
2. Two distinct kinds of families occur with reference to the frequencies of light and medium variegated individuals; the proportion of light

variegated plants is high in one group and low in the other, as shown by the values in the fifth column.

3. The families containing a high proportion of light variegated plants were derived from light variegated ears (class 1 or 2) and those with few such individuals arose from medium variegated ears (class 3 or 4).
4. The frequency of occurrence of self colored ears is of the same order as that of light variegateds among the offspring of medium variegateds.
5. The results in the first and second backcross generations are alike.

The similarity in breeding behavior of medium variegateds descended from light and medium variegateds, respectively, is illustrated by the data from families 62-9 to 62-21 in table 1. Each of these 13 twice backcrossed families was grown from the seeds on a medium variegated (class 3) ear. All were descended from a single F_1 hybrid family, 6-1, containing both light variegated and medium variegated plants. Families 62-9 and 62-10 were descended from a light variegated (class 1) ear, families 62-11 to 62-15 from a medium variegated (class 3) ear, and families 62-16 to 62-21 from a medium variegated ear of class 4, in the F_1 generation. It will be noted that the three groups of twice backcrossed families are similar in the proportions of light variegated,

TABLE 2

Distribution of offspring of VV/WR-22 light and medium variegated plants backcrossed once and twice to inbred line 22.

Family	Var. grade seed parent	Offspring		% among colored		
		Total	Colored	Light var.	Med var.	Self
		First backcross generation				
6-80	1	181	85	97.6	0	2.4
6-81	1	200	98	63.3	34.7	2.0
6-82	2	286	142	12.7	84.5	2.8
6-83	4	240	130	1.5	93.1	5.4
6-84	4	313	157	0.6	96.2	3.2
6-85	4	195	94	2.1	91.5	6.4
6-86	4	340	167	2.4	94.0	3.6
		Second backcross generation				
62-22	2	200	102	69.6	21.6	8.8
62-23	2	113	53	54.7	43.4	1.9
62-24	2	131	72	79.2	16.6	4.2
62-25	2	150	77	55.8	37.7	6.5
62-26	2	87	44	79.5	18.2	2.3
62-27	3	168	91	36.3	62.6	1.1
62-28	3	159	77	2.6	93.5	3.9
62-29	3	149	80	2.5	95.0	2.5
62-30	3	134	74	12.2	85.1	2.7
62-31	3	173	81	2.5	92.6	4.9
62-32	3	157	75	21.3	78.7	0.0
62-33	3	109	56	3.6	92.8	3.6
62-34	3	247	126	2.4	92.8	4.8

medium variegated, and self colored ears which they contain. This means that medium variegated plants of the same kind as those produced directly by medium variegated parents may be extracted from light variegated individuals.

Unfortunately, corresponding data bearing on the comparability of light variegated plants derived from light and medium variegated parents, respectively, are not available. This gap in the evidence is due to failure in the first backcross generation of numerous plants to form ears usable for seed as a result of severe insect injury. Evidence of another kind, however, presented in table 5, which will be considered later, indicated that light variegateds originating in these two ways are probably alike.

Backcrosses to inbred 22

The first and second backcross generation data from hybrids between inbred line 22 and S1937-10 are brought together in table 2. Again, two distinct kinds of families, distinguishable on the basis of the proportion of light variegated plants which they contain, are represented. There is much more heterogeneity between families within each of the two main groups, however, than was found in the backcrosses to line 8 discussed above. Family 6-80 in the first backcross generation, for example, comprised 83 light variegated, no medium variegated, and two self colored ears. That is to say, 97.6 percent of the colored offspring were light variegated. One sister family, 6-81, contained 63.3 percent light variegateds, and a second sister family, 6-82, only 12.7 percent. All three of these families were based on light variegated (class 1 or 2) seed ears. It is evident, however, that in spite of this wide variation between families, the group as a whole differs sharply from the families derived from medium variegated (class 4) sister ears. Among the latter progenies, the proportion of light variegated plants in no case exceeds 2.4 percent.

The data from the second backcross generation are obviously heterogeneous also. Here too, however, the frequencies of light variegated plants from the light and medium variegated parent ears, respectively, do not overlap, although the results from family 62-27 raise some doubt on this point. The 62-27 parent seed ear was scored as grade 3 variegated, a classification which has been confirmed by reexamination of the remnant. The progeny contained 36.3 percent light variegated individuals, a value approximately midway between the average of the other families from class 3 ears and the average of families from the light variegated (class 2) parent ears. Furthermore, it will be noted that among the families based on class 3 ears, excluding 62-27, the proportion of light variegated offspring varies from 2.4 percent to 21.3 percent. The possible causes of these variations will be considered later.

Backcrosses to inbred 23

The third body of data bearing on the composition of the *VV* gametes formed by *VV/WR* heterozygotes is summarized in table 3. The initial cross in this case involved S1937-10 and inbred line 23. Two successive backcrosses to the red cobbed white inbred were made in accordance with the general plan.

TABLE 3

Distribution of offspring of VV/WR-23 light and medium variegated plants backcrossed once and twice to inbred line 23.

