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Chloroplast DNA evidence for non-random selection of females in an outcrossed population of soybeans [*Glycine max* (L.)] *

D. J. Lee, C. A. Caha, J. E. Specht, and G. L. Graef

Department of Agronomy, University of Nebraska, Lincoln, NE 68583-0915, USA

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Summary. Restriction fragment length polymorphisms (RFLPs) were used to assess chloroplast DNA (cpDNA) variation in a population of soybeans subjected to continuous cycles of forced outcrossing. This population was derived by crossing 39 female lines with four male-sterile (Ms_2ms_2) maintainer lines and advancing each generation by selecting only outcrossed seed borne on male-sterile (ms_2ms_2) plants. Analysis of the original 39 female lines revealed three groups based on cpDNA RFLPs. These three groups had been previously documented in soybeans, and the distribution of these groups among the female parents of this population was similar to that observed in germ plasm surveys of soybean. Thirty-four of the female parents had group I cpDNA, 3 had group II, and 2 had group III. Plants collected from this population after seven cycles of outcrossing were scored for four morphological traits (flower color, pubescence color, seed color, and pubescence type) known to be controlled by alleles at single nuclear loci. The frequencies of the phenotypes observed in this study indicated that the population underwent random mating with respect to flower and pubescence color, but deviated from random mating at the other two loci. Analysis of 158 of these same plants collected from the population after seven cycles of outcrossing revealed no individuals with group II or group III cpDNAs. The fixation of the group I cpDNA marker in this outcrossing population was judged to result primarily from selection against individuals in the population with the rare cpDNAs.

Key words: Glycine max – Chloroplast DNA – RFLP – Population – Male-sterile

Introduction

Population improvement in self-pollinated crop species such as soybeans (*Glycine max*) and barley (*Hordeum vulgare*) can be facilitated with the use of male sterility (Brim and Stuber 1973; St. Martin 1981; Suneson and Ramage 1963). The segregation of genetic male sterility in a population of ordinarily autogamous individuals provides a means for producing allogamous individuals because seeds produced on male-sterile (MS) segregants are the result of cross-fertilization events. New genetic combinations can therefore be generated without the labor-intensive task of performing hand emasculations and pollinations.

The value of male-sterile-facilitated population improvement methods may be limited by various short-term and long-term effects of the mating system on variation in the population (Jain 1969). In barley populations segregating for male sterility, natural selection changed the frequency of alleles at loci specific for isozymes and seed storage proteins (Alexander et al. 1990). Soybean populations segregating for male sterility have undergone successful recurrent selection for increased seed yield (Nelson 1987; Burton et al. 1990), changes in seed composition (Burton and Brim 1981), and other quantitative traits. Little is understood, however, about the effect of selection for genetic male sterility per se on the frequencies of specific alleles controlling other traits in soybeans. Selection for male sterility could affect allele frequencies at other loci as a result of linkage, epistasis, and/or pleiotropy. Natural selection on the phenotypic

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Correspondence to: D. J. Lee

variation found among male-sterile plants will also influence the frequency of alleles at other loci in these populations. These effects could dramatically shift allele frequencies at specific loci and dictate the utility of male-sterile facilitated recurrent selection. It is therefore important to assess the impact of the mating system on genetic variation in these populations.

Maintaining cytoplasmic variation can be a desired objective in population improvement programs (Weissinger and Albertson 1984). Because the inheritance of the mitochondria and plastids is maternal in most plant species, a continuous cyclic selection of female parents could affect cytoplasmic diversity in these populations. Cytoplasmic genes have been demonstrated for herbicide resistance, susceptibility to disease, and other agronomically or physiologically important traits (Oxtoby and Hughes 1989; Harvey et al. 1972; Robertson and Frey 1984; De Broux et al. 1990).

Extranuclear DNA markers are useful in assessing the effect of repetitive selection for cytoplasmic genotypes in a male-sterile population. Recent studies have demonstrated the existence of variation in the chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) among *G. max* lines (Close et al. 1989; Grabau et al. 1989) that can be used as markers defining specific cytoplasmic genotypes. Hatfield et al. (1985) demonstrated that the chloroplast DNA is inherited maternally in soybeans. Therefore, the number and frequency of cpDNA genotypes in populations with cpDNA variation can be used to assess changes in cytoplasmic diversity that occur within the population over time.

