exist. It is recognized that chromatographic analyses need to be repeated after further generations of backcrossing, so that the genotypes in the various cytoplasms are more nearly alike. But this may not be as essential as appears, for in our extensive investigations of T cytoplasm it was found that the chromatographic pattern of ninhydrin-positive spots in the early backcross generations was identical with the pattern of the later generations.

A second noteworthy feature of the results of the restorer tests concerns cytoplasmic sources F and H. According to the notation accompanying the original seed, the male sterility of source F was iojap induced by introducing the ij gene into the cytoplasm of a normal, fertile line. If this is correct, then S type cytoplasm can arise from the action of the ij gene. Alternatively, it may be suggested that S cytoplasm and S restorers were present in the original female parents, and that as a result of the crosses with iojap the restorer genes were lost, thereby allowing expression of male sterility. In this connection a peculiar feature of the S type sterility system should be pointed out. Since in plants with S cytoplasm the non-restoring allele is eliminated in the pollen, and all functional pollen carries the restorer allele, there is no way, in the normal course of events, whereby sterile plants can arise in a population with S cytoplasm, so long as selfs and intercrosses are confined within the population. The presence of sterile cytoplasm would not be suspected unless outcrosses were made to pollen parents which possessed non-restoring alleles associated with normal (or T) cytoplasm.

The second cytoplasm of interest, source H, is derived from Ind. 33-16. The finding that this is S type cytoplasm means that the peculiar behavior of Ind. 33-16 described by Josephson and Jenkins apparently lies with nuclear factors rather than with the cytoplasm.

Harry T. Stinson, Jr.

3. Chlorophyll variegation in normal and sterile WF9 lines.

In the 1959 MNL the mode of inheritance of a chlorophyll variegation in a WF9 stock was reported. The results were interpreted to indicate that the chlorophyll abnormality, characterized by streaks of pale to yellow-green leaf tissue, was cytoplasmically inherited. Additional crosses grown in 1959 support this interpretation. Three families of the third backcross generation of WF9 streaked ♀ x WF9 normal ♂ again produced all streaked plants. In contrast, the reciprocal backcross, WF9 normal ♀ x WF9 streaked ♂, now in the second generation, gave all green offspring. Also mentioned in last year's report was the fact that it had not been possible to eliminate the chlorophyll abnormality by selecting, from earlier generations, what appeared to be green plants. This statement still holds. Selfs of the greenest plants again produced some obviously streaked offspring, as did the second generation backcrosses of WF9 "green" ♀ x normal WF9 ♂. The intensity of streaking is, however, somewhat less severe in families of the "green" plants than in families of the obviously streaked plants. As previously mentioned, the families in which this chlorophyll abnormality appeared were derived from two WF9 plants which carried S type cytoplasm, but which had undergone a change from male sterility to male fertility. The event leading to the alteration in pollen behavior presumably occurred in the cytoplasm. As yet there is no indication of any causal connection between the chlorophyll aberration and the alteration in fertility.

In 1959 other WF9 lines were examined for signs of the chlorophyll disturbance. These lines included several normal WF9 stocks as well as cytoplasmic male sterile lines in various stages of conversion to a WF9 genotype. In an attempt to evaluate the chlorophyll variegation on a slightly more objective basis, the top 8 leaves of the plants in each family were scored for the extent (number and size of streaks) of yellow or pale green streaking on a scale from 0 to 5. By adding the values for the 8 leaves it is possible to get numerical expressions for the intensity of variegation for each streaked plant, and from these an average value for the family can be calculated. In scoring, relatively small

streaks that probably would not be noticed in a more superficial inspection were counted and given a value of 0.5. Thus, some plants tabulated as variegated would probably have been considered green in previous years. The results are summarized in the table below.

