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RENEWAL REQUEST

PROGRESS REPORT

for period

April 1, 1985 through March 31, 1989

THE ROLES PLAYED BY MITOCHONDRIAL DNA AND NUCLEAR GENES
IN REVERSION TO FERTILITY IN S-TYPE MALE-STERILE MAIZE

CURRENT CONTRACT

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Collaboration

Over the four-year period of the DOE grant, as before, we have continued collaborations with other laboratories where studies related to ours are underway. We have had a long-standing cooperative arrangement with Chris Leaver at the University of Edinburgh. A joint paper with Leaver and others comparing cytoplasmic revertants to fertility from different cms-S sources has recently been published.

In the course of the work in our nurseries we occasionally come across nonchromosomal stripe (NCS) mutants. NCS mutations are due to mtDNA alterations. We pollinate these mutants when possible and establish NCS strains. We are collaborating with Kathy Newton at the University of Missouri on the analyses of a number of NCS strains. Two papers resulting from these collaborations have recently been submitted for publication.

We have just begun a collaboration with Christiane Fauron at the University of Utah on a comparative study of cms-T and cms-P mtDNAs. She already has a cms-T WF9 library and map. We have supplied her with seed of cms-P WF9 with which to begin the studies. This laboratory has identified an inbred line which restores cms-P but not cms-T. This summer we will be making F1s of various isogenic cms-T and cms-P strains with this inbred line and Christiane will be studying the basis for the differential restoration at the DNA and RNA levels.

List of Publications from Laboratory
1985-1989

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The Project Description in the original research proposal included the following major categories of research:

1. Molecular Basis for Nuclear Reversion
2. Molecular Characterization of Cytoplasmic Revertants
3. Nuclear Control Over Cytoplasmic Reversion and Over Replication of S1 and S2
4. Developmental Studies
5. Transposition of Nuclear Restorer Elements

Introduction

Our research concerns the basic mechanisms responsible for cytoplasmic male sterility (CMS) in certain strains of maize and for spontaneous mutations (reversions) that result in recovery of male fertility (normal pollen function). The molecular determination of CMS is in the DNA of the mitochondrial chromosome(s) (mtDNA). We have shown that the organization (sequences) of mtDNA units is controlled by the nucleus of the cell, that significant changes in mtDNA organization occur when one nuclear genotype is substituted for another, that similar reorganizations of mtDNA accompany cytoplasmic reversion of CMS to male fertility, that the nuclear genes that restore fertility (Rf genes) in the S type of CMS are located at various sites

in the chromosomes of the nucleus, and that these Rf genes are transposable elements (transposons). We have also found that certain spontaneous mutations (reversions) to apparent male fertility carry newly-arisen Rf genes that are lethal to the male gametophyte (pollen) and provide a unique opportunity not only to identify, map and characterize, at the molecular level, genes that are indispensable for successful pollen function but also to similarly characterize the restorer (Rf) genes at the molecular level. A knowledge of the structure and function of these genes can lead to an understanding of the molecular basis for CMS in maize and, by extension of this knowledge, to higher plants in general.

Molecular Basis for Nuclear Reversion

On July 31, 1989 we conclude five years of support from the Competitive Grants Section of the USDA Program on Plant Biology and Human Nutrition. The field genetic studies supported by USDA were undertaken to identify cases of insertion of transposable controlling elements (CEs) into nuclear restorer-of-fertility (Rf) genes of cms-S and of insertion of transposable Rf genes into known maize loci. The goal of these studies is the isolation and identification of Rf gene sequences. It is anticipated that molecular characterization of the Rf gene will help determine the mechanism of nuclear reversion to fertility in cms-S plants. The USDA phase of the work has been done in collaboration with Dr. Patricia Bedinger of the University of North Carolina at Chapel Hill and she is analyzing the four putative cases identified in the USDA studies. The use of cloned probes developed in these studies is regarded as an important aspect of this DOE project even though the development of such probes was supported with funds from another agency. We intend to continue these studies under the auspices of DOE, using strains we have now developed for a much improved protocol as indicated in the RENEWAL PROPOSAL.