Family	Var. grade seed parent	Offspring		% among colored		
		Total	Colored	Light var.	Med. var.	Self
		First backcross generation				
6-89	2	194	100	44.0	53.0	3.0
6-90	2	267	129	36.4	56.6	7.0
6-91	2	245	134	38.1	56.7	5.2
6-92	3	274	139	5.0	82.8	12.2
6-93	3	237	124	5.6	80.7	13.7
6-94	4	218	101	6.9	84.2	8.9
6-95	4	177	93	6.5	84.9	8.6
		Second backcross generation				
62-43	2	135	68	36.8	58.8	4.4
62-44	2	142	60	40.0	53.3	6.7
62-45	2	225	103	48.5	42.8	8.7
62-46	4	132	69	5.8	89.9	4.3
62-47	4	90	44	11.4	77.2	11.4
62-48	4	142	72	5.6	76.3	18.1

The results are closely parallel to those obtained from the test matings involving inbred line 8, except in one respect. Whereas approximately 50 percent of the offspring from the light variegated seed ears (classes 1 or 2) were light variegated in the formed case, the average proportion of plants of this kind in both the first and second generation backcross families involving line 23 are closer to 40 percent. Contrariwise, the medium variegated ears in the line 23 group yielded families containing a higher frequency of light variegated plants than did the corresponding class of ears in the line 8 families. Since the differences mentioned occur rather regularly, and the respective bodies of data on which they are based are relatively homogeneous in contrast to those obtained from the line 22 backcrosses, the disparities in proportion of light variegated plants may be presumed to have a genetic basis. Aside from this point, the data from the line 23 backcrosses are in remarkably close accord with those from the line 8 test matings. Thus, the five basic characteristics of the backcross families to which attention was called on reviewing the results with line 8 are again clearly in evidence.

Backcrosses to inbred 4Co63

The white cobbed colorless pericarp inbred line 4Co63 was the recurrent parent in the backcrosses on which table 4 is based. The results given in the table are in conformity with those reported for the matings involving inbreds 8, 22, and 23 in showing that two categories of families occur, one with a high proportion of light variegated individuals and the other containing few plants of this class. The former type of families is derived from light variegated seed ears and the latter exclusively from medium variegated parent ears. The variation in proportion of light variegated plants between families within each of these categories, however, is relatively high.

Evidently homozygosity for the residual inheritance does not stabilize the proportion of light variegated plants appearing in the separate families within each of the two recognized types. The data in table 4 support this conclusion. The 4Co63 families were the most highly inbred among the four backcrossed series. This resulted from the fact that the foundation variegated plant, S1937-10, used in the original outcrosses to the several inbreds, derived 75 percent of its inheritance from 4Co63. The two successive matings subsequently made to this inbred line would result in a second backcross generation almost 95 percent of whose inheritance coincided with that of 4Co63. In spite of the high degree of convergence of the 4Co63 test families toward the recurrent 4Co63 parent inbred line, a considerable amount of heterogeneity remains in the families arising from ears of a given variegation class.

TABLE 4

Distribution of offspring of VV/WW-4Co63 light and medium variegated plants backcrossed once and twice to inbred line 4Co63.

Family	Var. grade seed parent	Offspring		% among colored		
		Total	Colored	Light var.	Med. var.	Self
		First backcross generation				
6-109	2	69	33	78.8	18.2	3.0
6-110	2	93	49	51.0	47.0	2.0
6-111	4	95	47	12.8	85.1	2.1
6-112	4	122	64	1.6	87.5	10.9
6-113	4	159	86	9.3	84.9	5.8
6-114	4	149	77	10.4	85.7	3.9
6-115	4	160	82	6.1	85.4	8.5
		Second backcross generation				
62-35	1	53	28	89.3	10.7	0.0
62-36	4	146	62	6.5	79.0	14.5
62-37	4	118	60	10.0	86.7	3.3
62-38	4	131	64	6.2	79.7	14.1
62-39	3	81	39	7.7	89.7	2.6
62-40	4	197	103	7.8	78.6	13.6
62-41	3	81	39	12.8	79.5	7.7
62-42	4	131	61	3.3	90.1	6.6

A few families have been grown from backcrossed self colored ears in each of the above four series. They have regularly shown equal proportions of self colored and colorless offspring, within the limits of random sampling.

The proportion of light variegated and self red offspring of medium variegated plants

A summary is given in table 6 of the frequencies with which light variegated and self red plants occurred in the families from medium variegated seed ears

TABLE 5

VV/WR segregates from light and medium variegated VV/WR F_1 plants pollinated by VV/WW.

