Theoretical and Applied Genetics 43, 109–116 (1973)

Mutations Leading to Nuclear Restoration of Fertility in S Male-Sterile Cytoplasm in Maize[1,2]

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Summary. Among the offspring of crosses involving S male-sterile *shrunken-2* inbred lines and their corresponding isogenic maintainer lines a number of exceptional male-fertile plants were identified. Some of these were plants with entirely fertile tassels but most were chimeras involving both sterile and fertile tassel elements. The majority of male-fertile exceptional plants, upon crossing with male-sterile testers, produced male-sterile test-cross progeny, indicating that the male-fertile trait is not pollen transmissible. However, there were four separate instances, involving three of the inbred lines, in which the crosses with S male-sterile testers produced male-fertile progeny, indicating that the newly arisen male-fertile trait is pollen transmissible. In three of these cases, the male fertility can be traced to a single plant in essentially male-sterile families. The fourth evidently involved a change in a maintainer plant whose progeny thereafter segregated for the ability to restore S sterile cytoplasm. In all cases, the results of progeny tests are consistent with the gametophytic pattern of restoration associated with S male-sterile cytoplasm.

The male-fertile exceptions described here can be accounted for formally as mutations at one or more restorer gene loci in the nucleus. Taking account of the fact that mutations of restorer genes have not been reported previously in maize, and that four such changes were encountered in the same strains in which we have identified other male-fertile exceptions involving change in the cytoplasm, we have suggested a common basis for the two kinds of events. According to this scheme, given the first appearance, by whatever process, of the male-fertile element in sterile cytoplasm, it may become established and continue to propagate either in the cytoplasm or in the nucleus. In the former case, the change registers as cytoplasmic and the new strain has the characteristics of a maintainer which transmits the male-fertile trait through the egg, but not the sperm; in the latter case, the change occurs in the nucleus and the new strain, now behaving as a restorer, transmits male fertility through both egg and sperm.

Professor Marcus M. Rhoades, whose outstanding contributions in the field of plant science, and of plant genetics in particular, are most appropriately celebrated in the publication of this issue, was the first to describe the inheritance of cytoplasmic male sterility in corn. His first paper (1931), published in Science, is a brief preliminary account of a graduate thesis study carried out in R. A. Emerson's laboratory at Cornell. It was followed by a comprehensive report on the same subject in the Journal of Genetics (Rhoades, 1933). We are delighted to have the opportunity to participate in this commemoration and, in view of Dr. Rhoades' pioneer contributions in this field, it is a particular pleasure to report here on investigations dealing with cytoplasmic male sterility and its genic restoration.

While the early investigations carried out by Rhoades led him to conclude that the trait with which he dealt "is determined entirely by the non-nuclear elements of the maternal gamete", it is now apparent, and has been for some time, that the expression of cytoplasmic male sterility in corn, and in a large number of other plant species, is a function of the nuclear genotype as well as of the condition of the cytoplasm (see reviews by Edwardson, 1956, 1970; Duvick, 1965). Nuclear genes which obviate the effect of sterilizing cytoplasms are known as restorers of fertility, designated *Rf*. In maize, such restorer genes have been identified in inbred lines, hybrids and open-pollinated varieties from a number of geographic sources (Duvick, 1965). So far as we are aware, there are no reported instances of mutation at restorer gene loci in maize. The present preliminary report deals with studies of four recent independent occurrences of mutations which restore fertility in S sterile cytoplasm and are pollen transmissible.

The cytoplasmic male-sterile strain studied by Rhoades has since been lost. Nevertheless, there have been repeated instances of independent discoveries of male-sterile strains which satisfy the criteria for involvement of the cytoplasm in the inheritance of the trait. Duvick (1965), for example, indicates a total of at least eighty-four separate discoveries of cytoplasmic male sterility in corn. Since the cytoplasm is involved, classical Mendelian procedures can not be used to categorize these traits. Nevertheless, the existence of nuclear genes, usually dominant in action, which restore male fertility in spite of the presence of sterile cytoplasm, has provided the opportunity to characterize and group the various strains. The usual procedure is to cross the cytoplasmic male-sterile individuals with pollen from a series of estab-

[1] Dedicated to Dr. M. M. Rhoades on the occasion of his 70th birthday.