Molecular Characterization of Cytoplasmic Revertants

Early studies on the mitochondrial DNA (mtDNA) of cytoplasmic revertants to fertility in cms-S strains indicated features that all had in common. Reversion was found to be associated with the disappearance of the S1 and S2 episomes (Levings et al., 1980; Laughnan et al., 1981), and with specific changes in the main mtDNA S2 sequences (Schardl et al., 1985). However, the revertants analyzed all arose in the inbred line M825 or in hybrid M825/Oh07 nuclear background. We have been characterizing revertants that arose in other inbred backgrounds, specifically, WF9, 38-11 and H95, as well as those arising in hybrid WF9/M825 backgrounds. Revertants in these nuclear backgrounds do not exhibit the same mtDNA changes as the M825 revertants. Revertants in the 38-11 and H95 nuclear backgrounds lose S1 and S2 upon reversion as do M825 revertants. Revertants in the WF9 background, however, retain S1 and S2 (Escote et al., 1985; Ishige et al., 1985). In an accompanying 1989 Maize Genetics Cooperation News Letter (MGCNL) we report that revertants arising in hybrid WF9/M825 backgrounds, all involving cms-RD WF9 (RD is a subtype of S) as the original female parent and crossed variously by M825 and some by WF9 following that, exhibit variation as to whether they lose or retain S1 and S2. Sequences homologous to S1 and/or S2 exhibit changes in revertants from all nuclear backgrounds compared to their sterile progenitors. These changes are different from those observed in M825 revertants and are different from one another. We do however observe changes which seem to be characteristic of a given nuclear background. This similarity of revertants from the same nuclear background indicates nuclear control. At least some of this control is exercised on the organization of

the mtDNA before reversion (see following section). Even though we would expect reversion to fertility to somehow correct the defect in S-type cytoplasm that causes male sterility, no common mtDNA sequence has been identified that is altered in all revertants. Confounding the analyses is the fact that mtDNA alterations upon reversion are detected using a number of different probes. It is as though the initial mtDNA rearrangement that results in reversion to fertility causes a series of "adjustments" to be made in mtDNA organization. We are continuing to characterize cytoplasmic revertants from different inbred backgrounds but will be focusing our efforts towards finding the one mtDNA alteration common to all reversion events.

Nuclear Control Over Cytoplasmic Reversion and Over Replication of S1 and S2

In most cms-S strains, the S1 and S2 episomes occur in equimolar amounts. In M825, the molar amount of S1 exceeds that of S2; in 38-11, the reverse is true. It has been shown (Laughnan et al., 1981) that, not only are S1 and S2 under nuclear control, they respond to the nuclear background independently of one another. In an accompanying 1988 MGCNL article we report on studies of the effect of nuclear background on S1 and S2 as carried in cms-S sterile plants and in fertile WF9 cytoplasmic revertants as well as on the related R1 and R2 molecules present in some fertile RU strains. These studies were essentially complete at the time of the MGCNL report except for the conversion of cms-S M825 to 38-11. We predicted that this conversion would exhibit the reduced S1 pattern characteristic of 38-11. Recent studies have shown, however, that the episomes in this strain are essentially equimolar, with perhaps a slight excess of S2, after nine recurrent crosses of cms-S M825 by 38-11. These converted strains do not exhibit the usual 38-11 S1 to S2 ratio. It is possible that the molar amount of S1 was reduced by the 38-11 nucleus as predicted but that S2 was not amplified or perhaps S2 was amplified as predicted but S1 not reduced. Alternatively, S2 may have been amplified somewhat and S1 reduced somewhat so that these molecules now appear essentially equimolar. These alternatives cannot be distinguished since equal amounts of mtDNA from the converted strain and the control cms-S M825 and cms-S 38-11 were not loaded on the gels. This will be done in the future.

Cytoplasmic revertant strains from inbred line M825 cms-S background are stable. No mutations back to the male-sterile condition have been observed in these strains, even following a backcrossing program designed to convert their nuclear backgrounds to ones that might favor such mutations. The mtDNA studies of these revertants (Schardl et al., 1985; Escote-Carlson et al., 1988) suggest that the changes occurring upon M825 reversion would be irreversible.

Sterile plants have been observed to occur from the cytoplasmic revertants that arose in the WF9 nuclear background. The mtDNA organization of one of these unstable revertant strains, and progeny descended from one of its exceptional sterile plants, is under intensive study by Carol Leja as part of her Ph.D. work. This sterile strain is unstable and frequently segregates fertile plants. This offers a unique opportunity to study the cycle of mtDNA alterations concomitant with changes from sterile organization to fertile, back to sterile and to fertile again. Since there are many mtDNA changes accompanying cytoplasmic reversion to fertility, it is hoped that this unstable sterile strain will help us to distinguish the change responsible for the male-fertile phenotype from those that are a consequence of this initial change.