Family	Seed Parent		VV/WW pollen parent	Poll. date	Variegated offspring					
					Light		Medium		Total	% light
	Genotype	Var. grade			No.	Mean grade	No.	Mean grade		
N156	VV/WR-8	1	7-5-106	7-22	26	1.61	14	3.14	40	65.0
N161	”	4	”	”	7	1.42	46	3.13	53	13.2
N164	”	1	7-5-106	7-23	27	1.37	19	3.21	46	58.7
N165	”	1	”	”	21	1.61	14	3.07	35	60.0
N168	”	4	”	”	3	1.33	46	3.34	49	6.1
N169	”	5	”	”	4	1.50	36	3.22	40	10.0
N30	VV/WR-22	2	S2559-16	7-28	35	1.48	23	3.04	58	60.3
N36	”	4	”	”	9	1.44	41	3.12	50	18.0
N49	VV/WR-22	2	S2559-16	7-27	15	1.40	5	3.00	20	75.0
N50	”	2	”	”	17	1.35	14	3.00	31	54.8
N53	”	4	”	”	19	1.99	25	3.08	44	43.2
N54	”	4	”	”	8	1.50	27	3.07	35	22.9
N58	VV/WR-23	1	S2559-18	7-23	10	2.00	18	3.16	28	35.7
N62	”	4	”	”	0	....	16	3.25	16	0.0
N63	”	5	”	”	2	2.00	32	3.21	34	5.9
N64	VV/WR-23	2	S2559-18	7-24	5	2.00	36	3.06	41	12.2
N65	”	2	”	”	28	1.89	29	3.03	57	49.1
N68	”	4	”	”	6	1.83	43	3.07	49	12.2
N69	”	4	”	”	6	1.83	51	3.11	57	10.5
N70	”	4	”	”	6	1.66	46	3.03	52	11.5
N72	VV/WR-23	2	S2559-18	7-25	16	1.75	3	3.00	19	84.2
N76	”	4	”	”	14	1.58	33	3.00	47	29.8
N77	”	4	”	”	6	1.66	29	3.06	35	17.1
N85	VV/WR-28	1	S2559-19	7-23	8	1.62	35	3.02	43	18.6
N90	”	4	”	”	5	1.60	41	3.04	46	10.9
N91	”	4	”	”	3	1.66	33	3.15	36	8.3
N92	VV/WR-28	1	S2559-19	7-24	8	1.75	40	3.02	48	16.7
N93	”	2	”	”	6	1.16	22	3.04	28	21.4
N97	”	3	”	”	2	2.00	21	3.10	23	8.7
N98	”	4	”	”	5	1.60	53	3.09	58	8.6
N118	VV/WR-70	1	S2560-5	7-23	18	1.94	14	3.57	32	56.2
N121	”	3	”	”	4	1.75	51	3.02	55	7.3
N123	”	4	”	”	4	1.50	28	3.53	32	12.5
N124	VV/WR-70	1	S2560-5	7-24	17	1.70	13	3.38	30	56.7
N125	”	1	”	”	10	1.60	25	3.28	35	28.6
N127	”	1	”	”	4	2.00	18	3.44	22	18.2
N130	”	3	”	”	4	1.75	31	3.64	35	11.4

in the four backcross series. The proportion of self reds to light variegateds in the total is 260 to 206, or a ratio of 1.3 : 1. The individual comparisons which may be made in the table disclose substantial variations in the ratio between both series and backcross generations, for which no explanation is at hand. Certain regularities, however, are in evidence. The agreement in the ratios between generation, for example, is relatively close in the case of the 8 and 23 backcross series. This fact is in accord with the comparative uniformity in behavior of these two groups in other respects also, including the percentages of light variegated offspring from family to family obtained from the light variegated seed ears. These ratios are of interest when considered in relation to the evidence from twin spots, to be presented later, which shows that mutations of medium variegated to light variegated and self color often occur concurrently.

Influence of WR gametes extracted from light and medium variegated VV/WR heterozygotes on the phenotypic expression of variegated in the next generation

The results of the experiments made to disclose the composition of *WR* gametes from light and medium variegated heterozygotes (*VV/WR*) with reference to factors conditioning variegated pericarp are assembled in table 5. All the hybrids tested in this series, except one, had plant S1937-10 as their variegated parent. A closely related variegated individual, S1930-1, was used in the cross with inbred 8. This series of F_1's is directly comparable, therefore, to the *VV/WR* hybrids whose *VV* offspring are recorded in tables 1 to 4 in so far as the inbred parent lines are the same. The 4Co63 inbred line was omitted from the present tests because its white cob made it unsuitable for the kind of testcross made; and two red cobbed inbred lines, 28 and 70, not used in the other experiments, were added.

A single type of test mating was employed. A given collection of pollen from an unrelated variegated plant heterozygous for white cob, colorless pericarp was distributed over the silks of a sufficiently large number of F_1 *VV/WR* sibs to include, in the cases reported, both light and medium variegated plants. Thus, *WR* gametes from otherwise closely comparable light and medium variegated plants were brought into association with a common array of *VV*

TABLE 6

Proportion of light variegated and self red offspring in families derived from medium variegated backcrossed ears.

Backcross series	No. of families	Number of plants		Ratio
		Light var.	Self red	R/V
		First backcross generation		
8	3	18	22	1.2
22	4	9	24	2.7
23	4	27	51	1.9
4Co63	5	28	23	.8
Totals:	16	82	120	1.5
		Second backcross generation		
8	13	43	58	1.3
22	7	36	19	0.5
23	3	13	21	1.6
4Co63	7	32	42	1.3
Totals:	30	124	140	1.1

gametes. The relevant class of offspring bore red cobbed variegated ears, and only this class was scored.

Sometimes pollen was taken from the same VV/WW individual on successive days. The results obtained with each such collection are entered separately in table 5.

It was a matter of great interest to find that the red cobbed variegated ears in the test cross families fell into two distinct groups, light variegated and medium variegated. That is to say, the same two types of variegation which characterized the F_1 pistillate parents from which the WR alleles were derived reappeared in the offspring, even though the latter carried VV genes from a different source. Table 5 shows the distribution of the ears between the light variegated and medium variegated classes in each family. The classification on this basis was clear cut except in the case of two families among the 37. The discontinuity between light variegated and medium variegated was not sharp in N53 and N127, so that the counts given in these families may err somewhat in either direction.

The ears within the respective main variegation classes in each family were scored also for variegation grade against the standard set of ears. The mean values thus obtained are included in the table.