[2] Research supported in part by CSRS grant 177-15-04, PL 89-106, and by NSF GB 29488.

lished inbred lines and note the pattern of restoration among the offspring. On this criterion, two kinds of male-sterile cytoplasms have been recognized for some time (Duvick, 1965). One of these is designated T (Texas), the other S (USDA). Beckett (1971) analyzed a number of cytoplasmic male-sterile strains by crossing and backcrossing repeatedly with a series of inbred lines used as pollinators. Thirty male-sterile strains from a variety of independent sources, and eighteen inbreds were involved in the study. On the basis of the pattern of restoration, most of the male-sterile cytoplasms were assignable to either the T or S groups. Two of the sources, however, cmsC and cmsRB, had restoration responses which fit neither the T nor the S pattern. The sterility-inducing cytoplasm which they carry has been designated C. It seems not unlikely that still other male-sterile cytoplasms will be identified.

Two gene loci, Rf_1 and Rf_2, have been identified as restorer factors for the T male-sterile cytoplasm (see Duvick, 1965). In this system, restoration is determined by restorer genotype at the sporophytic (diploid) level. Thus $Rf_1\,rf_1\;Rf_2\,rf_2$ plants with T cytoplasm produce all normal pollen in spite of the fact that only one-fourth of the pollen grains produced by these plants carry the $Rf_1\;Rf_2$ genotype. On the other hand, as Buchert (1961) has shown, restoration of fertility to plants carrying S-type cytoplasm is determined by the genotype of the gametophyte. Thus, a plant with S cytoplasm that is heterozygous for the appropriate restorer locus, in this case $Rf_3\,rf_3$, is semifertile in that about half of the pollen grains, those carrying Rf_3, are normal and functional, while the other half, those carrying rf_3, are aborted and nonfunctional. In agreement with this interpretation, Buchert found that when such plants are used to pollinate S male-sterile individuals, all the offspring are semifertile, exhibiting 50% pollen abortion. He also showed that when plants with S cytoplasm and the genotype $Rf_3\,rf_3$ are self pollinated they produce equal numbers of two types of offspring: those, with all normal pollen, whose crosses with maintainer plants indicate that they are $Rf_3\;Rf_3$ in genotype, and those, with 50% aborted pollen, whose crosses with maintainer plants indicate that they carry the $Rf_3\,rf_3$ genotype. The suggestion is clear that the events which determine restoration in T-type cytoplasm occur at an earlier developmental stage than those responsible for restoration in S-type cytoplasm.

Because it has only recently been positively identified, male-sterile C cytoplasm has not been intensively investigated. On the other hand, the characteristics and behavior of S and T cytoplasms, including their responses to nuclear restorer genes, are well documented through numerous studies. Both S and T cytoplasms, in particular the latter, have been employed, along with appropriate restorer genes, in the commercial production of hybrid seed corn, and in the course of this enterprise there has been the opportunity to observe large numbers of cytoplasmic male-sterile progenies. From these and other observations, Duvick (1965) concluded that S and T male-sterile cytoplasms are notoriously stable. Moreover, there are no recorded instances of spontaneous mutation in the cytoplasm of maize from normal to male-sterile condition; the male-sterile cytoplasms so far identified were encountered either as already occurring male-sterile plants in families, or as segregants following a cross involving a male-fertile female parent having sterile cytoplasm (restored phenotype) with a male parent (maintainer) not carrying the restorer gene.

Singh and Laughnan (1972) reported on a number of cases of instability of S male-sterile cytoplasm. The exceptional male-fertile individuals encountered in these studies occurred among the otherwise male-sterile progenies of S male-sterile female parents crossed with maintainer (nonrestorer) pollinators. Subsequent analyses of the fertile exceptions, including 1. progenies from testcrosses with S male-sterile plants, 2. F_2, F_3 and F_4 progenies in the exceptional pedigree, and 3. progenies from repeated backcrosses of the exceptional fertile individual with maintainer pollinators, indicated that the exceptional male fertility could not be attributed to the action of either dominant or recessive nuclear restorer genes. The results were consistent with the hypothesis that the event responsible for the appearance of the unexpected fertile plants involved a change from male-sterile to male-fertile condition in the cytoplasm. Numerous instances of such inherited, cytoplasmic changes from S male-sterile to normal male-fertile condition have since been identified. We have shown that the events occur most frequently in certain pedigrees, that the tassels of exceptional fertile plants may either be entirely fertile or exhibit fertile chimeras, and that, on occasion, ear chimeras also occur. Whether or not the exceptional event involves a qualitative change, analogous to gene mutation, in a cytoplasmic entity or entities governing the expression of male fertility, we are uncertain. In the absence of evidence bearing on this question, we have considered it equally likely that it is the result of occasional transfer of normal cytoplasm through the male germ cells of maintainer parents routinely employed in these crosses.