The unstable revertant strain arose on an ear that gave rise to two other cytoplasmic revertants (sibling revertants) as well. These two are stable and are used as controls along with the sterile progenitor strain and the

exceptional sterile strain. Differences are detected between the revertant strains and their sterile progenitor strain using probes for the S episomes, coxI, atp9, atp α , IR and R regions of cms-S. Differences between the unstable revertant and its sibling revertants are also detected with some of these same probes. The unstable revertant strain retains some of the sterile arrangement of regions lost from its sibling revertants detectable with the probes for coxI, atp9, IR and R. The atp α probe detects band changes in intensity and rearrangement not easily interpreted. The mtDNA organization of the exceptional sterile strain that was derived from the unstable revertant is different from that of the progenitor sterile strain. It appears, therefore, that this is not merely a case of low level retention of the sterile mtDNA organization and its subsequent amplification and sorting out. These strains continue to be studied.

Because the mtDNA organization of cytoplasmic revertants from identical cms-S sources differs according to nuclear background, we investigated whether these differences could be a reflection of differences in organization of the mtDNA genome before reversion. This is the case; mtDNA organization is under nuclear control. Accompanying this report is a preprint giving our results to date concerning reorganization of cms-RD WF9 upon conversion to M825. We are in the process of examining reorganization with additional mitochondrial gene probes and in additional line-cytoplasm combinations. Sterile reorganization of cms-S appears not to be a general phenomenon. It is not exhibited in all cases in which the nuclear background is changed. It is not detected by all mtDNA gene probes (e.g. coxII, cob, coxIII and atp6) and therefore involves only localized regions of the genome. The mtDNA regions exhibiting reorganization when the nuclear-background is changed are the same ones exhibiting reorganization upon cytoplasmic reversion of the male-sterile cms-RD WF9 to fertility. These studies were part of the Ph.D. thesis of Loida Escote and are being continued by Carol Leja.

Developmental Studies

Studies of the time course for the disappearance of S1 and S2 associated with cytoplasmic reversion as outlined in the April 1, 1985 PROPOSAL have been postponed. We have ample supplies of seed for these studies but they are labor intensive and we have placed emphasis on other investigations in this category.

In maize the first vertical division of the embryo initial determines the right and left halves of the plant, a plant that is bilaterally symmetrical through the midribs of the leaves. This pattern of development was deduced by studying gamma- and X-ray induced losses of chromosome segments in dry seeds whose embryos were heterozygous for various marker genes (Steffensen, 1968; Coe and Neuffer, 1978).

Plants in the M825 inbred background have an average of over 35 tassel branches and ten percent of cms-S plants exhibit spontaneous cytoplasmic reversion to fertility (Laughnan et al., 1981), expressed as fertile-sterile tassel sectors or totally fertile tassels in families of plants expected to be all male sterile. Only plants with large tassel sectors in which the main rachis is included in the reversion event, or those with totally-fertile tassels have been observed to have correlated reversion events in their ears. We have carried out a study of plants with these two types of events in stocks of cms-VG, cms-I, cms-ML and cms-RD WF9, cms-S 38-11, cms-VG N6, cms-VG K55 and cms-ML M14, all of which have been converted to the M825 nuclear background by ten recurrent backcrosses using M825 inbred line as the male parent. Ears on such plants were pollinated to determine whether they were

included in the reversion events; the progeny were scored as male-fertile, male-sterile or mixed (both fertile and sterile). Tassels of plants with large sectors were mapped to determine the positional relationship of the sector with the ears borne on these plants. Ears subtending the fertile tassel sectors were considered to be "in", those subtending the sterile portion of a sectorized tassel were considered to be "out", and in a third class ears were judged near enough to the projected fertile-sterile tassel interface to be considered "on the border".

The studies by Steffensen and by Coe and Neuffer referred to above predict that fertile tassel sectors larger than one-half of the tassel are not likely to occur. Such fertile sectors were observed, however, in about 25% (12 cases) of the 52 plants with large tassel sectors that were studied intensively. Four plants with large tassel sectors had exactly 50% of the tassel fertile, and 33 plants had fertile sectors involving the main rachis but fewer than 50% of the tassel branches. In three cases, the total number of tassel branches on plants with large sectors (15, 16 and 17 branches fertile) was not determined, but based on the average number of tassel branches these would have involved 50% or less of the tassel.