Eleven groups are available within each of which a comparison may be made between the eventual effect on variegation of WR gametes derived, respectively, from light variegated and medium variegated VV/WR heterozygotes. The pistillate parents in each group are sibs to which pollen from a single collection were applied.

With due regard to the fact that the relations to which attention is called are much more clearly shown in certain groups than in others, the salient features of the data in table 5 may be summarized as follows:

1. Each of the 37 families, with one exception, which might be due to random sampling, comprises two classes of variegated plants, light and medium.
2. Comparisons on a within group basis show that progenies from light variegated seed ears contain a higher percentage of light variegated plants than progenies from medium variegated seed ears. The one exception is family N64, from a light variegated ear, in which the proportions of light variegated offspring is unaccountably low.
3. In a few of the groups there is a wide gap between the percentages of light variegated offspring from light and medium variegated seed ears, respectively. The two groups involving VV/WR-8 hybrids pollinated by plant 7-5-106 are the most clearly differentiated in this respect.
4. Within a group, the mean variegation grade of the light variegated offspring from light variegated seed ears is essentially the same as the mean grade of the same class of plants from medium variegated seed ears. The same relation holds for the medium variegated offspring of the two respective classes of parent ears.

5. Progenies from light and medium variegated ears of comparable parentage thus differ only in the relative percentages of light variegated and medium variegated plants which they contain.

The percentages of light variegated offspring in progenies from seed ears of a given variegation class vary widely from family to family. Possible reasons for the high variability both here and in the backcross series will be considered more fully later in the paper. Meanwhile it may be noted that part of the present variation may have arisen from the variegated plants from which the pollen was taken for the test crosses. At the time the pollinations in question were made it was thought that the test crosses would be sufficient in themselves to provide the evidence needed. Consequently, no matings were made by which the pollen parents could be characterized independently for their content of factors affecting variegation. In spite of the uncertainties thus created regarding the origin of some of the variability present, the evidence as a whole shows unmistakably that *WR* gametes arising from light variegated *VV/WR* heterozygotes, when recombined with *VV* gametes, yield families containing a higher proportion of light variegated plants than similarly tested *WR* gametes produced by otherwise comparable medium variegated plants. This result is comparable, in a broad statistical sense, to the observation which EMERSON (1929) made on the properties of *WR* gametes from variegated heterozygotes of different grades.

When the data in table 5 are considered in relation to the evidence obtained in the backcross series (tables 1 to 4) an additional fact of particular significance becomes apparent, namely, that the direction of the effect associated with variegation grade is the same for both the *WR* gametes and the *VV* gametes arising from a given *VV/WR* heterozygote. If the latter is light variegated, then both the *WR* and *VV* gametes, when appropriately tested, tend to give a high proportion of light variegated offspring. If the heterozygote is medium variegated, both the *WR* and *VV* gametes yield relatively low percentages of light variegated progeny.

Since the differential between light variegated and medium variegated pericarp thus assorts more or less freely with both the *VV* and *WR* alleles, it cannot reside in the *VV* gene and, indeed, must be separate from the *P* locus.

Twin spots

Mutations of variegated pericarp sometimes appear as twin spots. These consist of a self colored area adjacent to a sector showing a lighter grade of variegation than prevails elsewhere on the ear. Twin spots are thus complementary in the sense that one component involves an increase and the other a decrease in pigmentation, relative to the parent phenotype. The more readily identifiable twins vary in size from a single kernel upward. A relatively large one is illustrated in figure 3. The number of twins appears to differ widely between strains. The first estimate of frequency made in our laboratory was based upon a diverse assemblage of 2915 open pollinated variegated ears, heterozygous for red cobbed white. The individuals bearing self colored patches covering 10 kernels or more and not extending to the butt or tip of the ear were picked out. Within this sample, comprising 57 ears, the self colored mutant patches were found to be twinned in 26 percent of the cases. This observation suggested that twinning probably was an important aspect of the variegation phenomenon, and also raised the hope that twins of adequate size might be encountered frequently enough among suitably bred hand-pollinated ears to enable progeny tests of the three phenotypes concerned to be made.

