# Gametophyte Genetics in *Zea mays* L.: Dominance of a Restoration-of-Fertility Allele (*Rf3*) in Diploid Pollen

**T. L. Kamps, D. R. McCarty and C. D. Chase**

*Horticultural Sciences Department, University of Florida, Gainesville, Florida 32611*

Manuscript received August 23, 1995
Accepted for publication November 28, 1995

ABSTRACT

In *Zea mays* L. plants carrying the S-type of sterility-inducing cytoplasm, male fertility is determined by a gametophytic, nuclear restoration-of-fertility gene. Haploid pollen carrying the fertility-restoring allele (historically designated *Rf3*) is starch-filled and functional, whereas pollen carrying the nonrestoring allele (historically designated *rf3*) is shrunken and nonfunctional. Because restoration of fertility occurs in haploid tissue, the dominance relationship of restoring and nonrestoring alleles is unknown. We have tested the dominance relationship of the restoring and nonrestoring alleles at the *rf3* locus in diploid pollen. The meiotic mutant *elongate* was used to generate tetraploid plants carrying both *Rf3* and *rf3* alleles in the S cytoplasm. These plants shed predominantly starch-filled pollen, consistent with dominance of the restoring allele. Restriction fragment length polymorphisms linked to the *rf3* locus demonstrated cotransmission of *rf3* and *Rf3* alleles through heterozygous diploid pollen, providing conclusive genetic evidence that the restoring allele is the dominant or functional form of this restoration-of-fertility gene. We suggest that other S-cytoplasm restorers result from loss-of-function mutations and propose analysis of unreduced gametes as a test of this model.

CYTOPLASMIC male sterility (CMS) systems provide an opportunity for molecular and genetic investigations of mitochondrial-nuclear genome interactions in higher plants (HANSON and CONDE 1985). The mitochondrial genome encodes this maternally inherited failure to shed functional pollen (reviewed in HANSON and CONDE 1985; LEVINGS 1990; HANSON 1991). Nuclear restoration-of-fertility genes can, however, condition a male-fertile phenotype in the presence of a sterility-inducing cytoplasm (reviewed in DUVICK 1959; HANSON and CONDE 1985). Three different sterility-inducing cytoplasms of maize (C, T and S) were originally recognized by their genetically distinct nuclear restoration-of-fertility systems (DUVICK 1965; BECKETT 1971; GRACEN and GROGAN 1974). Investigations of these different fertility restoration mechanisms may provide insights into different aspects of mitochondrial-nuclear genome interactions.

Nuclear restoration of fertility in the CMS-S system of maize is gametophytic. Pollen carrying the restoring allele (designated *Rf3*) is starch-filled and functional, whereas pollen carrying the nonrestoring allele (designated *rf3*) is shrunken and nonfunctional. Heterozygous CMS-S plants (*Rf3/rf3*) are phenotypically semifertile, shedding 50% starch-filled and 50% shrunken pollen grains (BUCHERT 1961). Because *Rf3* acts in haploid tissue, the dominance or functional relationship of the restoring and nonrestoring alleles is unknown. The restoring allele might act to inhibit the expression of male sterility. Alternatively, the nonrestoring allele might induce the male-sterile phenotype. Knowledge of the functional or dominance relationship of restoring and nonrestoring alleles is fundamental to molecular and genetic investigations of restorer gene action. We have therefore investigated this relationship through the construction and fertility analysis of CMS-S tetraploid (4N) plants carrying both restoring and nonrestoring alleles. These plants produced functional heterozygous (*Rf3/rf3*) pollen grains, providing conclusive evidence that the restoring allele is the dominant or functional form for this restoration-of-fertility gene.

**This work is dedicated to the memory of John R. Laughnan.**

*Corresponding author:* Christine D. Chase, Horticultural Sciences Department, Box 110690, University of Florida, Gainesville, FL 32611-0690. E-mail: ctdc@gnv.ifas.ufl.edu

## MATERIALS AND METHODS

**Construction of 4N lines:** The plant lines employed in this study are described in Table 1. The *elongate* (*el*) mutation of maize was used to construct 4N plants. This mutation, in the homozygous condition, causes an elevated frequency of unreduced female gametes. The unreduced gametes result primarily from failure of the second meiotic division (RHOADES and DEMPSEY 1966). The restorer status of a normal cytoplasm, *el/el* stock (805E) was determined by using 805E plants to pollinate a CMS-S *rf3/rf3* inbred line (W182BN). The 805E plants were also self-pollinated. Progeny from the CMS-S W182BN × 805E pollinations were grown and scored for male fertility based on pollen shed. Male-sterile (*rf3/rf3*) plants exserted few anthers and shed no pollen. Semifertile (*Rf3/rf3*) plants exserted anthers and shed pollen. Families (of 16 plants) with only male-sterile plants and families segre-