The above account involving instances of change in S male-sterile cytoplasm is especially pertinent to the studies reported here inasmuch as the cases of mutation at restorer loci which we will discuss occurred in the very progenies which were being searched, successfully as it turns out, for cases of changes in the cytoplasm from male-sterile to male-fertile condition. We therefore call attention, at this point, to the fact that the unanticipated appearance of male-fertile individuals in otherwise S male-sterile progenies may

result from change at either the cytoplasmic or the nuclear-gene level, and that the question of which of these is involved can only be determined on the basis of subsequent appropriate progeny tests.

Materials and Methods

Male-sterile and male-fertile (maintainer) versions of five *shrunken*-2 (sh_2; shrunken endosperm) inbred lines were employed in these studies. Each of the fertile lines and its male-sterile counterparts are essentially isogenic, having been maintained by selfing and backcrossing to the male-sterile version for from twelve to fifteen generations. The inbred lines are R839, R851, R853, R853N and M825. The male-sterile cytoplasm incorporated into these inbred lines traces to a *Vg* (dominant vestigial glume) source which has been shown to be equivalent to the S (USDA) sterile cytoplasm (Stinson, 1960; Beckett, 1971; Singh and Laughnan, 1972).

Male-sterile plants from the sh_2 inbred lines indicated above were crossed by their corresponding maintainer inbred lines and the resulting progenies were searched for male-fertile exceptions among otherwise male-sterile offspring. Plants with entire tassels fertile, and those with fertile tassel chimeras were either self pollinated or crossed as egg parents by the corresponding maintainer inbred line. Exceptional male-fertile plants were also routinely crossed as pollinators with sibling male-sterile individuals and, in some instances, with male-sterile plants of other sh_2 inbred lines. In a few instances they were also crossed with an S male-sterile version of commercial inbred WF9. Exceptional male-fertile plants in the original progenies and in those from the crosses described above were, in a number of instances, examined for percentage of aborted pollen grains using a field pocket microscope (magnification 20×).

Results

Over 300 exceptional male-fertile plants which were identified in progenies during the 1971 summer growing season at Urbana were subsequently progeny tested to determine whether the fertility they exhibited occurred at the cytoplasmic or nuclear level. Included in this group were individuals with entirely fertile tassels and larger numbers of plants with sectors of fertility in the tassel. In all but five instances, the progenies of testcrosses with S male-sterile plants indicated that the male-fertile character of exceptional plants was not transmitted through the pollen. These results are consistent with those of previously tested cases in indicating that the sudden appearance of male fertility in otherwise S male-sterile progenies is based on a change in the cytoplasm. One of the five cases mentioned above has not yet been confirmed by further progeny tests and will not be dealt with here. It is clear, however, that the remaining four cases do not fit the usual pattern described above; in each instance, testcrosses of these exceptional male-fertile individuals with S male-sterile plants produced male-fertile offspring, suggesting a Mendelian, or nuclear, basis for the fertility. One of these cases occurred in inbred line R853N; two others occurred in R853 progenies; and the fourth was identified in M825, the inbred line which is particularly susceptible to corresponding changes at the cytoplasmic level (Singh and Laughnan, 1972). Because the circumstances accompanying the original appearance of these male-fertile exceptions vary somewhat, and because there is also some variation in their performance in progeny tests, they are given individual treatment in the following account. Results are presented under headings corresponding to the lines in which the exceptions appeared.

R853N Case

This apparent instance of restorer gene mutation is unique in that it is the only one of the four in which the first occurrence of fertility can not be traced to a single exceptional plant. During the 1971 growing season, nine families, involving 286 plants, resulting from crosses of R853N S male-sterile individuals with R853N isogenic maintainer (nonrestorer) plants were searched for cases of tassel fertility. Eight of these families, involving 224 plants were essentially male-sterile; in one of these, there were two plants with tassel fertility which could not be tested further; in another there were two plants with fertile tassel chimeras which outcrosses with male-sterile tester plants indicated were cytoplasmic in origin; and in still another family, among 31 plants, there was one, with an entirely fertile tassel, whose analysis again indicated that a change at the cytoplasmic level was involved. The ninth family, 71-741, consisting of 62 plants, had male-sterile and male-fertile plants in about equal numbers and field examination of a number of the male-fertile plants in this family indicated pollen abortion levels of from 50 to 70 per cent Further analyses of this unusual family are detailed below.