A population of soybeans segregating for a nuclear male-sterility gene was used in the study presented here. It had been subjected to forced outcrossing within the population for seven cycles by advancing only the progeny of male-sterile plants. The first objective of this research was to determine the number and frequency of distinct cpDNA variants among the original female parents of this population. The second objective was to determine the number and frequency of cpDNA genotypes in a random sample of individuals collected from the population after seven cycles of forced outcrossing.

Materials and methods

Population derivation

The derivation of the soybean population used in this study, SG1, has been described by Specht et al. (1985). The initial synthesis of SG1 was accomplished by making all possible two-way crosses between 39 female parental lines and four male parental lines. The pollen donors were male-fertile Ms_2ms_2 plants selected from near-isogenic, male-sterile maintainer lines of the adapted cvs 'Beeson' (Maturity Group II), 'Wells' (II), 'Williams' (III), and the genetic type T259H (III). The SG1 parents are listed in Table 1. Except for the Clark and Harosoy lines, Mandarin 13177, and PI 360.844 ('Raiden'), the female parents form the ancestral basis of contemporary soybean cultivars and cytoplasm (Specht and Williams 1984). The 156 parental matings were accomplished by manual pollinations made in summer field nurseries during the period of 1978–1981. The F_1 plants (genotypically 1 Ms_2Ms_2:1 Ms_2ms_2) were selfed and individually threshed to obtain F_2 seed.

Outcrossing cycles

For the first outcrossing cycle of SG1 in 1982, a composite of equivalent amounts of F_2 seed from each of the 156 matings (74,880 total seeds) were planted in an isolated nursery using the following procedure. The seed was divided into 240 planting packets, each containing two randomly selected F_2 seeds per cross (312 seeds). Half of these packets were planted in the 36.6×61.0 m nursery, which consisted of 120 two-row plots. The remaining 120 packets were planted 3 weeks later in the same nursery in a direction perpendicular to the first planting. The staggered planting dates created more opportunity for pollen transfer between plants of differing maturities and reduced the temporal limitations to effective random mating. The cross-hatched row pattern established by the planting method encouraged a more random, zig-zagging pattern of pollen movement by insect vectors. Insect-mediated pollen transfer from male-fertile (MF) plants generated the seeds produced on male-sterile (MS) plants. In subsequent cycles, a random selection of outcrossed seeds from the previous cycle were planted in an isolated nursery at a single planting date.

Harvest

At flowering, plants were randomly selected in the nursery, and a minimum of 200 MS plants were identified on the basis of the absence of pollen development. These plants were tagged and served as a reference for other MS plants at harvest. Because of the wide range of maturities among plants in this population, harvest was performed in late October after a hard freeze. Tagged MS plants exhibited a delayed senescence and reduced pod set compared to MF plants. Thus, additional MS plants could be identified and were randomly collected based on these criteria. No conscious selection was made on the basis of plant size, color, or architecture. Since disease also could have contributed to reduced pod set, plants with disease symptoms were discarded. MS plants were gathered, threshed, and this outcrossed seed was bulked to generate the seed used to produce the next cycle of the population. Approximately 2,000 MS plants were harvested during the first three cycles of this population. After the third cycle approximately 400 MS plants were harvested using the procedures described above. MF plants from each cycle were also gathered at random and threshed for storage as bulked seed in a cold room.