**TABLE 1**

**Maize lines used in the construction and analysis of CMS-S 4N plants**

| Line | Cytoplasm | Genotype | Source |
|---|---|---|---|
| 805E | N | *el/el* | Maize Stock Center No. 84-666 |
| 805E-rf3 | N | *el/el rf3/rf3* | This study |
| W182BN | CA[a] | *rf3/rf3* | D. R. PRING |
| Ky21 | S | *Rf3/Rf3* | S. GABAY-LAUGHNAN No. 87-509 |
| W23 2N | N | *rf3/rf3* | S. GABAY-LAUGHNAN No. 89-333 |
| W23 4N (N107B) | N | *rf3/rf3/rf3/rf3*[b] | Maize Stock Center No. 87-2239 |
| N104B | N | unknown 4N | Maize Stock Center No. 85-676 |

[a] CA is a subgroup of the S cytoplasm (SISCO *et al.* (1985).
[b] Genotype was assigned based upon RFLP and fertility analyses performed in this study.

gating male-sterile and semifertile plants were recovered. This demonstrated that the 805E stock was segregating for *Rf3* and *rf3* alleles and enabled us to identify an *rf3/rf3* 805E plant. Self-pollination of the *rf3/rf3* 805E plant established the *rf3/rf3 el/el* stock (805E-rf) used for the construction of CMS-S 4N plants.

The construction of CMS-S 4N plants is outlined in Figure 1. The CMS-S *Rf3/Rf3 El/El* maize inbred Ky21 was pollinated with the *rf3/rf3 el/el* stock. The heterozygous genotype of the resulting $F_1$ progeny with respect to *rf3* was confirmed by microscopic examination of pollen as described below. These plants (CMS-S *Rf3/rf3 El/el*) were self-pollinated. The predicted $F_2$ progeny are shown in Figure 1. Tissue samples (for DNA extraction) and pollen samples (for microscopic examination) were collected from 63 $F_2$ plants. Microscopic examination of pollen was used to distinguish between *Rf3/rf3* and *Rf3/Rf3* genotypes. An undetermined number of $F_2$ plants were pollinated with the 4N version of the maize inbred line W23, and seeds were recovered from ears borne by two fully male-fertile $F_1$ plants. The W23 diploid (2N) is known to be *rf3/rf3* (BECKETT 1971). The W23 4N stock was therefore expected to carry one or more *rf3* alleles. Restriction fragment length polymorphism (RFLP) analysis (described below) was consistent with this expectation. The predicted genotypes of the CMS-S 4N progeny are shown in Figure 1 for the case of a W23 4N parent carrying four *rf3* alleles. An unrelated 4N stock (N104B) was used as a female parent in testcrosses with the CMS-S 4N plants.

**Fertility analysis:** Male fertility was determined by microscopic examination of pollen. Pollen was collected from field or greenhouse grown plants by shaking freshly exserted anthers over a drop of acetocarmine stain (SISCO *et al.* 1985) on