Four of the exceptional male-fertile plants in family 71-741 were self pollinated and crossed with male-sterile sibling plants. All offspring from both types of cross were male-fertile; there were 74 male-fertile offspring from the four self pollinations and 75 male-fertile offspring from the male-sterile testcrosses. Another plant in family 71-741, which was not self pollinated, was testcrossed with a male-sterile sibling and all tested offspring, 38 in number, were male-fertile. This same plant was test crossed with a single S male-sterile plant in each of five strains of the inbred line WF9. There were 190 plants in the resulting five test-cross progenies and all were male-fertile.

The appearance of exclusively male-fertile offspring among the progeny of self-pollinated male-fertile exceptions does not bear directly on whether the basis for fertility lies in the cytoplasm or the nucleus (Singh and Laughnan, 1972). As noted earlier, we have encountered a number of cases, which turned out to represent cytoplasmic changes, in which male-fertile exceptions, when self pollinated, produced all male-fertile offspring, and when test crossed with male-sterile plants produced all male-sterile progeny. At the same time, the peculiarities of gametophytic restoration in the S male-sterile system (Buchert, 1961) are such that plants with S cytoplasm

that are heterozygous for the genic restorer are also expected to yield all male-fertile offspring from self pollinations. The progenies from male-sterile test crosses of the male-fertile exceptions are, however, critical for the question of a nuclear versus cytoplasmic event. If it is cytoplasmic, these test-cross progenies, through failure of transmission in the pollen, are expected to be male-sterile; if it is nuclear, that is, the result of change at a restorer locus, the test crosses are expected to yield male-fertile offspring. Moreover, again because of the gametophytic character of S restoration, all plants in such progenies are expected to be male-fertile. The male-fertile exceptions in inbred line R853N, family 71-741, fit this pattern. As noted above, five male-fertile exceptions in this family were crossed with male-sterile testers and all 303 plants in the progenies were male-fertile. These results are consistent with the hypothesis that a dominant nuclear restorer is responsible for the exceptional male-fertile individuals that appeared in family 71-741.

The results of advanced generation progeny tests in the 71-741 pedigree are also consistent with the restorer gene interpretation. It will be recalled that self pollination of four exceptional male-fertile plants in this family produced 74 male-fertile F_2 offspring. It was possible to classify the pollen characteristics of 54 of these plants into two groups: 23 plants had essentially normal pollen, and 31 plants were semifertile, exhibiting from 50 to 60 percent pollen abortion. On the restorer hypothesis, again taking note of the gametophytic nature of S restoration, the former would be considered homozygous, the latter heterozygous, for the newly arisen restorer allele. Three of the semifertile F_2 plants were testcrossed as female parents with pollen from maintainer inbred lines; one of the crosses involved the inbred line maintainer R853N itself, another the inbred line M825, and the last the commercial inbred line N6. Each of these crosses gave both male-fertile and male-sterile offspring, the total numbers of each for the three progenies being 19 and 21, respectively. Self pollinations and male-sterile testcrosses of both male-fertile and semifertile plants in these same progenies yielded only male-fertile offspring. These results are consistent with the behavior of a genic restorer in S cytoplasm; both restorer and nonrestorer alleles of heterozygotes in S cytoplasm are expected to transmit through the female gametophyte but the nonrestorer allele is not expected to transmit through the pollen of such plants.

The foregoing evidence indicates that the male-fertile exceptions in family 71-741 resulted from nuclear restoration. The occurrence of a number of such male-fertile exceptions in the same family raises the question of their origin. The possibility that they arose as a result of fertilization by contaminating pollen grains carrying a restorer gene is excluded on the following grounds. First, the male-fertile exceptions, as well as their male-sterile siblings both lacked vigor and exhibited the characteristic morphological traits of the R853N inbred line. Second, the fertile exceptions can not be accounted for either through contamination or a legitimate cross involving pollen from R853N plants carrying an S restorer gene since we have not developed an R853N restorer strain. We are obliged, therefore, to categorize the event as a mutation, at a restorer gene locus, from nonrestorer to restorer condition.