Plant material and DNA extraction

The seed source for the SG1 parental genotypes used in this study was the soybean germ plasm collection (R. Nelson, USDA-ARS, University of Illinois, Urbana, IL 61801 and E. Hartwig, USDA-ARS, Delta Branch Experiment Station, Stoneville, MS 38776). This collection was the same source for seeds used in the initial synthesis of SG1. No cytoplasmic heterogeneity was observed within a line, and cytoplasmic genotypes characterized in this study were consistent with those determined for parental lines in other studies (Close et al. 1989). Seeds of parental lines were grown in the greenhouse and growth chamber. Seeds harvested from MF plants of the initial cycle (cycle-0 selfed progeny) and seventh cycle (cycle-7 selfed progeny) of SG1 were grown in the field in 1990. Individual plants were tagged and young leaves were removed from parental

Table 1. Chloroplast genotypes, morphological trait phenotypes, and maturity groups of SG1 female parents

Name	Chloroplast group	Pubescence color	Pubescence density	Flower color	Seed coat	Maturity group
		Females				
Manitoba Brown	I	Tawny	Normal	White	Dark	00
Mandarin (Ottawa)	I	Gray	Normal	Purple	Light	00
PI 194.654	I*	Gray	Normal	Purple	Light	0
PI 180.501	I	Tawny	Normal	Purple	Light	0
Habaro	I	Gray	Normal	Purple	Light	0
Mandarin	I*	Gray	Normal	Purple	Light	0
Sac	I	Tawny	Normal	Purple	Light	0
Bansei (Ames)	I	Gray	Normal	Purple	Light	II
Harosoy-DT_2S	I	Gray	Normal	Purple	Light	II
Harosoy-Pd_1	I	Gray	Dense	Purple	Light	II
Harosoy-pa_1pa_2	I	Gray	Normal	Purple	Light	II
Kanro	I	Gray	Normal	Purple	Light	II
Korean	I	Tawny	Normal	White	Light	II
Mukden	I*	Gray	Normal	White	Light	II
Richland	I*	Gray	Normal	Purple	Light	II
Seneca	I*	Gray	Normal	White	Light	II
PI 65.338	I*	Gray	Normal	Purple	Light	II
A.K. Harrow	I*	Gray	Normal	White	Light	III
Dunfield	I*	Gray	Normal	White	Light	III
Illini	I*	Gray	Normal	White	Light	III
Jogun (Ames)	I	Gray	Normal	White	Light	III
Lincoln	I	Tawny	Normal	White	Light	III
Manchu	I*	Tawny	Normal	Purple	Light	III
Manchuria 13177	I	Gray	Normal	Purple	Light	III
PI 360.844	I	Tawny	Normal	Purple	Light	III
Clark-Dt_2S	I	Tawny	Normal	Purple	Light	IV
Clark-Pd_1	I	Tawny	Dense	Purple	Light	IV
Clark-pa_1pa_2	I	Tawny	Normal	Purple	Light	IV
Patoka	I	Gray	Normal	Purple	Light	IV
Sato-3	I	Tawny	Normal	White	Dark	IV
Haberlandt	I	Tawny	Normal	White	Light	VI
Hahto	I	Tawny	Normal	Purple	Light	VI
Ogden	I	Gray	Normal	Purple	Light	VI
Roanoke	I*	Gray	Normal	White	Light	VII
Aoda	II*	Gray	Normal	Purple	Light	III
Midwest	II	Tawny	Normal	Purple	Light	IV
Arksoy	II	Gray	Normal	White	Light	VI
Capital	III	Tawny	Normal	Purple	Light	0
Peking	III*	Tawny	Normal	White	Dark	IV
		Males				
Beeson Ms_2ms_2	I	Gray	Normal	Purple	Light	II
Wells Ms_2ms_2	I	Gray	Normal	Purple	Light	II
Williams Ms_2ms_2	I	Tawny	Normal	White	Light	III
T259H Ms_2ms_2	I	Tawny	Normal	White	Light	III

* First characterized by Close et al. 1989

genotypes, cycle-0, and cycle-7 selfed progeny. Leaf tissue was frozen after removal, lyophilized, and total DNA extracted according to the methods of Saghai-Maroof et al. (1984).

