

Carbon monoxide, which is found on comets may possibly be derived in part from the radical decomposition of acetone. An observation that acetone and water will yield reducing sugars on prolonged exposure to intense sunlight¹⁴ needs confirmation as well as a report on the occurrence of different hydrates of acetone.¹⁵ One of these hydrates of acetone with a composition of $C_3H_6O \cdot 17 H_2O$ has been shown to exist by Quist and Frank only recently.¹⁶ The formation of hydrates of small organic molecules in space is of considerable astronomical interest and has been discussed by Miller.¹⁷

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**THE STAGE OF THE GENOME-PLASMON INTERACTION IN
THE RESTORATION OF FERTILITY TO CYTOPLASMICALLY
POLLEN-STERILE MAIZE***

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The expression of various characters in many organisms is known to depend upon genome-plasmon interactions, but information on the time and location of these interactions is meager. In phenomena such as the killer trait in *Paramecium*,¹ the production of a normal cytochrome system in *Saccharomyces*,² the modified slow-growth (*poky*) character in *Neurospora*,³ and certain phenomena described by Michaelis,⁴ the interaction appears to be continuous; however, in many other cases the interaction possibly takes place in only certain tissues (or cells) and at only certain stages during the development of the organism. In view of the great

deal of work which has been done in the area of developmental genetics, time and place of interaction could be important factors in development. That time and location of a genome-plasmon interaction could also influence the heredity of nuclear genes would not be so readily anticipated. Time and place of such an interaction, their effect upon pollen development, and their effect upon the inheritance pattern of a chromosome segment have revealed themselves during a study of the restoration of fertility to cytoplasmically induced pollen-sterile maize.

In the process of combining a sterility-inducing plasmon and its restorer(s) (chromogenes capable of restoring fertility to plants with this plasmon) with various inbred nuclear genotypes during the course of seven years (1950–1956) at the Connecticut Agricultural Experiment Station, there occasionally appeared backcross progenies which contained all fertile plants. These progenies resulted from crosses which, according to Mendelian segregation, should have given rise to segregating families, for the male parents were theoretically heterozygous for a restorer gene(s). In view of the fact that the progenies of hundreds of similar crosses were grown during this period, that only from ten to eighteen representatives of an individual progeny were grown, and that many different genic ratios are possible, it was thought that these nonsegregating rows represented extreme variations due to chance, or were the expression of a complex genic system.⁵ The writer's attention was called to the problem by H. T. Stinson when in 1956 a few progenies, all with similar genic material, had only fertile plants where segregation was expected. A pedigree search involving all relatives of all the nonsegregating cultures since 1949 was undertaken by the writer. When the search was completed and all the crosses and their resulting progenies were organized in pedigree fashion, it immediately became evident that many of the families with this behavior could be placed into one group. The pedigree of the progenies of the group displayed a somewhat consistent behavior pattern, and all entries fell into place fairly well. The study of this group revealed the phenomena presented below.

Table 1 shows the relationship and observations of some of the families in the group. The families have been listed according to the year grown, and in generation sequence. Specific families have been given numbers (in parentheses) so that they can be identified in subsequent crosses. The first entry listed reveals the source of restoration. Since $[ms_2]A158$ (see Table 1 for key) is sterile, the seven fertile segregates in the progeny must have resulted from the action of a gene(s) introduced by Ky21. When one of these fertile plants was backcrossed to the sterile line, instead of segregation, only fertile plants were recorded. While it is conceivable that some sterile plants were present but not recorded, the behavior of subsequent generations makes this unlikely.

All subsequent generations seem to follow the same pattern. Whenever an $[ms_2]$ -sterile was pollinated by a plant of the type $[ms_2]Rf$ heterozygous for the restorer(s), only fertile plants resulted. The one sterile plant in the progeny of $[ms]A73^4 \times [ms_2]RfA158^4(5)$, which appears to be an exception, could have been due to any one of a number of nonpertinent factors, and shall be tentatively disregarded. Likewise when selfed, such heterozygous plants produced only fertile progeny; that is, except the progeny of $[ms_2]RfA158^4(5)$ selfed. One of the three sterile plants in this culture was definitely "off type," indicating that it was an outcross. Since outcrosses usually do not occur singly, the other two may have