Since both fertile and sterile plants appeared in family 71-741, it is reasonable to consider that the restorer gene mutation occurred somatically during the development of the male-sterile female parent of this progeny such that the ear borne on this plant was a chimera with both sterile (nonrestored) and fertile (restored) female reproductive elements. However, evidence now at hand does not support this interpretation. The male parent of exceptional family 71-741 was 70-108A-3. Four fertile siblings of this plant were crossed with isogenic R853N male-sterile plants. One of these crosses, involving the 70-108A-7 sibling male parent, also produced both male-fertile and male-sterile progeny, 12 and 10, respectively. The other three gave only male-sterile offspring. These results suggest that the restorer gene mutation occurred in the R853N maintainer strain which was involved as the male parent of progeny in family 71-741. Further tests support this conclusion. We have routinely propagated isogenic male-sterile and maintainer versions of inbred lines by selfing an individual maintainer plant and crossing it onto an isogenic male-sterile plant in a companion family. Paired male-sterile and male-fertile maintainer families are planted from these crosses and the pollination procedure described above is repeated. The male parent, 70-108A-3, of exceptional family 71-741, was self pollinated and three plants among the progeny (family 71-741A) were testcrossed with isogenic male-sterile plants in family 71-741. Two of the test-cross progenies produced both male-fertile and male-sterile offspring, while the third gave exclusively male-sterile plants. These findings thus exclude the possibility that the exceptional male-fertile plants in family 71-741 arose by mutation in the R853N male-sterile female parent of that progeny; they indicate, rather, that the change, presumably a mutation at a restorer gene locus, occurred in the male-parent R853N maintainer strain and that it continues to be propagated in that strain through self pollination.

R853 Case #1

Two additional cases of presumptive restorer gene mutation were identified among the progenies of crosses between S male-sterile plants of inbred line R853, and R853 maintainer plants. Twelve such progenies, involving 457 plants, were grown and observed for instances of tassel fertility. In nine of these families all plants, 220 in number, were male-

sterile. In another of these progenies, all but one of the plants were male-sterile. In the case of the single exceptional plant, anthers with normal appearing pollen were exserted in a portion of one lateral branch of the tassel. This pollen was used in a cross with a male-sterile sibling plant; all 33 plants in the test-cross progeny were male-sterile, indicating that the fertile tassel sector was not attributable to mutation of a restorer gene. In each of the two remaining families, 71-737 (81 plants) and 71-739 (49 plants), there was a single exceptional plant exhibiting some degree of tassel fertility. Further analyses of both of these cases indicate that nuclear restoration was involved in the first appearance of male fertility. Progeny test data on the first of these exceptions, plant number 71-737-16, is presented in the following paragraphs. The second case, 71-739-37, is treated in the next major section.

Plant number 71-737-16 had a fertile chimera in the tassel involving the florets on one side of the main rachis and all florets of twelve lateral branches on that side of the tassel. Thus, this plant exhibited a vertically oriented fertile sector involving from one-third to one-half of the tassel elements. The single tiller of this plant had an entirely fertile tassel. In this connection it is worth noting that the 80 male-sterile siblings in family 71-737 had a total of 136 tillers, all male-sterile.

The ear borne on exceptional plant 71-737-16 was crossed with pollen from the R853 maintainer source and among the offspring there were 19 male-fertile and 32 male-sterile plants. Crosses of these male-fertile offspring with male-sterile testers have been made but progeny test results are not yet available. Hence it is not yet possible to say whether the male fertility transmitted through the female elements of exceptional plant 71-737-16 is attributable to nuclear or cytoplasmic change. However, pollen from the fertile tassel chimera of this plant was used in crosses with two male-sterile sibling plants. All 32 offspring from these crosses were male-fertile, and two of these, in turn, when self pollinated produced only male-fertile F_2 progeny. A third, upon crossing with pollen from the M825 inbred-line maintainer source, gave five male-fertile and ten male-sterile offspring. Again, these results, as in the R853N case described in the previous section, indicate the action of a dominant nuclear restorer allele in S-type cytoplasm.

The male-fertile tiller of plant 71-737-16 had no ear but its pollen was used in a cross with a male-sterile sibling. The small test-cross progeny had seven male-fertile and no male-sterile plants. Although these data are limited, the transmission of male fertility through the male germ cells of this fertile tiller suggests that here too the male-fertile condition is assignable to nuclear restoration.

Since exceptional plant 71-737-16, whose siblings were male-sterile, had both male-sterile and male-fertile tassel elements, it must be assumed that the exceptional event, which we conclude from the foregoing evidence is equivalent to restorer-gene mutation, occurred during ontogeny. Since transmission of male fertility through the fertile pollen elements of both the main plant and its tiller fits the pattern of a restorer gene in S-type cytoplasm, we think it most likely that a single somatic event was responsible for the male-fertile phenotypes of the main plant and its tiller.