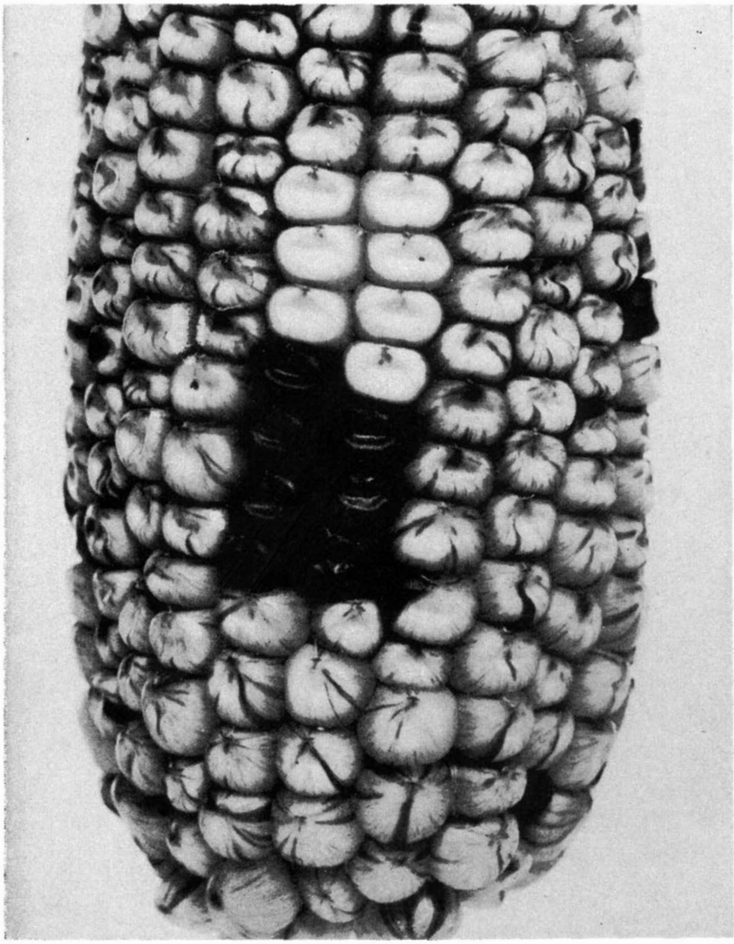


FIGURE 3.—Twin spots, consisting of light variegated and self colored kernels, on a medium variegated ear.

The frequency of twin spot formation was estimated in 23 of the second generation backcross families listed in tables 1, 3, and 4. The medium variegated ears in these families were sorted for individuals bearing a self colored area covering five kernels or more. This somewhat arbitrary lower limit for size was chosen on the grounds that it probably excluded most of the cases in which it is difficult to determine satisfactorily whether a given self colored patch is or is not twinned. It will be seen from the data, which are summarized in table 7, that in all three backcross series, the frequency of twinning exceeds 50 percent, and in the case of the 4Co63 group, reaches approximately 90 percent. These values should not be generalized because they are based upon a sample which includes only the few mutations to self color which occurred early enough in ontogeny to give relatively large mutant patches. Since it is evident, however, that many smaller self colored areas are twinned also, even though scoring in this size range is less certain, it can be concluded that a significant proportion of the mutations of variegated result in two complementary phenotypes. Only a beginning has been made in the genetic analysis of such twins. The few data now available are presented in table 8.

Each of the five parent plants represented in table 8 was heterozygous for colorless pericarp, and the pollen applied to them carried colorless pericarp also. One-half of the offspring, within sampling limits, were found to have colorless pericarp, as expected. The remaining data are too few to be conclusive on the point, but they support the view that the light variegated, medium variegated, and self colored sectors agree in genotype with entire ears of the three respective kinds (cf. tables 1 to 4 for progenies from light and medium variegated ears). The colored offspring from self colored kernels are regularly self colored.

TABLE 7

Frequency of twin spot formation on medium variegated ears in second generation backcross families involving inbreds 8, 23, and 4Co63.

Backcross group	Number of medium variegated ears			Percent twinned
	Total examined	Bearing 5-kerneled red patches	Twinned	
8	908	57	33	57.9
23	151	20	11	55.0
4Co63	354	28	25	89.3

Tests are in progress on the inheritance conditioning variegated pericarp transmitted through the *WW* or *WR* gametes from the five groups of plants represented in table 8.

If it is proved eventually that light variegated–self colored twin spots on otherwise medium variegated ears correspond genotypically to the same respective classes of offspring from medium variegated parents, a conclusion to which the present limited evidence points, the fact is highly important. It means that, in medium variegated plants two mutations, one to light variegated and the other to self color, may arise concurrently from the same single event. The fact was long since established by EMERSON that the change of variegated to self color involves a mutation at the *P* locus. The present investigation shows that the differential between light variegated and medium variegated, on the other hand, rests upon a factor at a site distinct from *P* and

TABLE 8

Phenotypes of plants reared from light variegated, medium variegated, and self red kernels from ears bearing twin spots.

Family	Phenotype of parent sector	Colored offspring			Colorless	Total
		Light var.	Med. var.	Self red		
Ear 1[1]						
63-12	Light var.	1	0	1	2	4
63-13	Med. var.	0	6	0	6	12
63-14	Self red	0	0	2	1	3
Ear 2[1]						
63-15	Light var.	1	1	1	7	10
63-16	Med. var.	0	6	1	4	11
63-17	Self red	0	0	0	4	4
Ear 3[1]						
63-18	Light var.	0	0	0	2	2
63-19	Med. var.	1	7	0	9	17
63-20	Self red	0	0	1	2	3
Ear 4[2]						
63-21	Light var.	1	1	0	3	5
63-22	Med. var.	0	0	0	2	2
63-23	Self red	0	0	1	0	1
Ear 5[2]						
63-24	Light var.	1	0	0	1	2
63-25	Med. var.	0	4	0	5	9
63-26	Self red	0	0	5	5	10

[1] Parent genotypes = VV/WW × WW.
[2] Parent genotypes = VV/WR × WR.

often, at least, remote from the latter locus. The general conclusion to which the evidence leads is that a single germinal change takes place which affects two loci.

DISCUSSION

The leading fact disclosed by these experiments is that the mutable character variegated pericarp in maize may be differentiated regularly into two distinct phenotypes depending, not upon the variegated allele present, but upon a genetic unit separable from the *P* locus. Proof of the occurrence of such a unit is afforded by the data in tables 1 to 5 which show that the capacity to differentiate between light and medium variegated assorts more or less independently of *VV* and *WR* in the gametes formed by *VV/WR* heterozygotes.

It is proposed, for purposes of discussion, to call the newly recognized element Modulator, as descriptive of its regulatory effect on the variegated phenotype, and to apply the symbol *Mp* to it. The justification for considering Modulator as a genetic entity is that the phenotype from which the presence of the element is inferred is of a distinctive and recurring kind. If this criterion alone were applied, the unit might be classified appropriately as a major modifying gene.