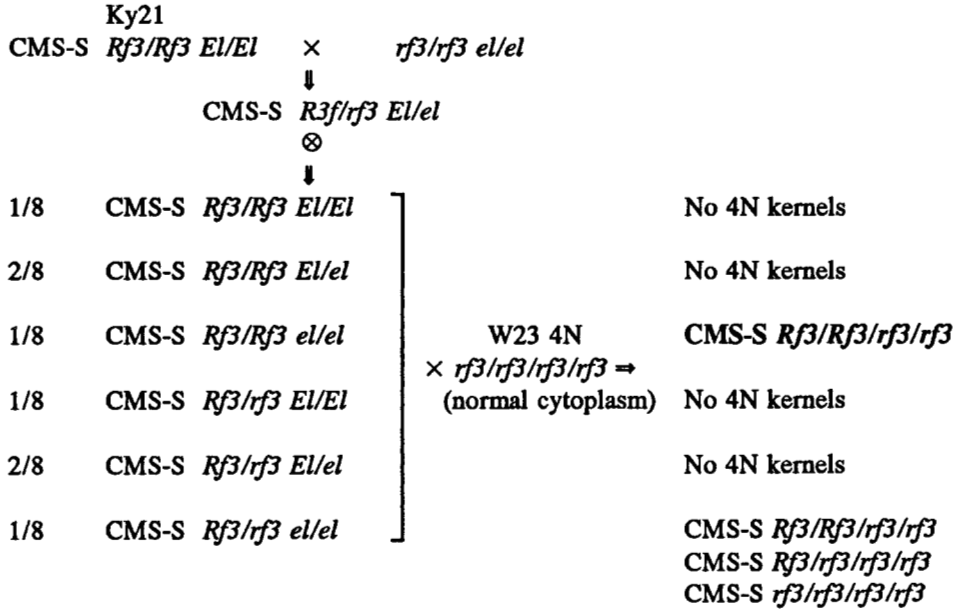


FIGURE 1.—Crossing scheme for the production of CMS-S tetraploid plants. The CMS-S Ky21 inbred was pollinated with an *el/el* plant known to carry the *rf3/rf3* genotype in normal cytoplasm. The resulting $F_1$ was self-pollinated to produce an $F_2$ population. The predicted $F_2$ genotypes and frequencies are shown. The *rf3* and *el* loci are unlinked, mapping to chromosomes *2* and *8*, respectively. The *rf3/rf3* genotype is absent due to failure of *rf3* pollen in the presence of the S cytoplasm (BUCHERT 1961). *Rf3/rf3* $F_1$ and *Rf3/Rf3* $F_2$ individuals were verified by microscopic examination of pollen and confirmed by RFLP analysis as described in the text as shown in Figure 3, lanes 3–4. $F_2$ plants were pollinated with the 4N version of maize inbred W23 in normal cytoplasm. Only *el/el* $F_2$ genotypes will produce 2N eggs and 4N kernels in this cross. Kernels resulting from the pollination of haploid eggs by 2N pollen fail due to imbalance of maternal and paternal genomes in the developing endosperm (LIN 1984). The predicted progeny genotypes are shown for the case of a W23 4N parent carrying four *rf3* alleles.

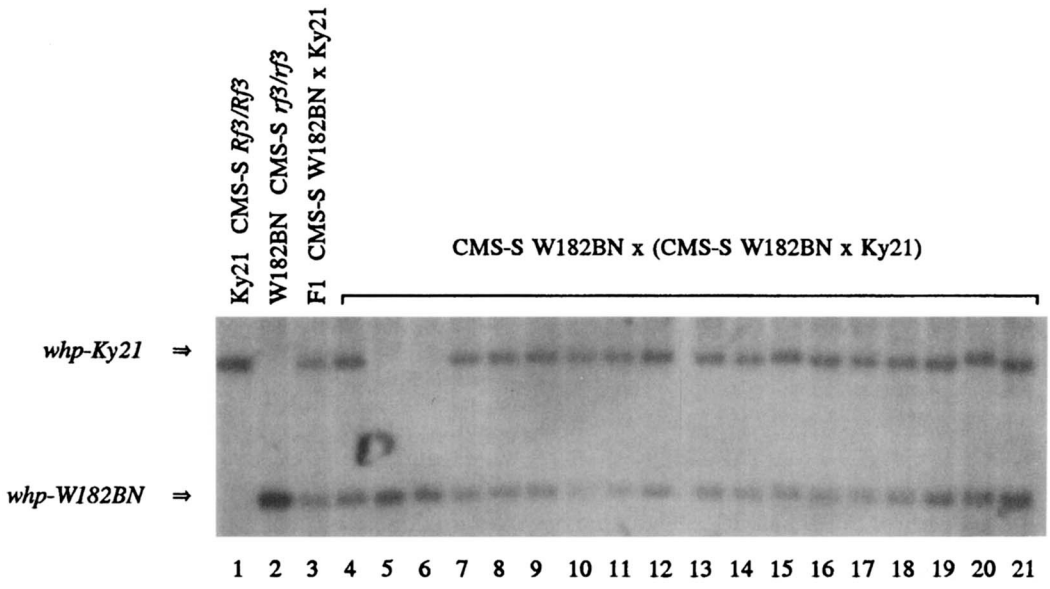


FIGURE 2.—Cosegregation of *whp* and *rf3* alleles in a backcross population. A backcross population [W182BN/CA × (W182BN/CA × Ky21/S)] was analyzed for male fertility by microscopic examination of pollen grains and for segregating *whp* RFLP markers by Southern blot hybridization. The *Sst*I-digested DNA of the 18 semifertile (*Rf3-Ky21/rf3-W182BN*) progeny shown above demonstrated cosegregation of the 10.2-kb *whp-Ky21* RFLP with the *Rf3-Ky21* allele. Two semifertile recombinant individuals (lanes 5 and 6) did not carry the *whp-Ky21* RFLP; the total population of 47 semifertile plants contained three recombinants in the *whp-rf3* interval.

a microscope slide. Pollen grains were examined under a light microscope at ×100 magnification. At least 500 grains were counted for each plant. Rounded grains that stained with acetocarmine were scored as functional. Shrunken grains without staining were scored as aborted.

**DNA blot analysis:** Alleles of *Rf3* were identified and tracked with linked RFLPs. We obtained a white pollen (*whp*) gene-specific clone (pwhpC6) from U. WEINAND, Max Plank Institut, Koln. Plasmid DNA was prepared by the triton lysis procedure (LONSDALE *et al.* 1981). The cloned *whp* sequence was purified by agarose gel electrophoresis (HEERY *et al.* 1990) and radiolabeled *in vitro* by randomly primed DNA synthesis (FEINBERG and VOGLESTEIN 1984). Maize nucleic acids were prepared from frozen leaf tissues or etiolated seedlings as described by DELLAPORTA *et al.* (1983). Nucleic acids were digested with restriction endonucleases, fractionated by agarose gel electrophoresis, transferred to nylon supports and hybridized with radiolabeled probes. Hybridizations and washes were performed at 65° in the buffers described by CHURCH and GILBERT (1984).