Family	Pedigree	No. Plts.	No. Variegated	% Variegated	Avg. intensity of variegation
1	WF9 normal	19	4	21%	0.75
2	" "	16	5	31%	0.60
3	" "	20	7	35%	0.57
4	" "	18	9	50%	0.72
5	" "	17	4	24%	2.60
6	" "	20	4	20%	1.60
7	" "	20	4	20%	2.00
8	" "	17	5	29%	1.40
	Totals	147	42		
			Avg.	29%	1.29

Family	Pedigree	No. Plts.	No. Variegated	% Variegated	Avg. intensity of variegation
9	WF9 A5	22	19	86%	11.3
10	" B7	17	17	100%	1.9
11	" C4	17	11	65%	1.2
12	" D4	18	12	72%	2.1
13	" E5	14	14	100%	2.1
14	" F4	17	17	100%	2.3
15	" G3	17	13	76%	1.7
16	" H6	16	11	69%	11.5
17	" S12	17	10	59%	2.2
	Totals	155	125		
			Avg.	81%	4.03
18	WF9 T11	32	0	0	0

Streaked plants appeared in all 8 families of normal WF9. In 4 of these families, as seen from the values in the last column of the table, (Nos. 1 - 4), the variegated plants were only slightly variegated, with only 1 or 2 leaves showing a small non-green streak. Such plants are essentially green, and these 4 families may be considered to be virtually free of variegated plants. Although the proportion of streaked plants is no greater, the values expressing the average degree of variegation are higher in the remaining 4 WF9 normal families (Nos. 5-8). In each family this is the result of the presence of one plant with fairly extensive streaking, i.e. families 5-8 contained one obviously streaked plant in addition to some slightly streaked plants like those in families 1-4.

The various sterile versions of WF9 with cytoplasmic sources A through H, and with type S, also contained streaked plants. In all families the proportion of streaked plants was greater than in any of the normal WF9 lines. The average intensity of variegation observed in all sterile WF9 lines was also relatively high, none of the values reaching the low readings obtained in 4 of the normal WF9 families.

The best comparison in this sense is between the sterile WF9 lines and family 1 of the normal WF9 stocks, which was the recurrent parent used for the conversion of all sterile lines.

These results tend to suggest that the chlorophyll abnormality occurs with a higher frequency and with greater severity in the WF9 sterile families. Since in terms of pollen abortion, cytoplasmic sources A through H are of the S type (see preceding page), the readings for the 9 sterile families have been pooled for comparison with the pooled data from the 8 WF9 normal families. It will be noted that approximately 3 times more plants were streaked in the sterile families, and also that the intensity of streaking was about 3 times greater in the sterile families. However, it is apparent that not all sterile families were identical with respect to intensity of variegation, for the plants in WF9 A5 and WF9 H6 were clearly more severely streaked.

No streaked plants were observed in a single family of WF9 T sterile. We have, however, noted in past years an occasional streaked individual among WF9T plants, and Brown and Duvick have also reported chlorophyll streaking in their WF9 T material. Variegation in WF9T stocks probably occurs with about the same frequency as in WF9 normal.

Although the precise explanation of the origin and inheritance of the chlorophyll abnormality in WF9 is perhaps uncertain, there are some features about the phenomenon that seem fairly well established from our own studies and from those of other investigations (see Brown and Duvick, MNL 1958, p. 120). The chlorophyll abnormality appears sporadically and "spontaneously" in WF9 stocks, and few lines of the inbred seem to escape entirely the production of an occasional streaked plant. Whatever its mode of origin, the character, once initiated, is then maternally inherited. Rarely, however, a streaked plant may appear in the progeny from a cross of the type normal WF9♀ x streaked WF9♂. While this might indicate male transmission of the character, it is difficult to be certain of this interpretation, since the streaked phenotype also occurs in selfs of normal WF9 and in crosses between normal lines.

One possible interpretation of the variegation phenomenon would be that the WF9 genotype from time to time induces alterations in the cytoplasm or plastids, the altered cytoplasmic constituents subsequently being maternally transmitted and remaining in the altered condition throughout sexual generations in the absence of the specific WF9 genotype which produced the alteration. On this view the abnormal chlorophyll condition in WF9 is analogous to the variegation brought about by the iojap gene, except that homozygous ij/ij plants are regularly variegated, whereas only an occasional WF9 plant is variegated. This explanation focuses attention on the WF9 genotype as the primary cause of the abnormal plastid condition.