TABLE 1
STEPS IN THE CONVERSION OF A158 TO ITS RESTORED-STERILE COUNTERPART, AND
OBSERVATIONS OF THE FAMILIES*

	Family no.	No. of ears	Year grown	Observations
$[ms_2]A158^4 \times (Ky21 \times A158)$	(1)	2	1951	7+, 5-
$[ms_2]A158^5 \times [ms_2]RfA158^1(1)$	(2)	1	1952	MAP
$[ms_2]A158^6 \times [ms_2]RfA158^2(2)$	(3)	3	1953	MAP about normal
$[ms_2]A158^7 \times [ms_2]RfA158^3(3)$	(4)	?	1954	All MAP
$[ms_2]RfA158^3(3) \times [ms^+]A158$	(5)	3	1957	All 29+
			1958	23+, 4-
			1958	21+, 17-
$[ms_2]RfA158^4(4) \times [ms_2]RfA158^4(5)$	(6)	1	1955	All 19+
			1955	17+, 3-?
$[ms_2]RfA158^4(5)$ selfed	(7)	3	1955	11+, 5-
			1957	30+, 24-
$[ms_2]A73^4 \times [ms_2]RfA158^4(4)$..	1	1955	+
$[ms_2]A73^4 \times [ms_2]RfA158^4(5)$..	3	1955	Remainder +, 1-
$[ms_2]A374^4 \times [ms_2]RfA158^4(4)$..	3	1955	All +
$[ms_2]A374^4 \times [ms_2]RfA158^4(5)$..	4	1955	All +
$[ms_2]M14D^7 \times [ms_2]RfA158^4(4)$..	?	1955	All +
$[ms_2]M14D^7 \times [ms_2]RfA158^4(5)$	(8)	1	1955	All +
$[ms_2]RfA158^4-S_1(6)$ selfed	(9)	1	1956	All +
			1957	All 35+
$[ms_2]RfA158^4-S_1(6)$ selfed	(10)	1	1956	All +
			1957	All 42+
			1956	All +
$[ms_2]RfA158^8(7)$ selfed	(11)	1	1956	All +
			1957	All 68+

* Crosses and observations made prior to 1957 were made by D. F. Jones. MAP = many anthers extruded, pollen; + = fertile; - = sterile; $[ms^+]$ = normal plasmatype; $[ms_2]$ = sterility-inducing plasmatype (sometimes referred to as "S"); Rf (not italicized) = restorers present but genotype not known; Ky21, A158, A73, A374, and M14D = residual genotypes (inbreds). (Superscripts designate the number of backcrosses to the nbred.)

had that origin also. When heterozygous restored-sterile plants were pollinated by the inbred ("reciprocal" cross), however, the two families segregated.

These data suggested the operation of a type of male gametophytic selection which insures fertilization by pollen grains carrying the restoring allele(s). To investigate this problem further, samples of remnant seed of the previously grown families were planted again in 1957. Observations of these samples (Table 1) were similar to those made by D. F. Jones. Many plants in each culture were used in making one or two of the three types of "crosses": $[ms_2] \times [ms_2]Rf$, $[ms_2]Rf \times [ms^+]A158$, and $[ms_2]Rf$ selfed. Portions of the tassels of many plants were taken and preserved for later analysis.

Offspring of the crosses made the previous year were planted and observed in 1958. Whenever an $[ms_2]RfA158$ plant (heterozygous for the restorer(s)) was used as a male, no segregation occurred; while in crosses where these were not used as the male segregation always resulted. The presence of an equal number ($P = 1.0$ and $0.9-0.8$) of fertiles and steriles in the segregating families clearly demonstrates that the difference between the steriles and fertiles in these progenies was brought about by a single gene (Rf^2) introduced by Ky21. As determined by microscopic examination of anther contents, the amount of normal pollen produced by randomly chosen plants which were theoretically heterozygous for the restorer was about 50% of that produced by the normal counterpart ($[ms^+]A158$) on the same day.

It appears, then, that all the pollen grains bringing about fertilization had the restorer. Consequently the inheritance pattern can be explained by assuming that

only pollen grains with Rf^2 function, while those with the alternate allele (rf^2) abort. The hypothesis was tested as follows: (1) Anthers from plants in families resulting from a self-pollinated plant of the type $[ms_2]Rf^2rf^2A158$ were checked for degree of normal-pollen production. It was found that half of the plants in such families produced pollen 50% of which was aborted, while about an equal number ($P = 0.3-0.2$) of plants exhibited no such abortion of pollen. This is to be expected if each viable pollen grain of the parent carried Rf^2 and only half the egg nuclei contained the restoring allele. The plants in these "F₂" families exhibiting no abortion would, therefore, be Rf^2Rf^2 , while those with 50% abortion would be the heterozygous offspring. (2) When these "F₂" plants with about normal pollen production were used as females in crosses with $[ms^+]A158$ the progenies contained only fertile plants; in contrast, when the segregates with 50% pollen abortion were used in similar crosses with $[ms^+]A158$, the progenies gave a 1:1 sterile-to-fertile ratio. This would also be expected if indeed the plants with no abortion were Rf^2Rf^2 , and those with the excess abortion were heterozygous for the restorer.