Restriction endonuclease digestion, gel electrophoresis

Total DNA samples were quantified using a fluorometer and DNA-specific dye, Hoechst 33258. DNA samples were digested to completion with *Eco*RI or *Cla*I (Promega) according to suppliers' instructions. Approximately 10 µg of digested total DNA per lane were loaded onto a gel containing 8 gl^{-1} agarose, and electrophoresis was carried out on a horizontal apparatus in TBE gel buffer (TBE = 0.089 *M* TRIS, 0.089 *M* boric acid, 0.002 *M* EDTA). Gels were stained with ethidium bromide and photographed under ultraviolet light. Mobilities of the *Hind*III-digested Lambda DNA markers were measured from the photograph.

Southern transfers and hybridizations

The transfer of restriction fragments from agarose gels to nylon membranes was performed according to the methods of Reed and Mann (1985). After the transfer was completed, filters were baked at 80 °C for 2 h. A library of mung bean (*Vigna radiata* L.) cpDNA *Pst*I and *Sal*I restriction fragments cloned into pBR322 served as hybridization probes for the filter-bound DNA frag-

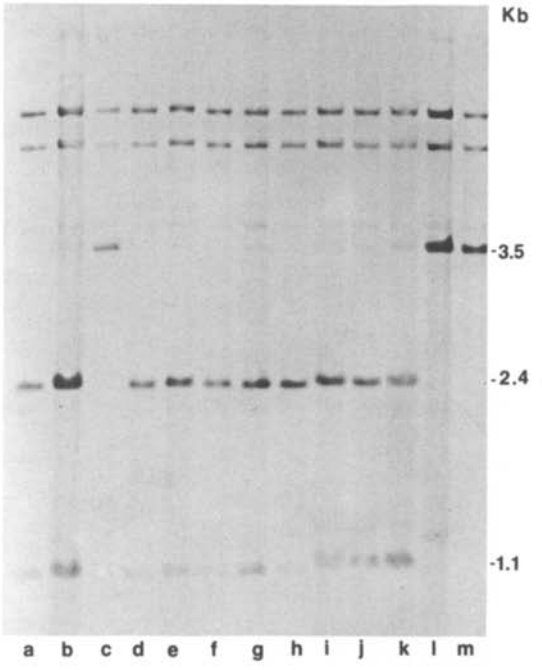

Fig. 1. Chloroplast DNA restriction fragment patterns that differentiate SG1 female parental genotypes. Mung bean cpDNA probe 4b hybridized to filter-bound *Cla*I-digested total DNA from the following genotypes: *a* Hahto, *b* PI 65.338, *c* Arksoy *d* Ogden, *e* Haberlandt, *f* Clark Dt_2S, *g* Clark Pd_1, *h* Clark pa_1pa_2, *i* Harosoy Dt_2S, *j* Harosoy Pd_1, *k* Harosoy pa_1pa_2, *l* Capital, *m* Peking. Genotypes with group I cpDNA have 2.4- and 1.1-kb fragments hybridized with this probe/enzyme combination while a 3.5-kb fragment is hybridized in lines with group II (Arksoy) or group III (Capital and Peking) cpDNAs