Modulator, however, is not a modifying gene of the usual sort; the second generation backcross data given in tables 1 to 4 rule out this possibility. One-half the families in this generation, from either the light variegated or the medium variegated seed ears, should have given one class of variegated offspring only on any of the standard assumptions which may be made concerning the distribution of a stable modifier among the parents. This is not what occurred. Among the 18 families from light variegated ears and the 30 families from medium variegated ears, there were none which did not include both kinds of offspring. Furthermore, the frequencies with which light and medium variegateds appear in these families are not compatible with the view that the differential between the two classes is a gene so incorporated in the hereditary complement that it is transmitted in a conventional manner.

Modulator may belong to the obscure category of germinal substances, termed heterochromatin, which has been associated through the work of MULLER (1930), SCHULTZ (1936), DEMEREC (1940), and others, with different variegated phenotypes in Drosophila. Variegated pericarp now is seen to conform in certain respects also to the genetic behavior of the mutable loci in the short arm of chromosome 9 in maize ingeniously analyzed by MCCLINTOCK (1950) and interpreted by her in terms of chromatin material accessory to the gene. The type of explanation of variegation which MCCLINTOCK has advanced may prove to be applicable to variegated pericarp, but our data are too limited to show how far the parallel extends.

The present results are understandable on the assumption that a common variegated gene is involved in the F_1 and backcross families, and that light variegated differs from medium variegated in the possession of Modulator, as a factor separate from the *P* locus. Derivation of the *VV* gene from a single heterozygous individual in the near ancestry of the foundation plant, S1937-10, and the uniformity of the two respective variegated phenotypes, light and medium variegated, are in accord with the view that one *VV* allele is present throughout.

The origin and germinal basis of Modulator are almost wholly obscure at present. No data are available concerning transmission of the element except in relation to *P*. The stability of the factor has not been studied, nor have the dosage effects been established. These are problems which can be clarified only by further experiments. There are certain regularities, however, in the rather complex body of data now at hand. It is important to inquire into the possible meaning of these facts if for no other purpose than to discover the directions in which a fuller understanding of Modulator may be sought.

The data from twin spots serve as an appropriate point of departure in constructing a working hypothesis. Adjacent self colored and light variegated areas on medium variegated ears heterozygous for colorless pericarp are so frequent that they must be regarded as the outcome of a single mutational event. Emerson (1917) demonstrated that the change from variegated to self color was due to a mutation at the *P* locus. The data presented in tables 1 to 5 show that light variegated, the co-twin to self color, differs from the parent phenotype, medium variegated, not in the *VV* allele present, but in the possession of Modulator at a locus separate from *P*. One may infer from this evidence that Modulator is a product of the transformation of *VV* to the stable self colored allele, *RR*.

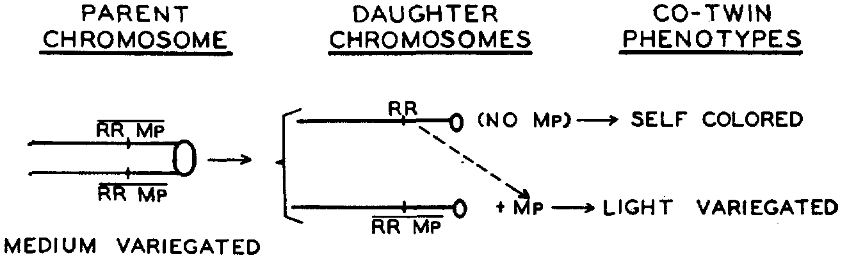
It might be supposed that the variegated allele is an *RR* gene with which Modulator is associated at the *P* locus, the effect of *Mp* in this position being to suppress pigment formation. That is to say, *VV* may be equated to $\overline{RR\ Mp}$. Mutation of variegated to self color, on this hypothesis, would consist in the loss of Modulator from the *P* locus, thus restoring the pigment producing activity of the *RR* gene. Loss of Modulator at the *P* locus, when variegated mutates to self color, may be coincident, however, with acquisition of the same element elsewhere in the genome. It is assumed that Modulator at the new site plus the *VV* allele at the *P* locus gives rise to the light variegated phenotype. The medium variegated phenotype is the result of the action of the *VV* allele at the *P* locus, in the absence of *Mp* elsewhere in the complement. Modulator, except when present at the *P* locus, has no effect on the stable, self colored allele, *RR*. It is necessary to postulate also that the point at which Modulator becomes affixed, after removal from the *P* locus, varies.

The three colored phenotypes occurring in segregating families thus may be represented as follows:

1) Self colored = *RR*
   Mp may be present in the genome, but not at the *P* locus and does not affect *RR* action.
2) Medium variegated = $\overline{RR\ Mp}$
   Modulator is present at the *P* locus only, and inhibits pigment formation.
3) Light variegated = $\overline{RR\ Mp} + Mp$
   This class differs from medium variegated in possessing a second Modulator unit which is not directly associated with the *P* locus, although it may lie in the same chromosome or in another chromosome.

If the new site which *Mp* occupies after removal from the *P* locus is at least 50 crossover units from *P* in chromosome 1, or is in another chromosome, then equal numbers of light and medium variegated offspring should result from mating heterozygous light variegateds with standard red cob colorless plants. On the other hand, if the new position to which *Mp* is transposed is near *P*, the resulting linkage will distort the ratio of light to medium variegated.