Tassels of these 52 plants with large fertile sectors were mapped and ears on these plants were pollinated by M825 maintainer (nonrestoring) plants. In 21 cases the ears were mapped "out" and in all 21 cases the crosses by maintainer pollen produced male-sterile offspring plus the usual minority of plants with fertile tassel sectors resulting from newly-occurring reversion events. The sizes of fertile sectors on tassels of these 21 plants ranged from 17.2% to 51.2% of the tassels. Only two of these fertile sectors, however, involved 50% or more of the tassel. In 15 cases mapped either "on the border" (9 cases) or "in or on the border" (6 cases), 13 gave male-sterile progeny, one gave mixed progeny and one gave all fertile progeny. The two cases producing fertile progeny were among the six considered "in or on the border" so this result is not unexpected. The 15 cases ranged in size from 19.0% to 54.3% of the tassel. Only three of these sectors involved 50% or more of the tassel and these produced male-sterile progeny. The case that produced mixed progeny involved 27.0% of the tassel and the case producing all fertile progeny 31.4%. Of the 16 cases mapped "in" (25.6 to 95% of the tassel) only eight (25.6% to 95%) gave entirely male-fertile progeny, while three (35 to 86.7%) gave mixed progeny and five (39.4 to 84.6%) gave male-sterile progeny. This result from the 16 "in" cases was unexpected but, as it turned out, not inconsistent with the results obtained from ears borne on plants with totally fertile tassels. These were also expected to produce entirely fertile progeny. Of 36 such plants analyzed, only 22 produced entirely male-fertile progeny. Four produced mixed progeny and ten produced male-sterile progeny. A total of 31% of the ears considered to be "in" large fertile sectors, and 28% of the ears borne on plants with totally fertile tassels gave male-sterile progeny.

The above observations indicate a correspondence between male-fertile "in" tassel sectors or entirely fertile tassels and male-fertile progeny from ears on such plants crossed with nonrestoring pollen; in other words, in these cases the male-fertile products of the cytoplasmic reversion event are found in both tassel and ear of the same plant. Even so, in about 30% of plants with this type of tassel fertility the progeny from their crossed ears were male-sterile. Why is there not perfect correspondence between tassel and ear fertility in these cases? We believe the answer lies in the timing of the primary event that leads to fertility. If it occurs in the mother cell that divides to produce the two daughter cells that define right and left halves of the mature plant, or in one of those two daughter cells, both tassel and ear

initials should carry the reversion, and correspondence of male-fertile elements in tassel and subtending ear is expected. On the other hand, if the reversion event occurs much later, after cells ancestral to tassel primordia and those ancestral to ear initials are defined, noncorrespondence between male-fertile elements in tassel and ear is expected. This model is consistent with numbers of instances in which ears on male-sterile plants crossed by maintainer plants have produced male-fertile progeny.

The theory of midrib symmetry for the corn plant is not consistent with the position of all fertile tassel sectors, numbers of which, including large ones, have been found to overlap the midrib borderline. It might be argued that "sorting out" of mitochondria can explain these observations since the theory was developed as a result of studies of sectors that occurred following the loss of nuclear genes. We think this is unlikely since such "sorting out" of organelles should still exhibit correspondence with cell lineage patterns; it is more likely that these "nonconforming" fertile tassel sectors result from discrepant or noncoincidental patterns of cell division during very early stages of embryo development.

As a result of independent spontaneous reversion events, we have established eight nuclear revertant strains, in the five CMS inbred line backgrounds WF9, Oh51A, B37, M14 and Mo17, that are phenotypically male fertile but functionally male sterile. These were termed "pseudorevertants" in the 1985 PROPOSAL at which time there were six such strains in four inbred backgrounds. We now refer to them as "pseudorestorer" genes and use the symbol Rf-nf for nonfunctional restorer gene. The inheritance of these Rf-nf genes through the egg is that expected of a nuclear restorer gene. All eight strains exhibit 50% pollen abortion as expected if they are heterozygous for a nuclear restorer (Rfrf). In five of the strains the remaining 50% of pollen grains appear normal and in the other three strains the remaining 50% of pollen grains are only partially filled. When used as pollen parents all eight strains produce essentially barren ears.