As an alternative explanation which does not involve the WF9 genotype, it could be suggested that the plastid alterations arise directly from mutations in the plastids, or other cytoplasmic elements capable of self-duplication. On this interpretation, there is apparently an unstable condition specifically characteristic of certain cytoplasms (including the cytoplasm in many WF9 stocks), but not characteristic of other cytoplasms. There are reasons for considering this second explanation less likely. First, the variegation which was so frequent in the WF9 S, and A through H stocks, has not appeared in a similar series of these same cytoplasms with an A158 genotype. Second, variegation in WF9 plants has been observed by investigators working with various selected and reworked lines of WF9. It seems questionable that all of these lines would have the same source of cytoplasm. With available evidence, there is thus reason for believing that the WF9 genotype is somehow intimately associated with the origin of the chlorophyll variegation, although this genotype may not be required for maintenance of the condition.

If the WF9 genotype has the property of inducing or allowing the expression of cytoplasmic alterations affecting the chloroplasts, the results reported above may indicate an additional basis for dis-

tinguishing among cytoplasms. If it is true that variegation is more frequent in the WF9 S steriles, this would mean that the normal and sterile cytoplasms differ not only in their effects on pollen viability, but also in the cytoplasmic factors whose response to a WF9 nucleus produces the chlorophyll abnormality. Also, there is some indication of differences in the response of cytoplasms A - H. In short, it is suggested that the relative frequency with which cytoplasmically inherited plastid alterations occur in the presence of a WF9 genotype may be used as a criterion for characterizing different cytoplasms. This is only a speculation, and further investigation is needed to establish the validity of this approach.

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4. The origin of cytoplasmic sterility in maize.

So far all of the many different sources of cytoplasmic male sterility or pollen abortion fall into two distinct groups which we have designated S and T. These two plasmatypes together with the usual cytoplasm, which may be called the M type, found in most of the cultivated maize varieties commonly grown throughout the world, form three distinct classes of cytoplasmic differences. Their classification is based on their interaction with fertility restoring genes. It is possible that these cytoplasms originated in the different species that are considered to have had a part in the development of cultivated maize. These are the primitive pod corn or pro-maize described by Mangelsdorf, Tripsacum (gama grass) and Euchlaena (teosinte). Mazoti has shown that chlorophyll genes that are aberrant in maize cytoplasm are normal in teosinte cytoplasm. This is evidence that teosinte cytoplasm is different from that of Zea mays.

If this conjecture should be borne out by more complete evidence it would show that cytoplasmic differences are permanent over very long periods of time and that they are more important in the origin and separation of species than is generally realized.

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5. The performance of restored-sterile hybrids.

Many double crossed hybrids made on sterile seed parents with various non-restoring and restoring pollinators have been compared in yield, time of maturity, and stalk quality. The results are given in our annual corn report (Conn. A. E. S. Progress Report G1, 1960). Where natural restoring inbreds are used with either normal fertile or sterile inbreds as pollinator single crosses, the final double crosses are equal in performance in all characters measured. The actual ratio of fertile and sterile plants in percent is 56:44 where the pollinator is fertile x restorer and 58:42 where the pollinator is sterile x restorer. The excess of fertile plants in both cases is probably due to minor modifying factors.

Twelve different double crosses were made with restoring pollinators nearly alike in genotype but differing in plasmatype. One series is a normal fertile inbred x a restored sterile inbred. The other series is the sterile version of the same inbred by the same restored sterile inbred. In both series the segregation of fertile and sterile plants is practically the same; 47:53 in the normal x restored sterile, and 46:54 in the sterile x restored sterile. In both series there is a slight excess of sterile plants. The excess of steriles in this series and an excess of fertiles in the other is probably due to residual gene differences in the different inbreds used for the sterile seed parents and restoring pollinators. For practical purposes the differences are not important since adequate pollen production is supplied in both series.