Twin spots can be assumed to originate in a mitotic division in which one of the two strands arising from the *VV* parent chromosome is *VV* (i.e., $\overline{RR\ Mp}$) and the other *RR*, at the *P* locus. That is to say, the Modulator component of *VV* is lost from one only of the two daughter chromosomes. Modulator is transposed in this process to a different site in the same or another chromosome. Also, the chromosome strand to which *Mp* is conveyed from the *P* locus must pass to the same daughter nucleus as the *VV* chromosomes, thus giving rise to the light variegated phenotype. The *RR* sector in a twin spot will thus lack Modulator. The following diagram illustrates this hypothesis:



The available twin spot data serve to test only one of the assumptions on which this model of the origin of Modulator is based. Is the supposition correct that the light variegated co-twin to self color is identical with the light variegated genotype to which the data in tables 1 to 4 apply?

The few data on hand on the genotype of the kernels in twin spots are presented in table 8. The plants bearing the five ears containing these twin spots were heterozygous for colorless pericarp, and were mated to colorless pericarp lines. The evidence bears on the composition of the *VV* and *RR* gametes only. Another generation is required to complete the separate test being made on the distribution of Modulator in the gametes bearing colorless pericarp. Twenty-three plants were obtained from the light variegated twin spot kernels, of which 15 proved to have colorless pericarp. Four of the plants with colored pericarp were light variegated, two were medium variegated, and two were self red. The numbers of plants are too few to be conclusive on the point, but the relatively high proportion of light variegated offspring is as expected if the twin spot kernels of this phenotype correspond to those on entire light variegated ears. Twenty-five plants with colored pericarp were obtained from the medium variegated control kernels on the twin spot ears. One of these plants had self colored pericarp, 23 were medium variegated, and only one was light variegated. The 21 self colored kernels from the twin spots gave nine self colored and 12 colorless offspring. It seems reasonably likely, therefore, that both the phenotypes appearing as co-twins are identical genetically with the same respective classes of whole ears. If the results from the tests now in progress on the constitution of the gametes bearing *WW* or *WR*, with reference to the differential between light and medium variegated, accord with those given above, it may be considered as proven that self color and light variegated arise concurrently from a single mutational event affecting two different loci.

It is apparent from the relevant data in tables 1 to 4, which are summarized in table 6, that among the offspring of medium variegated plants, self colored ears appear with about the same order of frequency as light variegateds. This fact may be understood on the assumption that the light variegated and a corresponding number of the self colored ears are the result of complementary somatic mutations. The self colored class, however, is usually in excess. The difference may be due to Modulator frequently failing to become reestablished elsewhere in the chromosome complement after removal from the *P* locus. A mutational event of this kind would also explain the occurrence of self color mutant areas which are not twinned. An additional condition must be met, however, in the formation of a twin spot. The transposition of Modulator from the *P* locus to another site must be followed by passage of the recipient chromosome to the daughter nucleus which receives the *VV* strand, rather than the *RR* strand, of the dividing chromosome in which the mutation occurs. Presumably, Modulator is without effect on the self colored phenotype. Thus the appearance of light variegateds among the offspring of medium variegateds may be explained as the result of newly arisen mutations. Medium variegated does not contain Modulator as an element separate from the *VV* allele. It possesses the capacity to generate it, however, in conjunction with the transformation of *VV* to the self colored allele.

Consideration may next be given to the frequencies with which light and medium variegated and self colored ears occur among the offspring of light variegated plants. We have attempted below to account for the breeding behavior of the light variegateds in terms of segregation of Modulator considered as a relatively stable unit. It is clear that segregation is an operative factor in these families, but the extent to which the frequencies of lights and mediums are affected by somatic mutations of Modulator as well is not known. Mutations of light variegated to medium variegated occurring relatively early in ontogeny would be disclosed by the presence on the light variegated parent ears of large sectors of rather heavily variegated tissue. Such readily detectable mutant patches were not present on the ears in question. Late occurring somatic mutations of light to medium variegation, however, giving rise to mutant sectors involving a part of a kernel, often would not be recognizable because of the comparatively coarse nature of the variegated pericarp pattern. The effect of such late occurring mutations on the progeny of light variegated heterozygotes would be confounded with that of segregation at meiosis. Independent data on the stability of Modulator are needed to clarify this point. Meanwhile the possibility must be recognized that the factual basis for an interpretation of the breeding behavior of light variegateds may be incomplete in an essential respect.

The high proportions of light variegated progeny from heterozygous plants of this phenotype backcrossed to standard colorless pericarp (tables 1 to 4) are interpreted as the result mainly of segregation of Modulator, whose presence distinguishes this class of plants. If it were assumed that in light variegateds, *Mp* occupies a position 50 crossover units from *P* on chromosome 1, or were located on a non-homologous chromosome, and that no new mutations were occurring, then one-half the colored offspring of these test matings should be light variegated. The frequencies observed in several families, particularly in the first and second generations of backcrosses to inbred line 8, approximate this value rather regularly. Thus it may be concluded that the site which *Mp* occupies after removal from the *P* locus frequently is remote from *P*.