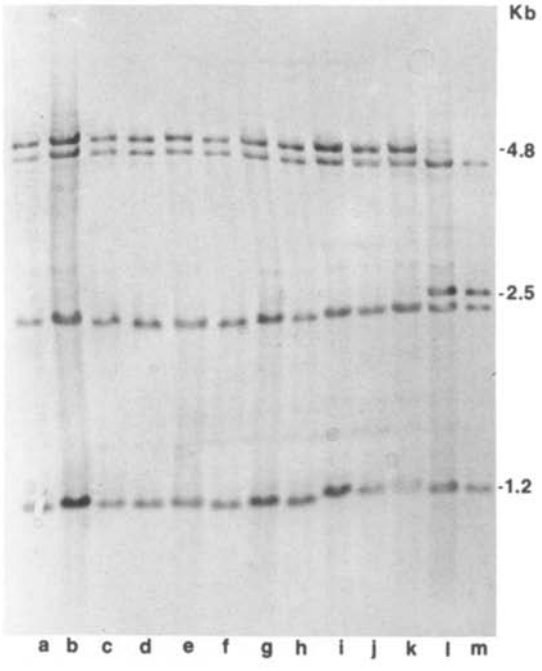

Fig. 2. Chloroplast DNA restriction fragment patterns that differentiate SG1 female parental genotypes. Mung bean cpDNA probe 4b hybridized to filter-bound *Eco*RI-digested total DNA from the following genotypes: *a* Hahto, *b* PI 65.338, *c* Arksoy, *d* Ogden, *e* Haberlandt, *f* Clark Dt_2S, *g* Clark Pd_1, *h* Clark pa_1pa_2, *i* Harosoy Dt_2S, *j* Harosoy Pd_1, *k* Harosoy pa_1pa_2, *l* Capital, *m* Peking. Genotypes with group III cpDNA have a 2.5-kb fragment hybridized by this probe/enzyme combination, while group I and group II cpDNAs have a 4.8-kb fragment

ments. This library covers the entire mung bean chloroplast genome (Palmer and Thompson 1981). Plasmids were isolated from *E. coli* hosts using the miniprep procedure of Birnboim and Doly (1979). CpDNA inserts were cut from low-melt agarose gels, and the DNA labeled by random priming (Feinberg and Vogelstein 1983) with digoxigenin-11-dUTP. Prehybridization, hybridization, filter washing, and detection of hybridized filter-bound fragments were performed in accordance with the instructions provided with the "Genius" nonradioactive system (Boehringer Mannheim). Sizes of the hybridized filter-bound fragments were determined by regression analysis using the method described by Schaffer and Sederoff (1981).

Morphological traits

Cycle-0 and cycle-7 selfed progeny were tagged and scored for three morphological traits, pubescence color, flower color, and pubescence density. Cycle-0 and cycle-7 seeds were also scored for seed coat color pattern. These traits are known to be controlled by alleles at single nuclear loci, with tawny pubescence (*T*), purple flower color (*W1*), dense pubescence (Pd_1), and light seed coat (*I*) being the dominant traits. Phenotypes of the parents for these traits are given in Table 1. All parental lines were homozygous at the loci controlling these morphological traits.

Results and discussion

SG1 female parental line cpDNA genotypes

Cytoplasmic diversity was present among the female parents of SG1. These 39 lines were classified into three cpDNA groups (Table 1) on the basis of restriction fragment length polymorphisms (RFLPs) visualized by two probe/enzyme combinations (Figs. 1, 2). A mung bean cpDNA *Pst*I/*Pvu*II restriction fragment designated as probe 4b (see Close et al. 1989 for probe designation) hybridized to *Cla*I fragments that were 2.4 + 1.1 kilobase pairs (kb) in group I cpDNAs and a 3.5-kb *Cla*I fragment in groups II and III (Fig. 1). Probe 4b hybridized to a 4.8-kb *Eco*RI fragment in groups I and II and a 2.5-kb *Eco*RI fragment in group III (Fig. 2). The chloroplast DNA RFLPs observed in this study confirmed the variation documented by Close et al. (1989) in 13 of the 39 female parents. The 26 soybean genotypes that had not been previously characterized were assignable to the group I, II, or III cpDNA marker classes based on the same probe/enzyme combinations utilized by Close et al. (1989). These accessions were primarily group I (23 accessions) compared to groups II and III (2 and 1 acces-