## RESULTS

**Restriction fragment polymorphisms cosegregating with the *rf3* locus:** The *rf3* locus was mapped to the distal region on the long arm of maize chromosome *2*, flanked by the *whp* and *bnl17.14* RFLP loci. Recombination frequencies placed *rf3* ~6 cM from each of these flanking loci (T. L. KAMPS and C. D. CHASE, unpublished data, Maize Genetics Cooperation Newsletter 66:45). Of the two flanking RFLP markers, only *whp* was informative for all materials used in this study (not shown). The cosegregation of *whp* and *rf3* loci in a mapping backcross is shown in Figure 2.

**Construction of 4N plants:** The construction of 4N plants, as outlined in Figure 1, was successful. Seeds were recovered from pollination of two *Rf3/Rf3* $F_2$ plants by the W23 4N line. The *whp* probe distinguished alleles carried by CMS-S Ky21 (original donor of the *Rf3* allele), the *el/el rf3/rf3* stock and the W23 2N stock (also *rf3/rf3*) (Figure 3, lanes 1, 2 and 5). These alleles were designated *whp-Ky21*, *whp-el*, and *whp-W23*, respectively. Alleles at the *whp* locus were used to confirm genotypes of the $F_1$ and $F_2$ plants used in the construction of CMS-S 4N plants (Figure 3, lanes 3 and 4).

**Male fertility of CMS-S 4N plants:** Fertility analysis was performed on the CMS-S 4N plants derived from the pollination of CMS-S *Rf3/Rf3 el/el* plants with the 4N version of inbred W23 (Figure 1). Because the W23 2N is known to have the *rf3/rf3* genotype (5), we expected the CMS-S 4N plants to have the *Rf3/Rf3/rf3/rf3* genotype. RFLP and male fertility analyses (described below) were consistent with this expectation. Table 2 presents the predicted genotypes and phenotypes of pollen grains shed by CMS-S *Rf3/Rf3/rf3/rf3* plants in the case of a dominant (or functional) restoring allele and in the case of a dominant (or functional) nonrestoring allele. Because the *rf3* locus was unlinked to the chromosome *2* centromere, alleles of *rf3* on the eight chromatids were expected to segregate independently, giving rise to 28 possible combinations of two alleles in 2N pollen. Of the 28 combinations, 22 contain an *Rf3* allele. If the restoring allele is dominant, plants are predicted to shed 79% (22/28) starch-filled grains. If the nonrestoring allele is dominant, plants are pre-

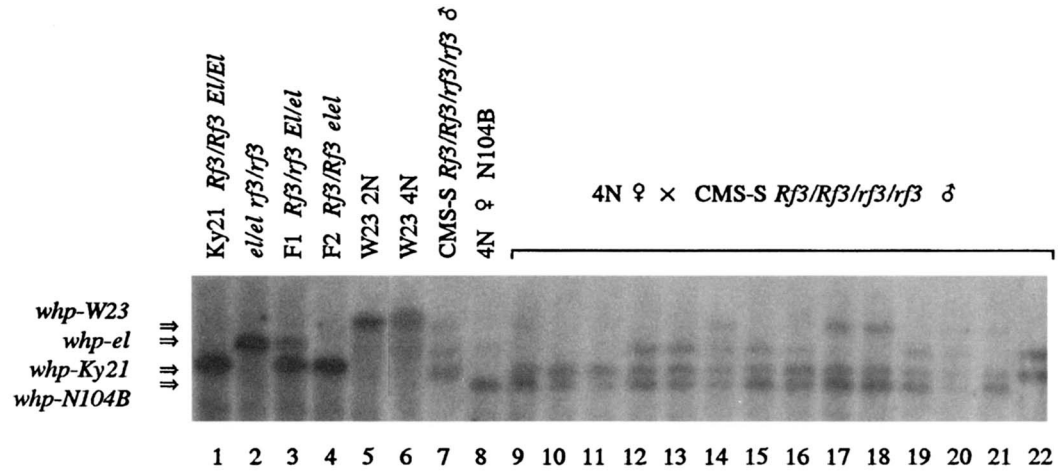


FIGURE 3.—Transmission of *Rf3* and *rf3* alleles through 2N pollen. A 4N female (from stock N104B) was pollinated with a CMS-S plant carrying *Rf3* and *rf3* alleles. Alleles of *Rf3* and *rf3* were identified and tracked with RFLPs at the linked *whp* locus. Genomic DNAs prepared from progenitor stocks, parent plants, and testcross progeny plants were digested with *Bam*HI, fractionated by agarose gel electrophoresis, blotted to nylon supports and hybridized with a radiolabeled *whp* probe. The *whp-W23, whp-el, whp-Ky21* and *whp-N104B* RFLPs were 26.3 kb, 17.8 kb, 13.2 kb and 11.7 kb, respectively. Gel lanes were loaded as follows: 1, CMS-S Ky21 stock; 2, *el/el rf3/rf3* stock; 3, $F_1$ plant from CMS-S Ky21 × *el/el rf3/rf3*; 4, CMS-S *Rf3/Rf3 el/el* $F_2$ plant, female parent of lane 7 plant; 5, W23 2N stock; 6, W23 4N stock; 7, CMS-S *Rf3/Rf3/rf3/rf3* male testcross parent; 8, N104B 4N stock; 9–22, progeny of N104B 4N × CMS-S *Rf3/Rf3/rf3/rf3* lane 7 plant.