Inspection of all the relevant data in tables 1 to 4, however, shows that the ratios from family to family in some backcross groups are far from homogeneous. Evidently *Mp* does not always assort independently of *VV*. Linkage between Modulator and *P* could account for the ratios obtained in certain families. On this hypothesis, the additional assumption must be made, however, that the site which Modulator occupies, after transposition from the *P* locus, varies widely. For example, in family 6-80 entered in table 2, all the variegated offspring are of the light class, indicating very close linkage between *VV* and *Mp*. The percentage of light variegated offspring in family 6-81 is 63.3 percent, corresponding to a rather loose linkage. The value drops to 12.7 in the third sister family, namely, 6-82. It is necessary to assume in the latter case that Modulator is coupled with *WR*, rather than *VV*.

The data in table 5, showing the distribution of Modulator in relation to *WR* among the offspring of light variegated *VV/WR* plants mated with *VV/WW* individuals are explainable in the same terms. The proportions of light variegated plants, although clearly set off, for the most part, from the corresponding group of data from the medium variegated sibs, are notably variable, even between certain sister families. In spite of the very close relationship between N124, N125, and N127, for example, these families contained 56.7 percent, 28.6 percent, and 18.2 percent, respectively, of light variegated plants. The average scores for variegation grade of the progeny ears show clearly that the same two classes of offspring, light and medium variegated, are present throughout. That is to say, the three families differ only in the proportions in which these two phenotypes occur. On the supposition that linkage is the principal factor determining the distributions it is necessary to postulate that Modulator occupied a different site in each case.

It is quite clear from these several considerations that Modulator cannot be explained in conventional genetic terms either in respect to origin or mechanism of inheritance. There are other facts, not presented in this report, which must be brought into relationship with each other and with the present evidence before a general interpretation of the phenomenon can be undertaken profitably. For example, ANDERSON (1924) has shown that variegated mutates to colorless pericarp as well as to self color, although much less often. These white mutations have not been studied, and the mating plan used in the present investigation would not disclose them. Furthermore, it is evident from unpublished data which we have obtained that more than one kind of *P* allele occurs conditioning the so-called calico type of variegation which characterizes the present stocks. The basis for any general conclusions obviously will remain incomplete until the effects on the variegated phenotype of major variations both at the *P* locus and elsewhere in the genome can be integrated. A further well established fact which remains unexplained is the higher rate of mutation of *VV* to *RR* in *VV/WR* heterozygotes than in *VV/VV* homozygotes. These are some of the reasons why discussion of the problem is necessarily speculative at this stage. A certain orderliness in the experimental results appears, nevertheless, when the difference between light and medium variegated pericarp is looked at from the point of view outlined above. No more is claimed for the hypothesis offered, however, than that it serves currently as a basis for continuing the analysis.

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SUMMARY

1. Three distinct pericarp phenotypes, light and medium variegated, both of which are unstable, and self colored, which is stable, were obtained in each F_1 family resulting from the application of a single collection of pollen from a particular homozygous variegated (*VV/VV*) maize plant to a series of different inbred strains having either colorless pericarp, red cob (*WR/WR*) or colorless pericarp, white cob (*WW/WW*). The average proportions in which the three phenotypes appeared were 10.7 percent, 72.4 percent, and 16.8 percent, respectively.
2. Breeding tests were made designed to disclose the inheritance conditioning variegated pericarp transmitted through (1) the *VV* gametes and (2) the *WR* gametes, formed by the light variegated and medium variegated F_1 sib plants. The data on the *VV* gametes were obtained by backcrossing through two successive generations to the appropriate inbred line. Composition of the *WR* gametes was inferred from the character of the red cobbed offspring arising from the F_1 *VV/WR* plants after mating with unrelated *VV/WW* individuals.
3. Most of the offspring within the relevant classes, obtained from the medium variegated plants, were medium variegated, following both types of test matings. That is to say, the potentiality for determining the medium variegated phenotype was transmitted through a high proportion of both the *VV* and *WR* gametes. A few light variegated plants also appeared regularly in these families, together with a somewhat higher proportion of self colored individuals.
4. In contrast, the light variegated plants gave relatively high proportions of light variegated offspring in both kinds of test matings. The frequency of medium variegated plants was correspondingly reduced; and the proportion of self colored individuals also was lowered.
5. Counts made on medium variegated ears showed that a patch of mutant self colored tissue was frequently twinned with a light variegated area. The limited breeding data available indicate that light variegated and self colored

co-twins correspond to these respective classes of kernels as they occur on entire ears. If this finding is confirmed it means that self color and light variegated arise concurrently from a single mutational event affecting two different loci.

6. As a working hypothesis, it may be assumed that the unstable variegated allele, *VV*, is a modified form of the stable *RR* gene, the difference between them being that *VV* embodies a discrete element, termed Modulator, *Mp*, which inhibits pigment formation. Mutation of *VV* to *RR* consists in the loss of *Mp* from the *P* locus.

7. Following removal from the *P* locus, *Mp* may become attached at one or another position elsewhere in the chromosome complement. Modulator thus situated, plus *VV*, gives the light variegated phenotype. *VV* alone conditions the medium variegated phenotype.

8. Modulator cannot be explained in conventional genetic terms either with respect to origin or mechanism of transmission. At this stage an interpretation on any other basis is necessarily speculative.

9. The relatively few light variegated and self colored offspring of medium variegated plants are interpreted as carrying newly arisen mutations. The character of the families from light variegated plants is conditioned both by such mutations and the segregation of *Mp* as a unit separate from the *P* locus.

10. The varying proportions of light and medium variegated offspring of light variegated plants are interpreted in terms of linkage or independence of Modulator relative to the *P* locus.

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