These observations seem to establish firmly the idea expressed in the foregoing hypothesis.

Apparently, then, the mechanism of selection is the interaction of the $[ms_2]$ plasmagene and the Rf^2 chromogene: the abortion is due to the presence of the $[ms_2]$ factor, and the normal pollen grains produced by plants carrying $[ms_2]$ are not aborted because of a type of epistatic effect of Rf^2 upon $[ms_2]$. To test this further, Rf^2 was combined with two other types of cytoplasm: $[ms_1]$, another type of sterility-inducing plasmon; and $[ms^+]$. Plants heterozygous for the restorer gene with these types of plasmon exhibited no selection for Rf^2 .

Since the restoration process takes place only in those spores with Rf^2 , it can be concluded that there is no way for a sufficient amount of the essential product of the restoring gene to get into the cytoplasm of the spores which abort. This condition could only result if the critical restoring action takes place at a place and time when the cytoplasm of the different spores are separated by a barrier to the transfusion of the essential substance. Such circumstances only exist in the microspore or maturing pollen grain, i.e., after the formation of the crosswalls during microsporogenesis, the onset of the gametophytic generation. Further, also the critical time of production of the primary diffusible gene product must occur after the barrier is formed. This is because all other conditions necessary for the completion of the restoration process exist in both types of spores, for the residual genotype is the same in each spore.

Knowing the approximate time of critical restoring action helps to establish also the time when the critical step in the abortion mechanism occurs in $[ms_2]$ -steriles. Several steps must precede this critical stage: among them, the synthesis of the primary diffusible chromogene product; and the more direct restoration process. Since the critical times for these two steps occur after microsporogenesis, the critical stage determining the abortion must occur only a short time before the deterioration can be seen, i.e., in the early stages of spore maturation.

The type of nuclear-cytoplasmic interaction, and consequently the inheritance pattern and degree of pollen abortion, encountered with the $[ms_2]$ plasmatype is different than that encountered with the $[ms_1]$ type (sometimes referred to as "T"). Plants with $[ms_1]$ which are heterozygous for the restorers may produce as

much pollen as their normal counterparts,⁵ and the restorers (Rf_1^1 and Rf_2^1) segregate (by progeny observation) normally.^{6, 7}

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ENZYME COMPLEMENTATION IN MIXED EXTRACTS OF MUTANTS FROM THE SALMONELLA HISTIDINE B LOCUS*

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Imidazoleglycerol phosphate dehydrase, the enzyme in the pathway of histidine biosynthesis (see Table 1) which converts imidazoleglycerol phosphate (IGP) to

TABLE 1
TERMINAL STEPS IN HISTIDINE BIOSYNTHESIS

	Reaction	Related <i>Salmonella</i> gene
(1)	IGP $\xrightarrow{\text{IGP dehydrase}^3}$ IAP + H ₂ O	<i>his B</i>
(2)	IAP + L-glutamate $\xrightleftharpoons{\text{IAP transaminase}^4}$ HLP + α -ketoglutarate	<i>his C</i>
(3)	HLP + H ₂ O $\xrightarrow{\text{HLP phosphatase}^5}$ Histidinol + PO ₄	
(4)	Histidinol + 2DPN ⁺ + H ₂ O $\xrightarrow{\text{Histidinol dehydrogenase}^6}$ Histidine + 2DPNH + 2H ⁺	<i>his D</i>

These reactions, based upon observations in *Neurospora*, in *E. coli*, and in yeast, also occur in *Salmonella*.¹ Genetic,² and biochemical studies¹ have correlated 3 of the reactions with the genes indicated; no specific gene was correlated with reaction (3). Abbreviations used are: IGP (imidazoleglycerol phosphate); IAP (imidazoleacetol phosphate); HLP (histidinol phosphate), DPN and DPNH (diphosphopyridine nucleotide and reduced diphosphopyridine nucleotide, respectively).

imidazoleacetol phosphate (IAP), has been correlated with the *his B* genetic locus in *Salmonella typhimurium*.¹ This locus is complex in that tests by abortive transduction have indicated four subgroups with the capacity for genetic complementation.² This paper reports that complementation also occurs between cell extracts, i.e., that dehydrase activity appears in mixtures of extracts from appropriate dehydraseless *his B* mutants. Biochemical and immunochemical data thus far obtained indicate that this complementation is effected through interaction of protein components. The capacity, or lack of the capacity, of *his B* mutants to yield IGP dehydrase activity in extract mixtures correlates well with the genetic com-