dicted to produce 21% (6/28) starch-filled, functional grains, which might be inadequate for anther exsertion and dehiscence. Two families of 4N plants (the progeny of two different *Rf3/Rf3* $F_2$ plants) were examined for pollen fertility. In one family of 14 progeny, individual plants shed from 88–92% starch-filled pollen. In a second family of six progeny, individual plants shed 78–88% starch-filled pollen. As a control, we analyzed 22 normal cytoplasm W23 4N plants. These shed 88–98% starch-filled pollen. Pollen analysis was therefore consistent with a dominant fertility restoration allele, given a CMS-S *Rf3/Rf3/rf3/rf3* genotype.

**Transmission of restoring and nonrestoring alleles through 2N pollen:** To confirm the production of functional *Rf3/rf3* 2N pollen grains, a CMS-S 4N plant with the predicted genotype of *Rf3/Rf3/rf3/rf3* (as shown in bold in Figure 1) was used to pollinate an unrelated 4N plant (stock N104B). The resulting progeny were grown and analyzed for alleles at the *whp* locus (Figure 3). In addition to the *whp-Ky21, whp-el,* and *whp-W23* alleles, the *whp* probe distinguished an allele in the N104B 4N stock (*whp-N104B*) (Figure 3, lanes 1, 2, 5, and 8). The N104B and the W23 4N stocks were also segregating for the *whp-el* allele (Figure 3, lanes 6 and 8). The presence of this allele suggests that the *elongate* mutation may have been used in the construction of these 4N stocks.

The CMS-S 4N plant used as a male parent in the transmission experiment carried *whp-Ky21, whp-W23* and *whp-el* alleles (Figure 3, lane 7). The two *whp-ky21* alleles were contributed by the $F_2$ parent (Figure 3, lane 4) of this CMS-S 4N plant, while the *whp-el* and *whp-W23* alleles were contributed by the W23 4N parent (Figure 3, lane 6). The analysis of 14 4N progeny plants is shown in Figure 3, lanes 9–22. Each of the 14 progeny plants inherited the *whp-Ky21* allele from the 4N male parent. Six of the progeny (lanes 9, 14, 17, 18, 20 and 21) also inherited the *whp-W23* allele from the 4N male parent. Cotransmission of *whp-Ky21* and *whp-W23* alleles indicated cotransmission of *Rf3-Ky21* and *rf3-W23* alleles through heterozygous diploid pollen. The presence of the *whp-Ky21* allele in all progeny examined (including 11 additional progeny not shown in Figure 3) was consistent with a requirement for the *Rf3* allele in functional pollen.

As we did not construct the W23 4N line, which contributed a *whp-el* allele to our CMS-S 4N parent, we could not directly verify the linkage of this *whp-el* allele to an *rf3* allele. However, the results shown in Figure 3 argue strongly against the presence of an additional restoring allele (*i.e.,* an *Rf3-Ky21/Rf3-Ky21/Rf3-el/rf3-W23* genotype) in our CMS-S 4N male parent. If an

**TABLE 2**

**Predicted genotypes and phenotypes of diploid pollen grains shed by CMS-S *Rf3/Rf3/rf3/rf3* plants**

| Genotype | Frequency[a] | Phenotype | |
|---|---|---|---|
| | | Dominant restorer | Recessive restorer |
| *Rf3/Rf3* | 6/28 | Starch filled | Starch filled |
| *Rf3/rf3* | 16/28 | Starch filled | Aborted |
| *rf3/rf3* | 6/28 | Aborted | Aborted |

[a] Frequencies are based upon random segregation of alleles on eight chromatids.

additional restoring allele was present, functional pollen without the *Rf3-Ky21* allele (and linked *whp-Ky21* allele) would be produced and result in progeny lacking the *whp-Ky21* allele. Such progeny were not observed among the 25 plants we examined by RFLP analysis. The probability of recovering a *whp-Ky21* allele in 25 progeny of a CMS-S *Rf3-Ky21/Rf3-Ky21/Rf3-el/rf3-W23* male parent by chance alone is $(22/27)^{25}$ or 0.006 (assuming *rf3/rf3* pollen to be inviable). While we consider an *Rf3-Ky21/Rf3-Ky21/Rf3-el/rf3-W23* 4N male parent to be improbable, the presence of an *Rf3-el* allele in the male parent would in no way alter our conclusion regarding the dominance of the *Rf3* allele based upon the cotransmission of *Rf3-Ky21* and *rf3-W23* alleles through 2N pollen, as demonstrated by cotransmission of *whp-Ky21* and *whp-W23* alleles (Figure 3, lanes 9, 14, 17, 18, 20 and 21).