Special mention is made at this point of two additional types of test crosses incvolving the R853 exceptional plant 71-737-16. One of these involved an S male-sterile plant from the M825 inbred line as the female parent and produced 16 male-fertile and one male-sterile offspring. Other instances of occasional male-sterile plants from this type of test cross have been encountered in these studies. Although Buchert (1961) found no such "escapes" in this original studies of S-type restoration, Duvick (1965) does call attention to the appearance of occasional male-sterile offspring from crosses of this type which he has made. The possible significance of these cases is treated in the DISCUSSION section of this report. The second special cross to be discussed involves exceptional plant 71-737-16 and S male sterile inbred line R839. Surprisingly, all 32 offspring from two such test crosses were male-sterile. Thus, the exceptional R853 male-fertile plant 71-737-16 restores fertility in crosses with male-sterile inbred lines R853 and M825, but fails to do so in crosses with male-sterile inbred line R839 which ostensibly carries the same type of male-sterile cytoplasm as do R853 and M825.

R853 Case # 2

As noted in the foregoing section, this case, like the previous one, appeared among the progeny of an S male-sterile plant from the R853 inbred line crossed with an isogenic R853 maintainer plant. There were 48 male-sterile offspring from this cross; there were also 84 tillers on these plants, all of them male-sterile. The single exceptional plant, 71-739-37, had an entirely fertile tassel, as did the single tiller produced by this plant. The possibility that this plant arose as a result of fertilization by a contaminating pollen grain from a strain carrying the restorer gene is excluded on the same grounds as those discussed above in the case of the R853N exceptional family 71-741. That it does not represent seed contamination involving the R853 maintainer line is clear from the evidence presented below which indicates that the exceptional plant has genic restorative properties.

The ear of male-fertile exceptional plant 71-739-37, crossed with R853 maintainer pollen, produced a sample progeny of 49 plants, of which 13 were male-fertile and 36 were male-sterile. A test-cross progeny from a cross of one of these male-fertiles with a male-sterile tester plant produced only three surviving offspring, but all were male-fertile, suggesting the action of a nuclear restorer gene. The progeny of 13 male-

fertile and 36 male-sterile plants produced by the maintainer cross on the ear of the original exceptional plant deviates significantly from the 1:1 ratio expected if this plant was heterozygous for a genic restorer. It is possible, though now not subject to verification, that the ear borne on the exceptional male-fertile plant was a chimera involving two types of megasporocytes, those homozygous for the nonrestoring genotype, and those heterozygous for the newly arisen restorer allele.

The exceptional male-fertile plant *71-739-37* was used as pollen parent in test crosses with six male-sterile plants. Test crosses with three male-sterile R853 siblings produced a total of 89 offspring, all male-fertile. Likewise, testcrosses with a single S male-sterile plant from each of the inbred lines M825, R839 and WF9 gave 8, 9 and 42 male-fertile offspring, respectively; there were no male-sterile progeny. A number of the fertile offspring from these male-sterile testcrosses were self pollinated but data from only two such progenies are available at this time. One F_2 progeny, from the self pollination of a male-fertile plant from one of the testcrosses with a sibling male-sterile plant, had three male-fertile and no male-sterile plants; the other, from the test cross with the S male-sterile plant from inbred line WF9, had 13 male-fertiles and one male-sterile plant.

The male-fertile tiller of exceptional plant *71-739-37* was crossed with a male-sterile sibling; there were 16 male-fertile and one male-sterile offspring. Another test cross of this tiller plant with an R851 male-sterile plant gave three male-fertile and no male-sterile offspring.

These male-sterile test-cross and F_2 data indicate that the male fertility of R853 exception *71-739-37*, and of its tiller, is pollen transmissible. This is consistent with the interpretation that the newly arisen male fertility is the result of the action of a nuclear restorer gene and is not anticipated from a corresponding change at the cytoplasmic level. Moreover, the appearance of all male-fertile offspring in progeny of most of the crosses with male-sterile testers is again reminiscent of the characteristic action at the gametophytic level of the restorer gene in S-type cytoplasms. Attention is called to the unexpected appearance of a single male-sterile plant in one of the test-cross progenies and of another in an F_2 population. As noted above, one such case also appeared in the *71-737-16* exceptional pedigree.

The circumstances surrounding the origin, and subsequent behavior in progeny tests, of the two exceptional plants, *71-737-16* and *71-739-37*, are similar. Both were identified as single male-fertile individuals in otherwise male-sterile progenies of inbred line R853. Both appear to be male-fertile by reason of a nuclear restorer gene rather than a change at the cytoplasmic level, since both exhibit male-germ cell transmission of the fertile trait in crosses with male-sterile testers. They differ, however, with respect to restorative properties in crosses with male-sterile plants of inbred line R839; tested in this manner, *71-737-16* gave all male-sterile offspring, whereas *71-739-37* gave all male-fertile progeny.