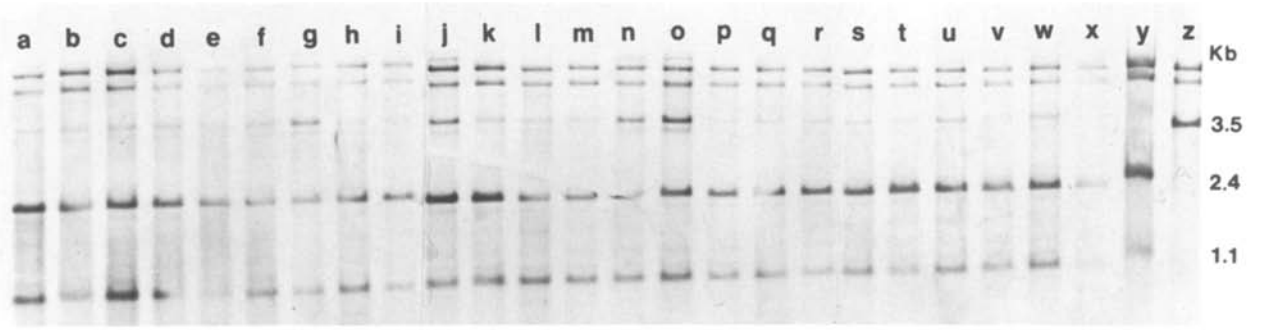

Fig. 3. Chloroplast DNA restriction fragment patterns of cycle-7 plants. Mung bean cpDNA probe 4b hybridized to filter-bound *Cla*I-digested total DNA from 25 representative plants and 1 female parental genotype (Peking, *lane z*). All cycle-7 plants have 2.4+1.1-kb fragments hybridized with this probe/enzyme combination, placing them in cpDNA group I. Group II and III cpDNAs have 3.5-kb fragments as demonstrated by Peking (*lane z*)

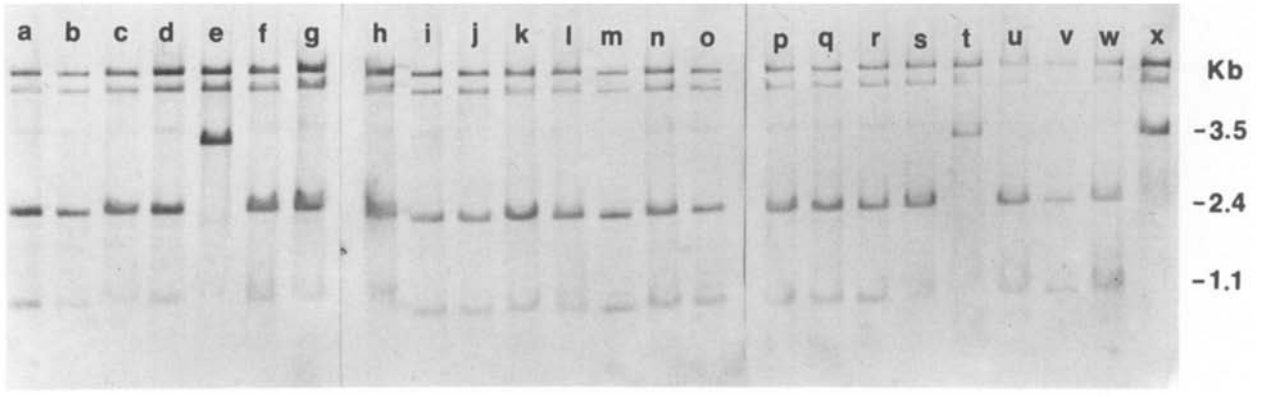

Fig. 4. Chloroplast DNA restriction fragment patterns of cycle-0 plants. Mung bean cpDNA probe 4b hybridized to filter-bound *Cla*I-digested total DNA from 23 cycle-0 plants and 1 female parental genotype (Peking, *lane x*). Cycle-0 plants with group I cpDNA have 2.4+1.1-kb fragments hybridized with this probe/enzyme combination. Peking and cycle-0 plants with group II or III cpDNA (lanes e and t) have 3.5-kb *Cla*I fragments hybridized with probe 4b

Table 2. Chi-square goodness-of-fit test of observed (O) versus expected (E) numbers of plants in cpDNA groups from cycle 0 and cycle 7 populations in SG1

Chloroplast type	Cycle 0			Cycle 7		
	O	E	χ^2	O	E	χ^2
Group I	49	46	1.48	158	137	24.29[b]
Group II+III	4	7		0	21	

[a] Expected numbers based on allele frequencies in the original parents and the assumption of no selection during selfing generations and random mating during outcrossing cycles
[b] Deviations significant at the 0.005 level

sion respectively). The female parents of SG1 were therefore representative of the cpDNA diversity and distribution in *G. max* documented in the germ plasm survey of Close et al. (1989).