Collectively, our observations argue against an active role of the nonrestoring allele (*rf3*) in pollen abortion. The restoring allele (*Rf3*) must be dominant to the nonrestoring allele and function in some way to suppress the CMS phenotype.

## DISCUSSION

The collapse of CMS-S *rf3* pollen grains occurs suddenly and late in pollen development (LEE *et al.* 1980). The expression of the CMS-S sterility determinant may therefore be restricted to this stage of gametophyte development. Expression of the mitochondrial protein associated with CMS in *Phaseolus vulgaris* is restricted to sporophytic and gametophytic reproductive tissues (ABAD *et al.* 1995). Restoration-of-fertility alleles for sterility determinants expressed only in the gametophyte would, of necessity, exhibit a gametophytic mode of inheritance. However, expression of gametophytic restoration-of-fertility genes is not necessarily limited to the gametophyte. A class of genes controlling male fertility through expression in both sporophytic and gametophytic tissues has been identified in Arabidopsis (XU *et al.* 1995).

In unusual circumstances (such as haplo-insufficiency), a dominant phenotype can result from the loss of gene function. In the case of *Rf3,* the plant phenotype is based upon gene action in haploid tissues, and an active role for the *Rf3* gene product in restoration of fertility is the simplest interpretation of a dominant *Rf3* allele. The expression of novel mitochondrial genes has been associated with CMS in a number of systems (LEVINGS 1990; HANSON 1991; GRELON *et al.* 1994; KRISHNASAMY and MAKAROFF 1994; MONEGER *et al.* 1994; ABAD *et al.* 1995). Identification of sterility determinants has allowed the characterization of fertility restoration mechanisms including the destabilization of CMS transcripts (MONEGER *et al.* 1994), altered processing of CMS transcripts (DEWEY *et al.* 1986; KENNELL *et al.* 1987; SINGH and BROWN 1993), and effects on either the translation or stability of CMS gene products (KRISHNASAMY and MAKAROFF 1994; ABAD *et al.* 1995). While most fertility restoration mechanisms characterized to date affect the expression of CMS genes within the mitochondria, a dominant restoring allele could also compensate for the presence of a functional CMS gene product. A mitochondrial reading frame has recently been associated with the maize S cytoplasm (GABAY-LAUGHNAN *et al.* 1995). Further characterization of this reading frame and its expression will facilitate investigations of the *Rf3* restoration mechanism.

Characteristics of other S-cytoplasm restorers of fertility suggest that restoring alleles for this cytoplasm are not always dominant. In CMS-S maize plants, nuclear reversion to fertility gives rise to additional fertility restorers. The new restorers (*Rf1-RfX*) are gametophytic and each maps to a different genome location (LAUGHNAN and GABAY 1973, 1978; LAUGHNAN *et al.* 1981). With the exception of *Rf1V,* all of the new restorers are associated with deleterious effects including decreased transmission through the female gamete and lethality in the homozygous condition (LAUGHNAN and GABAY 1975). New restorers arise at relatively high frequencies and their molecular-genetic basis is currently unknown.

The new restorers of fertility may result from loss-of-function mutations in nuclear genes affecting mitochondrial gene expression. This class of mutations would alter expression of the mitochondrial CMS determinant as well as other mitochondrial genes. A general effect on mitochondrial gene expression would account for the deleterious, sporophytic phenotype associated with homozygosity for a new restoring allele. The large number of nuclear genes involved in mitochondrial gene expression (TZAGOLOFF and DIECKMANN 1990) may contribute to the relatively high frequency with which new restorers are recovered.

The loss-of-function model implies tissue-specific differences in requirements for mitochondrial gene expression, with plant growth and pollen development requiring a higher level of function than pollen maturation and fertilization. Tissue-specific differences in the level of mitochondrial expression have been observed in a number of higher plant species (PIECHULLA 1988; EHRENSHAFT and BRAMBL 1990; MONEGER *et al.* 1992; CONLEY and HANSON 1994; SMART *et al.* 1994). The expression levels of nuclear genes encoding mitochondrial proteins (PIECHULLA 1988; EHRENSHAFT and BRAMBL 1990; CONLEY and HANSON 1994; HUANG *et al.* 1994) also exhibit tissue-specific differences.

S-cytoplasm restorers of fertility are in many ways analogous to the high chlorophyll fluorescence (*hcf*) mutations of maize (MILES 1982). The *hcf* phenotype results from mutations in nuclear genes that affect chloroplast gene expression. Many *hcf* mutations are recessive and homozygous lethal. They are recovered at high

frequency and affect processes such as plastid RNA processing and translation. These mutations can result in decreased accumulation of all chloroplast proteins or characteristic sets of chloroplast proteins (BARKAN *et al.* 1986; BARKAN 1993). One difference between the restorers of fertility and the *hcf* mutants is the stage of lethality in homozygotes. Mutants affecting plastid function are lethal at the seedling stage, whereas the S-cytoplasm restorers are lethal in the zygotic stage. Lethality in the zygotic stage is consistent with the requirement for functional mitochondria in seed development and germination.