M825 Case

As indicated earlier, exceptional male-fertile events occur more frequently in male-sterile inbred line M825 than in the other male-sterile *shrunken-2* inbred lines. In the 1971 summer nursery, 29 progenies produced by crossing plants of M825 male-sterile inbred line with isogenic M825 maintainer plants were searched daily during the flowering period for individuals with fertile tassels or with sectors of fertility in the tassel. Among the total of 780 plants, five with entirely fertile tassels, and 67 with sectors of fertility in the tassels were identified. Progenies from crosses of 27 of the male-fertile exceptions with male-sterile sibling plants were grown and scored for male fertility. In all but one case there was a failure of transmission of the male-fertile trait through the male germ cells of the exceptional plant, thus indicating a nonnuclear, or presumably cytoplasmic, basis for the occurrence of fertile tassels elements. The only departure from this pattern occurred in family *71-727* in which six of the 60 plants had fertile sectors in the tassel. One of these six plants, *71-727-37*, gave a test-cross progeny which indicated that nuclear restoration was involved.

The exceptional plant *71-727-37* had a tassel in which the main rachis and most lateral branches were sterile (no anthers exserted). However, eight contiguous lateral branches on one side of this tassel were fertile and produced abundant pollen. Pollen from this fertile sector was crossed onto two male-sterile siblings in family *71-727* and all 16 plants in the test-cross progenies were male-fertile. Pollen from the same source was also crossed with a single S male-sterile plant of inbred line WF9 and again all progeny, 20 in number, were male-fertile. The ear of plant *71-727-37*, crossed with pollen from a fertile tassel sector of a sibling plant gave a progeny with no male-fertile and 39 male-sterile plants; four plants had fertile tassel sectors. The absence of male-fertile offspring indicates that the event which led to fertility in a portion of the tassel of the exceptional plant was not shared by reproductive elements of its ear. This is not surprising since the main rachis of the tassel of this plant was sterile.

Although data from advanced generations are not yet available, the results from male-sterile test crosses presented above clearly indicate pollen transmission of the male-fertile phenotype which appeared as a chimera in the M825 exceptional plant *71-727-37*.

Discussion

The evidence presented above indicates a genic restorer basis for the appearance of male fertility in four separate instances. Three of these involve cases

in which the male fertility can be traced to a single plant in families of essentially male-sterile plants. In two of these cases, the fertile event was apparently postzygotic since the tassels of these plants were chimeras involving fertile and sterile portions. The third involved a plant with an entirely fertile tassel. In the fourth case, involving family 71-741, the exceptional event occurred in a fertile maintainer plant of inbred line R853N, so that upon self pollination the progeny of this plant segregated for the ability to restore fertility in S sterile cytoplasm.

In each of the four cases, crosses with S male-sterile testers produced male-fertile progeny, thus indicating that the newly arisen male-fertile trait is pollen transmissible and therefore can not be attributed to a change in the cytoplasm. This behavior, in fact, departs from that of numerous other instances of newly arisen male fertility in male-sterile pedigrees in which male-sterile test-cross progenies, and other evidence, indicate that the male-fertile event resulted from a change in the cytoplasm (Singh and Laughnan, 1972).

A number of inbred lines which restore S or S-like sterile cytoplasm have been identified (Duvick, 1965; Beckett, 1971). In the studies by Buchert (1961), which provided the first evidence that restoration of S male-sterile cytoplasm takes place at the gametophytic level, inbred line Ky21 was the source of the genic restorer. The pattern of restoration which we observed for the four cases of newly arisen male fertility reported here appears also to be gametophytic rather than sporophytic. Further studies, now underway, are designed to determine whether the presumptive genic restorers of these four strains involve the same gene locus, and if so, whether they are allelic with the standard S restorer gene of inbred line Ky21 and of other inbred lines which restore fertility in S cytoplasm.

The male-fertile exceptions described here can be accounted for formally as mutations at one or more restorer gene loci in the nucleus. So far as we are aware, these are the first reported instances of mutation in restorer genes. That we should have encountered four such male-fertile exceptions seems highly coincidental. We think it may be significant, also, that these changes were encountered in the same strains in which we have identified numerous additional cases of male-fertile exceptions involving cytoplasmic "mutations". We have no direct evidence that these two kinds of events are connected, but if they are we might consider the following as a basis for their common origin.