SG1 cycle-7 progeny cpDNA genotypes

The effect of seven cycles of forced outcrossing on cpDNA variation in SG1 was assessed by analyzing randomly selected selfed progeny from cycle-7 plants. DNA from these 158 individuals was digested with *Cla*I and hybridized with the mung bean clone 4b. This revealed that all of the plants sampled had the group I restriction fragment pattern (Fig. 3). Consequently, an analysis of these individuals with the probe/enzyme combination that differentiated groups II and III was not necessary. A chi-square test for goodness-of-fit for this cpDNA data is given in Table 2. Expected cpDNA genotypes were based on the assumption of strict maternal inheritance of cpDNA and random selection of females during the seven cycles of forced outcrossing. The chi-square test for goodness-of-fit indicated that deviations between numbers of observed and expected cpDNA genotypes were significant at the $P<0.005$ level. This analysis demonstrated that the cpDNA variation present among the female parents of SG1 was absent in the population after seven outcrossing generations.

SG1 cycle-0 progeny cpDNA genotypes

The absence of cpDNA variation among cycle-7 individuals could be attributed to forces imposed on the popula-

tion during outcrossing or the loss of the infrequent group II and group III cpDNA variants prior to the onset of outcrossing. Consequently, restriction fragment patterns of cpDNA from 53 randomly selected cycle-0 selfed progeny were determined. *Cla*I digests were hybridized with probe 4b to differentiate group I from group II or III chloroplast genotypes. Pooling the two smaller chloroplast groups allowed for an expected number greater than five in the rare class for a chi-square test for goodness-of-fit (Table 2). Deviations between observed and expected numbers in the two classes indicated that the frequencies of chloroplast genotypes in the population prior to forced outcrossing were not significantly different from the frequencies found among the female parents of SG1. The plants analyzed from cycle 0 were F_2-derived F_3 progeny from the original parental matings. The presence of group II and III cpDNA genotypes among these randomly sampled plants (Fig. 4) revealed that the population maintained cpDNA diversity during it's initial assembly and that there was no apparent shift in cpDNA genotpye frequencies. Therefore, the fixation of SG1 for the group I cpDNA genotype occurred as a result of forces imposed on the population during the cycles of outcrossing.

Table 3. Chi-square goodness-of-fit test of observed (O) versus expected[a] (E) numbers of plants for morphological traits in SG1

	Cycle 0			Cycle 7		
	O	E	χ^2	O	E	χ^2
Pubescence color						
Tawny *T*___	193	196	0.10	233	230	0.09
Gray *tt*	185	182		165	168	
Flower color						
Purple *W1*___	164	179	3.42	237	243	0.48
White *w1w1*	119	104		114	108	
Pubescence type						
Dense *Pd1*___	25	12	14.46[b]	50	15	84.9[b]
Normal *pd1pd1*	354	367		346	381	
Seed coat color						
Light *I*___	397	388	6.57[c]	400	392	8.37[d]
Dark *ii*	3	12		0	8	

[a] Expected numbers based on allele frequencies in the original parents and the assumption of no selection during selfing generations and random mating during outcrossing cycles
[b] Deviations significant at the 0.005 level
[c] Deviations significant at the 0.05 level
[d] Deviations significant at the 0.01 level

Morphological traits

The effect of the mating system on nuclear alleles was examined by scoring SG1 plants for morphological traits. Random mating was observed at loci controlling two of these traits in SG1. Pubescence color and flower color phenotypes scored on cycle-0 and cycle-7 selfed progeny are shown in Table 3. Calculations of expected numbers for the phenotypes were based on the known genotypes of the SG1 parents (Table 1) and the assumption of no selection during two selfing generations and random mating during the cycles of outcrossing. Analysis of progeny from both cycles of this population demonstrated that the observed numbers of phenotypes for both morphological traits did not deviate significantly from expected numbers at the 0.05 level (Table 3). This analysis indicated that SG1 had the distribution of flower color and pubescence color phenotypes expected at the onset of outcrossing (cycle 0) and underwent random mating for seven cycles with respect to the nuclear alleles controlling flower color and pubescence color.