The ability to construct tetraploid lines and track the transmission of alleles with linked RFLPs will enable us to test dominance relationships of restoring and nonrestoring alleles at the new S-cytoplasm restoration of fertility loci and critically test the loss-of-function model. In addition to the S-cytoplasm fertility restorers, there are a number of maize loci exhibiting gametophytic patterns of inheritance (NELSON 1994). The tetraploid approach may also prove useful in studies of other gametophyte factors in maize.

We acknowledge the technical assistance of V. M. ORTEGA and thank L. C. HANNAH for helpful discussions and critical reading of this manuscript. We thank S. GABAY-LAUGHNAN, D. R. PRING, U. WEINAND and the Maize Genetics Cooperation Stock Center for providing maize stocks and probes. T.L.K. was supported in part by a Graduate Research Assistantship from the University of Florida Department of Horticultural Sciences. This is paper number R-04689 in the University of Florida Agricultural Experiment Station Journal Series.

## LITERATURE CITED

ABAD, A. R., B. J. MEHRTENS and S. A. MACKENZIE, 1995 Specific expression in reproductive tissues and fate of a mitochondrial sterility-associated protein in cytoplasmic male-sterile bean. Plant Cell **7:** 271–285.

BARKAN, A., 1993 Nuclear mutants of maize with defects in chloroplast polysome assembly have altered chloroplast RNA metabolism. Plant Cell **5:** 389–402.

BARKAN, A., D. MILES and W.C. TAYLOR, 1986 Chloroplast gene expression in nuclear photosynthetic mutants of maize. EMBO J. **5:** 1421–1427.

BECKETT, J. B., 1971 Classification of male sterile cytoplasms in maize (*Zea mays* L.). Crop Sci. **11:** 724–726.

BUCHERT, J. G., 1961 The stage of the genome-plasmon interaction in the restoration of fertility to cytoplasmically pollen-sterile maize. Proc. Natl. Acad. Sci. USA **47:** 1436–1440.

CHURCH, G. M., and W. GILBERT, 1984 Genomic sequencing. Proc. Natl. Acad. Sci. USA **81:** 1991–1995

CONLEY, C. A., and M. R. HANSON, 1994 Tissue-specific protein expression in plant mitochondria. Plant Cell **6:** 85–91.

DELLAPORTA, S. L., J. WOOD and J. B. HICKS, 1983 A plant DNA minipreparation: version II. Plant Mol. Biol. Reptr. **1:** 19–21.

DEWEY, R. E., C. S. LEVINGS III and D. H. TIMOTHY, 1986 Novel recombinations in the maize mitochondrial genome produce a unique transcriptional unit in the Texas male-sterile cytoplasm. Cell **44:** 439–449.

DUVICK, D. N., 1959 The use of cytoplasmic male-sterility in hybrid seed production. Econ. Bot. **13:** 167–195.

DUVICK, D. N., 1965 Cytoplasmic pollen sterility in corn. Adv. Genet. **13:** 1–56.

EHRENSHAFT, M., and R. BRAMBL, 1990 Respiration and mitochondrial biogenesis in germinating embryos of maize. Plant Physiol. **93:** 295–304.

FEINBERG, A. P., and B. VOGELSTEIN, 1984 A technique for radiolabeling DNA restriction fragments to high specific activity. Anal. Biochem. **137:** 266–26.

GABAY-LAUGHNAN, S., G. A. ZABALA and J. R. LAUGHNAN, 1995 S-type cytoplasmic male sterility in maize, pp. 395–432 in *Advances in Cellular and Molecular Biology of Plants Volume II—The Molecular Biology of Plant Mitochondria*, edited by C. S. LEVINGS and I. K. VASIL. Kluwer, Dordrecht.

GRACEN, V. E., and C. O. GROGAN, 1974 Diversity and suitability for hybrid production of different sources of cytoplasmic male sterility in maize. Agron. J. **66:** 654–657.

GRELON, M., F. BUDAR, S. BONHOMME and G. PELLETIER, 1994 Ogura cytoplasmic male-sterility (CMS)-associated *orf138* is translated into a mitochondrial membrane polypeptide in male-sterile *Brassica* cybrids. Mol. Gen. Genet. **243:** 540–547.

HANSON, M. R., 1991 Plant mitochondrial mutations and male sterility. Annu. Rev. Genet **25:** 461–486.

HANSON, M. R., and M. F. CONDE., 1985 Functioning and variation of cytoplasmic genomes: lessons from cytoplasmic-nuclear interactions affecting male fertility in plants. Intl. Rev. Cytol. **94:** 214–267.

HEERY, D. M., F. GANNON and R. POWELL, 1990 A simple method for subcloning DNA fragments from gel slices. Trends Genet. **6:** 173.