The nonfunctional nature of these Rf-nf strains, and their allelism and viability as homozygotes, are being studied in a hybrid background with M825 inbred line. Preliminary results of these studies are described in an enclosed report which appeared in the 1989 MGCNL. Two alternative hypotheses have been proposed to explain the characteristics of the Rf-nf strains. These are also described in an enclosed report in the 1989 MGCNL. To distinguish between the two hypotheses we have developed a procedure which, by analogy with the behavior of normal and defective lambda particles in E. coli (Appleyard, 1956) we have called the "helper" experiment. This protocol, along with preliminary results, is described in this same MGCNL report.

Transposition of Nuclear Restorer Elements

Most cms-S rfrf (male-sterile) inbred lines we have studied exhibit spontaneous nuclear reversion to fertility (cms-S Rfrf), some with greater frequency than others; only inbred lines N6 and IllA, among the 15 lines we have studied, have given no revertants. The origin of these mutations is still obscure. In this case, to say that the Rf revertants arise de novo is to imply that their male-sterile progenitors carry quiescent Rf sequences and that perhaps Rf revertants occur when these are activated by an infrequent homologous or nonhomologous recombination event. These silent Rf sequences may be absent in nonreverting inbred lines or they may carry the sequences but lack the activation event(s).

As indicated earlier in this PROGRESS REPORT there is indication that the nuclear reversion event is attended or caused by transposition of Rf sequences. In particular, two of the Rf-nf (nonfunctional) pseudorestorer

revertants occurred in association with failure of function(s) necessary for normal male gametophyte development, suggesting that transposition of Rf sequences into genes vital for pollen function has occurred.

Even the spontaneous Rf revertants that have normal pollen function have other deleterious effects. Earlier studies of ten such revertants indicated that they occupy different chromosomal sites and that, compared with the natural cms-S restorer (Rf3) located in chromosome 3L, they have unusual properties:

- (1) Seven of the spontaneous Rfs are homozygous lethal, that is, when heterozygous (S) Rfrf plants are self pollinated the ears exhibit 50% ovule abortion and no plants with all-normal pollen (RfRf) are found among the offspring.
- (2) The same seven Rf strains have a deleterious effect on endosperm development when they are present in two doses in that tissue.
- (3) Two others of the 10 restorers, RfV and RfX, are homozygous viable but kernels with RfRfRf endosperm genotype are smaller than RfRfrf kernels on the same ear; in these two cases RfRf sporophytes are also less vigorous and later to mature than their Rfrf siblings.
- (4) Normal-cytoplasm heterozygotes, (N) Rfrf, of all ten spontaneous revertants, crossed as male parents onto (S) rfrf male-sterile testers, exhibit a greatly reduced male transmission of the Rf allele as indicated by the excess of male-sterile vs. male-fertile plants among offspring.

We were led initially to undertake studies on transposition of the spontaneous nuclear Rf revertants when mapping experiments indicated that they could occupy different chromosomal sites, and when the variety of deleterious effects associated with these Rfs were identified. The hypothesis that these revertants result from transposition of Rf sequences with insertion mainly into genes coding for functions that are indispensable for sporophyte development appeared consistent with the Rf gene properties described above. If the revertants are Rf-carrying transposons they must be highly selective regarding their insertion sites as all but RfIV have a deleterious effect on endosperm development, but visible mutants involving the kernel, seedling and mature plant are not associated with them.

As described in previous PROGRESS REPORTS the studies on transposition involved three of the studied revertants, Rfs I, III and VI, all of which are homozygous lethal with deleterious endosperm effects, and RfIV, which is homozygous viable and without apparent damaging endosperm effects. RfIII and RfVI are located in chromosome 2L (they are allelic), RfI is in chromosome 8, and RfIV is in 3L about 8 map units from lg2. We had identified wx-translocation stocks with breakpoints in close linkage with wx and with the particular Rf gene involved. An initial cross was made between an Rf stock, say RfI, and the appropriate translocation stock:

CROSS 1: (S) RfI N Wx/rf N Wx X (N) rf T8-9d wx/rf T8-9d wx

where (N) refers to normal (fertile) cytoplasm, N to noninterchanged chromosome and T to a particular translocation (8-9d) whose breakpoint is located favorably close to RfI in chromosome 8. In the translocation F1 heterozygote produced from this cross the RfI and wx recombination is about 10-12%. The F1 plants, all heterozygous for Rf, wx and T are testcrossed to cms-S waxy homozygous, nonrestorer, noninterchanged testers:

CROSS 2: (S) rfrf wxwx X (S) RfI N Wx/rf T8-9d wx

Since pollen grains carrying the nonrestoring allele rf abort, only RfI grains function so that the percentage of waxy kernels on these testcross ears provides a direct estimate of RfI-wx recombination for each male parent testcrossed. Without transposition, about 10% of kernels on the testcrossed ear should be waxy. If the male parent carries a transposed RfI gene at a more distant site than in the control, the event would be identified by a recombination rate significantly higher than 10%. Transposition to a site in another chromosome would register 50% recombination, while insertions into a more distant site in chromosome 8 are expected to give intermediate recombination rates.

In the previous PROGRESS REPORT we indicated that evidence for transposability was found for all four Rf genes tested according to the protocol described above. In some cases the Rf-wx recombination rates are significantly below 10%, suggesting that the Rf gene has moved closer to the translocation breakpoint in chromosome 8, and to wx in chromosome 9. Many tested plants showed intermediate Rf-wx recombinations and there were cases of recombination not significantly different from 50%. Overall, from 10 to 15% of plants tested (see CROSS 2) gave Rf-wx recombination rates significantly different from the control rate of 10%.

We have been cautious, perhaps overly so, about publishing these results. We found it difficult to accept such high frequencies of transposition, and wanted not only to repeat the experiments but to seek corroboration through other approaches. In the previous RESEARCH PROPOSAL we indicated experiments would be undertaken to determine whether the restorer elements transpose to preferred chromosomal sites, are the genetic properties of the transposed Rf changed in transit, is transposition replicative or nonreplicative, and does the standard restorer Rf3, located in the long arm of chromosome 2 undergo transposition?

We have repeated the transposition experiment described above; the results corroborated the findings in the original experiments, indicating that all four Rfs exhibit unusually high rates of transposition.

We had planned to use the homozygous-lethal trait to determine whether the genetic properties of the transposed restorer are changed, and whether the Rf genes transpose to preferred sites. Thus, as indicated above, Rfs I, III and VI are homozygous lethal; do they retain this trait when transposed? RfIV is homozygous viable; are its transposed versions viable or lethal? Surprisingly when self pollinations of transposed and control versions of Rfs I, III, IV and VI were made, almost all were found to be homozygous viable. Likewise, for Rfs I, III and VI, in which tests for allelism are based on results of crosses of transposed and control versions with the corresponding Rf tester stocks, ear sets were normal, indicating lack of allelism. Since both transposed and control cases gave the same result we have considered that different background genotypes may be responsible. The genetic background of our tester stocks may favor lethality of the Rfs when homozygous, while the genetic background of the translocation heterozygotes in the transposition experiments may be permissive in this regard. We have tested this possibility and have found that Rfs I, III and VI are homozygous lethal in the translocation background. We have also made heterozygotes between these Rfs and numbers of inbred lines whose backgrounds might permit viability of the Rf homozygotes. Preliminary results (with many more F1 combinations to be selfed this summer) suggest that the inbred line backgrounds do not influence the lethality of Rf homozygotes. We are left with the possibility, pending this summer's results, that the translocation backgrounds, mainly of W23 and M14 origin, promote high rates of transposition so that even cases with control

rates of recombination in the experiment have transposed to nearby sites and no longer give a positive test for allelism with the parental Rf, or exhibit homozygous lethality when self pollinated.

We have carried out experiments designed to determine whether the naturally-occurring Rf3 restorer carried by the inbred line CE1 is transposable. The results indicate that it is. The title of the relevant 1989 MGCNL abstract submitted with this PROGRESS REPORT is: "Evidence for transposition of the naturally-occurring cms restorer in inbred line CE1".

A second 1989 MGCNL abstract submitted with this proposal is titled: "Naturally-occurring restorers of cms-S are located at various chromosomal sites in different inbred lines and appear to be transposable". The title gives the gist of this report; these findings suggest that the Rf3 natural restorer gene has a history of transposition, such that different inbred lines we have today carry Rf3 at different sites in chromosome 2 and possibly even in different chromosomes. The results from tests of inbred-line sibling plants suggest very high transposition rates for Rf3 in at least two inbred lines, R177 and H95.

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