The frequency of rare alleles studied in the population, however, changed significantly. Individuals with the *ii* genotype were not observed in the cycle-7 population. In contrast, the frequency of plants with dense pubescence (*Pd1*_) increased relative to the parental frequencies in this population (Table 3). Consequently, rare alleles could be maintained in the population or lost, emphasizing that random mating (or non-random mating) is locus specific.

Basis of changes in cpDNA marker frequency

Changes that occur in the frequency of cytoplasmic types during the course of several generations of outcrossing in SG1 were judged to result primarily from selection. Migration, mutation, and drift were not forces with a significant influence on allele frequencies in SG1 based on the following arguments. (1) Frequencies of maternally inherited cpDNA restriction fragment patterns would not be changed by migration. Pollen-transferred alleles could be subjected to changes in frequencies imposed by migration if population isolation was not adequate. (2) The mutation of all group II and III cpDNAs to the group I genotype is unlikely for several reasons. Mutagenic conversion of the group II and III restriction fragment pattern to that of group I would require a specific nucleotide sequence change to the *Cla*I recognition site in a large number of independent events. Furthermore, the cpDNA is a highly conserved genome in plant species, and no new cpDNA RFLPs were detected in our analysis. (3) Fixation of this population by drift alone would be extremely unlikely. A population this size (minimum of 400 females) with organelle allele frequencies of 0.87 (group I cpDNA) and 0.13 (group II + group III cpDNA) would be expected to reach fixation for the predominant allele from drift alone in 244 generations (Birky et al. 1983). The observed fixation of SG1 to group I cpDNAs after seven generations of outcrossing would require forces imposed on this population in addition to that of drift.

By inference then, selection was the force that would have had the largest influence on the deviations in the cytoplasmic allele frequencies observed in SG1. Selection may have been direct, based on the expression of cytoplasmic alleles, or indirect, based on nuclear or cyto-nuclear effects.

The fixation of SG1 for group I cpDNAs may have been a consequence of the presence of unfavorable nuclear alleles in the original female parental lines with group II and group III cpDNAs. Selection against these alleles in the early cycles of outcrossing in SG1 would have led to correlated selection against the cytoplasms associated with these alleles as a result of genome linkage (Clegg et al. 1978). Nuclear alleles that influence maturity are likely to undergo selection in this population. Gutierrez and Sprague (1959) noted that time of flowering was a major factor causing departure from random mating in maize. The wide range of maturity groups found among the initial females of SG1 could impose limits to effective random mating between early and late individuals in this population. The group II and group III cpDNA types, however, were distributed among a range of maturity groups (Table 1). Thus, a loss of cpDNA diversity cannot be explained entirely by selection against plants in maturity groups that were too early (00, 0, and I) and/or too late (IV, V, and VI) for the growing environment at Lincoln, Nebraska.

Changes observed in SG1 cpDNA genotypic frequencies may reflect incompatibilities between nuclear and cytoplasmic genes. Nuclear-cytoplasmic incompatibilities could manifest themselves as phenotypic effects that limit outcrossed seed production on some MS plants in SG1. Cytoplasmic types that undergo cyto-nuclear interactions which restore male fertility in ms_2ms_2 genotypes would be selected against in the first cycle of outcrossing in this population.

The examination of intervening cycles of SG1 would reveal whether or not fixation for group I cpDNAs occurred immediately after one cycle of outcrossing or was a result of incremental increases in cpDNA group I frequency over successive cycles of mating. Polymorphisms in mtDNA (Grabau et al. 1989, 1992) have been used to further resolve the cytoplasmic types among SG1 parents. Additional studies with these markers will allow for a more definitive analysis of cytoplasmic genotype frequency in cycles of SG1.

The assessment of cpDNA and mtDNA diversity can be a critical objective in crop breeding programs concerned with cytoplasmically controlled traits such as susceptibility to disease, herbicide resistance, and male-sterility. The maintenance and utilization of cytoplasmic diversity could also be important in protecting a crop from vulnerability to new environmental challenges. The loss of different cytotypes in SG1 may therefore influence its utility in variety development. Further studies of SG1 should provide insight into the nature of changes in cytoplasmic diversity in male-sterile-facilitated breeding schemes.

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