HUANG, J., F. STRUCK, D. F MATZINGER and C. S. LEVINGS III, 1994 Flower-enhanced expression of a nuclear-encoded mitochondrial respiratory protein is associated with changes in mitochondrion number. Plant Cell **6:** 439–448.

KENNELL, J. C., R. P. WISE and D. R. PRING, 1987 Influence of nuclear background on transcription of a maize mitochondrial region associated with Texas male sterile cytoplasm. Mol. Gen. Genet. **210:** 399–406.

KRISHNASAMY, S., and C. A. MAKAROFF, 1994 Organ-specific reduction in the abundance of a mitochondrial protein accompanies fertility restoration in cytoplasmic male-sterile radish. Plant Mol. Biol. **26:** 935–946.

LAUGHNAN, J. R., and S. J. GABAY, 1973 Mutations leading to nuclear restoration of fertility in S male-sterile cytoplasm in maize. Theor. Appl. Genet. **43:** 109–116.

LAUGHNAN, J. R., and S. J. GABAY, 1975 An episomal basis for instability of S male sterility in maize and some implications for plant breeding, pp. 330–349 in *Genetics and the Biogenesis of Cell Organelles*, edited by C. W. BIRKY, JR., P. S. PERLMAN and T. J. BYERS. Ohio State Univ. Press, Columbus.

LAUGHNAN, J. R., and S. J. GABAY, 1978 Nuclear and cytoplasmic mutations to fertility in S male-sterile maize, pp. 427–446 in *Maize Breeding and Genetics*, edited by D. B. WALDEN. John Wiley, New York.

LAUGHNAN, J. R., S. GABAY-LAUGHNAN and J. E. CARLSON, 1981 Characteristics of cms-S reversion to male fertility in maize. Stadler Symp. **13:** 93–114.

LEE, S. J., E. D. EARLE and V. E. GRACEN, 1980 The cytology of pollen abortion in S cytoplasmic male-sterile corn anthers. Am. J. Bot. **67:** 237–245.

LEVINGS, C. S., III, 1990 The Texas cytoplasm of maize: cytoplasmic male sterility and disease susceptibility. Science **250:** 942–947.

LIN, B.-Y., 1984 Ploidy barrier to endosperm development in maize. Genetics **107:** 103–115.

LONSDALE, D. M., R. D. THOMPSON and T. P. HODGE, 1981 The integrated forms of the A1 and S2 DNA elements of maize male-sterile DNA are flanked by a large repeated sequence. Nucleic Acids Res. **9:** 3657–3669.

MILES, D., 1982 The use of mutations to probe photosynthesis in higher plants, pp. 75–106, in *Methods in Chloroplast Molecular Biology*, edited by M. EDELMAN, R. B. HALLICK and N.-H. CHUA. Elsevier, New York.

MONEGER, F., P. MANDARON, M. F. NIOGRET, G. FREYSSINET and R. MACHE, 1992 Expression of mitochondrial genes during microsporogenesis in maize. Plant Physiol. **99:** 396–400.

MONEGER, F., C. J. SMART and C. J. LEAVER, 1994 Nuclear restoration of cytoplasmic male sterility in sunflower is associated with the tissue-specific regulation of a novel mitochondrial gene. EMBO J. **13:** 8–17.

NELSON, O. E., 1994 The gametophyte factors of maize, pp. 496–

503 in *The Maize Handbook*, edited by M. FREELING and V. WALBOT. Springer-Verlag, New York.

PIECHULLA, B., 1988 Differential expression of nuclear and organelle-encoded genes during tomato fruit development. Planta **174:** 505–512.

RHOADES, M., and E. DEMPSEY, 1966 Induction of chromosome doubling at meiosis by the elongate gene in maize. Genetics **54:** 505–522.

SINGH, M., and G. G. BROWN, 1993 Characterization of expression of a mitochondrial gene region associated with the *Brassica* "Polima" CMS: developmental influences. Curr. Genet. **24:** 316–322.

SISCO, P. H., V. E. GRACEN, H. L. EVERETT, E. D. EARLE, D. R. PRING *et al.*, 1985 Fertility restoration and mitochondrial nucleic acids distinguish at least five subgroups among cms-S cytoplasms of maize (Zea mays L.) Theor. Appl. Genet. **71:** 5–15.

SMART, C. J., F. MONEGER and C. J. LEAVER, 1994 Cell-specific regulation of gene expression in mitochondria during anther development in sunflower. Plant Cell **6:** 811–825.

TZAGOLOFF, A., and C. L. DIECKMANN, 1990 PET genes of *Saccharomyces cerevisiae.* Microbiol. Rev. **54:** 211–225.

XU, H., R. B. KNOX, P. E. TAYLOR and M. B. SINGH, 1995 *Bcp1*, a gene required for male fertility in Arabidopsis. Proc. Natl. Acad. Sci. USA **92:** 210–2110.

Communicating editor: M. R. HANSON