The male-fertile exceptions which have been identified as cytoplasmic changes, rather than changes in nuclear restorer genes, may be regarded, for descriptive purposes, as mutations of an S cytoplasmic entity. Depending upon one's current notions regarding the cause of sterility-inducing cytoplasms, such a qualitative change to male-fertile status might involve loss or suppression of a sterilizing virus, or a mutation which corrects the missense message of a cytoplasmic cistron associated with male sterility. However, our data are not sufficient to support the notion that the exceptional event involves a qualitative change in a cytoplasmic entity of S male-sterile cytoplasm. It is equally plausible that the exceptional male fertility is the result of occasional transfer of normal cytoplasm through the male germ cells of maintainer pollen parents. Whether the first introduction of fertile elements into sterile cytoplasm follows from transfer of normal cytoplasm through pollen of the maintainer parent, or alternatively, comes about through mutation of an S sterile element, we presume that the first expression of male fertility is the result of the favorable outcome of a sorting-out process involving mixed cytoplasmic entities. Leaving aside the question of the mechanism by which a mixture of sterile and fertile elements appears initially in the S cytoplasm of exceptional plants, we would suggest that subsequent male-fertile expression may arise in two ways. 1. The fertile element is "established" in the cytoplasm and continues to be replicated there, thus giving rise to male-fertile exceptions which transmit the male-fertile trait through the egg, but not through the sperm. In ensuing progeny tests, these cases register as cytoplasmic changes, and have the characteristics of a maintainer strain since they now carry normal cytoplasm but have a nuclear constitution not capable of restoration. 2. The fertile element is "established" in the nuclear genome and continues to be replicated there, giving rise to a male-fertile exception which transmits this trait through both egg and sperm. Such strains, like the ones described in this report, would be restorers of S male-sterile cytoplasm.

According to this scheme, the introduced male-fertile element is episomal in nature. It may be fixed in either cytoplasm or nucleus. Hence, in the newly arisen restorer strains the fertility element would be fixed and carried in the nucleus rather than in the cytoplasm. Another way of viewing this, although only changed terminology may be involved, is to consider that the fertile element, by whatever means, may be transposed from cytoplasm to nucleus, and vice versa. Naturally occurring restorer strains would thus be viewed as the product of an evolutionary process involving the fixation of fertility elements in nuclear genomes, a process which would have strong selective advantage in cytoplasmic male-sterile strains here defined as those lacking the fertility element, or carrying a defective one, in the cytoplasm. Many cases of cytoplasmic male sterility are known to arise from interspecific crosses (see reviews by Edwardson, 1956, 1970) and must be presumed to result from incompatibilities involving the cytoplasm of one species and the nuclear genome of another. Nuclear restoration in these instances may, on the scheme entertained here, amount to an introgression of nuclear

genetic material which renders the hybrid derivative male-fertile.

While most of this is speculation at this point, we note that there are interesting opportunities to test the scheme. In this connection, we return to the several cases in which male-sterile test crosses of exceptional male-fertile restorer plants produced occasional male-sterile individuals among male-fertile siblings. These, to be sure, could result from contaminating pollen grains from line maintainer sources, but we note that Duvick (1965, page 6) also calls attention to them: "In some genotypes a small percentage of fertile pollen grains of rf_3 genotype are produced by Rf_3rf_3 plants in USDA cytoplasm in addition to the fertile Rf_3 pollen grains." It seems not too farfetched to consider that the occasional male-sterile "escapes" result from loss of an episomal-like fertility element (Rf_3) during the development of male gametophytes involved in these fertilizations, or during early stages of the developing zygote. We hope to test this hypothesis.

Another obvious approach to the problem involves more intensive investigation of the four exceptional pedigrees now in hand, and a search for additional cases of "mutations" of restorer genes. If these events are connected with transposition of episomal elements, we might anticipate that the newly arisen restorer function is labile; instability of such strains might register either as loss of restoring ability and of the male-fertile trait (total loss of the fertility element), or as loss of restoring ability but not of the male-fertile trait (transposition of fertility element from nucleus to cytoplasm). Needless to say, the tests for allelism among the exceptional restorer strains and standard restorers of S cytoplasm will aid in interpretation. If these changes result from mutation of a nonrestoring allele to a restoring form, we might expect that a single gene locus is involved whereas this constraint does not apply if the phenomenon is episomal in nature. That differences do exist in the restorer strains we have dealt with is already apparent from the observation that some do, and some do not, restore fertility in the S cytoplasm of inbred line R839.

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Received November 28, 1972

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